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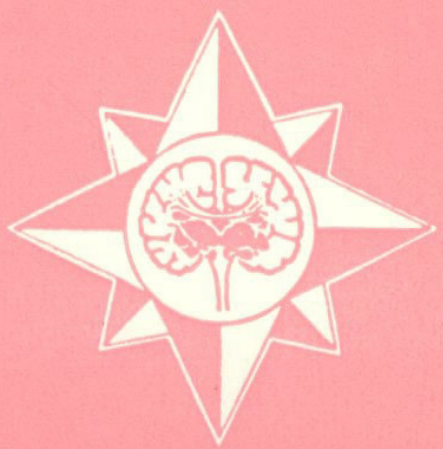

HOMAGE TO INDIA

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Editorial

Professor Chopra, Professor Toole, Colleagues:

Before beginning the scientific sessions we wish to rend due homage to our host country, India: which aiming to a brilliant evolution in Science and technology, has kept alive the spiritual values by which it has always been admired among the nations of the world.

A country is made by men, and its greatness derives from the outstanding personalities who like precious gems shine and enhance its history.

We shall mention just three of thems Rabindranath Tagore, Mahatma Gandi and Krishnamurti.

Rabindranath Tagore was a dreamer endowed with a clear inteligence, and a fine sensibility, he was a poet, a singer, an essay writer, actor and novelist, nationalist and internationalist, and a great patriot.

His fruitful life has trascendended the limits of India to belong to the whole humanity.

The charm of magic of his words has conquered every human heart, and one of his most beautiful songs "Jana Gana Mana" has become India's National Anthem.

He did not share the opinion that in order to attain peace it was necessary to avoid the pleasures of life, expressing his opinion in this poem:

Deliverance is not for me in renunciation,
"I feel the embrace of freedom
in a thousand bonds of delight.
No, I will never shut the doors of my senses.
The delight of sight and hearing
and touch will bear Thy delight.
Millions of living beings make
up the vast fair of this world
And you ignore it all as a child's play!"

Another gigantic personality who embodied the best of the Indian's spiritual virtues was Mahatma Gandhi, a brilliant man with a timid and humble personality, who with his two squalid arms held like pillars the moral of his whole country.

Gandhi said "The light persists among the shadows".

His greatest achievement was to demonstrate how the armed jorces may be defeated without arms.

He taught the people of his country that to inflict sufferings is less effective than to endure them.

He was a great conductor and a passionate patrict, step by step this man who combined an iron will with a fine moral sensibility rose to trascend all human standards.

Of him Einstein said "Future generations will have difficulty to believe that somebody like him of bone and flesh has walked on the Earth.

We will refer now to another outstanding man who honored his country, and this is Krisnamurty, whose history is a fascinating one.

During the twenties, rumors were spread throughout the world that in India has appeared an extraordinary beautiful adolescent destined to be a Messiah, and to incarnate God on earth, he received a careful education in England and at the same time devoutly trained to fulfill his task which he accepted. But as the years passed by, he firmy refused to be agod, to become one of the most famous and brilliant philosophers and educators being a real guide for the young people of his country who were fascinated by the intelligence with which he mantained dialogues with them stimulating their power of thinking.

He like Rabindranath Tagore believed that this beautiful world was created to be admired, enjoyed, being fully aware of every detail in nature which may bring delight to the senses, trascending in its material form to become a mystical experience.

Now I am ready to welcome all of you to the XIVth International Symposium of the Fulton Society on "The Neurobiology of Brain Implants".

I am most grateful to the authorities of the XIV th World Congress of Neurology for their kind cooperation and to the brilliant hosts of speakers who have honored us accepting our invitation to enhance our Symposium with their outstanding contributions to this topic.

Our Chairman's address is aimed to introduce those not familiar with the subject emphasizing some important facts connected with this new therapeutic tool to bring some hope to several intractable diseases.

It is evident that the purpose of restoring cerebral functions which are deteriorated by irreversible lesions that cannot be improved by medical or surgical treatment, have a better perspective by means of brain grafting.

During the last decade it has been possible to restore the function to the injured brain in animals, with grafting techniques.

The first brain graft experiment in a human being was performed at the Karolinska Institute in Stockholm in a patient with Parkinson's disease, during the winter of 1982.

Since the beginning of this century it could be observed that in certain mammals the grafting in the brain of foreign tissues is not rejected as it happens when the same tissue is grafted to other parts of the same body. The anterior chamber of the eye and the brain are organs in which tissue grafting is successfu-

lly made. This tolerance is due to the failure of graft associated antigens to insentivate the immune system of the host animal.

On the other side the blood brain barrier excluded substances from the brain and possibly also excludes certain substances from the rest of the body and protects the brain from the action of the body's immune systems.

The decrease in acetylcholine that appears to be present in Alzheimer's Disease has always been a real concern. The cholinergic neurons which are of utmost importance are those with cell bodies in the nucleus basalis and septum.

Experiments have been performed producing bilateral fimbria-fornix lesions, originating a disconnection of the cholinergic fibers passing from the septum to the hippocampus.

If these animals are submitted to learn a maze they show less ability than those rats without these kind of lesions. Septal grafts placed next to the hippocampus produces a partial restoration of acetylcholine input to the hippocampus, determines in the rats some recuperation of their capacity to learn mazes.

Taking into account if the same sphere of influence exists in the human as in the rat and graft influence it is a must throughout the striatum for recovery. Thus it would be necessary to implant many more grafts to correct the disability in the human being than those needed in rats.

In order to increase the benefits it is necessary to perform techniques so that the influence of the graft may be augmented. To recognise important growth factors which allows neuronal processes to become more widely distributed. If a method of dispersed cells is employed a better distribution of the graft tissue may be also obtained. To achieve that the neurons of the graft accede into the host brain.

For the grafting may be used the embyro or cultured cells. The first ones may be obtained from non-human primates.

We will remark that the experiments show that the tissues of the Central Nervous System are able to receive grafts, but the animal behavior is altered by such grafting while the normal neurotransmitter continues being manufactured.

It may be stressed the fact that other dopamine producing tissue introduced into the brain preserve its aptitude to originate It. This kind of research has demonstrated that it may be possible to make functional grafts.

The substantia nigra is the area of cells loss in Parkinson disease. The lack of dopamine innervation to the striatum is the reason why the symptomatology of the Parkinson disease appears, the tremor, rigidity, and mask-like facies.

Dopamine may be a neuromodulator setting the tonus or regulating thresholds for postsynaptic firing rather than triggering the firing of neurons. Important technological advances were developed aiming to the study of intracerebral implant of nucleus of the brain containing monoamines, Olson, Seiger and Hoffer made transplant to the anterior chamber of the eye which is endowed with immu-

nological properties similar to those of the brain and in this way the evolution of the tissue transplanted to the anterior eye chamber may be monitored by external observation intraccular locus coeruleus grafts were able to reinnervate the host iris.

With this technique Olson and colleagues practiced implants of more than one embryonic brain region to the anterior eye chamber with the purpose of studying developmental and trophic interactions between brain regions. Substantia nigra grafts may originate a vigorous growth response in the presence of a denervated target tissue even after they have matured.

In experiments performed in rats in which unilateral lesions of the substantia nigra was done, afterwards a transplant of the embryonic substantia immediately adjacent to the striatum was practiced in order to obtain a reinnervation. With this purpose in mind two different transplantation techniques were employed.

In the first, embryo's substantia nigra was transplanted to the lateral ventricle which is adjacent to the striatum. In these experiments substantia nigra demonstrated an excellent survival and gave origin to a partial dopaminergic reinnervation of the dorsal-medial caudate putamen adjacent to the grafts.

The second technique used to implant embryonic substantia nigra to the striatum implicates the use of previously prepared transplantation cavities.

By anspiration a small area of the cerebral cortex and corpus callosum that covers the striatum are extirpated. After a certain lapse of time in this cavity embryonic substantia is implanted. In these conditions a good survival of the implanted brain tissues is observed that may be related to a vascular bed formation in the cavity and to the secretion of trophic substances produced by the damaged brain tissue adjacent to the transplantation cavity.

I has been observed that many of the properties of transplanted substantia nigra neurons are similar to those of normal neurons of the substantia nigra and are inhibited by dopamine agonists and excited by dopamine antagonists.

When the embryonic brain tissue is dissociated into individual cells and directly injected into the host brain tissue good graft survival is achieved.

In the experiments performed by Dannett and his collaborators it has been observed that the grafts implanted into more than one site into the striatum were more successful than a single one which is in accord with the criterium that dissociated cell transplantation is a more appropiate method of increasing the behavioral efficacy of embryonic substantia nigra grafts. In animals suffering from unilateral lesions of substantia nigra it is originated spontaneous motor assymetry. Dannett and his colleagues observed that this disturbance is also diminished by substantia nigra grafts, rats with unilateral lesions of substantia nigra have been also observed that the animals are prone to ignore sensory stimuli controlateral to the lesion.

Performing substantia nigra grafts in such a way that the reinnervation of ventrolateral parts of striatum may be obtained, and improvement of the disturbance is observed.

It has been varified that when the grafts are placed in dorsomedial and ventral-lateral striatum using the dissociated cell technique the motor and sensitive disturbances are improved.

If bilateral lesion of substantia nigra is done the animals show severe perturbances, developing profound aphagia and adipsia, akinesia, rigidity and impaired grooming.

In these conditions it is more difficult to improve the aphagia and adipsia than the other manifestations observed. Even if substantia nigra grafts were placed into multiple locations in the striatum in animal whem it has been practiced bilateral substantia nigra lesions any kind of benefits concerning the aphagia and adipsia were observed.

Experiments performed in aged rats that received grafts of dissociated substantia nigra cells into the striatum or grafts of dissociated septal cells into the hippocampus have shown substantial improvement of the motor coordination in those who have received substantia nigra grafts. Regarding animals with septal grafts have not demonstrated any kind of change in their general behavioral activity.

Studies performed infering lesions of the mesolimbic dopaminergic neurons which innervate the nucleus accumbens generate deficiency in the exploratory behavior. It has been also observed a diminution in amphetamine - induced hyperactivity and an augmented response to apomorphine. Motor impairments in activity have not been observed. If the rats with these lesions are submitted to dissociated embryonic substantia nigra graft to the nucleus accumbens, may be appreciated an increase of exploratory behavior, spontaneous activity and responses to amphetamine.

Investigations performed by Olson have demonstrated that adrenal cromafin cells grafted to the anterior eye chamber partially reinnervated the host iris.

It has been observed that Adrenal cromaffin cells containing dopamine when they are transplanted to the lateral ventricle generate processes that did not reinnervate recipient brain.

It has been ascertained that grafts contain a large amount of catecholamines and these are disseminated into the host brain; while intraventricular adrenal medulla grafts are rich in high concentrations of dopamine.

In fact the adrenal medulla grafts in their functional activity produce dopamine which diffuse all over the brain, but lack of anatomical connections with the brain's cells.

With the aim of increasing the amount of adrenal chromaffin cells lasting longer into intrastriatal implantation, with this purpose several techniques have been elaborated.

Chronic infusion of nerve growth factor into the striatum were by Stromberg et al. using osmotic minipumps and dialysis fibers. In this way it is achieved that

a greater number of chromaffin cells are kept alive into striatal implantation and thus the effieciency of these grafts are better.

These success of these grafts depend in great measure on the age of the donor. The younger donors assure better results.

Adrenal medulla grafts obtained from ageing rat donors have meager results when they are implanted into the lateral ventricle. But when they are implanted directly into the striatum they become behaviorally effective but they do not reach the level obtained from young donors.

Probably there is a diminution in the capacity of cromaffin tissue from ageing donors to shift catecholamine production in favor of dopamine after transplantation.

Adrenal medulla could be successfully implanted into the striatum of primates, even if the number of surviving cells have been reduced.

The number of cromaffin cells which survive have also been studied, implanted into cortical cavities of primates and a great number of them hade been detected.

Casting a retrospective lock we will refer now to the first essays of intraparenchimal implantation of adrenal medulla in patients suffering from Parkinson's disease.

In 1985 Backland, E. E. et als. published their findings. In the two first patients cromaffin tissue was implanted into a single site in the striatum. Transient improvements were observed in both patients which disappeared after a few days.

In the cerebro spinal fluid the catecholamines showed also transient elevations. This may lead to the conclusion that the grafted cells survived for only a few days.

Adrenal medulla grafts have a long term survival after intraventricular transplantation. PC 12 Pheochromocytoma cell line was developed from a rat's adrenal medullary tumor.

These cells contain only norepinephrine and dopamine and for this reason they were considered for transplantation into the brain.

Investigations have been performed in order to obtain that the implanted grafts do not endure a complete rejection and to achieve that the implanted cells survive.

It is known that the nerve growth factor inhibits division and elicits differentiation of PC 12 cells, for that reason treating these cells with nerve growth factor after transplantation it is expected that long term survival of the grafted cells may be obtained.

The question has been posed if the neurites from substantia nigra grafts

develop synaptic contacts with host neurons or if they function just through a non specific release of dopamine.

It has been observed that the substantia nigra grafts do not induce a considerable reinnervation of the striatum but it may originate a more or less complete reinnervation within a very restricted anatomical sector. Substantia nigra grafts are integrated by dopaminergic cells which are akin to those of normal substantia nigra; in some aspects they resemble immature substantia nigra cells.

The criterion may be admitted that the reinnervation of the striatum, even if it is anatomically restricted, is quantitatively substantial.

If a single substantia nigra graft is implanted in the lateral ventricle, cortical cavities, or directly into the striatum, dissociated cells scarcely reinnervate a sector of the striatum.

The depth of penetration of catecholaminergic neurites from a substantia nigra graft in the lateral ventricle into the striatum may reach about 1 mm. in 3 weeks, after 2 years depths of penetration very seldom surpass 1.5 mm.

I has been observed that brain injury may exert a trophic effect upon the growth of substantia nigra dopaminergic neurites. It is feasible that in cavity transplantation experiments, the damage inflicted to the brain in the process of performing the cortical cavities may have stimulatory effect on the growth of graft derived neurities.

Brain graft may be rejected but normally they survive because they are unable to sensitive the host immune system. This is due to the fact that brain tissue contains very scarce histocompatibility antigens which are principally responsible for graft rejection.

There exists the possibility that brain tissues grafted deep into the brain are often out of reach by the effector cells of the immune system.

Parkinson's disease is originated by a lack of dopaminergic innervation of the striatum.

But in Huntington's disease neurons into the striatum die instead of losing their inputor.

An animal model of Huntinton's disease may be obtained by stereotactic injection of the neurotoxins kainic acid or ibotenic acid in a direct way into the striatum. In this way a loss of striatal neurons may be induced.

In order to obtain a recovery in the model animal, with striatal lesion Deckel and coworkers, Isacson and co-wokers have performed grafts of embryonic striatum tissue into the striatum of these animals. They observed that there was a clear survival of the grafted tissue.

It is presumed that the transplant may have restored local circuits that were damaged by the lesion. Longer pathways were not replaecd.

It is admitted that the transplant may only elicit a chemical substance which acts upon the host brain or may exert some mechanical or trophic action in the neighboring tissues.

It is feasible that implanted tissue sheds important neurotrophic substance which kept alive axotomized host neurons and the reinervation of normal target neurons. Another possibility could be that the transplanted tissue may be a provider of neurotransmitter substances that may improve the functioning of the host brain.

Prof. Dr. VICTOR SORIANO.

The Use of Fetal Neocortical Transplants in the Repair of Corttcal lesions in Newborn Rats. An Anatomical and Electrophysiological Study.

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INTRODUCTION

Numerous studies demonstrating the successful grafting of fetal neuronal tissue into the central nervous system of newborn or adult animals suggest the therapeutic potential of transplantation techniques in the repair of nervous system dysfunction. Indeed, enthusiasm for this potential has been sparked recently by clinical reports showing a marked allevation of Parkinsonian symptoms after the grafting of fetal dopamine neurons (Freed et al., 1990; Lindvall et al., 1990; Lindvall et al., 1989). These clinical findings may be considered to represent the culmination of intensive research efforts from several laboratories around the world. While the many studies done in this global effort have focused on the use of neuronal grafts in transmitter replacement therapy, additional studies have examined the use of neuronal transplants in the reconstruction of damaged neuronal circuits. In this regard several laboratories including our own have grafted fetal neocortical tissue into the cerebral hemisphere of newborn or adult rats that previously had sustained neocortical lesions. This report summarizes our anatomical and electrophysiological findings concerning the connectivity of such transplants.

METHODS

Transplantation procedures. Long-Evans black-hooded or Wistar albino rats were used in this work. Newborn rats were anesthetized by hypothermia and adult rats with sodium pentobarbital (50 mg/kg) or ketamine hydrochloride (100 mg/kg). Donor tissue was obtained from rat fetuses surgically removed their mothers primarily at 14-16 days gestation. With the aid of a surgical microscope the membranous skull and meninges overlying the fetal cerebral cortex were carefully removed. A 2-3 mm^2 plate of presumptive sensorimotor cortex was excised and placed in sterile Ringer's solution. The fetal tissue was then aspirated into a glass cannula attached to a 50 µl Hamilton syringe and then injected into a small neocortical lesion cavity made in the *newborn* recipient immediately before transplantation. In the 0-1 day old neonate, lesions were made by aspiration 1-2 mm from the midline just rostral to the coronal suture. The grafts were held in place by a bone flap. In some cases the transplants were placed into the host cortex immediately caudal to the lesion cavity.

In addition to the newborn hosts used in most of our studies, current ongoing experiments involve the placement of neocortical block grafts into small neocortical aspiration cavities made in *adult* rats. Also, neocortical cell suspension grafts were placed into cortical lesions made 3-5 days previously by multiple 0.4 µl injections of the excitotoxin N-methyl-D-aspartate (NMDA; 36 mg/ml/injection) into the cerebral cortex just rostral to the coronal suture and 2-5 mm from

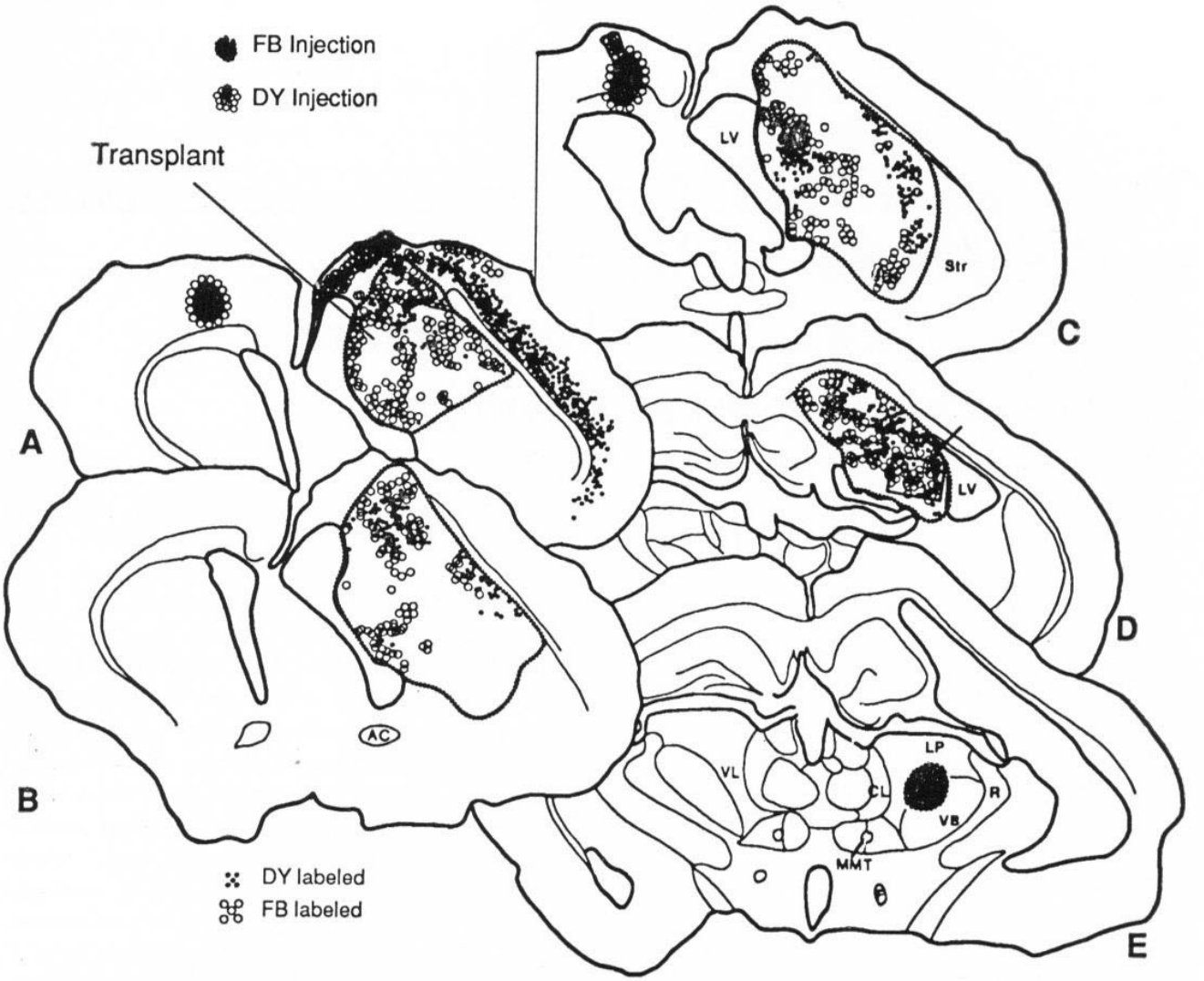

Fig. 1. — *Selected camera lucida drawings in rostral-caudal (A-E) sequence showing retrogradely-labeled transplant neurons after fluorescent dye injections. Host rerograde cortical labeling is depicted only in section. A. (Modified from (Castro et al., 1985).)*

the midline. The suspension grafts were made from blocks of fetal cortical tissue by incubating the blocks in a trypsin solution for 20 min at 37°C (Björklund et al., 1983). The blocks were then placed into a glucosesaline medium where they were repeatedly and gently pipetted with two fire-polished Pasteur pipettes of decreasing diameters. Several injections of the resulting cell suspension were then placed into the host cortical lesion area using a Hamilton syringe fitted with a glass cannula.

After transplantation, the dams were subsequently killed by an intracardiac injection of sodium pentobarbital.

Anatomical procedures. The host-transplant connectivity of grafts placed into newborn host was examined by methods on the retrograde transport of the fluorescent dyes Fast Blue (FB) and Diamidino Yellow (DY). At maturity the rats were secured in a stereotaxic instrument, and the skull reopened and inspected for a transplant. Using a microsyringe fitted with a glass pipette tip drawn to a diameter of 30-50 μm, 0.01-0.03 μl of 2 % FB or DY was be injected into the transplant or various areas of the host brain including the cerebral cortex opposite the graft, the ipsilateral thalamus, the spinomedullary motor decussation or the cervical spinal cord enlargement. Animals were sacrificed 2-8 days later and the brains removed and processed histologically according to routine methods. The distribution of retrogradely-labeled fluorescent neurons in the host brain or in the transplant was plotted using and X-Y

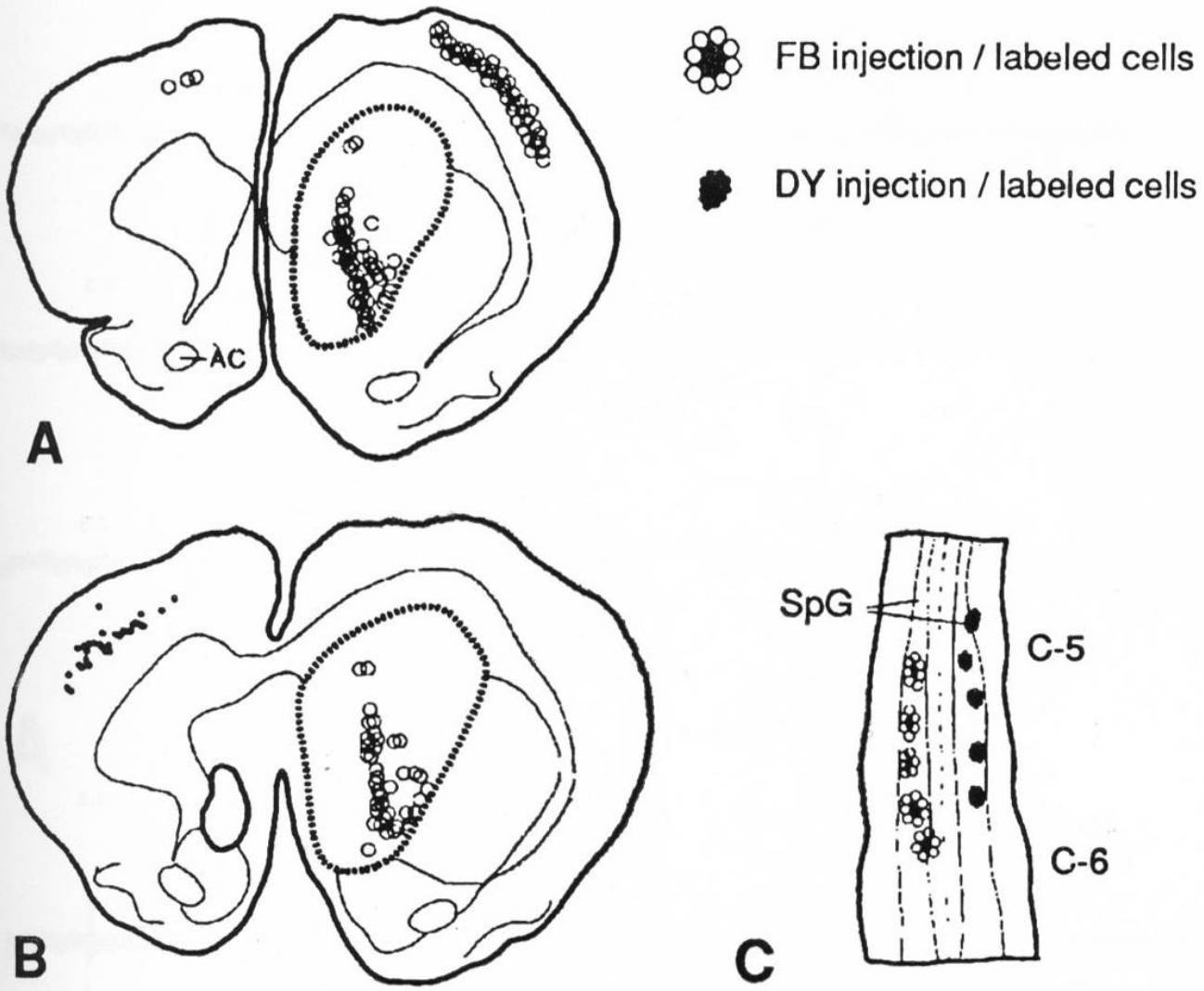

Fig. 2. — *Camera lucida drawings showing host and transplant retrograde neuronal labeling seen in two selected sections (A and B) after fluorescent dye injections into the spinal cervical enlargement as illustrated in longitudinal section (C). (Modified from (Castro et al., 1987).)*

digitizing system (Minnesota Datametrics) mounted on a Leitz Orthoplan microscope interfaced to an Apple computer.

Electrophysiological procedures. Animals receiving transplants as neonates or adults two or more months previous were examined for transplant unit activity evoked by thalamic or peripheral stimulation. A sharpened, glass-insulated tungsten wire with a 5-15 μm tip exposure served as the recording electrode inserted into the transplant, and a similar eletcrode with a 100 μm tip exposure served as the stimulating electrode inserted into the host ipsilateral thalamus (Neafsey, 1981). Transplant single unit activity was recorded in the transplant and adjacent host cortex while the thalamus or forepaws were electrically stimulated. A more thorough explanation of these methods has recently been published (Neafsey et al., 1989).

RESULTS

Neocortical transplants placed into newborn hosts demonstrated an 80-90 % survival in the several hundred cases done in our laboratory. When grafted into cortical lesions, they typically filled the lesion cavity, and in Nissl stain preparations they often displayed characteristic bands and whorls of neurons (Castro et al., 1987; Castro et al., 1988; Castro et al., 1985; Chang et al., 1984). Block grafts placed into lesion cavities or suspension grafts

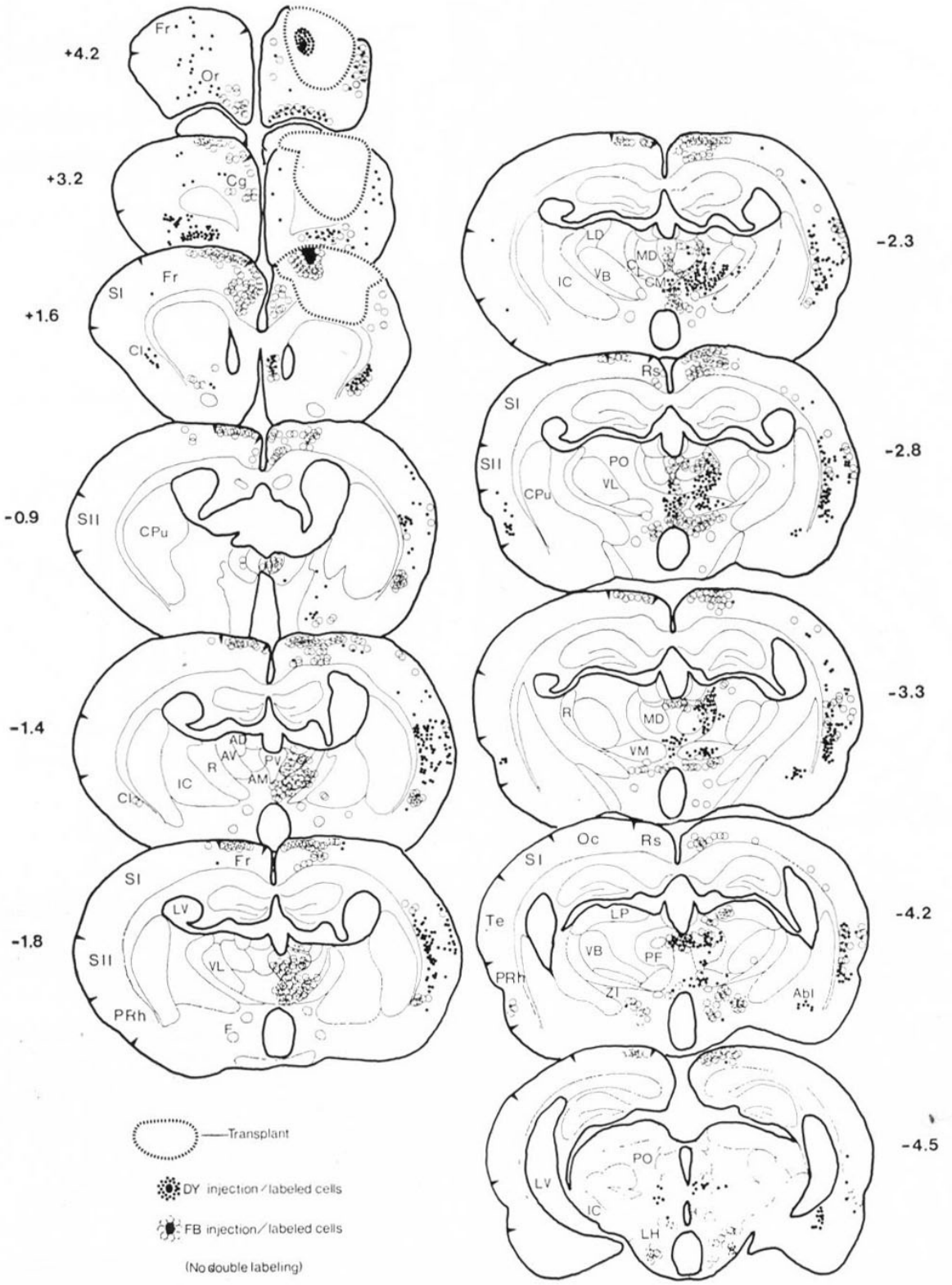

Fig. 3. — *Camera lucida drawings showing host retrograde neuronal labeling after fluorescent dye injections into a neocortical transplant placed into a cortical lesion cavity made at birth. Numbers are in reference to mm from bregma. (From (Castro et al., 1989). Figure used with permission).*

injected into excitotoxic lesions also showed good survival but we have not done a sufficient number of cases to provide a meaningful transplant survival rate. However, as with grafts placed into neonates they can be readily recognized in Nissl stain.

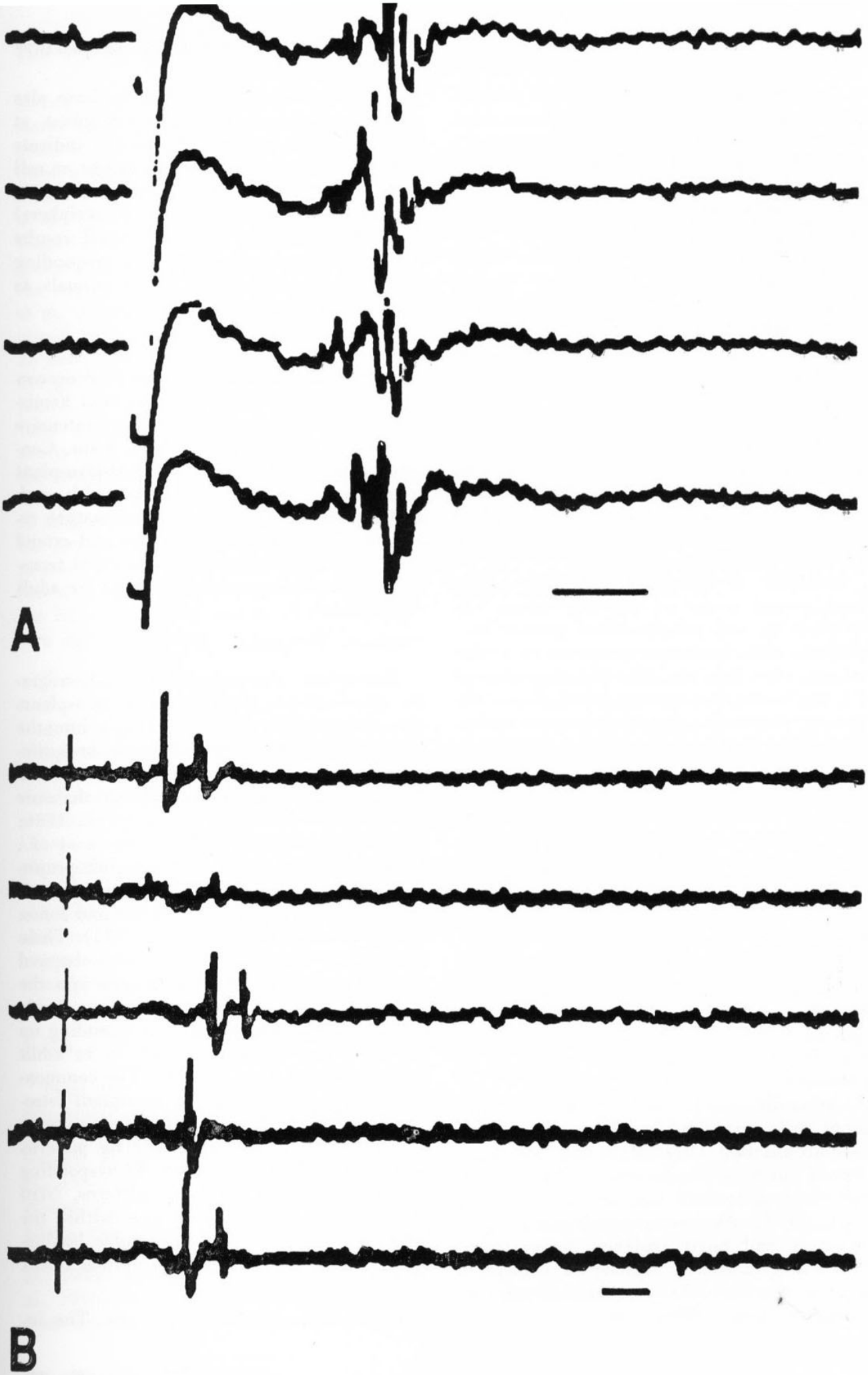

Fig. 4. — *Representative raster displays of responses of single units in transplants to electrical stimulation of the thalamus (A) or contralateral forepaw (B). Time bar in each panel equal* E *ms. (Modified from (Neafsey et al., 1989).)*

Anatomical Studies of transplant connectivity done in our laboratory have been confined so far to animals receiving neuronal grafts at birth. Transplant *efferent* projections into the host brain were demonstrated by the presence of numerous retrogradely-labeled neurons within the grafts after tracer injections into the recipient cortex and thalamus (Fig. 1). Similar to the striking supra- and infragranular layering pattern seen in the host cortex after such injections (Fig. 1A), transplant labeling shows evidence of this topography in some regions of the graft. Transplant efferent labeling was also observed after tracer injections into the spinomedullary motor decussation or the cervical spinal cord (Fig. 2). Resembling the host certical labeling observed after such injections, transplant labeling in some cases was confined to a linearly arranged band of cells.

Evidence of transplant *afferents* arising from several areas of the host brain was revealed by the presence of labeled host neurons after tracer injections into the transplants (Fig. 3). This labeling showed a wide variation in number between the various areas labeled and between individual animals. Relatively dense labeling was found in several thalamic nuclei and the ipsilateral cerebral cortex with less labeling found in the contralateral cortex. Sparse to light labeling was found in several other areas including the claustrum, zona incerta, lateral hypothalamus, basal forebrain, amygdala, ventral tegmentum, locus coeruleus and midline raphe.

Electrophysiology. Transplant unit activity has been examined primarily in animals receiving grafts at birth. Electrical stimulation of the host ventral thalamus evoked activity in approximately 90 % of the 63 transplant neurons sampled (Fig. 4A). Additionally, nearly two-thirds of the transplant cells responded to contralateral forepaw stimulation (Fig. 4B), and the latencies of these responses were comparable to recordings of normal host neurons (Neafsey et al., 1989). Analyses of median interspike intervals and burst indices demonstrated that the spontaneous activity of transplant neurons was not different than host neurons (Neafsey et al., 1989).

In current ongoing studies we have also examined animals receiving transplant at maturity. Our preliminary results indicate that block grafts into lesion cavities or cell suspension grafts will respond to thalamic stimulation and in a few cases to peripheral stimulation (Fig. 5). These initial results demonstrate considerably fewer responding units after grafting into adult animals as compared to newborn recipients.

DISCUSSION

Our results demonstrate that fetal neocortical tissue grafted into the cerebral hemisphere of newborn rats will form extensive interconnections with the host brain. Continuing studies indicate that host-transplant connections are also established with such grafts placed into the brains of mature recipients. These findings confirm and extend several other studies on fetal cortical transplants placed into the newborn or adult recipients.

Newborn recipients

Transplant efferent projections. Retrograde neuronal labeling within transplants after injection of fluorescent dyes into the host contralateral cerebral cortex or ipsilateral thalamus corresponds to previous work demonstrating transplant callosal, thalamic and striatal efferents (Chang et al., 1986; Floeter and Jones, 1984; Fonseca et al., 1988). Similarly, evidence for graft projections to the spinal medullary junction concurs with previous work (Floeter and Jones, 1984; Stanfield and O'Leary, 1985). These projections and particularly those observed after fluorescent dye injections into the cervical spinal cord represent a remarkable growth of transplant efferents extending up to 25-30 mm when measured in an adult animal (Castro et al., 1987). The commonly observed distribution of transplant retrogradely-labeled neurons into band patterns resembled the laminar layering patterns seen within the host cortex. Corresponding to these apparent banding patterns, Nissl stained sections revealed areas within the transplants that showed discernible lamination patterns quite similar to normal cortex (Castro et al., 1987).

Transplant afferent projections. The in-

jection of tracers into transplants demonstrated graft afferents arising from many areas of the host brain. These results indicated a more extensive pattern of transplant afferents than previously reported (Chang et al., 1984; Chang et al., 1986; Sharp and Gonzalez, 1986). These inputs originated in general from areas that normally project to the cortical location of the grafts (Castro et al., 1989) suggesting some measure of circuit reconstruction after cortical ablation. In cases where two dyes were injected into the grafts, different retrograde labeling patterns corresponding to each of the injections indicates that different host brain areas may distribute differentially within the transplants. This apparent topography of inputs is further reflected in the paucity of double-labeled neurons. In this regard, the occasional double-labeled neuron found primarily within the basal forebrain, locus coeruleus and midline raphe corresponds to the highly branched nature of these projection systems (Castro et al., 1988).

The possible functional significance of cortical transplantation is demonstrated by transplant unit recordings. Transplant activity evoked by ventral thalamic stimulation indicates the presence of electrophysiologically active inputs corresponding to the anatomical evidence for these connections. Of special interest, however, is the transplant unit activity evoked by electrical stimulation of the contralateral forepaw. The observed normal response latencies and the absence of abnormal spontaneous activity suggest that the grafts have become functionally integrated into the host's somatosensory pathways.

Adult recipients

Fetal neocortical tissue grafted as blocks into small lesion cavities were observed to survive and grow as reported in other experiments (Bragin et al., 1988; Dunnett et al., 1987; Labbe et al., 1983). Anatomical studies have demonstrated that such transplants will receive inputs from the host thalamus (Gonzalez et al., 1988; Labbe et al., 1983) and basal forebrain (Sorensen et al., 1990). Additionally, cell suspensions of fetal neocortex were also found to survive injection into excitotoxic cortical lesions and to receive cholinergic inputs (Sofroniew et al., 1986).

Our preliminary electrophysiological work reported here indicates that block or suspension cortical grafts placed into the ablated adult cortex will respond to thalamic or peripheral electrical stimulation. These findings concur with other work in which similar grafts were found to be responsive to vibrissae and tactile stimulation (Bragin et al., 1988). The apparent paucity of such units found in our studies as compared to the number found using newborn hosts may reflect a less well integrated transplant. Since rather short lesion-transplant intervals were used in our initial experiments, continuing studies are examining whether longer intervals may increase host-transplant connectivity.

Additional considerations

The use of cortical transplants in lesion repair is also suggested by studies of thalamic atrophy. An important aspect of the effects of central nervous system lesion is the secondary neuronal degeneration that commonly occurs in areas having a dense input to the lesion area. An example of this is the striking thalamic degeneration that occurs after neonatal cortical lesions. However, the grafting of fetal neocortical tissue has been found to ameliorate this atrophy, presumably by providing the appropriate target and trophic factors needed to rescue the thalamic projections deprived of their normal target by the lesion (Sharp and Gonzalez, 1986; Sorensen et al., 1989). In similar work using adult hosts, cortical grafts were able to prevent the lesion-induced atrophy of basal forebrain neurons (Sofroniew et al., 1986; Sorensen et al., 1989).

The transplant technique unavoidably disrupts the host vasculature particularly when grafts are placed into lesion cavities. Therefore the observed vascularization of cortical transplants and the re-establishment of a blood-brain barrier (Klausen et al., 1990; Swenson et al., 1989) is critical to transplant survival. The formation of the blood-brain barrier as also observed with other intracerebral transplants (Azcoitia et

al., 1989; Broadwell et al., 1989; Wiegand and Gash, 1988) is believed to reflect the lack of immunological rejection of neuronal homografts.

Speculations concerning the functional contributions of neuronal transplants imply the presence of appropriate cellular receptors. In this regard estrogen receptor density in fetal neocortical grafts placed into the newborn host was found to be considerably higher after two weeks survival in comparison to four weeks survival (Pedersen et al., 1990). Interpretation of these data in terms of the theoretical age of the transplants shows that the early surge in transplant estrogen receptor density corresponds to the surge found in normal cortical development (O'Keefe and Handa, 1990). These findings suggest that the receptor density was regulated by mechanisms intrinsic to the grafts and not by the host brain environment. Previous work similarly demonstrated that estrogen receptors in hypothalamic grafts developed independently of the gonaaal steroid environment (Paden et al., 1985). Other work on neocortical grafts placed heterotopically into the fourth ventricle of adult rats showed high densities of receptors for the peptides bombesin and vasoactive intestinal peptide (Getz et al., 1987). While abnormally high in density as compared to normal cortex, the associated peptides were suggested to play a role in the growth and vascularization of the transplants.

Perhaps the most important question regarding neuronal transplants concerns their potential positive or negative effects on behavior. Using newborn hosts, cortical grafts were found to recover deficits in skilled forelimb-digital usage induced in rats by neonatal cortical lesions (Plumet et al., 1990; Sandor et al., 1990). Also of importance is work showing that neocortical transplants did not disrupt the skilled motor movements displayed by rats walking an elevated narrow beam (Swenson et al., 1990). Additionally, cortical grafts placed into adult hosts reversed cortical lesion-induced deficits on spatial alternation or visual discrimination tasks (Dunnett et al., 1989; Labbe et al., 1983; Stein et al., 1985).

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SUMMARY

Fetal neocortical tissue was grafted into newborn or adult rats. In newborn recipients, anatomical studies showed transplant efferents to the contralateral cortex, thalamus and spinal cord. Numerous afferents from many areas of the host brain were also observed. Inputs from the thalamus were confirmed by unit activity evoked by electrical stimulation of the thalamus and forepaw. Similar unit activity was also seen in transplants placed into adult rats.

RESUMEN

Tejido fetal neocortical fué injertado en ratas recien nacidas o adultas. En los recipientes recién nacidos los estudios anatómicos demostraron el tejido injertado en el hemisferio cerebral opuesto, el tálamo y la médula espinal. Numerosos aferentes que provenían de varias regiones del cerebro anfitrion han sido observados. Las descargas originadas en el tálamo han sido identificadas por una actividad unitaria provocada por estimulación del tálamo y sector anterior. Una actividad unitaria similar ha sido igualmente observada entre los injertos colocados en ratas adultas.

RÉSUMÉ

Du tissu foetal provenant du neocortex fut greffé sur des rats nouveau nés ou adultes. Chez les nouveau nés des études anatomiques ont démontré le tissu greffé dans l'hémisphère cerebral opposé, le thalamus et la moelle épiniére. De nombreaux

afferents provenant de plusieurs régions du cerveau hôte ont aussi été observés. Des décharges originant du thalamus ont été identifieés par une activité unitaire provoqueé par stimulation du thalamus et de la patte d'avant. Une activité unitaire similaire a également été observeé parmi les greffes placées dans les rats adultes.

ZUSAMMENFASSUNG

Fetaler Neocortex wurde in das Gehirn von neugeborenen und erwachsenen Ratten transplantiert. Die anatomischen Untersuchengen an erwachsenen Teiren, die Transplantate als Jungtiere erhielten, zeigten efferente Fasern von den Transplantaten zum contralateralen Cerebrum, zum Thalamus, und zum Rückenmark. Zahlreiche afferente Fasern von verschiedenen Gebieten des Empfängergehirns wurden auch in den Transplantaten festgestellt. Electrophysiologische Untersuchungen der "unit activiti" bestätigten die thalamischen Fasern, die durch electrische Reizung des Thalamus und der Vorderpfote im Transplantat abgeleitet wurden. Die gleiche Art von Strömen wurde auch in den Transplantaten in erwachsenen Empfängern abgeleitet.

ABBREVIATIONS

Abl	basolateral amygdala
AC	anterior commissure
AD	anterodorsal nucleus of the thalamus
AM	anteromedial nucleus of the thalamus
AV	anteroventral nucleus of the thalamus
Cg	cingulate cortex
Cl	claustrum
CL	central lateral nucleus of the thalamus
CM	central median nucleus of the thalamus
CPu	caudate-putamen
F	fornix
IC	internal capsule
LD	lateral dorsal nucleus of the thalamus
LH	lateral hypothalamus
LP	lateral posterior nucleus of the thalamus
LV	lateral ventricle
MD	medial dorsal nucleus of the thalamus
MMT	mammillothalamic tract
Oc	occipital cortex
Or	orbital cortex
PO	posterior nucleus of the thalamus
PRh	perirhinal cortex
PV	paraventricular nucleus of the thalamus
R	reticular nucleus of the thalamus
Rs	retrosplenial cortex
SpG	spinal cord gray
SI	primary somatosensory cortex
SII	secondary somatosensory cortex
Te	temporal cortex
VB	ventrobasal nucleus of the thalamus
VL	ventrolateral nucleus of the thalamus
VM	ventromedial nucleus of the thalamus
ZI	zona incerta

REFERENCES

1. *Azcoitia, I., Sorensen, T., Zimmer, J., Alvarez, A.* and *Aznar, S.*: The blood-brain barrier in intracerebral grafts of nervous tissue. Euro. J. Neurosci., Suppl. 2: 138. 1989.
2. *Björklund, A., Gage, F., Stenevi, U.* and *Dunnett, S.*: Intracerebral grafting of neujronal cell suspensions. I. Introduction and general methods of preparation. Acta Physiol. Scand., Suppl., 522: 1-7. 1983.
3. *Bragin, A. G., Bohne, A.* and *Vinogradova, O. S.*: Transplants of the embryonal rat somatosensory neocortex in the barrel field of the adult rat: responses of the grafted neurons to sensory stimulation. Neurosci., 25: 751-758. 1988.
4. *Broadwell, R., Charlton, H., Ganong, W., Salcman, M.* and *Sofroniew, M.*: Allografts of CNS tissue possess a blood-brain barrier

I. Grafts of medial preoptica area in hypogonadal miced. Exp., Neurol., 105: 135-151. 1989.

5. *Castro, A., Tonder, N., Sunde, N.* and *Zimmer, J.*: Fetal cortical transplants in the cerebral hemisphere of newborn rats. A retrograde fluorescent analysis of connections. Exp. Brain Res., 66: 533-542. 1987.

6. *Castro, A., Tonder, N., Sunde, N.* and *Zimmer, J.*: Fetal neocortical transplants grafted to the cerebral cortex of newborn rats receive afferents from the basal forebrain, locus coeruleus and midline raphe. Exp. Brain Res., 69: 613-622. 1988.

7. *Castro, A. J., Sorensen, J. C., Tonder, N. Bold, L.* and *Zimmer, J.*: Fetal neocortical transplants grafted into cortical lesion cavities made in newborn rats receive multiple host afferents. A retrograde fluorescent tracer analysis. Restor. Neurol. Neurosci., 1: 13-23. 1989.

8. *Castro, A. J., Zimmer, J., Sunde, N. A.* and *Bold, E. L.*: Transplantation of fetal cortex to the brain of newborn rats: A retrograde fluorescent analysis of callosal and thalamic projections from transplant to host. Neurosci. Lett., 283-288. 1985.

9. *Chang, F., Steedman, J.* and *Lund, F.*: Embryonic cerebral cortex placed in the occipital region of newborn rats makes connections with the host brain. Dev. Brain Res., 13: 164-166. 1984.

10. *Chang, F. F., Steedman, J. G.* and *Lund, R. D.*s The lamination and connectivity of embryonic cerebral cortex transplanted into newborn rat cortex. J. Comp. Neurol., 244: 401-411. 1986.

11. *Dunnett, S. B., Ryan, C. N., Levin, P. D., Reynolds, M.* and *Bunch, S. T.*: Functional consequences of embryonic neocortex transplanted to rats with prefrontal cortex lesions. Behav. Neurosci., 4: 489-503. 1987.

12. *Floeter, M.* and *Jones, E.*: Connections made by transplants to the cerebral cortex of rat brains damaged in utero. J. Neurosci., 4: 141-150. 1984.

13. *Fonseca, M., DeFilipe, J.* and *Fairen, A.*: Local connections in transplanted normal cerebral cortex of rats. Exp. Brain Res., 69: 387-398. 1988.

14. *Freed, C. R., Breeze, R. E., Rosenberg, N. L., Schneck, S.A., Wells, T. H., Barrett, J. N., Grafton, S. T., Huang, S. C., Eidelberg, D.* and *Rottenberg, D. A.*: Transplantation of human fetal dopamine cells for Parkinson's disease - results at 1 year. Archives of Neurology, 47: 505-512. 1990.

15. *Getz, R. L., Moody, T. W.* and *Rosenstein, J. M.*: Neuropeptide receptors are present in fetal neocortical transplant. Neurosci. Lett. 79: 97-102. 1987.

16. *Gonzalez, M., Sharp, F.* and *Loekn, J.*: Fetal frontal cortex transplanted to injured moor/sensory cortex of adult rats: reciprocal connections with host thalamus demonstrated with WGA-HRP. Neurol., 99: 154-165. 1988.

17. *Klausen, B., Swenson, R. S., Shaw, P. L., Zimmer, J.* and *Castro, A. J.*: Development of the blood-brain barrier in neocortical transplants grafted into cortical lesion cavities in newborn rats. Europ. J. Neurosci., In press: 1990.

18. *Labbe, R., Firl, A., Mufson, E.* and *Stein, D.*: Fetal brain transplants: reduction of cognitive deficits in rats with frontal cortex lesions. Science, 221: 470-472. 1983.

19. *Lindvall, O., Brundin, P., Widner, H., Rehncrona, S., Gustavii, B., Frackowiak, R., Leenders, K. L., Sawle, G., Rothwell, J. C., Marsden, C. D.* and *Björklund, A.*: Grafts of fetal dopamine neurons survive and improve motor function in Parkinson's disease. Science, 247: 574-577. 1990.

20. *Lindvall, O., Rehncrona, S., Brundin, P., Gustavii, B., Astedt, B., Widner, H., Lindholm, T., Björklund, A., Leenders, K. L., Rothwell, J. C., Frackowiak, R., Marsden, C. D., Johnels, B., Steg, G., Fredman, R., Hoffer, B. J., Seiger, A., Bygdeman, M., Stromberg, I. and Olson, L.*s Human fetal dopamine neurons grafted into the striatum in two patients with severe Parkinson's disease. A detailed account of methodology and a 6-month follow-up. Arch. Neurol., 46: 615-631. 1089.

21. *Neafsey, E. J.*: A simple method for glass insulating tungsten microelectrodes. Brain Res. Bull., 6: 95-96. 1981.

22. *Neafsey, E. J., Sorensen, J. C., N. Tonder* and *Castro, A. J.*: Fetal cortical transplants into neonatal rats respond to thalamic peripheral stimulation in the adult. An electrophysiological study of single-unit activity. Brain Res., 493: 33-40. 1989.

23. *O'Keefe, J. A.* and *Handa, R. J.*: Transient elevation of estrogen receptors in the neonatal rat hippocampus. Develop. Brain Res., In press: 1990.

24. *Paden, C. M., Gerlach, J. L.* and *McEwen, B. S.*: Estrogen and progestin receptors appear in transplanted fetal hypothalamus-preoptic area independently of the steroid environment. J. Neurosci., 5: 2374-2381. 1985.

25. *Pedersen, E. B., O'Keefe, J. A., Handa, R. J.* and *Castro, A. J.*: Intrinsic regulation of estrogen receptro concentration in neocortical transplants during postnatal development. Europ. J. Neurosci., In press: 1990.

26. *Plumet, J., Cadusseau, M.* and *Roger, M.*: Fetal cortical transplants reduce motor deficits resulting from neonatal damage to the

rat's frontal cortex. Neurosci. Lett., 109: 102-106. 1990.

27. *Sandor, R., Gonzalez, M. F., Moseley, M.* and *Sharp, F. R.*: Motor deficits produced by the removal of cortical transplants grafted into injured fronal cortex of neonatal rats. Behav. Brain Res., In press: 1990.

28. *Sharp, F.* and *Gonzalez, M.*: Fetal cortex transplants attenuate thalamic atrophy caused by frontal cortex lesions: WGA-HRP and amino acid studies of connections between motor cortex and thalamus of neonatally lesioned rats with and without transplants. Neurosci. Abst., 12: 1473. 1996.

29. *Sharp, F.* and *Gonzalez, M.*: Fetal cortical transplants ameliorate thalamic atrophy ipsilateral to neonatal frontal cortex lesions. Neurosci. Lett., 71: 247-251. 1986.

30. *Sofroniew, M. V., Isacson, O.* and *Björklund, A.*: Cortical grafts prevent atrophy of cholinergic basal nucleus neurons induced by excitotoxic cortical damage. Brain Res., 388: 409-414. 1986.

31. *Sorensen, J., Zimmer, J.* and *Castro, A.*: Fetal cortical transplants reduce thalamic atrophy induced by frontal cortical lesion in newborn rats. Neurosci. Lett., 98: 33-38. 1989.

32. *Sorensen, J. C., Wanner-Olsen, H., Tonder, N., Danielsen, E., Castro, A. J.*, and *Zimmer, J.*: Axotomized, adult basal forebrain neurons can innervate fetal frontal cortex grafts. A double fluorescent study. Exp. Brain Res., In press. 1990.

33. *Stanfield, B.* and *O Leary, D.*: Fetal occipital cortical neurones transplanted to the rostral cortex can extend and maintain a pyramidal tract axon. Nature (Lond.)., 313: 135-137. 1985.

34. *Stein, D. G., Labbe, R., Attella, M. J.* and *Rakowsky, H. A.*: Fetal brain tissue transplants reduce visual deficits in adult rats with bilateral lesions of the occipital cortex. Beh. Neural Bio., 44: 266-277. 1985.

35. *Swenson, R., Shaw, P., Alones, V., Kozlowski, G., Zimmer, J. and Castro, A.*: Neocortical transplants grafted into the newborn rat brain demonstrate a blood-brain barrier to macromolecules. Neurosci. Lett., 100: 35-39. 1989.

36. *Swenson, R. S., Danielsen, E. H., Klausen, B. S., Erlich, E., Zimmer, J.* and *Castro, A. J.*: Deficits in beam-walking after neonatal motor cortical lesions are not spared by fetal cortical transplants in rats. J. Neur. Transplant., (In press): 1990.

37. *Wiegand, S.* and *Gash, D.*: Characteristics of vasculature and neurovascular relations in intraventricular anterior hypothalamic transplants. Brain Res. Bull., 20: 105-124. 1988.

Autologous Adrenal Medullary Transplant to the Striatum of Patients with Advanced Parkinson's Disease: 18 month follow-up

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INTRODUCTION

Madrazo and colleagues [1987] reported dramatic improvements in two patients with Parkinson's disease (PD) after neurosurgical treatment with a transplant of adrenal medulla to the right caudate nucleus. The operation developed by Madrazo was subsequently evaluated in 19 patients in three U.S.A. medical centers (our center and two others) [Goetz et al, 1989A]. Six months after surgery, these patients showed moderate improvement, but morbidity was severe in some. One year after surgery, the 7 patients treated in our center showed persistent clinical improvement in selected measures [Goetz et al, 1989B]. We now report the 18 month followup data in these 7 patients.

METHODS

Patient selection: Patients with PD who were under the care of physicians from the movement disorder center at Rush-Presbyterian St. Luke's Medical Center, Chicago, were selected for inclusion based on the following criteria: age between 35 and 65, PD onset after age 25, at least three of the four cardinal signs of PD (rigidity, rest tremor, bradykinesia and postural reflex impairment), no significant medical illness, no previous history of encephalitis, no other neurologic signs or history indicative of secondary parkinsonism or multisystem degeneration; good response to dopaminergic therapy during at least part of each day; full therapeutic trials on higher and lower doses of antiparkinsonian medication in

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order to establish optimal medication; parkinsonian disability of at least Hoehn and Yahr Stage IV (bilateral disease with severely impaired postural reflexes) during part of the day in spite of optimal medical therapy. Exclusion criteria were dementia (Mini-Mental) state < 24), a history of psychiatric illness prior to the diagnosis of PD and psychiatric illness within the prior 12 months. No patient had undergone prior neurosurgical procedures for Parkinson's disease. An abdominal CT verified the presence of two adrenal glands, and any abnormalities of adrenal gland function or structure excluded a potential patient. All patients had been followed in our center for at least two years, and all had been tried on all other available pharmacotherapeutic regimens before being considered for the surgery.

Patient group: Seven patients underwent adrenal medullary transplant surgery between June 1987 and February 1988. This group was characterized by: mean age at surgery 50.7 yrs (SD 5.6), mean PD duration 11.6 yrs (SD 3.7) mean duration of levodopa therapy 11.0 yrs (4.0). All received chronic carbidopa/levodopa, mean dose 946.4 mg/day (SD 535.1) and all but one received an agonist, mean dose of bromocriptine equivalent (10 mg bromocriptine = 1 mg pergolide) 42.5 mg/day (SD 8.2). *Surgery*: The surgical procedure was performed as developed by Madrazo procedure and is described in more detail elsewhere [Penn et al, 1989]. In brief, a right frontal craniotomy and right subcostal abdominal adrenalectomy were performed simultaneously by neurologic and general surgery teams respectively. Using ultrasound localization, a balloon catheter was guided into the right lateral ventricle just medial to the head of the caudate nucleus at the foramen of Monro. A small cavity was made in the caudate with biopsy forceps, using a surgical microscope. Simultaneously, the right adrenal gland was removed, fat and connective tissue were dissected, and adrenal tissue was cooled with iced saline. Medullary tissue was dissected under the microscope, and 4-5 pieces, each measuring approximately 3x3x3 mm were selected for transplant. The transplant pieces were placed into the caudate cavity and held to the ependymal surface with non-magnetic metal clips placed with an angled clip applier, so that the graft was exposed to the cerebrospinal fluid. *Clinical monitoring*: Patients were assessed by the same movement disorder neurologist at each visit. Parkinsonian function was quantified using: the motor and activities of daily living subscales from the Unified Parkinson's Disease Rating Scale (UPDRS) [Koller, 1987], Hoehn and Yahr stage [Hoehn and Yahr, 1967] and Schwab and England scale [Schwab and England, 1969]. All ratings were obtained for "on" and "off" periods. "On" was defined as those times during the waking day when the patient was most independent and responding well to medication. "Off" referred to those times when the patient functioned least independently. "On" and "off" were determined by patient report that they were experiencing a typical episode. Prior to program entry, patients were trained in keeping on/off records to assess the percent of the waking day spent "off", "on" with chorea and "on" without chorea. These records were used for calculating the mean hours spent "on" (with and without chorea) and "off".

Timed motor, psychometric and behavioral assessments: Timed motor tasks were fingertip tapping for motor speed and dexterity (FT) [Reitan and Davison, 1974] and the Purdue Pegboard (PB) [Purdue Research Foundation, 1948]. Fingertip tapping speed was recorded as the mean number of key presses during five 10-second trials for each hand on a Western Psychological Services electronic tapping device with digital readout. PB score was recorded as the sum of pegs placed during three 30-second trials for each hand. Both tasks were tested when the patient was "on".

Psychometric measures were used to quantify global mental status (Mini-Mental State, MMS) [Folstein et al, 1974], depression (Beck Depression Inventory, BDI) [Beck et al, 1961], and verbal fluency (VF) [Benton and Hamsher, 1978]. Behavioral and psychiatric symptoms were based on direct observation and structured interview. Delusions, hallucinations, and affecti-

ve disorders were defined in accordance with DSM-III criteria [American Psychiatric Association, 1980].

Statistical analysis: Since most of the data were ordinal, changes in patient functioning from baseline through 18 months after surgery were analyzed using non-parametric methods [Siegel, 1956]. Changes occurring through the entire post-operative period were analyzed using the Friedman ANOVA test with data sampling at baseline, 6, 12 and 18 months. To determine whether improvement was maintained at 18 months, all variables with a significant change during the overall period were analyzed using Wilcoxon signed-ranks tests (two-tailed) comparing scores at baseline and 18 months. Alpha was set at $p<0.05$.

RESULTS

Baseline data:

Before surgery, all patients had severe parkinsonian signs and marked fluctuations in motor response to levodopa/carbidopa. Mean "off" time was 39.3 % of the waking day, and "on" time was largely accompa-

TABLE I

COMPARISON OF CLINICAL DATA
18-MONTH VS. BASELINE

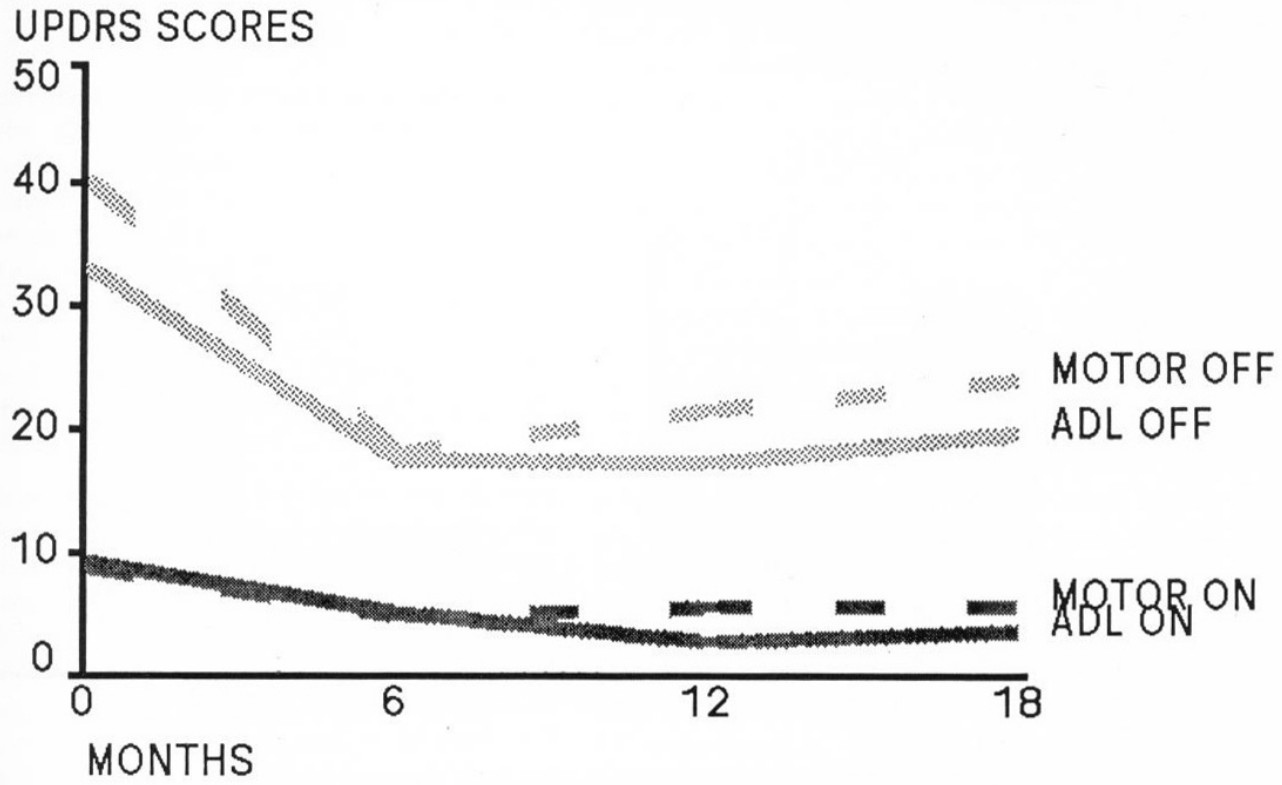
Measure	Baseline (S.D.)	18 month (S.D.)	Comment	P *
Quantity of On Time				
% Total On	60.7 (13.9)	70.6 (13.6)	improved	0.5541
% With Chorea	39.1 (25.1)	18.9 (15.0)	improved	0.0747
% No Chorea	21.6 (22.2)	51.7 (26.7)	improved	0.0796
Quality of On Time				
ADL	9.3 (3.7)	3.6 (3.3)	improved	0.0180
Motor	9.0 (5.1)	5.6 (4.8)	improved	0.0431
Hoehn & Yahr	2.9 (0.4)	2.0 (0.0)	improved	0.0277
Schwab/England	81.4 (6.3)	87.8 (2.5)	improved	0.0679
Quality of Off Time				
ADL	33.1 (7.6)	19.7 (10.8)	improved	0.0180
Motor	40.0 (6.8)	24.1 (15.4)	improved	0.0280
Hoehn & Yahr	4.3 (0.5)	3.1 (0.9)	improved	0.0277
Schwab/England	33.6 (19.3)	60.8 (25.0)	improved	0.0277

* Wilcoxon 2-tailed matched pairs signed rank test

ADL = activities of daily living
Motor = motor subscale
Both of the UPDRS

Fig. 1: MEAN UPDRS SCORES

BASELINE - 18 MONTHS POST OP



Friedman's ANOVA; $p < .05$ for MOTOR on, ADL on & off

nied by dyskinesias. Mean Hoehn and Yahr stage fluctuated from 2.83 while "on" to 4.29 while "off".

Post-operative medical course:

The mean duration of hospital stay was 30.1 days (SD 19.4), with a mean stay in the intensive care unit of 6.86 days (SD 3.6). Pneumonia occurred in 3 patients and cystitis in 2 patients. In all cases, patients responded to antibiotics. No permanent medical complication occurred.

Post-operative changes in PD function:

Medication doses did not change significantly after surgery. Parkinsonism began to improve within 3 to 4 months after surgery, reached a stable plateau at about 6 months, and maintained this level at 12 months. By 18 months after surgery, improvement had waned, although significant improvements were observed in most measures when compared to baseline (Table I).

When results for the whole period were analyzed, significant improvement was seen in UPDRS motor scores while "off" (Figure 1), UPDRS ADL scores while "on" and "off" (Figure 1), total time "off", and total time "on" without dyskinesias (Figure 2). Hoehn and Yahr stage was significantly improved while "on" ($p = 0.037$), and Schwab and England ratings while "off" ($p = 0.034$).

Post-operative psychometric and behavioral changes: There were no changes in Mini-Mental state, Beck Depression Inventory scores or verbal fluency over the study. Although no patient had a prior history of psychiatric problems before PD onset or in the year preceding surgery, transient behavioral changes developed after surgery. These are presented elsewhere [Tanner et al, 1988] and summarized in Table II. These behavioral changes were short-lived and resolved in all but one patient by 6 months. One patient with no prior psychiatric history became depressed after surgery,

Fig. 2:

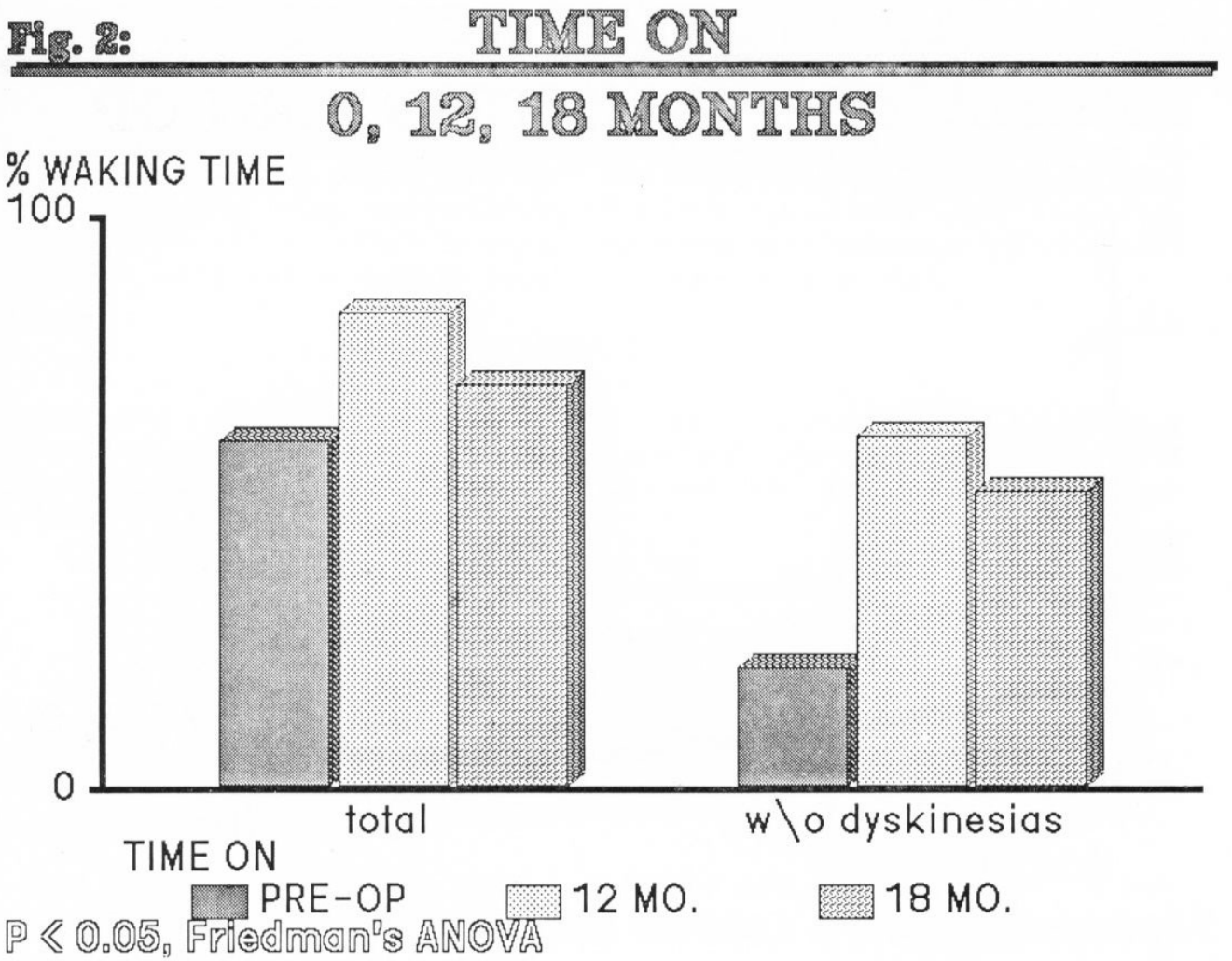


TABLE II

MEDICAL AND BEHAVIORAL COMPLICATIONS OF INTRASTRIATAL ADRENAL MEDULLA TRANSPLANT SURGERY IN PARKINSON'S DISEASE

WEEK	1	2	4	8	16	24	52	TOTAL
MEDICAL:								
PNEUMONIA	1	3	1	0	0	0	0	3
CYSTITIS	0	0	2	0	0	0	0	2
BEHAVIORAL								
SOMNOLENCE	4	0	0	0	0	0	0	4
DELUSIONS	6	6	6	0	0	0	0	6
DISINHIBITION/ HYPOMANIA	3	5	5	1	1	0	0	5
HALLUCINATIONS	2	2	1	1	1	1 *	1	6
DEPRESSION	0	0	0	0	1	1 *	1	1

* RESOLVED AFTER ECT

despite motor improvement. His symptoms did not improve after antidepressant treatment, but twice responded to electroconvulsive therapy, only to recur after several months.

DISCUSSION

Improvement first observed in the early months after surgery in our patients persisted, with some diminution, for 18 months. The improvements involved not only a decrease in "off" time, but also improved function as measured by ADL scores both while "on" and while "off". Our patients did not experience the dramatic improvements reported in Madrazo's series [1987], and none could decrease or stop their antiparkinsonian medications.

Maximum improvement occurred during the first six months after surgery, beginning about 4 to 6 weeks post-operatively. Over the next 12 months, improvements were stable, or waned slightly. While Madarzo et al [1987] originally reported an "excellent amelioration of most of the clinical signs of PD", we observed more modest benefit. All patients remained seriously parkinsonian when "off", and all continued to have fluctuations in motor response to antiparkinsonian medications.

Medical complications were rare in our series, but transient behavioral changes were observed in all cases. Although behavioral changes are common in parkinsonian patients treated chronically with dopaminergic agents, in this situation the patients' behavior was most abnormal when they were receiving no dopaminergic medications. Reinstitution of pre-operative therapy did not cause an exacerbation of behavioral signs. We conclude that overactivity of central dopaminergic systems plays little or no role in these behavioral changes.

The care of our patients after transplant surgery required a highly specialized team, including movement disorder neurologists, neurologic and general surgeons, intensivists, internists, psychologists and neurologic and intensivist nurses. Despite this, some morbidity did occur. Since morbidity is always a risk in such severely ill patients, we recommend that further assessment of the safety and efficacy of this surgical procedure be limited to centers with a similar multidisciplinary expertise.

The number of patients studied in our series is small. We have previously combined our patients with those of two other centers using similar methods, to allow analysis of a larger group [Goetz et al, 1989]. When we compared patient function at six months to that at baseline in this larger series, we found that the pattern of improvement was consistent across the three centers, with improved duration of "on" time and improved function during "off" time. These results continue in our seven patients at 12 [Goetz et al, 1989B, *In Press*] and 18 months.

No controlled, blinded studies have assessed the safety or efficacy of adrenal transplant surgery in Parkinson's disease. Given the significant investment of time, energy and funds on the parts of both patients and investigators, many have argued that the observed results merely represent a placebo effect. However, the gradual development of improvement, and its persistence for 18 months, suggest that a simple placebo effect is unlikely. Also, the similar course in patients treated in different centers argues in favor of a treatment effect.

The mechanism of intrastriatal implantation is unknown. Transplanted adrenal medullary cells have been proposed to survive in the brain and make dopamine. We could not assess this hypothesis in our patients, since all required treatment with levo-dopa, rendering CSF measurements of dopamine uninterpretable. At autopsy, two patients showed necrotic adrenal tissue and no definitively viable medullary cells [Peterson et al, 1988; Jancovic et al, 1989], suggesting that survival of graft tissue may not be necessary for a therapeutic response.

An alternative mechanism for the improvement we observed could be the transfer, secretion or stimulation of the production of trophic factors which may enhance neuronal sprouting, possibly leading to increased dopamine terminals and consequently increased dopamine storage [Bohn et al, 1987]. This hypothesis is consistent with the improved "on" time in response to do-

paminergic therapy observed in our patients, but not the inability to significantly reduce drug dose.

McRae-Deguerce and colleagues [1988] recently described an IgG antibody in the CSF of Parkinson's disease patients which cross-reacted with rat substantia nigra. In 6 of our 7 patients, this immunologic reactivity disappeared within the first six months after adrenal transplant surgery, coincident with but not exactly parallel to clinical improvement. It is possible that neuroimmunologic factors play some role in the efficacy of adrenal transplant surgery.

The persistence of clinical improvement 18 months after surgery provides a basis for cautions optimism. In our experience, the efficacy of adrenal transplant surgery is not transient. Although major surgery is always a risk in patients with severe Parkinson's disease, we did not observe long-term medical complications of surgery. Additional carefully controlled studies, incorporating the observations of basic and animal researchers, are essential to determine the final role of intrastriatal adrenal medullary implantation in the treatment of Parkinson's disease.

SUMMARY

We studied motor and psychometric changes after 18 months in seven patients with severe idiopathic Parkinson's disease (PD) who underwent intrastriatal autologous adrenal medulla transplant. Improvement began at four to six weeks after surgery, was maximal at about six months and was stable or waned slightly in the following 12 months. Eighteen months after surgery, significant clinical improvements were maintained in United Parkinson's Disease Rating Scale (UPDRS) motor and activity of daily living (ADL) scores and Hoehn and Yahr stage while "on" and "off" (Wilcoxon matched pair signed ranks test, baseline vs. 18 months). Compared to 12 months, improvement in individual motor and ADL scores had waned. The percent of total time spent "on" was no longer significantly different from baseline. Medications did not change significantly after surgery, and fluctuations in motor response to medication persisted. Persistent changes in cognitive function were not observed. Severe, recurrent depression, of uncertain relationship to the surgery, occurred in one case. Adrenal transplant surgery can produce functional improvement for at least 18 months. This long duration makes the placebo response an unlikely explanation for improvement.

RESUMEN

Siete pacientes afectados gravemente de Enfermedad de Parkinson, recibieron un autoinjerto de la médula suprarrenal en el cuerpo estriado. Presentamos los resultados clínicos observados durante los primeros 18 meses después de la operación.

Datos demográficos antes de la cirugía: término medio de edad 50.7 años, término medio de duración de la enfermedad de Parkinson 11.6 años término medio de tratamiento con levodopa 11.0 años. Todos recibieron una dosis de carbidopa/levodopa de 946.4 mg diaria y entre 6 y 7 recibieron una dosis de una agonista dopaminérgico 42.5 mg por día de equivalentes de bromocriptina (19 mg bromocriptina = 1 mg pergolide).

Una mejoría del parkinsonismo comenzó entre 4 y 6 semanas después de la operación y llegó al más alto nivel a los 6 meses y a continuación se mantuvo estable o disminuyó levemente. A los 18 meses se constató mejorías significativas para las fases "ON" y "OFF" según las pruebas regulares (Unifield Parkinson's Diseases Rating Seate motor subscale, Actividades of Daily Living subscale y la Hoehn and Yahr scale). Sin embargo en relación de los beneficios. El dosage de los medicamentos quedó sin modificaciones después de la cirugía y continuaron las fluctuaciones motrices.

Varias alteraciones transitorias de la conducta de las cuales delirias y una falta de inhibición, se desarrollaron después del acto quirúrgico, pero las mismas desaparecieron espontaneamente. No hubieron cambios cog-

nitivos permanentes, pero un enfermo sufrió de una recurrente depresión.

Este estudio muestra que los beneficios de los autoinjertos persisten por lo menos 18 meses. Esta prolongada duración de la mejoría asociada a los autoinjertos sugiere que el mecanismo está por encima de un efecto de placebo.

RESUMÉ

Sept malades atteints de cas graves de la Maladie de Parkinson ont recu une autogreffe de la médullo-surrénale dans le corps strié. Nous présentons les résultats cliniques des premiers 18 mois après l'opération.

Données démographiques avant la chirurgie: age moyen 50.7 ans, durée moyenne de la maladie de Parkinson 11.6 ans, durée moyenne du traitement avec levodopa 11.0 ans. Tous recevaient une dose de carbidopa /levodopa de 946.4 mg/j en moyenne, et 6/7 recevaient une dose d'une agoniste dopaminergique 42.5 mg/j en moyenne d'equivalents de bromocriptine (10 mg bromocriptine = 1 mg pergolide).

Une amélioration du parkinsonisme a commencé entre 4 et 6 semaines après l'opération, est devenu maximale à 6 mois, et ensuite, est restée stable ou a diminué legèrement. A 18 mois, on a constaté des améliorations significatives pour les phases "ON" et "OFF" selon le tests standards (Unified Parkinson's Disease Rating Scale motor subscale, Activities of Daily Living subscale et le Hoehn and Yahr scale). Cependant, par rapport à 12 mois, il y avait une réduction des bénéfices. Le dosage des médicaments restait inchangé après la chirurgie et les fluctuations motrices continuaient.

Plusieurs abérations transitoires du comportement, dont des délires et un manque d'inhibition, se sont developées après l'opération, mais elles ont disparu spontanément. Il n'y avait pas de changements cognitifs permanents, mais un malade a subi une dépression récurrente.

Cette étude montre que les bénéfices des autogreffes persistent au moins 18 mois. Cette longue durée d'amélioration associée aux autogreffes suggère que le mécanisme concerne plus qu'un effet de placebo.

ZUSAMMENFASSUNG

Sieben ernste Parkinsonkranke wurden einer Autotransplantation von suprarenalem medullären Gewebe in dons Corpus Striatum unterzogen. Hier sind die Ergebnisse, die 18 Monate nach der Operation beobachtet worden sind.

Praeoperative Daten: Mittleres Alter 50,7 Jahre; mittlere Krankheitsdauer 11,6 Jahre; mittlere Behandlungsdauer mit Levodopa 11 Jahre. Alle erhielten eine Dosis von carbidopa/levodopa von 946,4 mg taeglich, und sechs oder sieben erhielten die Dosis eines Dopaminergischen Agonisten von 42,5 mg taeglich eines Gleichwertigen von Bromocriptina (19 mg Bromocriptina = 1 mg Pergolide).

Die Besserung der Parkinschen Krankheit begann 4 bis 6 Wochen nach der Operation und erreichte ihren hoechsten Grad nach sechs Monaten. Danach hielt die Besserungan oder verminderte sich etwas. Nach 18 Monaten bemerkte man signifikante Besserungen fuer die Phasen "on" und "Off", mit den ueblichen Proben (Unifield Parkinson's Diseases Rating state motor subscale, Activities of Daily Living subscale y Hoehn and Yahr scale). Jedoch bezueglich der Vorteile, bliebe die Dosierung der Medikamente die Gleiche nach den Operationen und blieben die motorischen Fluktuationen die Gleichen.

Man beobachtete verschiedene voruebergehende Veraenderungen des Verhaltens wie Delinen und Fehlens der Inhibition, die sich nach der Operation einstellten. Aber diese verschwanden spontan. Man beobachtete keine permanenten cogniteiven Veraenderungen; aber ein Kranker litt an rekurrenter Depression.

Diese Arbeit zeigt, dass die Besserungen durch die Autotransplantate mindestens 18 Monate anhalten. Diese Dauer der Besserung durch die Autotransplantate, laesstr erkenen, dass dieser Heilungsmechanismus ueber dem Effek eines Placebos steht.

REFERENCES

1. *American Psychiatric Association.* Diagnositic and Statistical Manual (3rd edition), Washington DC: American Psychiatric Association, 1980.
2. *Beck A.T., Ward C.H., Mendelson M., Mock J., Erlbaugh J.*: An inventory for measuring depression. Arch Gen Psychiatry 1961; 4: 561-71.
3. *Benton A.L., Hamsher K.*: Multilingual Aphasia Examination. Iowa City: AJA Associates. 1978.
4. *Bohn M. C. Cupit L., Marciano F., et al.*: Adrenal medulla grafts enhance recovery of striatal dopaminergic fibers. Science 1987; 237: 916.
5. *Folstein M. F., Folstein S. E., McHugh P. R.*: Mini-Mental State. Journal of Psychiatric Research 1974; 12: 189-98.
6. *Goetz C. G., Olanow C. W.. Koller W. C., et al.*: Adrenal medullary transplant to the striatum of patients with advanced Parkinson's disease: multicenter study. N. Engl. J. Med 1989 A; 320: 337-340.
7. *Goetz, C. G., Tanner, C. M., Penn, R. D. et al.*: Adrenal medullary transplant to the striatum of patients with advanced Parkinson's disease: 1 year motor and psychomotor data. Neurology 1989B; 39: *In Press.*
8. *Hoehn M. M., Yahr M. D.*: Parkinsonism: onset, progression and mortality. Neurology 1967; 17: 427-42.
9. *Jankovic, J., Grossman, R., Goodman, C. et al.*: Clinical, biochemical and neuropathologic findings following transplantation of adrenal medulla to the caudate nucleus for treatment of Parkinson's disease. Neurology 1989; 39: 1227-1234.
10. *Koller W. C.*: *Handbook of Parkinson's Disease.* New York: Decker, 1987: 482-488.
11. *Madrazo I., Drucker-Colin R., Diaz V., et al.*: Open microsurgical autograft of adrenal medulla to right caudate nucleus in two patients with intractable Parkinson's disease. N. Engl. J. Med. 1987; 316: 831-4.
12. *McRae-Degueurce A., Klawans H. L., Penn R. D., et al.*: An antibody in the CSF of Parkinson's disease patients disappears following adrenal medulla transplantation. Neuroscience Letters 1988; 94: 192-197.
13. *Penn R. D., Goetz C. G., Tanner C. M., et al.*: Adrenal medullary transplant operation for Parkinson's disease. Neurosurgery 1988; 22: 1020-1027.
14. *Peterson D. I., Price M. L., Small C. S.*: Autopsy findings in a patient that who an adrenal-to-brain transplant for Parkinson's disease. Neurology 1988; 38, suppl 1: 144.
15. *Purdue Research Foundation.* Examiner's manual for the Purdue Pegboard. Chicago: Science Research Assoc., 1948.
16. *Reitan R. M., Davison L. A.*: Clinical neuropsychology: current status and applications. New York, Hemisphere Publ. 1974.
17. *Schwab R. S., England A. C.*: Projection technique for evaluating surgery in Parkinson's disease. In: Gillingham F. J., Donaldson M. C., eds. *Third Symposium on Parkinson's disease.* Edinburgh, Livingston, 1969; 152-157.
18. *Siegel S.*: *Nonparametric Statistics.* New York; McGraw-Hill, 1956.
19. *Tanner C. M., Goetz C. G., Gilley D. W. et al.*: Behavioral aspects of intrastriatal adrenal medulla transplant surgery in Parkinson's disease. Neurology 1988; 38 (suppl 1): 143-144.

Motor Cortex Transplantation in Adult Rats - Attempt to Cure the Paralysis

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INTRODUCTION

Reviewing the literature on neural transplantation (Azmitia and Bjorklund, 1987; Bjorklund and Stenevi, 1985; Das and Wallace, 1986; Gash, et al. 1985; Gash and Sladek, 1988; Sladek and Gash, 1984), studies conducted in a number of laboratories have used the techniques of grafting nerve cells into a host brain to study fundamental processes in neural development and regeneration. Evidence has accumulated indicating that fetal neurons can reestablish damaged connections in the host brain and substitute functionally for elements lost or damaged as a result of proceeding lesion. It has led to the realization that, contrary to traditional views, the adult mammalian CNS has a potential to incorporate new neuronal elements into already established neuronal circuitry and that implanted neurons can modify the function and behavior of the recipient.

In this study, the methods of neural transplantation was extended to the motor cortex of adult rats in order to address the following questions: (1) Can homotypic transplants compensate for locomotor functions impaired by motor cortex injury? (2) Can transplant cells develop a normal laminar pattern in an adult host cortex? (3) Can transplants replace damaged cortical projections and rebuild normal cortical cir cuits in the adult host brain?

MATERIAL AND METHODS

35 adult male Sprague-Dawley rats, wei ghing approximately 250-300 g, were used as both donor and recipient animals. Twenty of these animals received homotopic neural transplants, ten sustained cortical aspiration lesions only, and five underwent sham operations and served as shamcontrols.

Lesioning Procedure: With the host animals deeply anesthetized by sodium pentobarbital (40 mg/kg body weight, I. P.), they were placed securely in the stereotaxic instrument. The craniectomy was made unilaterally on the left side of the frontal bone. The opening was approximately 16 mm^2 in area and corresponded to the forelimb, trunk, and hindlimb motor cortical areas as electrophysiologically mapped by Hall and Lindholm (1974). A superficial lesion was then made on the surface of the motor cortex by gentle aspiration. After hemostasis, the cortical cavity was carefully cleared in order to facilitate transplant attachment to the host.

Transplantation Procedure: Donor embryos of 15 gestational days were removed from the uterus of timed pregnant females under sodium pentobarbital anesthesia (40 mg/kg body weight, I. P.) and placed into icecold lactated Ringer's solution. Under the surgical microscope, the frontal cerebral cortex was dissected away from the other structures. Mesenchymal tissue and vasculature were then meticulously teased away

from the actual neural tissue. The cortical fragments, further trimmed to 2 mm^2 in area, were ready to be transplanted to the host site.

The sheet of donor tissue was carefully transferred to the prepared cavity of the host brain by using a fine curette. The scalp was immediately repositioned and pressure applied for 30 seconds on the transplant site. The cranial incision was then closed with a 6-0 silk suture.

Animals serving as sham controls sustained cranial incisions identical to those of the animals receiving the transplants and lesions. The underlying cranial bone was likewise scraped with a scalpel and the incision then simply closed with 6-0 silk sutures. The preoperative and postoperative procedures were identical to those already mentioned.

Behavioral Evaluations: The open field examination used in this study was possible to observe general activity of the animal and any general locomotor dysfunction. Animals of transplant group were behaviorally tested beginning 5 weeks postoperatively; this allowed adequate time for the transplants to grow and differentiate. The test was carried out in those of lesion and sham operation groups 1 week postoperation; this allowed optimal opportunity to observe any motor deficits. Each group was tested over an 8 to 10 week period in order to determine the effects of the transplants on the recovery of cognitive functions.

The open field was a square enclosure made of white formica. The walls were 40 cm high, and the floor 1 x 1 m, subdivided into 25 equal 20 cm squares by lines on the floor. Four days following reinstatement of adlibitum feeding, each rat was tested for 10 min sessions on 5 consecutive mornings. On each test, the rat was placed in a corner square facing into the corner. Behavior was monitored by two observers sitting quietly at one side of the open field. A counting device tabulated the number of blocks crossed (all 4 paws entering a square) in the center and peripheral squares, the number of normal rears, frequency of urination and defecation. Various objects were also placed in the bottom of the box to provide manipulanda for the animals while being observed. 70 % ethyl alcohol was used to quickly swab out the inside of the instrument between each animal to eliminate possible olfactory cues from previously tested subjects.

Fiber Connection Tracing: Following behavioral testing, 12 animals of transplant group were selected to trace graft connections to the host brain, using anterograde axonal transport of PHA-L.

The plant lectin *Phaseolus vulgaris*-leucoagglutinin (PHA-L) tracing technique developed by Gerfen and Sawchenko (1984) was used in tracing retina transplant fibers two to six months after the transplantation. PHA-L (Sigma, L-4138) was injected iontophoretically into the transplant site of the host animal under anesthesia with the sodium pentobarbital (45 mg/kg, I. P.). Iontophoretic application is achieved through a glass micropipette (10 to 15 μm in diameter) using a positive-pulsed 5 mA DC current (7 s on, 7 s off) for 15 min. Then the animal was allowed to survive for 6 days, reanesthized and perfused transcardially at a constant hydrostatic pressure with 100 ml of physiological saline solution at room temperature, followed immediately by 350 ml fixative #1 (2.5 % paraformaldehyde and 0.5 % glutaraldehyde in 0.1M phosphate buffer) and then by 500 ml fixative #2 (4.0 % parafomaldehyde and 0.75 % glutaraldehyde in 0.1M phosphate buffer). The brain was removed and immersed in 0.1M phosphate buffer overnight at 4ºC.

Vibratomed sections at 80 μm were made in 0.05M TBS (Tris-buffered saline) and every 4th section was taken for PHA-L immunohistochemistry reaction procedure. These sections were transferred to a solution of TBS to which 0.1 % Triton X-100 with 1 % normal rabbit serum (NRS) was added (TBS-T). Sections were rinsed 3 x 15 min. After washing the sections were incubated in 1 % H_2O_2 in water for 1 hour at room temperature. Wash the sections again 3 x 15 min in TBS-T and then incubated them for 1 hour at room temperatu-

re in 10 % NBS using TBS-T as diluting solution. NRS was sucked out of wells without washing and replace with in primary antibody (goat anti-*Phaseolus*, Vector Labs, USA) at a dilution of 1:3000 in TBS-T. The sections were incubated in this solution for 1 hour at room temperature, then put in a refrigerator (4ºC) overnight (about 12 to 15 hours), subsequently remove tissue from refrigerator and incubated again at room temperature for 1 hour. Wash the sections in TBS-T for 4 x 15 min and then incubated in the secondary antibody (rabbit anti goat serum, Sigma) in TBS-T (1:200 dilution) for 2 hours at room temperature. After a through (4 x 15 min) rinse in TBS-T, the sections was transferred to goat peroxidase-antiperoxidase (PAP) complex in TBS-T at a dilution of 1:200 for two hours at room temperature. After rinsing in 4 x 15 min of TBS, the sections were incubated for 15 min with a solution of 0.04 % 3,3'-diaminobenzidine tetrahydrochloride (DAB, Sigma) and 0.003 % H_2O_2 in TBS. When staining was sufficient, sections were washed with TBS for 4 x 15 min and then mounted on subbed slides, cleared in xylene and covered. The adjacent sections were stained with cresyl violet for observing the cytoarchitecture of transplants.

The sections were examined with a light microscope (Leitz, Model Dialux 20).

Histological Examination: The cytoarchitecture of the transplant was determined by viewing the series of cresyl violet stained sections mentioned above and Golgi impregnant-stained sections under a light microscope.

Rapid Golgi method was used for the classification of neurons according to shape, dendrites, and origin or collateralization of the axon. 5 animals of transplant group were sacrificed with lethal dose of sodium pentobarbital. The transplant was then exposed from each rat as soon as possible, removed out carefully, and dissected into small slices (1 mm thick). These fresh and small tissue slices were hardened in a solution with 4 parts 3.5 % potassium dichromate and 1 part 1 % osmic acid, at room temperature for 2 to 7 days and then transferred to 0.75 % silver nitrate for 1 to 2 days in the dark. The impregnated blocks of tissue were dehydrated and embedded in Paraplast Plus (Fisher Co. USA) and sectioned at 10 μm.

RESULTS

Open Field Testing: Table 1 shows a summary of the open field data. Significant differences were noted between lesion group and control group in the following areas: lesioned animals displayed a decrease in the mean number of blocks crossed ($p<0.05$), a decrease in the mean number of normal rears ($p<0.05$), and an increase in the

TABLE 1. SUMMARY OF OPEN-FIELD DATA

	Control (n=5)	Lesion (n=10)	Graft (n=17)
Blocks crossed	116.2 ±21.19	75.4 ±38.56 *	127.4 ±35.36 **
Rears	21.5 ± 6.10	10.4 ± 6.90 *	23.7 ± 6.28 **
Urinations	0.49± 0.53	0.60± 0.48	0.51± 0.59
Defecations	1.33± 1.32	2.26± 1.91 *	0.95± 0.60 **

The values above reflect means and standard deviations.
n = animal number
p values were obtained using a Mann Whitney Rank Sum Test.
* significantly different from control, $p < 0.05$
** significant difference between lesion and graft rats, $p < 0.01$

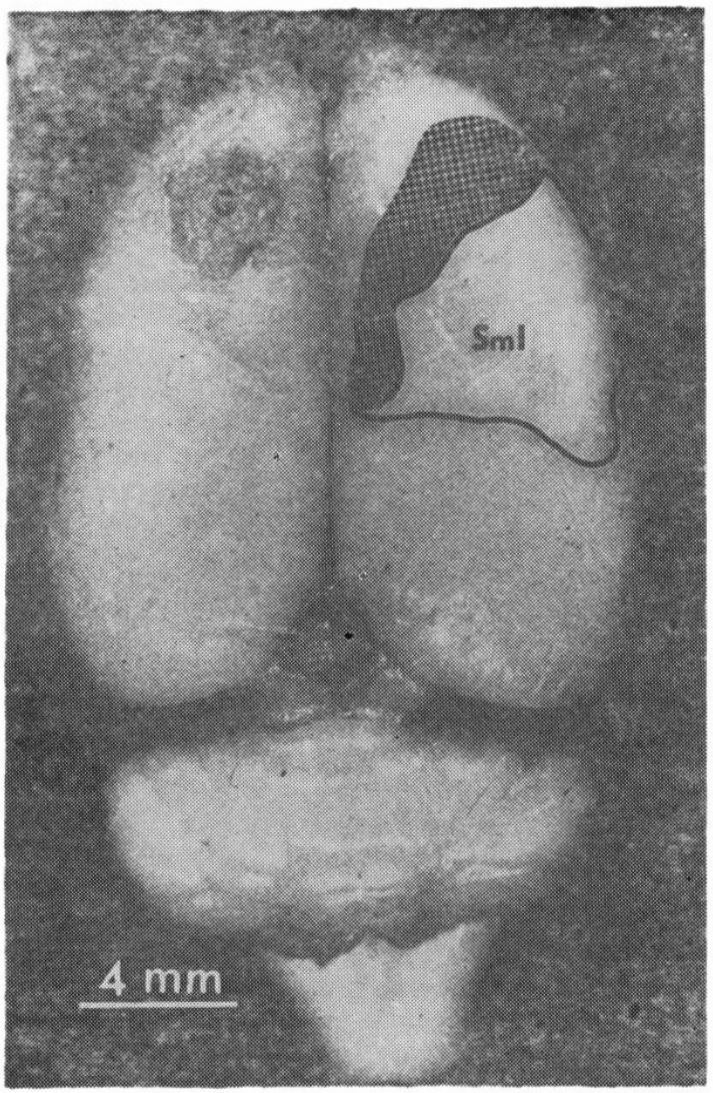

Fig. 1. — *Neocortical graft (G) in the motor cortex (shadowy area). SmI: primary sensory area.*

number of defecations ($p<0.05$). Animals receiving transplants showed a significant greater mean number of block crossed than lesioned animals ($p<0.01$). Also transplant animals showed significantly more activity than lesioned ones ($p<0.01$). The number of defecations were more less in transplant group than that in lesion group. Transplant animals were found not to be significantly different from controls on these subtests.

Survival Rate: The transplants were easily recognized from the other parts on either brain surface (Fig. 1) or sections (Figs. 2). 85 % of the transplants (17 of 20 animals) survived in adult hosts.

The transplants showed considerable post-transplantation growth and they were variable in size. In most cases, cortical transplants fully filled in the whole lesion cavity of motor cortex, which represents a six to eight fold increase from the volume of the tissue (2 mm^3) was placed in the hosts. Most of the surface apposing the host brain parenchyma was integrated with the host brain (integrated surface, i.e. interface between the transplant and the host parenchyma), however, an apposing surface separated from the host brain by the intervening *pia mater* (Fig. 2). The interface was found to be more than 80 % of the apposing surface. The surface protruding outside was facing the meningeal membranes and the cranium. Many blood vessels ran between the transplant and host brain or between the transplant and meninges.

Histological Analysis: The cresyl violet staining Golgi preparations of the transplants appeared to have more cells than ad-

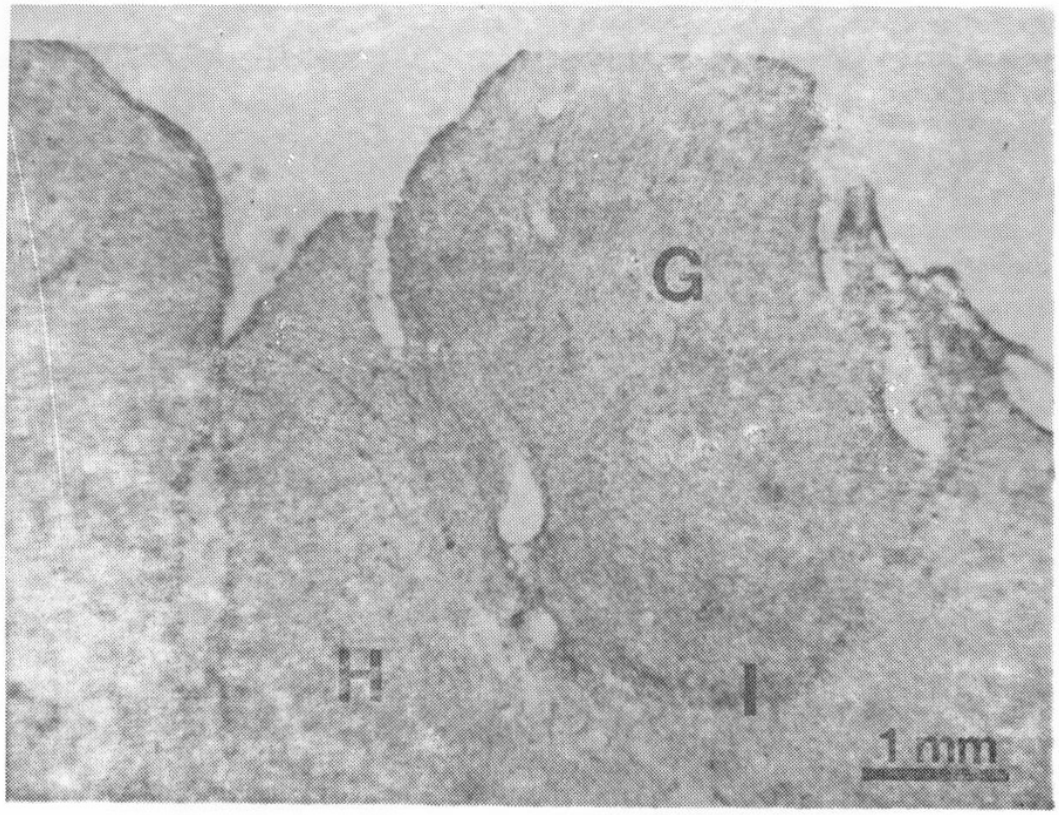

Fig. 2. — *Section view on the motor cortex graft. The interface (I) between the graft (G) and the host motor cortex (H) were seen. Cell density was higher in the transplant than that in the host cortex.*

jacent motor cortex. The transplants showed the presence of well-differentiated, normal-looking neurons (Figs. 3 and 4), neuroglial elements, and neuropil. Although the neurons were not arranged in distinct layers, but were dispersed and arranged in clusters, and the largest pyramidal cells were closed to the border between the graft and host tissue, this is a normal location for these cells. A molecular layer was clearly seen on the surface of the surviving transplants. In cell morphology, the neuron appeared large, with centrally located nuclei and clearly find nucleoli, and clearly stained Nissl bodies. They showed long and differentiated dendrites and their collateral with many spines on them (Fig. 4). Characteristically the dendrites and their branches appeared smooth and tapered along their length. The apical dendrites of the pyramidal cells oriented toward molecular layers. Non-pyramidal cells were of two types: spiny and aspiny.

Histological examination of the lesioned brains, eight brains were seen to have involvement of only neocortex. The other two lesioned brains showed a little involvement of callosal fibers in addition to neocortex, but lesions did not extend to the deep structures.

No evidence of any cortical or subcortical abnormality was seen in the five control brains by histological examination.

Fiber Connections: In 8 cases of 12 transplant animals small iontophoretic injections of PHA-L were successfully placed in a cortical transplant without involving any of the host brain. PHA-L was confined in the transplant without spreading into the host brain area.

Some pyramidal cells were filled with PHA-L (Fig. 5). Their labelled fibers ran through the transplant and projected further into several host brain targets (Fig. 6). In all cases, labelled fibers were found in the contralateral motor-sensory cortex and ipsilateral thalamus nuclei. The labelled fibers were seen at bilateral septum in three rats, at bilateral hipothalamus and ipsilate-

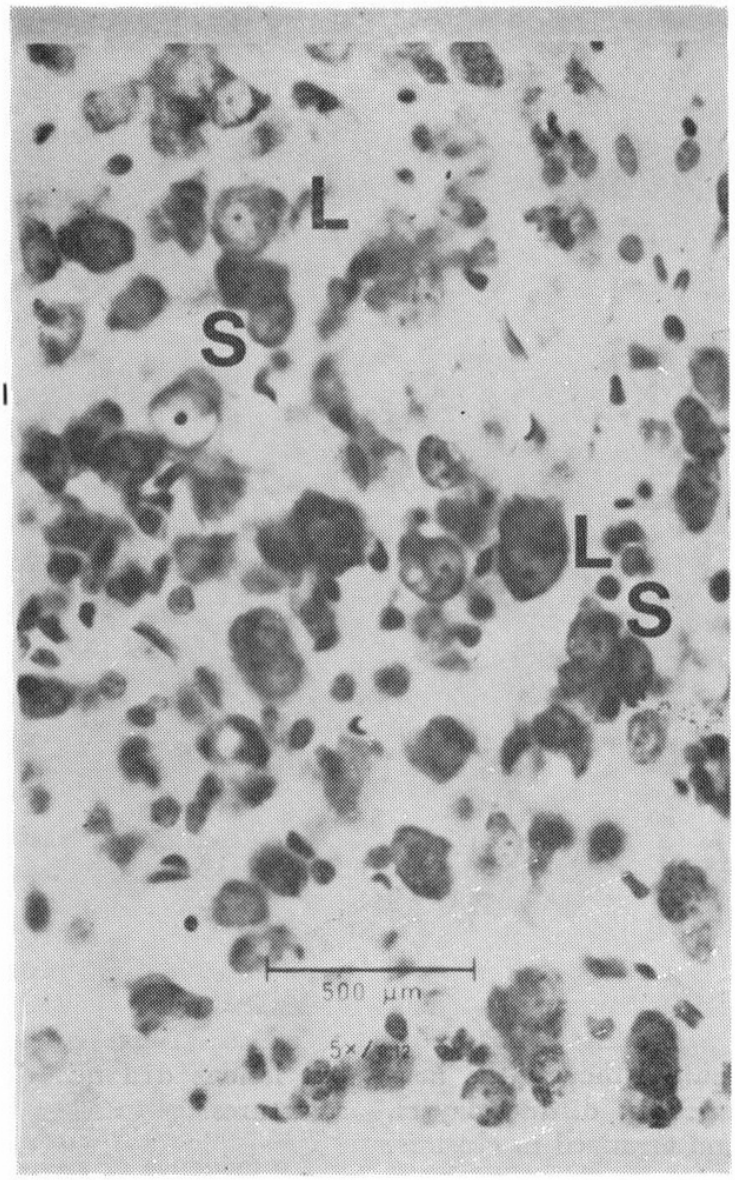

Fig. 3. — *Cytology of the transplant in cresyl violet stain. Some large cells (L) and small cells (S) were seen in clusters.*

ral reticular formation of the brain stem in two animals.

DISCUSSION

Recently several studies have shown that extending the methods of neural transplantation to the motor cortex of rats appears technically feasible and fetal homotypic cortical grafts survive and grow well in lesioned motro cortex of newborn or junior host rats (Harnsberger and Wallace, 1986; Jiao, et al. 1986a,b; Sharp and Gonzalez, 1984). What would happen when fetal brain tissue is implanted into the motor cortex of adult recipients? The present study indicated that transplants obtained from the rat embryos of 15-day developmental stages show very good survival rate in adult hosts. It was similar to the one seen after implanting immature cortical tissue to newborn or junior rats (Chang, et al. 1986; Jiao, et al. 1986a). The present results provided the further demonstration that fetal cerebral grafts can ameliorate lesion-induced impairments in locomotor function of adult hosts. In comparison with controls, lesioned animals became sluggish and had more defecation in the open field, however, rats with transplants were hyperactive and normal defecation. The grafting did cause a marked

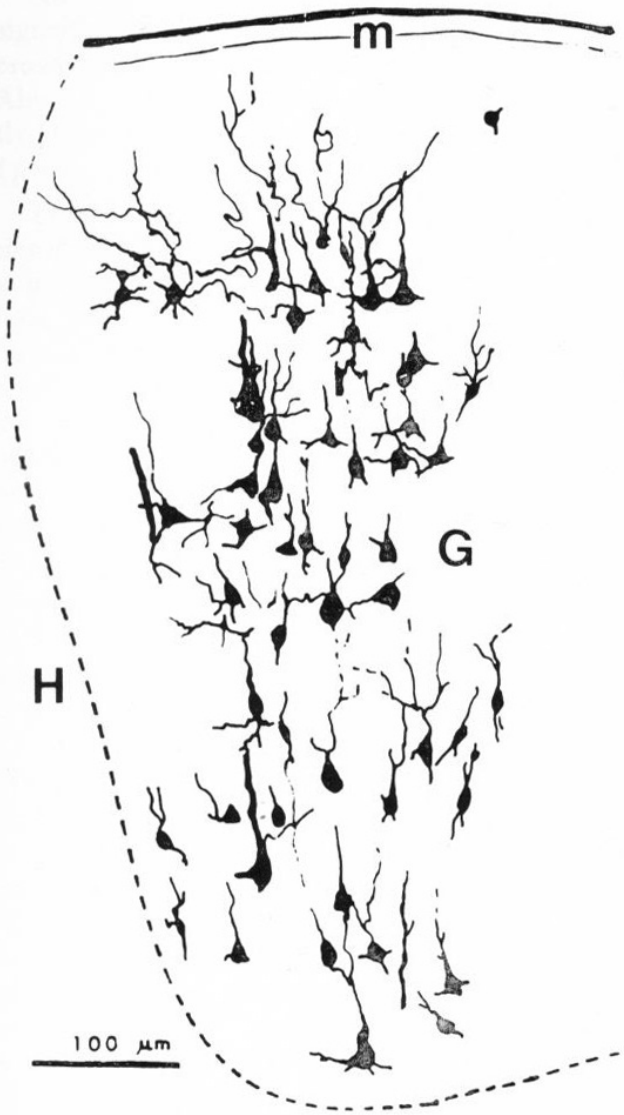

Fig. 4. — *Camera lucida drawing of the transplant neurons from Golgi preparation. The dendritic growth of pyramidal cells directed towards the molecular surface (M) of the graft. The boundary between graft (G) and host (H) is indicated by a broken line.*

improvement in their open field performance and made them similar to controls. The behavioral improvement depends on the structure of the transplants. Neurons typical for the motor cortex, including pyramidal cells and non-pyramidal cells were present in the grafts. The pyramidal cells tended to be arranged in small clusters but not in distinct laminar pattern as seen in normal rat brain. Most of large pyramidal cells or clusters were aligned along the graft-host interface. This arrangement might be because a dendritic growth directed towards the center or the molecular surface of the graft, but it might also be the trophic factors in the lesioned host brain attracted the graft neurons or in particular supported the survival of the most adjacent ones (Jiao, et al. 1986a; Tonder, et al. 1989). Our motor cortical transplants send efferents to several host brain targets which are the right sites receiving projections from normal motor cortex. Jaeger and Lund (1979) noted that projecting fibers from cortical transplant in host tectum follow existing host pathways such as the stratum opticum of the superior colliculus and the ascending and descending fibers of the dorsal longitudinal fasciculus. So it is considerable that the projections of grafts in motor cortex might be using the host efferent pathways. But we can not explain why no efferent fiber was found in the spinal cord.

In conclusion, the present results demonstrated that fetal homotopic cortical tissue can survive and grow after grafting to lesioned cavity in the adult rat motor cortex. The transplants can establish a good parenchymal integration and efferent connections with the host brain. They thereby at least partially replace the lesioned motor cortex. It is confirmed by the accompany locomotor behavioral recovery. Whether this recovery means the motor impulse to be arising from the grafts and conducting through their efferents is as yet unknown. A growing number of electrophysiological (Buzsaki, et al. 1987; Neafsey, et al. 1987) and behavioral studies (Bolam, et al. 1987; Dunnett, et al. 1985; Fine, et al. 1985; Isacson, et al. 1986; Low, et al. 1982) have shown that graft-host interconnections are indeed functional. Thus further studies are required to do the electrophysiological investigations on the grafts and their efferent pathways.

The damage of cerebral motor cortex caused by stroke or brain trauma can result in lasting paralysis and prolonged disability. The success with transplants in rat models has led to suggestions that neural grafts might be of therapeutic value in treating stroke or cortical trauma.

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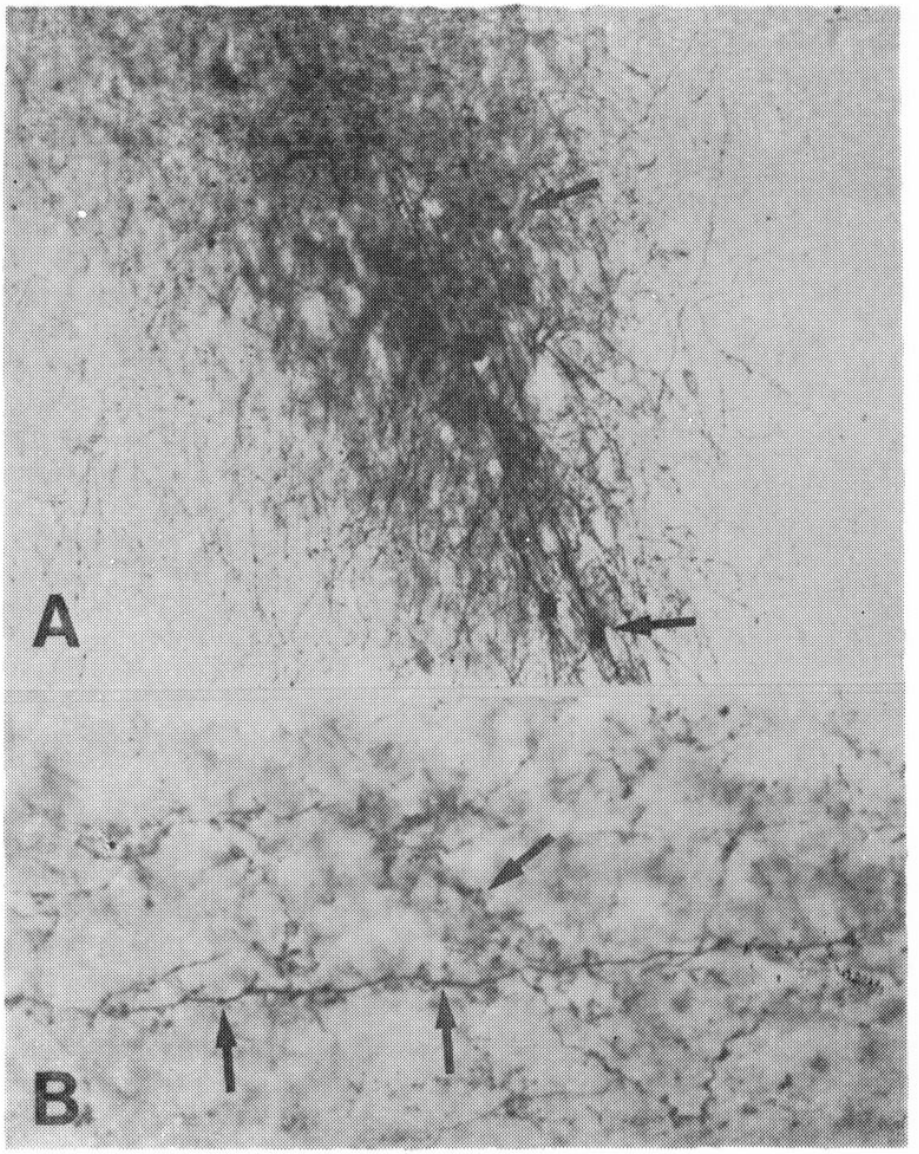

Fig. 5. — *A. Photomicrograph of PHA-L labeled cells (arrows) in an injection site in the graft, revealing the soma and entire extent of the dendritic trees. B. High-power light micrograph of the PHA-L labeled axons showing en passant varicosities (some are indicated by arrows).*

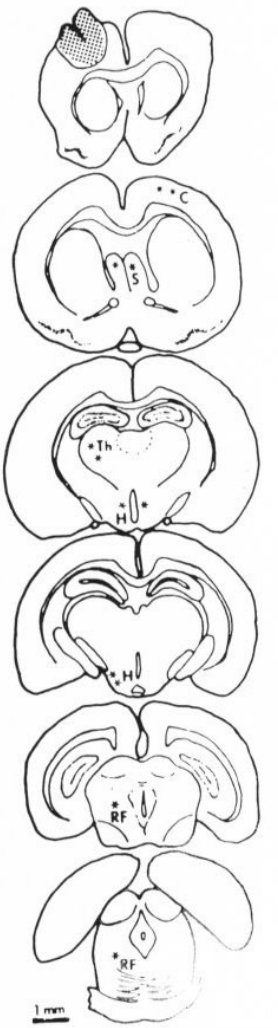

Fig. 6. — *Drawing of serial sections of a rat brain showing the distribution of PHA-L labeled fibers from a cortical graft (shadow area). C: motor-sensory cortex; S: septum; Th: Thalamus; H: hypothalamus; RF: reticular formation.*

SUMMARY

The purpose of this study was to investigate the replacement of damaged motor cortex by homotypic transplants of fetal brain tissue. Sprague-Dawley rats were anesthetized by sodium pentobarbital (40 mg/kg, I. P.) and the motor cortex, corresponded to the forelimb, trunk and hindlimb area, was aspirated completely. A piece of frontal cortical fragment was dissected away from a donor embryo of 15 gestational days and implanted into the lesioned cavity of the host brain. The lesion group didn't receive any donor tissue. The sham controls passed all procedures done in that two groups mentioned above except cortical aspiration and transplantation. The results of the open field testing showed significant differences between lesion group and control group in the following areas: lesioned animals displayed a decrease in the mean number of blicks crossed ($p<0.05$), a decrease in the mean number of normal rears ($p<0.05$).

Animals receiving transplants showed a significant greater mean number of block crossed than lesioned animals ($p<0.01$). Also transplant animals showed significantly more activity of rears than lesioned ones ($p<0.01$).

Transplant animals were found not to be significantly different from controls on these subtests. These results demonstrated that fetal cortical grafts can ameliorate lesion-induced impairments in locomotor function of adult hosts. Then animals were sacrificed for histological examination and fiber connection tracing. Up to 85 % of transplants survived and filled in the whole lesion cavity of motor cortex, which represents a 6 to 8 fold increase from the volume of the tissue (2 mm^3) was placed in the hosts.

The cresyl violet staining and Golgi preparations of the transplants appeared to have more cells than adjacent motor cortex. The transplants showed the presence of well-differentiated, normal-looking neurons, neuroglial elements, and neuropil. Although the neurons were not arranged in distinct layers, but were dispersed and arranged in clusters, and the largest pyramidal cells were closed to the border between the graft and host tissue, this is a normal location for these cells. A molecular layer was clearly seen on the surface of the surviving transplants. In cell morphology, the neuron appeared large, with centrally located nuclei and clearly find nucleoli, and clearly stained Nissl bodies. They showed long and differentiated dendrites and their collateral with many spines on them. Characteristically the dendrites and their branches appeared smooth and tapered along their length. The apical dendrites of the pyramidal cells oriented toward molecular layers. Non-pyramidal cells were of two types: spiny and aspiny. There was an interface between the transplant and host tissue. It suggests that the transplant has a good parenchymal integration with the host brain. A small iontophoretic injections of PHA-L were successfully placed in a cortical transplant without involving any of the host brain. Some pyramidal cells were filled with PHA-L. Their labelled fibers ran through the transplant and projected further into several host brain targets: the ipsilateral thalamus, the contralateral motor-sensory cortex, the bilateral hypothalamus and reticular form. The present data suggest that the cortical transplants can replace the damaged motor cortex and rebuild normal cortical projections in the host brain.

The motor cortex transplantation would be expected to be of therapeutic value in treating paralysis caused by stroke or cortical trauma.

KEY WORDS: neural transplant, motor cortex, brain damage, locomotor recovery, regeneration, nerve connections, rat.

RESUMEN

El propósito de este estudio fue investigar la restitución de corteza motora dañada por transplantes de tipo similar de tejido cerebral fetal. Ratas Sprague-Dawley fueron anestesiadas por fenobarbitúricos y la corteza motora correspondiente a áreas de miembros anteriores, posteriores y tronco fué completamente aspirada. Una porción del fragmento cortical frontal fue extirpada de un embrión a los 15 días de gestación e implantada en la cavidad lesionada del cerebro del lesionado. El grupo de lesión no recibió ningún tejido donante. Los simulados controles pasaron todos los procedimientos hechos en esos dos grupos antes mencionados, excepto aspiración cortical y transplante. Los resultados de las pruebas a campo abierto mostraron significativas diferencias entre grupo de lesión y grupo control en las siguientes áreas. Animales lesionados evidenciaron una disminución en término medio de bloques cruzados, una disminución en el término medio de retaguardias normales. Animales que recibieron transplante mostraron en significativo mayor término medio de bloques cruzado que en animales lesionados. También animales de transplante mostraron significativamente más actividad de reta guardia que los lesionados. Animales de transplante fueron encontrados sin ser significativamente diferente de controles en distintas pruebas. Estos resultados demostraron que injertos fetales corticales pueden mejorar defectos inducidos por le-

sión en la función locomotora de adultos. Entonces animales fueron sacrificados para examen histológico y trazado de conexión de fibras. Hasta 85 % de transplantes sobrevivieron y ocuparon la totalidad de la cavidad lesional de la corteza motora lo mal representa un 6 a 8 veces de aumento del volumen de tejido que fue colocado en el animal receptor.

La coloración vesil violeta y preparaciones de Golgi de los transplantes mostraron más células que la corteza motora adyacente. Los transplantes mostraron la presencia de neuronas de aspecto normal bien diferenciadas, elementos neurológicos y neuropila. Aunque las neuronas no estaban dispuestas en distintas capas, estaban dispersas y acomodadas en grupos y las mayores células piramidales estaban próximas al borde entre el injerto y el tejido receptor, es una localización normal para estas células. Una capa molecular fue vista en la superficie de los sobrevivientes transplantes. En morfología celular las neuronas aparecían amplias con núcleos localizados en el centro y nucleolos claramente vistos y cuerpos de Nissl claramente coloreados. Mostraron largas y diferenciadas dendritas y su colateral con muchas espinas en ellas.

En sus características las dendritas y sus ramas aparecen informes en su extensión. Las dendritas apicales de las células piramidales orientadas hacia capas moleculares. Células no piramidales eran de dos tipos: espinoso no espinos. Hubo un intermedio entre tejidos de transplante y del receptor. Sugiere que el transplante tiene una buena integración parenquimatosa con el cerebro receptor. Pequeñas inyecciones iontoforética de PHA-L fueron colocadas con éxito en un transplante cortical sin afectar el cerebro receptor. Algunas células piramidales fueron vencidas con PHA-L. Sus fibras marcadas fueron desplazadas a través del transplante y proyectadas ulteriormente a varios lugares del cerebro receptor: el tálamo homolateral, la corteza motora sensitiva contralateral, el hipotálamo bilateral y la formación reticular. Los hechos presentes sugieren que los transplantes pueden reemplazar la corteza motora dañada y reconstruir proyecciones corticales normales en el cerebro receptor.

El transplante de corteza motora tendría que ser de valor terapéutico en el tratamiento de parálisis causada por accidente vascular o trauma cortical.

Palabras claves: transplante neural, corteza motora, daño cerebral, recuperación locomotora, regeneración, conexiones nerviosas, rata.

RÉSUMÉ

Le motif de cette investigation a été d'étudier la regénération du cortex moteur endomagé, par le moyen de transplants de tissu foetal type similaire. Sur des Rats Sprague-Dawley anestesiées au fenobarhital, le cortex correspondant aux zones motrices des menbres anterieurs, posterieurs et tronc furent aspirées.

Il fut implanté a cette place un fragment de cortex frontal d'un embryon de 11 jours,

Les résultats des épreuves a champ ouvert ont montré des differences importants entre le group de lesion et le groupe de controle.

Les animaux du groupe de lesion ont montré une diminution moyenne des bloc croisés, et une diminution moyenne d'arrie-regarde.

Les animaux transplantés ont montré un nombre superieur de blocs croisés, ils montrére aussi une plus grande activité de arriéregarde.

Les animaux transplantés n'ont por été sensiblement differents des animaux de controle.

Ce qui démontre que des implants de foetus peuvent ameliorer des defaute par lesion du cortex moteur de l'adulte.

Les animaux furent sacrifiés pour examen histologique, et trace des conexion des fibres.

Le 85 % des implants ont survecus et ocupaient, la totalité de la cavité lesionelle, ce qui representait 6 a 8 fois le volume implanté.

Les coloration au crésyl-violet et des préparations de Golgi des transplants ont montrée plus de cellules qui cortex adyacent, rich des neurones bien differenciés et d'as-

pect normal et d'austres élements neurologiques. Les neurones n'étaient pas disposées en couches, mais dispersées en groupes et les grandes célules pyramidales etaient adyacentes a la couche limite avec le recepteur, ce qui est uné localization normale pour ces cellules. Une couche moleculaire fue trouvée a la surface des transplants survivants. Les neurones étaient grandes avec un noyau central et des nucleoles visibles, des corpuscules de Nissl nettement colorés. El possedaint des longues dendrites et un colateral tres epineu.

Les dendrites et leur rameaux ne sont pas systématisés. Les dendrites apicalles des cellules pyramidales sont orientéss vers le couche moleculaire.

Les cellules non pyramidales sont de 2 types: épineuses et non epineuses. Il y a un espace intermediaire entre le transplant et le recepteur.

Inclusions iontopherétiques de PHA'furent faites avec succés dans un transplant cortical sans afecterle recepteur. Quelques celules pyramidales furent marquéss au PHA-L. Les fibres marqueés se deplacerent a travers le transplant et progetées a divers endroits du récepteur: Le talamus homolateral de cortex sensitivo moteur contralateral l'hypothalamus bilateral et la formation lenticulaire. Ceci menne a penser que les transplants peuvent reemplacer le cortex endomagé et reconstruire les proyections corcales normales dans le cerveau recepteur.

Ceci menne a penser que le transplant de cortex moteur peut etre une valeur therapeutique dans le traitment de la paralisie causée por AV ou traumatisms.

Mots clefs: transplant neuronal, cortex moteur, domage cerebarl, recuperation, locomotrice, regeneration, conexions nerveuses rats.

ZUSAMMENFASSUNG

Diese Arbeit beschaeftigt sich mit der Untersuchung ueber die Wiederherstellung der motorischen Hirnrinde die geschaedigt wurde, durch die Transplantationen von foetalemwirngewebe aehnlicher Art. Sprague-Dawleu Ratten wurden betaeubt mit Phenobarbitursaeurepraeparaten und die motorische Hirnrinde entsprechend den Zonen fuer die Vorderglieder, Hinterglieder und Rumpf wurde vollkommen aspiriert. Eine Portion der frontalen Rinde wurde bei einem Embrion von 15 Tagen extirpiert und ueberpflanzt in die Grube der Empfaenger Hirnrinde.

Die Gruppe der Geschaedigten erhielt kein Transplant. Die simulierten Kontrollen wurden mit allen Prozessen durchgefuehrt, wie sie elei beiden vorherge nannten durchgefuehrt wurden, ausser der Aspiration und den Ueberpllanzungen.

Die Resultate der Proben bei offenem Feld zeigten signifikative Unterschiede zwischen der Gruppeder Verletzten und der Kontrollgruppein folgenden Zonen. Die verletzten Tiere zeigten eine Verminderung im Burchschnitt der "gekreuzten Bloecke", eine durchschnitliche Verminderung der normalen Rueckfront. Die Tiere, die Transplantate erhalten hatten, zeigten in einem signifikativ hoeheren Durchschnitt die "Gekreuzten Bloecke" als die verletzten Tiere. Auch zeigten die Tiere mit Transplantaten signifikativ hoehare Aktivitaet der Rueckfront als die verletzten Tiere. Die Tiere mit Transplantaten waren nicht signifikativ verschieden von dien Kontrolltieren bei den verschiedenen Tests. Diese Resultate zeigen, dass die foetalen Hirntransplantate Defekte hervorgerufen durch die Excisionen die lokomotorische Funktion ausgewachsener Tiere bessern koennen. Darauf wurden die Tiere getoetet, fuer histologische Untersuchungen und zu durchforschen die Verbindungen der Nervenfibern. Bis zu 85 % der Transplantationen ueberberlebten und man fand, dass die ganze durch die Aspiration erzeugte Grube der motorischen Hirnrinde mit dem Transplantat ausgefuellt war, was bedeutete, dass das das ueberpflanzte Material uechs bis acht mal vermehrt war. Die Cresyl-Violett Faerbung und die Golgi Praeparationen der Transplantate zeigten mehr Zellen als die benachbarte motorische Rinde, die Neuronen hatten normales Aussehen und waren gut differenziert, esgab neurologische Elemente und Neuropila. (Netzwerk von Axonen und Dendriten). Obwohl die Neuronen

nicht in verschiedenen Schichten angeoerdte waren sondern Verstreut und inverschiedenen Haufen angeoerdnet waren, und die groessten Pyramidenzellenin der Naehe des Randes zwischen dem Transplantat und und dem Empfaengergewebe, bedeutet dies eine normale Lokalisation fuer diese Zellen. Es wurde eine Molekularschicht an der Oberflaeche der ueberlebenden Transplantate gesehen. Bezueglich der Zellulaeren Morfologie waren dieNeuronen gross mit dem Nukleus im Zentrum und deutlich wahrnembare Nukleoli und die Nissl'schen Koerper deutlich gefaeerbt. Die Dendriten waren lang und differenziert und ihre Kollateralen mit mit vielen Stacheln.

Die Dendriten undihre Abzweigungen erscheinen unfoermig. Die apikalen Dendriten der Pyramidenzellen sind in Richtung Molekularschicht orientiert. Die Nicht Pyramidalen Zellen waren von zwei Sorten, mit Stacheln undohne Stacheln. Man beobachtete zwischen Transplantat und Empfaenger gewebe eine Zwischenzone. Das will heissen, dass das Transplantat sich gut in das Epmfaengergehirn integriert. Man machte kleine iontophoretische Injektionen von PHA-L in ein Rinden-Transplantat mit gurem Erfolge ohne das Empfaengehirn zu affektieren. Einige Pyramidenzellen waren mit PHA-L gefuellt.

Ihre markierten Fibrillen durchliefen das Transplantat und erreichten weiter verschiedene Ziele des Empfaengerhirns; den ipsilateralen Thalamus, die Kontralaterale motorisch-sensorische Hirnrinde, den Hypothalamus bilateral, und die Formatio reticularis. Die vorliegenden Daten lassen erkennen, dass die Rindentransplatationen die geschaedigte motorische Rinde ersaetzen koennen und normale corticale Projektionen wiederaufpauen koenen im Empfaengurhirn. Die Transplantation von motorischer Hirnrinde kann therapeutischen Wert haben bei der Behandlung der Paralyse durch Schlaganfall oder Hirnrindentrauma. Schluesselworte: Neurale Ueberpflanzungen, Motorische Rinde, Hirnschaden, lokomotorische Wiederherstellung, Regeneration. Nervenverbindungen, Ratte.

REFERENCES

1. *Azmitia, E. C.* and *Bjorklund, A.*: Cell and Tissue Transplantation into the Adult Brain, New York: The New York Academy of Sciences, 1987.
2. *Bjorklund, A.* and *Stenevi, U.*: Neural Grafting in the Mammalian CNS, Amsterdam: Elsevier Science Publishers, 1985.
3. *Bolam, J. P., Freund, T. F., Bjorklund, A., Dunnett, S. B. and Smith, A. D.*: Synaptic input and local output of dopaminergic neurons in grafts that functionally reinnervate the host neostriatum, Exp. Brain Res., 68: 131-146, 1987.
4. *Buzsaki, G., Gage, F. H., Kellenyi, L.* and *Bjorklund, A.*: Behavioral dependence of the electrical activity of intracerebrally transplanted fetal hippocampus. Brain Res., 400: 321-333, 1987.
5. *Chang, F.-L.F., Steedman, J. G.* and *Lund, R. D.*: The lamination and connectivity of embryonic cerebral cortex transplanted into newborn rat cortex, J. Comp. Neurol., 244: 401-411, 1986.
6. *Das, G. D.* and *Wallace, R. B.*: Neural Transplantation and Regeneration, New York: Springer-Verlag, 1986.
7. *Dunnett, S. B., Toniolo, G., Fine, A., Ryan, C. N., Bjorklund, A.* and *Iversen, S. D.*: Transplantation of embryonic ventral forebrain neurons to the neocortex of rats with lesions of nucleus basalis magnocellularis. II. Sensorimotor and learning impairments, Neuroscience, 16: 787-797, 1985.
8. *Fine, A., Dunnett, S. B., Bjorklund, A.* and *Iversen, S. D.*: Cholinergic ventral forebrain grafts into the neocortex improve passive avoidance memory in a rat model of Alzheimer's disease. Proc. Natl. Acad. Sci. USA, 82: 5227-5230, 1985.
9. *Gash, D. M., Collier, T. J.* and *Sladek, Jr. J. R.*: Neural Transplantation: A review of recent developments and potential application to the aged brain, Neurobiol. Aging, 6: 131-150, 1985.
10. *Gash, D. M.* and *Sladek, Jr. J. R.*: Transplantation in the Mammalian CNS: Preclinical and Clinical Studies, Amsterdam: Elsevier Science Publishers, 1988.
11. *Gerfen, C. R.* and *Sawchenko, P. E.*: An anterograde neuroanaotmical tracing method that shows the detailed morphology of neurons, their axons and terminals: immunohistochemical localization of an axonally transported plant lectin, *Phaseolus vulgaris*-leucoagglutinin, Brain Res., 290: 219-238, 1984.
12. *Hall, R.* and *Lindholm, E.*: Organization of motor and somatosensory neocortex in the albino rat, Brain Res., 66: 23-38, 1974.

13. *Harnsberger, J.* and *Wallace, R. B.*: A behavioral and histological examination of young rats receiving homotopic embryonic neural transplants in motor cortex. Das, G. D. and Wallace, R. B. (eds), Neural Transplantation and Regeneration, pp. 287-326, New York, Spring-Verlag, 1986.

14. *Isacson, O., Dunnett, S. B.* and *Bjorklund, A.*: Graft-induced behavioral recovery in an animal model of Huntington's disease, Proc. Natl. Acad. Sci. USA, 83: 2728-2732, 1986.

15. *Jaeger, C. B.* and *Lund, R. D.*: Efferent fibers from transplanted cerebral cortex of rats, Brain Res., 165: 338-342, 1979.

16. *Jiao, S.S., Wang, Y. S., Cai, Q.* and *Li, N.*: Replacement of damaged motor cortex by homotopic transplants of fetus brain tissue in rats. I. Survival, growth, and cytoarchitecture of the grafts, J. Beijing Second Medical College, 7: 93-101, 1986a.

17. *Jiao, S. S., Wang, Y. S., Wang, H.* and *Li, N.*: Replacement of damaged motor cortex by homotopic transplants of fetus brain tissue in rats. II. Grafthost connections, J. Beijing Second Medical College, 7: 102-106, 1986b.

18. *Low, W. C., Lewis, P. R., Bunch, S. T., Dunnett, S. B., Thomas, S. R., Iversen, S. D., Bjorklund, A.* and *Stenevi, U.*: Function recovery following neural transplantation of embryonic septal nuclei in adult rats with septohippocampal lesions, Nature, 300: 260-262, 1982.

19. *Neafsey, E. J., Sorensen, J. C., Tonder, N.* and *Castro, A. J.*: Thalamic stimulation evokes single-unit activity in fetal neocortical tissue grafted into the cerebral hemisphere of newborn rats, Soc. Neurosci. Abstr., 13: 513, 1987.

20. *Sharp, F. R.* and *Gonzalez, M. F.*: Fetal frontal cortex transplant (^{14}C) 2-deoxyglucose uptake and histology: survival in cavities of host rat brain motor cortex, Neurology, 34: 1305-1311, 1984.

21. *Sladek, Jr. J. R.* and *Gash, D. M.*: Neural Transplants: Development and Function, New York: Plenum Press, 1984.

22. *Tonder, N., Sorensen, T., Zimmer, J., Jorgensen, M. B., Johansen, F. F.* and *Diemer, N. H.*: Neural grafting to ischemic lesions of the adult rat hippocampus, Exp. Brain Res., 74: 512-526, 1989.

Cellular Mechanisms Involved in Purkinje Cell Replacement in the Cerebellum of Mice With Heredo-Degenerative Ataxia

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INTRODUCTION

Neurons are highly differentiated cells that lose their proliferative ability at a distinct step of their development; their loss cannot be compensated by proliferation of the surviving neurons. One experimental way to palliate the loss of neuronal populations in the adult brain is to replace the missing neurons by grafting homotypic, young postmitotic neurons, taken from isogeneic embryos. In systems organized in a "point-to-point" manner (Sotelo and Alvarado-Mallart, 1986), such as the cerebellum, neuronal replacement is effective only when the grafted neurons succeed in reestablishing the anatomical and functional integrity of the deficient networks, by constituting an equivalent synaptic circuit. Such a reconstitution is only possible when the embryonic neurons, grafted into the adult deficient cerebellum, are able to pursue their developmental program in such a way that the cell-to-cell interactions between immature and adult neurons recapitulate those taking place during normal ontogeny.

The biological system we have used to determine the degree of neuronal replacement and, therefore, the degree of structural restoration of the cerebellar circuits resulting from neural grafting, has been the cerebellum of adult mice affected by the Purkinje cell degeneration (pcd) mutation, an autosomal recessive mutation, mapped on chromosome 13. In the *pcd* mouse (Mullen et al. 1976), the cerebellum develops normally until the end of the 2nd postnatal week. Between P14 and P45 virtually all Purkinje cells degenerate (less than 120 of these neurons remain in 3-month-old mutants, most of them located in the nodulus, Wassef et al. 1987). In spite of this lack of Purkinje cells in the 3-month-old mutants, a large proportion of synaptic inputs remains intact in the molecular layer, although about 40 % of inferior olivary neurons have already degenerated (Ghetti et al. 1987; Shojaeian et al. 1988). According to its histopathology, this mutant mouse can be proposed as an animal model of the form of human cerebellar ataxia reported by Holmes (1907) and known as cerebellar cortical degeneration or cerebellar-olivary degeneration.

In the experiments reported here, donor tissue was taken from cerebellar primordia of 12 day-old (E12) mouse embryos (C57BL), and the hosts were 3-to-4 month-old homozygous *pcd* mice with a C57BL genetic background. Two different grafting procedures were performed: either the ce-

rebellar primordia were mechanically dissociated in tissue culture medium, and the cell suspensions used for grafting, or the primordia were sliced into small pieces, and the individual pieces used in solid transplantation. In both instances, the embryonic material was injected, with small glass pipettes, at variable depths within the parenchyma of the host cerebellum. The obtained results were almost identical with the two procedures and, therefore, they have been pooled together.

PURKINJE CELL REPLACEMENT: LONG-TERM SURVIVAL

In order to repair the cerebellar circuits of the pcd mouse, the Purkinje cell substitution needs to achieve three essential prerequisites: (a) from all the postmitotic neurons and progenitors present in the grafts, only neurons of the Purkinje cell category must leave the implanted cellular mass, and move to the correct position previously occupied by the missing host Purkinje cells. (b) Once they reach the proper locations, the grafted neurons must not only follow their differentiation program and build up their dendritic trees, but must participate in synaptogenesis with specific host afferents. These developmental events would lead to the synaptic integration of the grafted Purkinje cells into the deficient cortical circuitry. (c) Finally, no functional improvement can be accomplished if the disrupted corticonuclear projection is not reestablished. For that, the grafted Purkinje cells need to grow axons that, navigating throughout the adult host cerebellum, reach their appropriate targets in the deep nuclei, and synaptically contact the proper host nuclear neurons.

The results obtained with long-term survivals (2 to 4 months after grafting) demonstrate that these prerequisites can be fulfilled but with important limitations (Sotelo and Alvarado-Mallart, 1986; 1987a; Gardette et al. 1988).

Purkinje cell invasion:

Grafted Purkinje cells, visualized with immunostaining using selective markers such as calbindin (Jande et al. 1981), could be seen at distances as far as 700 μm away from the graft/host interface. Their situation, however, differs from that of normal cerebellum, since they do not form a monolayer but invade the superficial 4/5ths of the host molecular layer. Despite the ectopia of the cell bodies, the dendritic trees of the grafted Purkinje cells succeed in spanning the entire host molecular layer but never intrude into the granular layer, suggesting that the microenvironment of the latter is not permissive for them.

When compared to other grafting systems in the adult mammalian CNS, this invasive behavior of immature Purkinje cell is rather unique. To our knowledge, only spinal motoneurons have a similar behavior (Sieradzan and Vrbová, 1989), and in both cases there is competition between host and grafted neurons. Only when neurons of the host have disappeared, the embryonic grafted neurons exert their maximum invasion.

Synaptic investment of grafted Purkinje cells:

Despite the ectopic position of the cell bodies, the dendritic trees of the grafted Purkinje cells —the receptive surface of these neurons— tend to remain confined to the sagittal plane of the host cerebellum, as do normal Purkinje cells. In addition, these dendritic trees are also formed by a proximal and a distal compartment, indicating that they have succeeded in properly segregating the incoming synaptic inputs arising from host neurons. The ultrastructural and electrophysiological study done on these cerebella (Sotelo and Alvarado-Mallart, 1987a; Gardette et al. 1988) show that the grafted Purkinje cells have been successful in their synaptic integration into the deficient cortical circuitry of the mutant cerebellum. The ectopically located perikarya receive inputs qualitatively similar to those of control Purkinje cells. Synaptic inputs to their dendrites also mimic normality. However, one important difference exists: basket fiber-pinceau formations are absent around the initial segment of the Purkinje cell axon.

Using an in vitro slice preparation and intracellular recordings of the grafted Purkinje cells, Gardette et al. (1988) have shown that, after white matter stimulation,

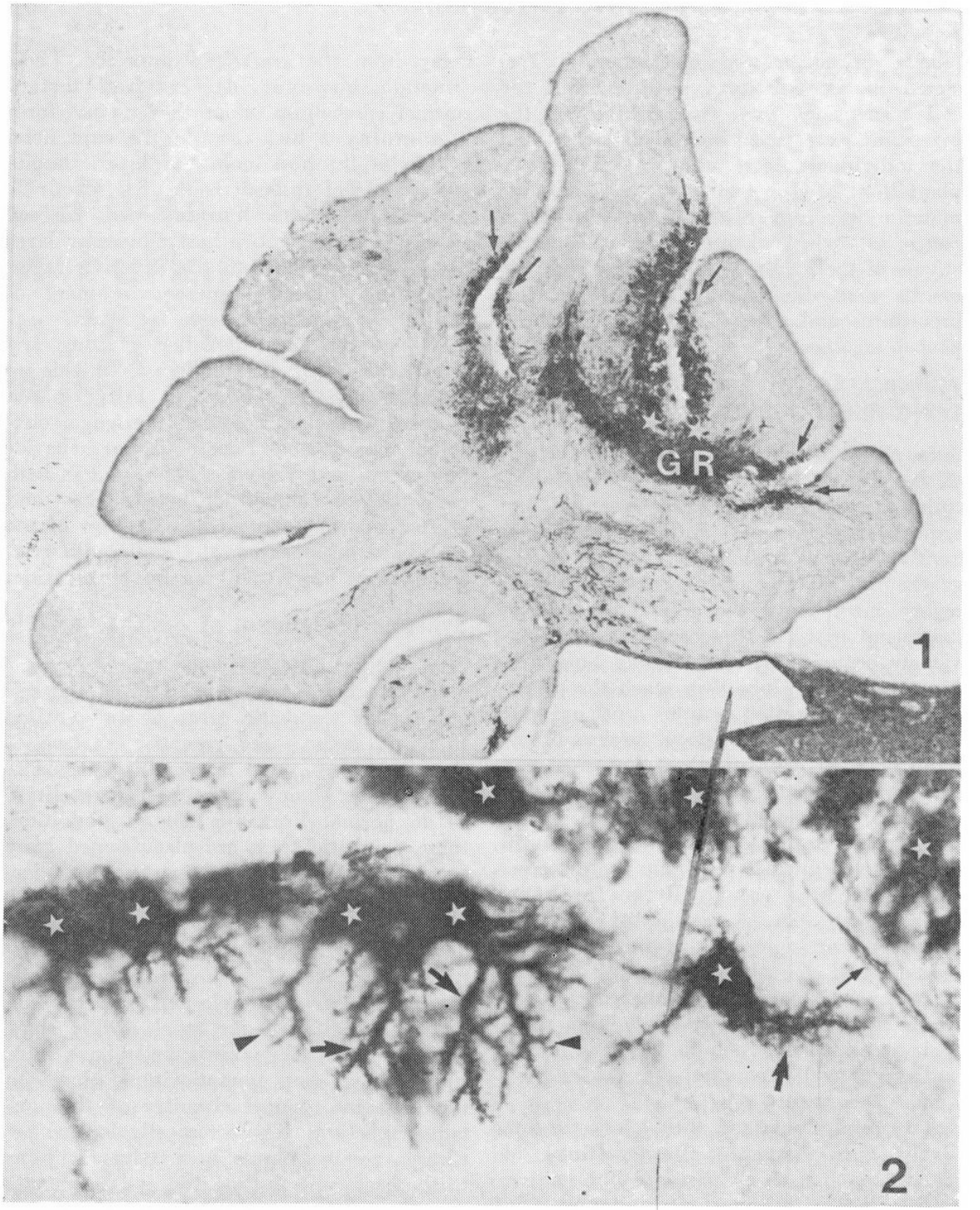

PLATE I

Fig. 1. — *Sagittal section of a pcd cerebellum 7 days after grafting. The graft remnant (GR) crosses two folia of the adult host cerebellum. The stars mark the interfaces between the graft and the host molecular layer. Note the presence of grafted Purkinje cells in the molecular layer of four folia of the host cerebellum (arrows). Calbindin immunostaining.*

Fig. 2. — *Sagittal section of a pcd cerebellum 14 days after grafting. This micrograph illustrates grafted Purkinje cells at the most distant end of their migration within the host molecular layer. The cell bodies (stars) occupy a superficial position in the host folia. Note the flattening of the dendrites (large arrows) and the presence of forming spiny branchlets (arrowheads). The small arrow points to a tiny bundle of axons located under the subpial basal lamina. Calbindin immunostaining.*

all the impaled cells responded by: I) typical all-or-none climbing fiber EPSPs (complex spike), II) simple spikes (corresponding to the disynaptic input of mossy fibers), as well as III) later inhibitory responses (corresponding to the trisynaptic pathway of mossy fiber - granule cell - basket or stellate cell - Purkinje cell). These IPSPs are of shorter duration than in control cerebellum. Thus, the electrophysiologic experiments confirm that the excitatory and inhibitory inputs forming the synaptic investment of the grafted Purkinje cells are functional, and have characteristics comparable to those in normal mouse cerebella.

Re-establishment of a cortico-nuclear projection:

The last and essential requirement for grafted Purkinje cells to reinstate the normal cerebellar circuitries is the formation of a cortico-nuclear projection. The fulfilement of this requirement is the most difficult to achieve. We have observed it in only a restricted number of cases, when the graft is close enough to the deep nuclei (less than 600 μm). Even in these cases, the amount of Purkinje cell axons reaching their targets in the host deep nuclei is very small, and the density of the axonic terminal arborization is much lower than would be expected normally. Thus, the third prerequisite for neuronal replacement in "point-to-point" systems is far from satisfied, although our results indicate that it can occasionally be achieved. Most of our present work in cerebellar transplantation is oriented to provide to the growing axons of grafted Purkinje cells a permissive substratum for a guided navigation towards the host deep nuclei. Without the reestablishment of this Purkinje cell output, the restoration of an improved cerebellar function is inconceivable.

PURKINJE CELL REPLACEMENT: DEVELOPMENTAL ISSUES

The results obtained with long-term survivals imply that, in the adult mutant cerebellum, the replacement of missing Purkinje cells occurs in a very precise manner, most probably recapitulating the processes followed by these neurons during their normal ontogeny. The principal events in cerebellar morphogenesis take place in sequential critical steps from the cellular proliferation of stem cells in the primitive cerebellar neuroepithelium to the functional validation and selective elimination of synaptic connections (Changeux and Danchin, 1976) resulting in the formation of the cerebellar cortex and its specific circuitry. These orderly steps (see refs. in Cowan, 1981) concern the following processes: (I) A phase of *cellular proliferation*, followed by (II) an outward migration of young postmitotic Purkinje cells from the subventricular zone of the cortical plate (Goffinet, 1983). (III) A phase of *cytodifferentiation* with the formation of neuritic expansions, which will result in the acquisition of tridimensionally arranged dendritic trees (see refs. in Sotelo, 1978) and in the establishment of highly organized efferent projections. (IV) Simultaneously with the formation of dendritic arborizations, Purkinje cells begin their *synaptogenetic period*, with the formation of redundant connections (Crepel et al. 1976), that will be followed by a secondary period of numerical adjustment, involving regressive processes resulting in (V) *the selective elimination of some synaptic connections* and the (VI) *synaptic stabilization* of the remaining ones. According to the hypothesis of Changeux and Danchin (1976) these last two processes are regulated by the function of the forming cerebellar circuits.

In order to determine (I) whether or not grafted Purkinje cells are able to recapitulate their developmental history within the adult host cerebellum, and (II) to disclose the cellular mechanisms underlying the successful neuronal replacement discussed above, the transplanted mutants have been analyzed in a timed sequence from 3 up to 21 days after grafting (3-21 DAG), to compare at a given age the phase of maturation of grafted Purkinje cells with that normally occurring in cerebellar ontogenesis. In the following description we shall summarize the results obtained for each of these developmental steps (Sotelo et al. 1989).

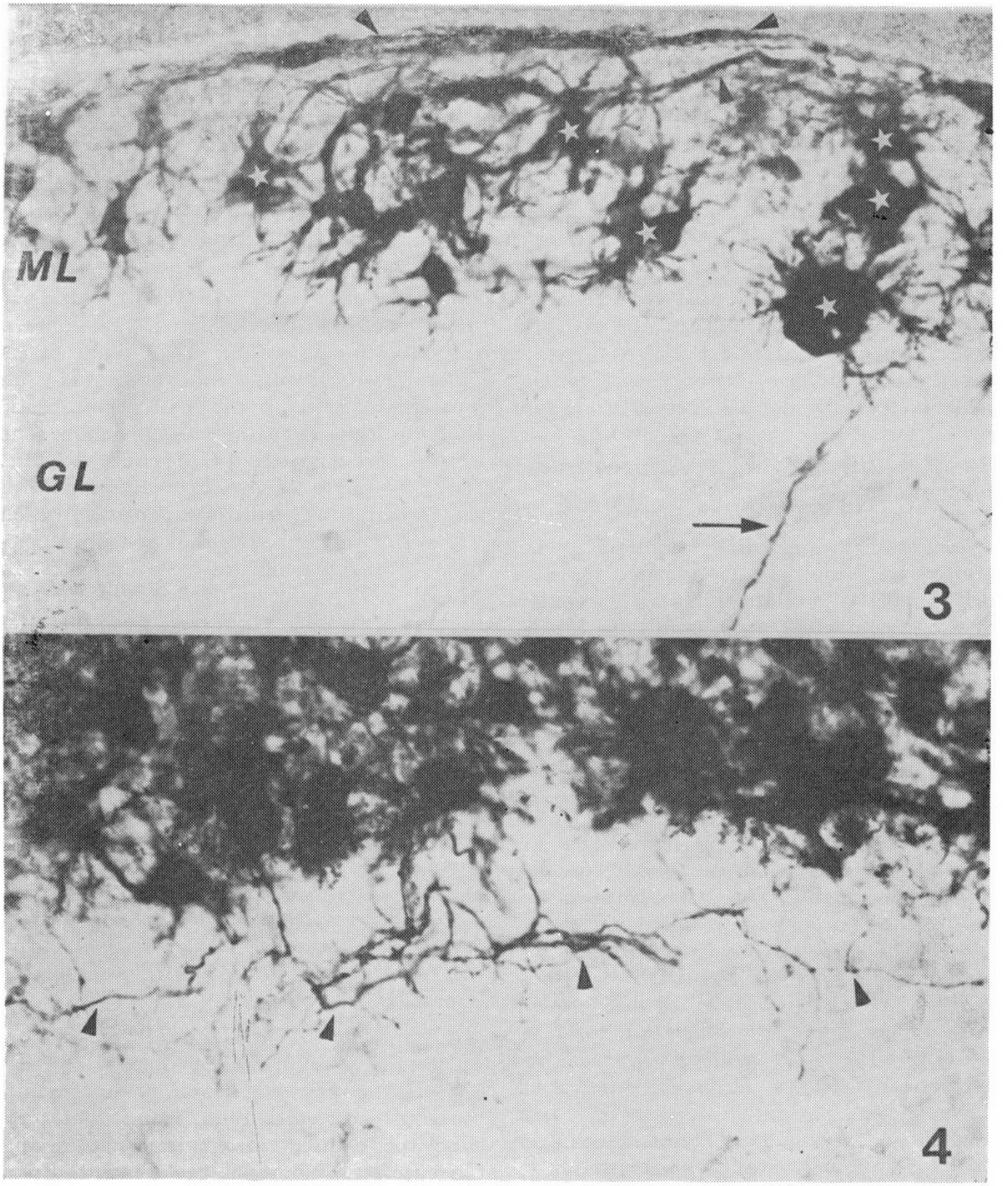

PLATE II

Fig. 3. — *Sagittal section of a pcd cerebellum 10 days after grafting. At this developmental stage most Purkinje cells (stars) are n the "phase of stellate cell with disoriented dendrons". The arrowheads mark the presence of bundles of Purkinje cell axons running at the folial surface, parallel to the subpial basal lamina. Note that one axon (arrow) is able to leave the molecular layer (ML), and enter the granular layer (GL). Calbindin immunostaining.*

Fig. 4. — *Sagittal section of a pcd cerebellum 11 dyas after grafting. The host molecular layer is filled with grafted Purkinje cells. At the interface between molecular and granular layer Purkinje cell axons form conspicuous plexuses (arrowheads) as if they could not enter the granular layer.*

PLATE III

Migratory routes of grafted Purkinje cells:

During the first days after transplantation, the grafts modify their shapes according to physical constraints. The pressure exerted by the parenchyma of the host cerebellum forces the grafts to adapt themselves to regions of less resistance either created by the cannula tract or already present in the host cerebellum like the cerebellar fissures. Thus, from three to four days after grafting (3-4 DAG), they have acquired irregular shapes consisting of a main elongated mass (Fig. 1), that crosses several folia of the host cerebellum, and one or several lateral swellings expanding between the surfaces of adjacent folia, at both sides of the main elongated mass. Owing to this special disposition, grafted Purkinje cells can invade the host parenchyma following two different migratory pathways, one originating from the graft-host interface and the other through the pial surface and its basal membrane. Indeed, 4-5 DAG a migratory stream has been formed at the periphery of the host folia directly attached to the graft. This stream emerges from the graft and moves off, between the subpial surface and the glial limiting membrane, in a funneling manner (at its emergence it consists of a five to seven-cell-deep layer and at its end, about 700 μm away, it is formed of a single row of elongated neurons), without disrupting the compact neuropil of the host cerebellar cortex. The stream is composed of undifferentiated bipolar neurons that express calbindin immunostaining as do migrating Purkinje cells in control fetuses (Wassef et al. 1985). The second early migratory pathway is formed by calbindin-immunoreactive, postmitotic Purkinje cells that, from the lateral swellings of the graft lying on the surface of the host folia, are able to pass directly into the host molecular layer through discontinuities in the subpial basal lamina. We have no precise information about the mechanisms underlying the breakage of the basal lamina but the migrating Purkinje cells could be responsable for this breakage. Indeed, migrating neurons are provided with a leading process and its terminal growth cone, and it has been proven that neuronal growth cones are able to release proteolytic enzymes, such as serine proteases and metallo proteinases that can be involved in the breakage of the basal lamina (see refs. in Monard, 1988).

It is important to recall that both types of early Purkinje cell migrations commonly coexist, and that both of them are at the origin of the inward migration of the grafted neurons. Indeed 6 to 7 DAG, grafted Purkinje cells of both origins massively penetrate the host molecular layer (Fig. 1). Those arising from the tangential migratory stream do so by changing polarity and adopting a bipolar radial or somewhat oblique position. Those directly passing form the lateral swellings of the graft do so by traversing the basal lamina and invading the host molecular layer. The inward-oriented processes of both groups penetrate the whole depth of the molecular layer, but do not enter the granular layer (Fig. 1), as if their permissive microenvironment abruptly stops at the molecular layer/granular interface. The inward migrating Purkinje cells can run parallel to the host Bergmann fibers, and be directly apposed to these glial axis as it seems to occur during normal ontogeny. By day 10 DAG, the grafted Purkinje cells have elaborated nascent dendritic trees which confer to the cells a stellate appearance, but maintain unchanged the position of their perikarya. Hence, the migratory behavior of these neurons, and the consecutive perikaryal translocation, is frozen when their leading processes reach the interface between molecular and granular layer, explaing the ectopic position of the grafted Purkinje cells in long-term survivals.

Dendritic differentiation:

The grafted Purkinje cells between 5 to 9 DAG exhibit a bipolar shape closely resembling normally developing Purkinje cells in their "phase of the fusiform cell" (Ramón y Cajal, 1926). The difference is that the long and asymmetrically branched dendritic segment has not an apical position but is inverted. The retraction of these long and smooth dendrites and the protrusion of numerous somatic filopodia also take place in the grafted neurons, so that on 10

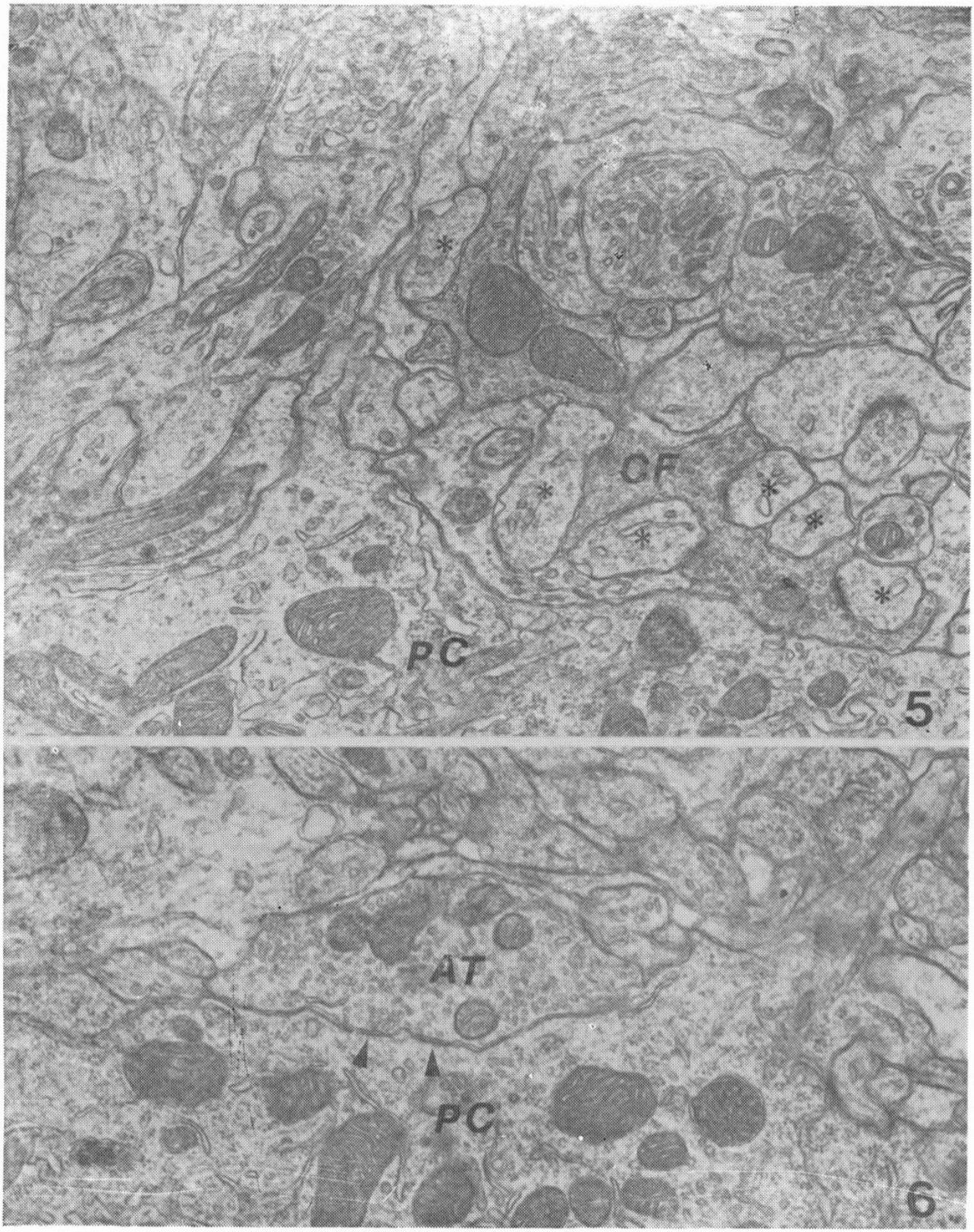

Fig. 5. — *Electron micrograph illustrating a grafted Purkinje cell body (PC) from which emerge long filopodial processes (asterisks). A convoluted axon terminal (CF) establishes numerous synaptic contacts on the somatic filopodia. The density of the axoplasm and the features of the synaptic complexes allow us to identify this axon terminal as belonging to a climbing fiber. pcd cerebellum 11 days after grafting.*

Fig. 6. — *This electron micrograph illustrates a synapse between a grafted Purkinje cell body (PC) and an axon terminal containing pleomorphic synaptic vesicles (AT). The axonal organelles, and the symmetric synaptic contacts (arrowheads) established on the smooth surface of the Purkinje cell body, indicate that the axon terminal belongs to a host molecular layer interneuron. pcd cerebellum 12 days after grafting.*

to 11 DAG they exhibit the appearance of Purkinje cells in their "phase of the stellate cell with disoriented dendrons" (Figs. 3 and 4). From 10 to 14 DAG, the perikarya of the grafted neurons have developed multiple primary dendritic segments, profusely branching into secondary and distal branches (Fig. 2). The latter, studded with spines, correspond to incipient spiny branchlets. At this age, despite the anomalies in number and orientation of the primary branches, most of the grafted Purkinje cells have reached the "phase of orientation and flattening of the dendrites" (Fig. 2). The conclusion of this study is that the biological age of grafted Purkinje cells in their transition from the first to the second maturational phases coincides with that of normally developing Purkinje cells, while the transition from the second to the third phase takes place earlier in the grafted cells.

Synaptogenesis between grafted and host neurons:

A careful analysis of the synaptic investment of the migrating Purkinje cells shows that, despite their proximity to the adult cerebellar neuropil containing abundant axon terminals, they practically lack synaptic inputs. Synaptogenesis between host axons and grafted Purkinje cells really starts 10-11 DAG, when the neurons reach the biological age of 2-3 postnatal days. One of the main features of this phase is the production of perikaryal filopodia, that in normal ontogeny of the cerebellum correspond to the post-synaptic elements of the synapses between climbing fibers and Purkinje cells (beginning of the stage of pericellular nest of Ramón y Cajal, 1911). Some of the somatic processes emerging from the grafted Purkinje cells are directly apposed to host beaded axons, with some of their varicosities establishing asymmetric synaptic contacts on the somatic filopodia (Fig.5). These beaded axons, in addition to the typical organelles of climbing fibers, can also contain tubular profiles of the smooth endoplasmic reticulum which resemble those in growth cones, and have been considered here to be axonal sprouts of host climbing fibers.

Shortly after the beginning of synaptogenesis between host climbing fibers and grafted Purkinje cells, some occasional axon terminals, belonging to host molecular layer interneurons, can be seen synapsing on the smooth surface of the grafted Purkinje cell perikarya (Fig. 6). Similarly, parallel fiber varicosities can synapse on the forming spines emerging from the growing dendrites of grafted Purkinje cells. Thus, the starting of synaptogenesis between host interneurons (granule, basket and stellate cells) and grafted Purkinje cells takes place earlier than during normal ontogeny.

Between 12 and 14 DAG, synaptogenesis is very active. The cell bodies of the grafted Purkinje cells are surrounded by abundant climbing fibers (pericellular nest stage) as well as by axon terminals emerging from inhibitory neurons (basket and/or stellate cells, or even collaterals of the axons originating from grafted Purkinje cells). Simultaneously, axonal varicosities of the host parallel fibers establish abundant asymmetric synapses with newly formed distal dendritic spines (Fig. 7). By 14 DAG, climbing fibers have started their translocation from their perisomatic to their peridendritic location, and more numerous boutons from the axons of the molecular layer interneurons are synapsing on the smooth surface of the perikarya as well as on the proximal dendrites (Fig. 8). At this age synaptogenesis between host parallel fibers and long-necked spines emerging from newly formed spiny branchlets proceeds at a very high rate (Fig. 8).

By 21 DAG, the synaptic investment of the grafted Purkinje cells is qualitatively similar to that observed in long-term survivals, indicating that after this stage of development only quantitative changes result from the continuing synaptogenesis. The morphological analysis summarized above indicates that timing and cellular mechanisms leading to the maturation of grafted Purkinje cells and their synaptic integration in the deficient cortical circuitry of the *pcd* cerebellum correspond to those occurring normally during ontogeny. One important point in the formation of the cerebellar circuitry is the transient passage through a phase of redundancy of the connections, particularly emphasized by the presence of a transient multiple innervation

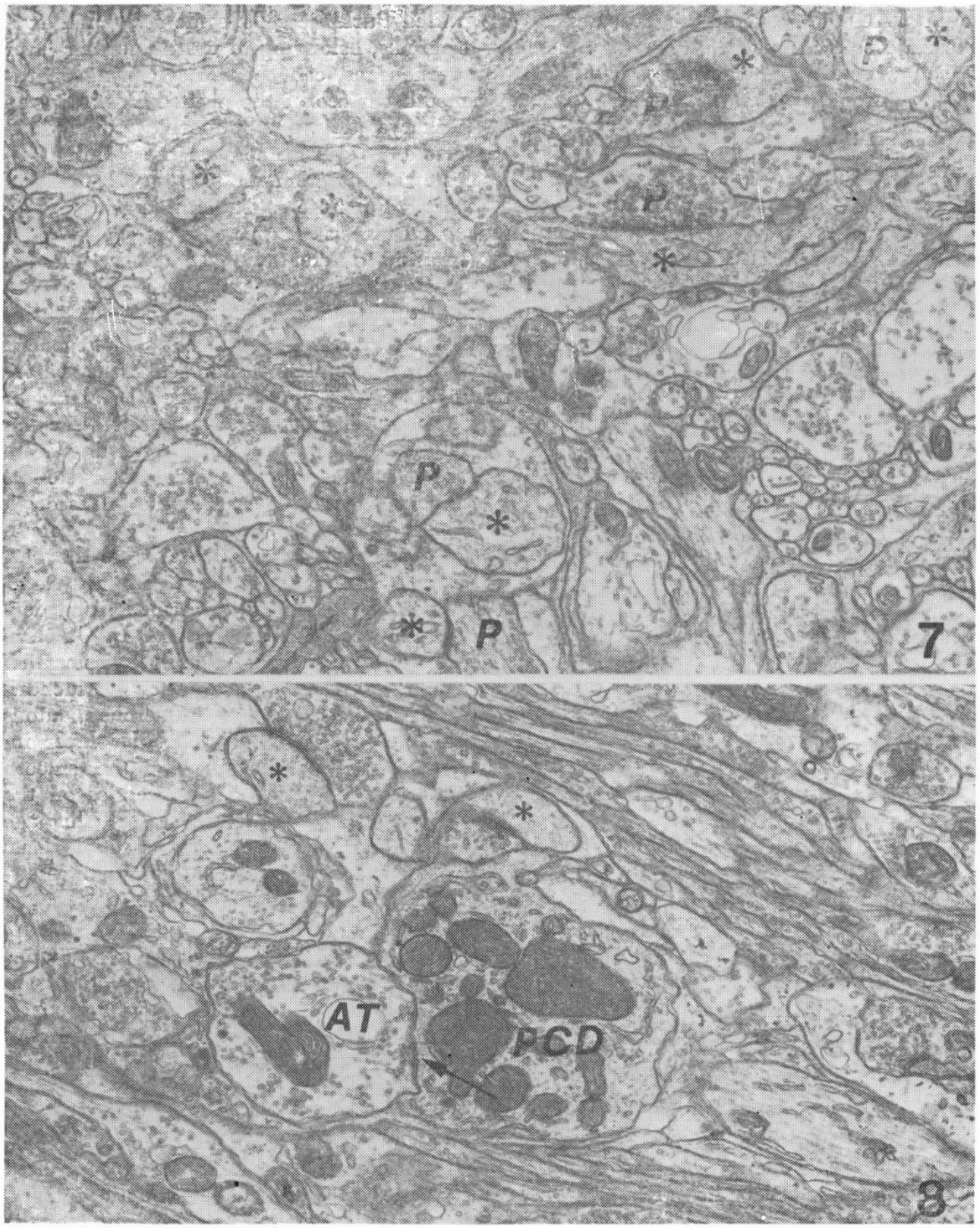

PLATE IV

Fig. 7. — *Electron micrograph of the molecular layer of a host pcd cerebellum 13 days after grafting. Note the presence of numerous spines (asterisks) belonging to nascent spiny branchlets of grafted Purkinje cell dendrites. All of them receive synaptic inputs from host parallel fiber varicosities (P).*

Fig. 8. — *Similar to figure 7 but taken from a transplanted mouse 14 days after grafting. In addition to synapses between parallel fibers and Purkinje cell spines (asterisks), this micrograph illustrates a synaptic contact (arrow) between a medium sized Purkinje cell dendrite (PCD) and an axon terminal (AT) belonging to a molecular layer interneuron.*

of Purkinje cells by climbing fibers (Crepel et al. 1976), followed by the selective elimination of the exuberant synapses and the stabilization of the remaining ones, to reach the adult one-to-one relationship. In our grafting situation, we have approached this problem with electrophysiological methods, in a collaborative study with Robert Gardette and Francis Crepel (Gardette et al. 1989). A transient stage of multiple innervation of grafted Purkinje cells by host climbing fibers exists between 10 and 15 DAG, as revealed by the stepwise variation in amplitude of the climbing fiber-mediated excitatory postsynaptic potentials, with increased intensity of stimulation. After 15 DAG, normal adulttype synaptic responses have been recorded in all tested Purkinje cells. Therefore, the transient phase of multiple innervation also exists on the grafted cells, but the time window for this phenomenon is much reduced when compared to normal development, since instead of lasting for 10 to 12 days, it only lasts for about 5.

Growth and navigation of the axons of grafted Purkinje cells

The calbindin immunostaining has allowed us to follow the axonogenesis of grafted Purkinje cells since, in the mutant, these axons are the only category of calbindin immunoreactive fibers present in the cerebellar cortex. During their radial migration (6-7 DAG), because of the inverted position of most of the bipolar Purkinje cells, their axons are oriented towards the subpial basal lamina at the surface of the folia, where they form bundles running parallel to the basal lamina. On 10-12 DAG, with the beginning of the formation of the ultimate dendritic trees, the Purkinje cell axons undergo extensive growth but remain almost exclusively confined to the host molecular layer, not only under the pial basal lamina (Fig. 3) but also participating with the synaptic investment of nearby grafted Purkinje cells. Between 12 to 14 DAG, some of these axons have reached the interface between molecular and granular layers, and end by forming highly dense plexuses (Fig. 4). In their behavior, these axons seem to follow similar influences as the Purkinje cell dendrites and avoid entering the granular layer. However, by some still obscure reasons, a few of the immunostained axons are able to penetrate and cross the granular layer (Fig. 3), reaching the white matter axis of the folium. There, these axons can either form tiny bundles or pursue their oriented growth in isolation from other Purkinje cell axons, to reach - if they are within the permissive 600 μm from the host deep nuclei - their targets. Within the host deep nuclei, the immunostained axons end forming poorly branched terminal arbors with a varicose configuration.

General conclusions

The results obtained in the series of short-term survivals indicate that grafted Purkinje cells are able to pursue their developmental program in the cerebellum of the adult host mutant mouse. Purkinje cell migration seems to be the result of the specific attraction of the deficient molecular layer of the host cerebellum. Despite the abnormalities of the early pathways followed by the young Purkinje cells, these neurons penetrate the host molecular layer through a radial and/or oblique migration, resembling that followed by postmitotic Purkinje cells during normal development, although they follow an inward instead of an outward direction. Our study also shows that the migratory phase lasts for four days and, therefore, that the biological age of grafted Purkinje cells at the end of their migratory period is E19, the age at which the migration of these neurons is also finished in mouse fetuses. The cellular mechanisms involved in normal Purkinje cell migration, although not well established, seem to be based on neuron-glial interactions as other cortical neurons do (Rakic, 1985). The grafted Purkinje cells during the last phase of their migration appear directly apposed to Bergmann fibers (Sotelo and Alvarado-Mallart 1987b), allowing us to conclude that their migration may follow cellular mechanisms similar to those used during the normal cerebellar morphogenesis.

At the end of their migration, the grafted Purkinje cells have typical bipolar shapes and begin to build up their ultimate dendritic trees. Our morphological analysis has revealed that the three developmental

phases described by Ramón y Cajal (1926) for Purkinje cell dendritogenesis are recapitulated by the grafted neurons. These observations indicate that the cell-to-cell interactions regulating the moulding of these extraordinary dendritic arbors in cerebellar ontogeny (see refs. in Sotelo, 1978) are also operative in the transplanted cerebella.

The timing in the maturation of dendritic trees from grafted Purkinje cells is slightly advanced when compared with that observed during normal dendritogenesis. This precocity is only effective from the passage from the second to the third phase in dendritic maturation. Indeed, the acquisition of the bipolar shape needed for migration and the regression of the long and smooth dendrites seem to be the consequence of intrinsic mechanisms regulated by the Purkinje cells themselves (Armengol and Sotelo, 1989) and independent from synaptogenesis. Conversely, as stated above, the formation of the ultimate dendritic tree depends on synaptogenesis, and the latter is somewhat more precocious in the grafts than in normal cerebellar ontogeny (see below).

It is also most remarkable to note that synapse formation between grafted Purkinje cells and adult host presynaptic axons proceeds according to a precise program, recapitulating all of the different steps described during normal cerebellar synaptogenesis (Larramendi, 1969). Despite the accelerated rate of synapse formation, explained by the fact that, in contrast to normal ontogeny, during reactive synaptogenesis all presynaptic elements are available for synaptic contact from the beginning of Purkinje cell invasion, the biological age of Purkinje cells at the initiation of the synaptogenesis is the same in both situations. Moreover, the electrophysiological results concerning climbing fiber-Purkinje cell synapses formation strongly suggest that the cell-to-cell interactions regulating the selective stabilization of these synapses are of the same nature that those taking place during normal development. Indeed, the abridgement of the transient period of multiple innervation, together with the precocity in the synaptogenesis between host parallel fibers and grafted Purkinje cells, suggests that the regression of the multiple innervation could be due to competition with parallel fiber-Purkinje cell synapses, as it seems to be the case during normal cerebellar development (Crepel et al. 1981).

The most important conclusion from all these studies on shorttime survivals is the striking similarity in the cellular mechanisms and chronology of the sequential steps leading to the maturation and synaptic investment of Purkinje cells during their normal ontogeny and when grafted to the adult *pcd* cerebellum. This remarkable correspondance allows us to postulate that maturation of grafted Purkinje cells follows an internal clock, which regulates all their developmental programs, independently of environmental signals. Thus, the presence of immature Purkinje cells in the deficient molecular layer of the host would allow adult neurons and glial cells to behave transiently as if they were young postmitotic cells, and to interact with the grafted Purkinje cells according to a tempo imposed by the latter. The most likely, but not necessarily unique, interpretation of this adaptive behavior is that the grafted Purkinje cells themselves regulate gene expression of adult neural cells by generating a transient permissive microenvironment. The end result of this plastic process will be the quasi-normal development of the grafted Purkinje cells and, above all, their synaptic integration into the deficient cortical circuitry. The interplay between hosts and grafts, allowing the specific migration of the missing Purkinje cells, and the regulatory role played by the latter in the plastic behavior of adult neurons of the host lead to the restoration of the impaired circuitry of the mutant cerebellar cortex.

SUMMARY

Missing Purkinje cells can be replaced in adult cerebellum of the Purkinje cell degeneration mutant mouse by grafting pieces of cerebellar primordium taken from 12 day-old isogeneic normal embryos. This replacement takes place with a complete sy-

naptic integration of the grafted neurons into the deficient cortical circuitry of the host. The cerebellar repair is only partial since: (I) the amount of cerebellar cortex receiving grafted Purkinje cells is small, and (II) the re-establishment of the cortico-nuclear projection is only achieved in few cases, and at a low density. The study of the cellular mechanisms underlying this successful replacement was performed 4 to 21 days after transplantation. Purkinje cells have an invasive behavior and migrate along stereotyped pathways to their final position in the host molecular layer. During this migration, the grafted neurons begin to build their dendritic trees and, somewhat later, they receive appropriate synaptic contacts from adult host neurons. Both the detailed timetable and the precise cellular interactions observed are remarkably similar to those taking place during normal cerebellar ontogeny. Our results suggest that the deficient molecular layer exerts a specific neurotropic effect on grafted Purkinje cells, and that the embryonic neurons are able to respond to this signal during a time window defined by their own internal clock. Once in the host cerebellum, they proceed with their developmental program, creating a permissive microenvironment that allows adult host neurons to establish appropriate synaptic connections, leading to the synaptic integration of the grafted embryonic neurons.

RESUMEN

Las células de Purkinje degeneradas del cerebro del ratón adulto afectado por la mutación "Purkinje cell degeneration" pueden reemplazarse, transplantando el primordio cerebeloso tomado de embriones de 12 días de ratones de la misma cepa.

Esta sustitución se lleva a cabo con la integración sináptica completa de las neuronas transplantadas dentro del circuito cortical deficitario del huésped. La restauración es sólo parcial: I) tan solo 15 % de la corteza cerebelosa del huésped va a recibir células de Purkinje, y II) el restablecimiento de una proyección corticonuclear solo ocurre en raros casos, y en ellos la proyección es muy poco densa. El análisis de los cerebelos de 4 a 21 días después de la transplantación, nos ha permitido estudiar los mecanismos celulares involucrados en esta sustitución. Las células de Purkinje son invasivas y, a traves de vias estereotipadas de migración, penetran en la capa molecular del cerebelo huésped. Durante la migración, las neuronas transplantadas comienzan a construir sus árboles dendríticos y, al final de este período migratorio, van a recibir las este período migratorio, van a recibir las aferencias sinápticas adecuadas procedentes de las neuronas adultas del huésped. La cronología y los tipos de interacciones celulares durante la sustitución neuronal son muy similares a los de la ontogénesis cerebelosa. Nuestros resultados sugieren que la capa molecular del mutante ejerce un efecto neurotrógico unicamente sobre las neuronas del implante de la misma categoría que las neuronas que faltan. Las células de Purkinje responde a esta señal durante el período de tiempo definido por su propio reloj interno como fase migratoria. Una vez en el cerebelo del huésped, estas células son capaces de continuar su programa de desarrollo creando un micro-ambiente permisivo que va a provocar el "sprouting" axónico y la sinaptogénesis con las células de Purkinje. El resultado final de todas estas interacciones celulares es la integración sináptica de las neuronas transplantadas y la restauración parcial del circuito cerebeloso deficitario.

RÉSUMÉ

Les cellules de Purkinje manquantes dans le cervelet adulte de la souris porteuse de la mutation "Purkinje cell degeneration" peuvent être remplacées par des greffes d'ébauches cérébelleuses d'embryons âgés de 12 jours. Cette substitution a lieu avec l'intégration synaptique complète des neurones greffés dans le circuit déficitaire cortical de l'hôte. La restauration cérébelleuse n'est que partielle car: I) la proportion de cortex cérébelleux recevant les cellules greffées est faible, et II) le réta-

blissement de la projection cortico-nucléaire n'a lieu que dans de très rares cas, et avec une très faible densité. L'analyse des cervelets greffés 4 à 21 jours après transplantation nous a permis d'étudier les mécanismes cellulaires qui sous-tendent ce remplacement. Les cellules de Purkinje sont invasives et, en suivant des routes stéréotypées, pénètrent dans la couche moléculaire du cervelet hôte. Pendant cette migration, les neurones greffés commencent à développer leurs arbres dendritiques et, à la fin de la migration, ils vont recevoir des afférences synaptiques appropriées émergeant des neurones adultes de l'hôte. La chronologie et les types d'interactions cellulaires observées pendant la substitution des cellules de Purkinje sont remarquablement semblables à celles qui ont lieu pendant l'ontogenèse cérébelleuse. Nos résultats suggèrent que la couche moléculaire déficitaire exerce un effet neurotrophique spécifique sur les neurones greffés de la même catégorie que les manquants. Ainsi, les cellule de Purkinje embryonnaires peuvent répondre à ce signal pendant une période de temps défini par leur propre horloge interne. Une fois dans le cervelet hôte, ces cellules sont capables de poursuivre leur programme de développement, en créant un microenvironnement permissif pour les neurones adultes de l'hôte, qui, de par la poussée collatérale axonale et la synaptogenèse, vont permettre l'intégration synaptique des neurones embryonnaires greffés.

ZUSAMMENFASSUNG

Die degenerierten Purkinjeschen Zellen des Kleinhirns der erwachsenen Maus, die leidet an der Mutation "Degeneration der Purkinjesschen Zellen" koennen ersetzt werden indem mane Kleinhirnanlage von Embryonen von 12 Tagen, ueberpflanzt von Maeusen des gleichen Stammes.

Diesen Ersatz fuehrt man aus durch die vollkommene synaptische Integration der ueberppflanzten Neuronen innerhalb des corticalen difizitaeren Kreislaufs des Empfaengers. Die Restauration ist nur teilweise. 1) nur 15 % der Kleinhirnrinde des Empfaengers wird die Purkinjeschen Zellen empfangen. 2) Die Wiederherstellung einer Cortico-nucleaeren Projektion ereignet sich in nur seltenen Faellen, und in diesam Faellen ist die Projektion sehr wenig dicht.

Die Analyse von Kleinhirnen 4 bis 21 Tage nach der Transplantation hat uns erlaubt, die zellulaeren Mechanismen bei diesem Ersatz zu studieren. Die Purkinjeschen Zellen sind invasiv, und ueber estereostipierte Migrationswege, dringen sie in die Molekularschicht des Empfaengerkleinhirns ein. Waehrend der Migration beginnen die transplantierten Neuronen, ihre dentritische Veraestelung zu konstruieren, und am Schluss dieser Migrationsperiode empfangen sie die geeigneten synaptischen Afferenzen, die von den erwachsenen Neuronen des Empfaengers herstammen. Die Chronologie und die Arten der zellulaeren Interaktioenen waehrend der neuronalen Ersatzes sind sehr aehnlich dem ontologischen Ent-Wicklungsgang des Kleinhirns. Unsere Ergebnisse lassen vermuten, dass die Molekularschicht des Mutierenden einen neurotropischen Effekt nur auf die Neuronen des Implantates der gleichen Kategorie wie die der fehlenden Neuronen ausuebt. Die Purkinjeschen Zellen reagieren auf dieses Signal waehrend dieser Zeitperiode, die durch die eigene innere Uhr als Migrationsphase erkannt ist.

Einmal im Empfaengerkleinhirn sind diese Zellen faehig, ihr Entwicklungsprogramm fortzusetzen, indem sie ein Mikro-Milieu schaffen, das tolerant ist und hervorruft die "Sprouting" der Axone und die Schaffung von Synapsis mit den Parkinjeschen Zellen. Das Endergebnis all dieser zellulaeren Interaktionen ist die sinaptische Integration der ueberpflanzten Neuronen und die teilweise Restauration des defizitaeren Kleinhirnkreislaufes.

REFERENCES

1. *Armengol J. A.* and *Sotelo C.*: Early dendritic development of the Purkinje cells in the cerebellum of the rat. II. A study after climbing fiber deafferentation (submitted for publication).
2. *Changeux J. P.* and *Danchin A.*: Selective

stabilization of developing synapses as a mechanism for the specification of neuronal networks. Nature 264: 705-712, 1976.

3. *Cowan W. M.*: The development of the vertebrate central nervous system: an overview. In: Development in the Nervous System (eds. D.R. Garrod and J. D. Feldman), pp. 3-33, Cambridge: Cambridge University Press, 1981.
4. *Crepel F., Delhaye-Bouchaud N.* and *Dupont J. L.*: Fate of the multiple innervation of cerebellar Purkinje cells by climbing fibers in immature control, X-irradiated and hypothyroid rats. Dev. Brain Res. 1: 59-71, 1981.
5. *Crepel F., Mariani J.* and *Delhaye-Bouchaud N.*: Evidence for a multiple innervation of Purkinje cells by climbing fibers in the immature rat cerebellum. J. Neurobiol. 7: 567-578, 1976.
6. *Gardette R., Alvarado-Mallart R. M., Crepel F.* and *Sotelo C.*: Electrophysiological demonstration of a synaptic integration of transplanted Purkinje cells into the cerebellum of the adult "Purkinje cell degeneration" mutant mouse. Neurosci. 24: 777-789, 1988.
7. *Gardette R., Crepel F., Alvarado-Mallart R. M.* and *Sotelo C.*: The fate of grafted embryonic Purkinje cells in the cerebellum of the adult "Purkinje cell degeneration" mutant moue. II. Development of synaptic responses: An "in vitro" study (submitted for publication).
8. *Ghetti B., Norton J.* and *Triarhou L. C.*: Nerve cell atrophy and loss in the inferior olivary complex of "Purkinje cell degeneration" mutant mice. J. Neurol. 260: 409-422, 1987.
9. *Goffinet A. M.*: The embryonic development of the cerebellum in normal and reeler mutant mice. Anat. Embryol. 168: 73-86, 1983.
10. *Holmes G.*: A form of familial degeneration of the cerebellum. Brain, 30: 466-489, 1907.
11. *Jande S. S., Maler L.* and *Lawson D. E. M.*: Immunohistochemical mapping of vitamin D-dependent calcium binding protein in brain. Nature, 294: 765-767, 1981.
12. *Larramendi L. M. H.*: Analysis of the synaptogenesis in the cerebellum of the mouse. In: Neurobiology of Cerebellar Evolution and Development (ed. by R. Llinas), pp. 803-843, Chicago: Am. Med. Assoc. 1969.
13. *Monard D.*: Cell-derived proteases and protease inhibitors as regulators of neurite outgrowth. Trends in Neurosci., 11: 541-544, 1988.
14. *Mullen R. J., Eicher E. M.* and *Sidman R. L.*: Purkinje cell degeneration, a new neurological mutation in the mouse. Proc. Natl. Acad. Sci. USA, 73: 208-212, 1976.
15. *Rakic P.* (1985): Contact regulation of neuronal migration. In: The cell contact: Adhesions and junctions as morphogenetic determinants (ed. by G. M. Edelman and J. P. Thierry), pp. 67-91, New York: Wiley and Sons, 1985.
16. *Ramón y Cajal S.*: Histologie du Système Nerveux de l'Homme et des Vertebres. Vol. 2, pp. 80-106, Paris: A. Maloine, 1911.
17. *Ramón y Cajal S.*: Sur les fibres moussues et quelques points douteux de la texture de l'ecorce cerebelleuse. Trab. Lab. Invest. Biol. Univ. Madrid 24: 215-251, 1926.
18. *Shojaeian H., Delhaye-Bouchaud N.* and *Mariani J.*: Stability of inferior olivary neurons in rodents. I. Moderate loss in adult "Purkinje cell degeneration' mutant mouse. Dev. Brain Res., 38: 211-218, 1988.
19. *Sieradzan K.* and *Vrbová G.*: Replacement of missing motoneurons by embryonic grafts in the rat spinal cord. Neurosci., 31: 115-130, 1989.
20. *Sotelo C.*: Purkinje cell ontogeny: formation and maintenance of spines. In: Maturation of the Nervous System (eds. M. A. Corner, R. E. Baker, N. E. van der Pol, D. F. Swaab and H.B.M. Uylings). Prog. Brain Res. Vol. 48, pp. 149-168. Amsterdam, Elsevier, 1978.
21. *Sotelo C.* and *Alvarado-Mallart R. M.*: Growth and differentiation of cerebellar suspensions transplanted into the adult cerebellum of mice with heredo-degenerative ataxia. Proc. Natl. Acad. Sci. USA, 83: 1135-1139. 1986.
22. *Sotelo C.* and *Alvarado-Mallart R. M.*: Reconstruction of the defective cerebellar circuitry in adult Purkinje cell degeneration mutant mice by Purkinje cell replacement through transplantation of solid embryonic implants. Neurosci., 20: 1-22, 1987a.
23. *Sotelo C.* and *Alvarado-Mallart R. M.*: Embryonic and adult neurons interact to allow Purkinje cell replacement in mutant cerebellum. Nature 327: 421-423, 1987b.
24. *Sotelo C., Alvarado-Mallart R. M., Gardette R.* and *Crepel F.*: The fate of grafted embryonic Purkinje cells in the cerebellum of the adult "Purkinje cell degeneration" mutant mouse. I. Development of reciprocal graft/host interactions (submitted for publication).
25. *Wassef M., Zanetta J. P., Brehier A.* and *Sotelo C.*: Transient biochemical compartmentalization of Purkinje cells during early cerebellar development. Dev. Biol., 111: 129-137, 1985.
26. *Wassef M., Sotelo C., Cholley B., Brehier A.* and *Thomasset M.*: Cerebellar mutations affecting the postnatal survival of Purkinje cells in the mouse disclose a longitudinal pattern of differentially sensitive cells. Dev. Biol., 124: 379-389, 1987.

Fetal Spinal Cord Overlays: Incorporation and Differentiation in Adult Host Spinal Cord

Condensed Title: Incorporation of Fetal Spinal Cord Overlayed onto Host Spinal Cord

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INTRODUCTION

The use of fetal tissue for transplantation into the central nervous system of adult hosts has become a promising technique for the repair of CNS lesions and as a tool in the study of development (Reviews Sladek and Gash, 1984: Bjorklund and Stenevi 1985). In spinal cord, it has been demonstrated that fetal rat CNS (spinal cord and brain) of appropriate age can survive in adult or immature host spinal cord (Das, 1983; Reir, 1985; Hallas 1984; Bregman and Reir 1986; Bregman 1987; Houle and Reir 1988; Patel and Bernstein 1983; Bernstein, Patel, Keleman, and Turtil 1984). Such implants may survive, grow, form differentiated neurons and synaptic connections within the implant and perhaps with the host spinal cord (Nornes, Bjojrklund and Stenevi 1984; Reir, Bregman, and Wujek 1986; Bregman 1987; Bjorklund, Nornes, and Gage 1986).

While the success of the transplantation of fetal CNS tissues into brain and spinal cord is established, the potential for use of the technique as mechanism for inducing CNS regeneration in adult host mammals remains unclear (Stenevi, Bjorklund, and Kromer 1984). It has been shown that some adult mammalian CNS neurons appear to be capable of regenerating axons in the environment of a transplant such as peripheral nerve (Aguayo, David, and Bray 1981; David and Aguayo 1985), but only limited observations suggest that fetal CNS tissues may aid in inducing or allowing axonal regeneration. Some evidence of axonal regeneration across transplants of fetal CNS for the bridging of host axons appears to be successful only to a limited extent in neonatal hosts (Bregman 1987). Still, such transplants may have other properties which promote functional recovery from spinal lesions (Berstein and Goldberg 1987).

One difficulty in the use of tissue transplants for spinal cord repair is how the transplanted tissue is introduced into the injured area. Typically this involves the creation of a cavity or lesion in the spinal cord followed by insertion of the transplant into the lesion. As this process produces considerable trauma, the development of implantation methods which are less traumatic may be more compatible with spinal cord repair. In the present study, we have investigated the ability of fetal spinal cord

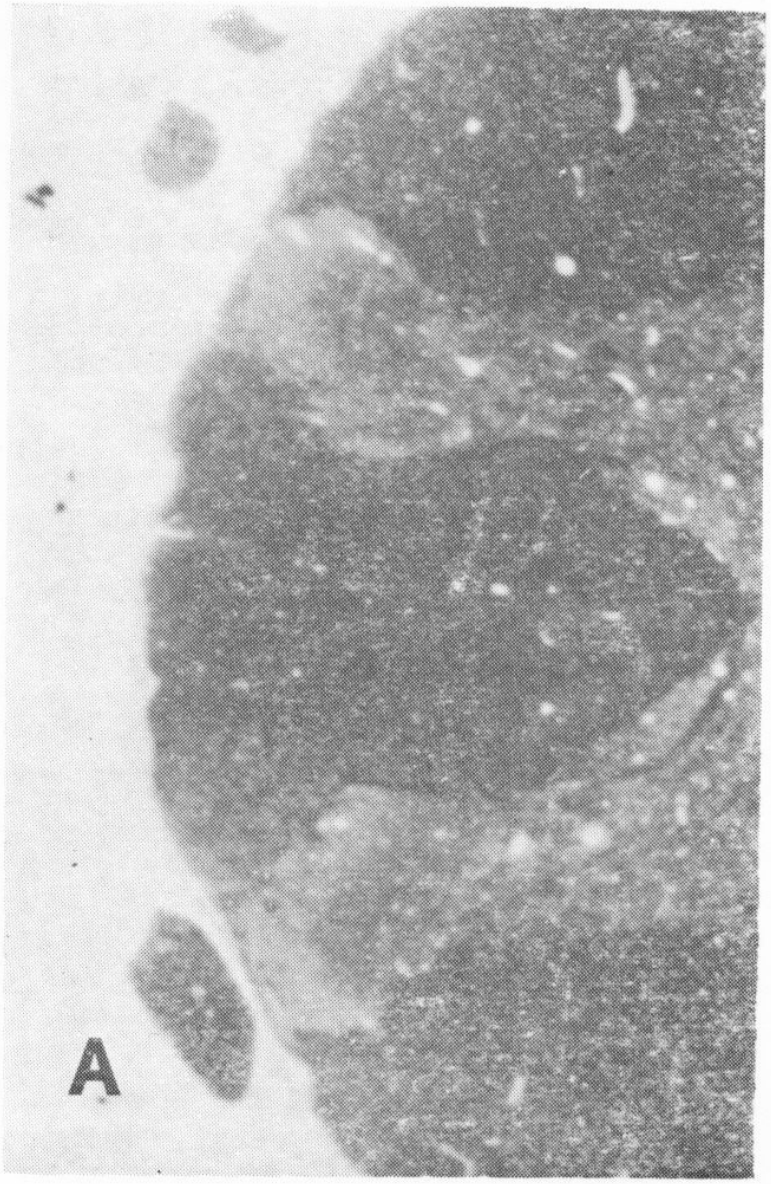

Fig. 1.A. — *A control animal in which the dura was cut and the pia punctured. No visible pathology is present. Toluidine blue.*

to incorporate itself into the host spinal cord with minimal trauma. We observed that fetal spinal cord overlayed onto reasonably intact uninjured adult host spinal cord can invade the host and form masses of differentiated cells.

MATERIALS AND METHODS

Fetuses (E11 or E15, crown rump-length 4-5 mm, 12-14 mm) were obtained from timed pregnant female Long-Evans Hooded rats (Charles River) which had been injected intraperitoneally with 5uCi/gm body weight of [^{3}H] thymidine (New England Nuclear) 24 hours prior to use. The uterine horns containing fetuses were removed from the donor under Chloropent (Fort Dodge) anesthesia (0.33 ml/100mg body weight) and placed on ice in cold sterile saline. The fetuses were carefully dissected free and the spinal cord removed. The fetal spinal cords were cleaned of meninges and adhereing tissue as completely as possible under a dissecting microscope.

Male host animals of the same strain (200 g average body weight) were anesthesized with Chloropent and a laminectomy made of half of two adjacent mid thoracic vertebrae (T5-T6). The dura was divided longitudinally in a small slit (3 mm). The pia on the dorsal surface of the spinal cord was carefully cut in several places with the

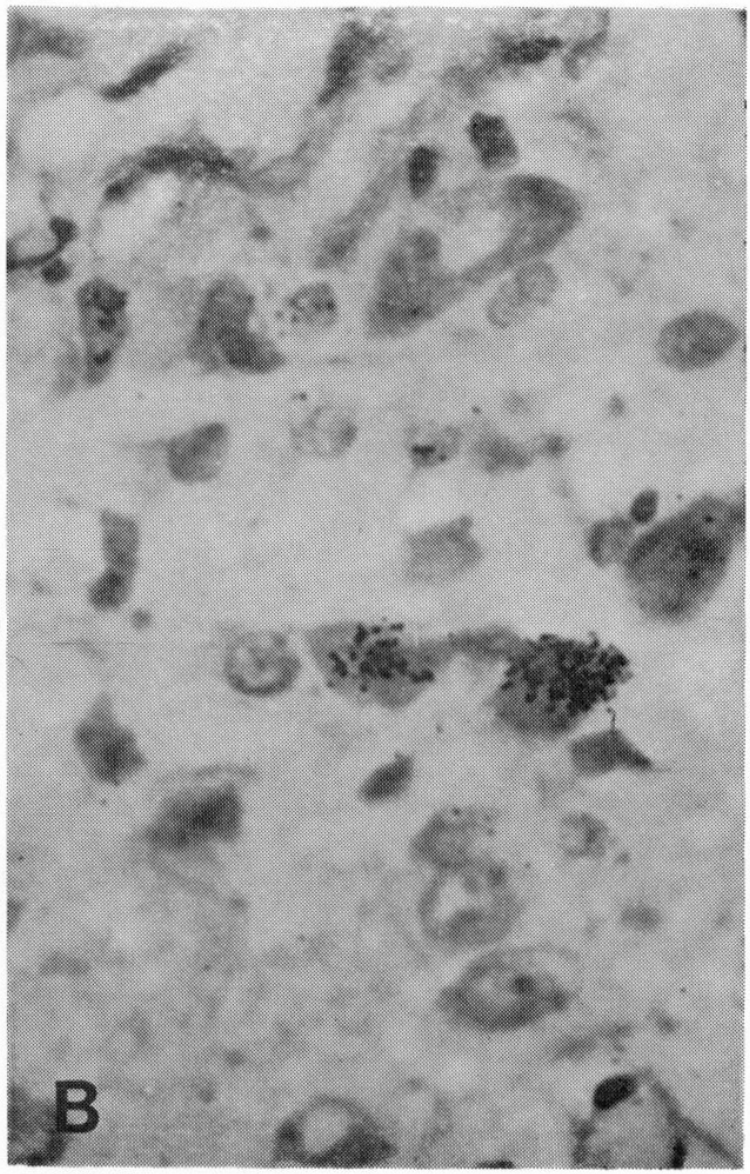

Fig. 1.B. — *Autoradiogram of an E11 spinal cord implant showing labelled nuclei in the implanted tissue at 2 weeks after implantation. Paraffin embedded tissue, neutral red stain.*

tip of a 27 G needle. Downward compression of the spinal cord during cutting of the pia was avoided by moving the needle longitudinally with respect to the spinal cord in a slicing action. The transplant was overlaid on the host spinal cord under the dura through the incision. The cut in the dura was covered with a small piece of gelfoam soaked in cerebrospinal fluid. The musculature and skin were sutured. Sham operated animals were subjected to the surgical procedures alone including cutting of the pia without transplantation.

At time intervals of 2, 3, and 4 weeks, 2, 3, 6 and 12 months at least 4 animals (2 receiving E11 and 2 receiving E15 implants) were perfused with 2 % glutaraldehyde, 2 % paraformaldehyde, 0.5 % acrolein, 0.5 % DMSO in 0.08 M cacodylate buffer pH 7.2. The entire spinal cord was removed and allowed to sit in the same fixative overnight at 4ºC. Sections 2-3 mm length were cut rostral and caudal from the site of implantation. In some instances where perfusion appeared to be poor, sections of spinal cord were embedded in paraffin and sectioned at 6 microns.

Samples of dissected embryonic spinal cord were collected from each donor to confirm the dissection procedure and to

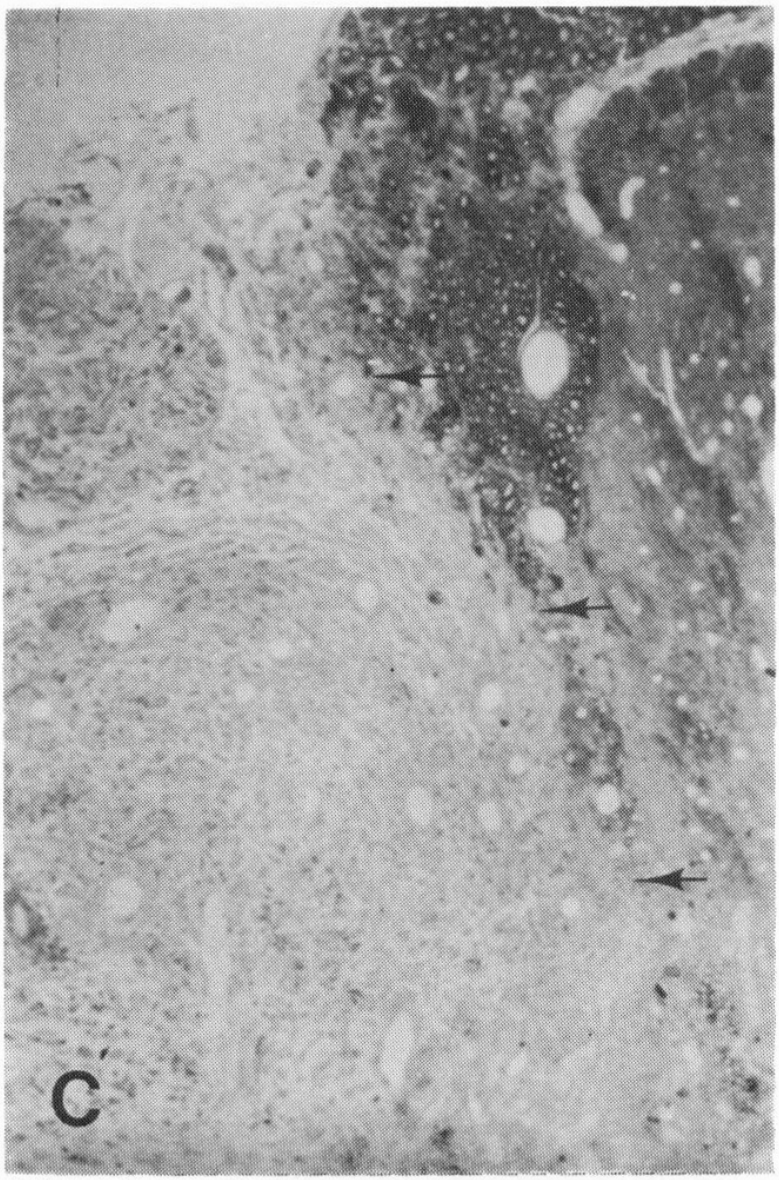

Fig. 1.C. — *An E11 spinal cord overlay 2 weeks after implantation. The implant is beginning to incorporate into the host dorsal columns. Arrows delineate the host-implant interface. The host dorsal horn is to the right side of the figure.*

examine the initial morphology of the transplant. Samples of spinal cord were also taken from postnatal animals from 3, 7, 10 and 14 days of age for comparison with some of the early transplanted material. Animals were anesthesized and perfused or immersed in fixative and processed for sectioning as described below.

Blocks to be embedded in plastic were postfixed with 2 % osmium before embedding. One micron sections of the whole cross section of the host spinal cord with the transplant and sham controls were made for light microscopy. Thin sections were made on identified areas of implants or transplant-host interface by trimming of blocks after 1 u sections were taken. The trimmed area was examined in 1 u sections before thin sections were taken. Thin sections on grids were stained with uranyl acetate and lead citrate and examined using a JEOL 100 CX electron microscope.

Autoradiography of 1 um sections from selected blocks were made using Kodak NTB-3 emulsion diluted 1:1 with distilled water. Dipped slides were air dried and exposed for 4-8 weeks. Slides were developed in D-19 at 15ºC and stained with neutral red (paraffin) or toluidine blue and basic fucsin.

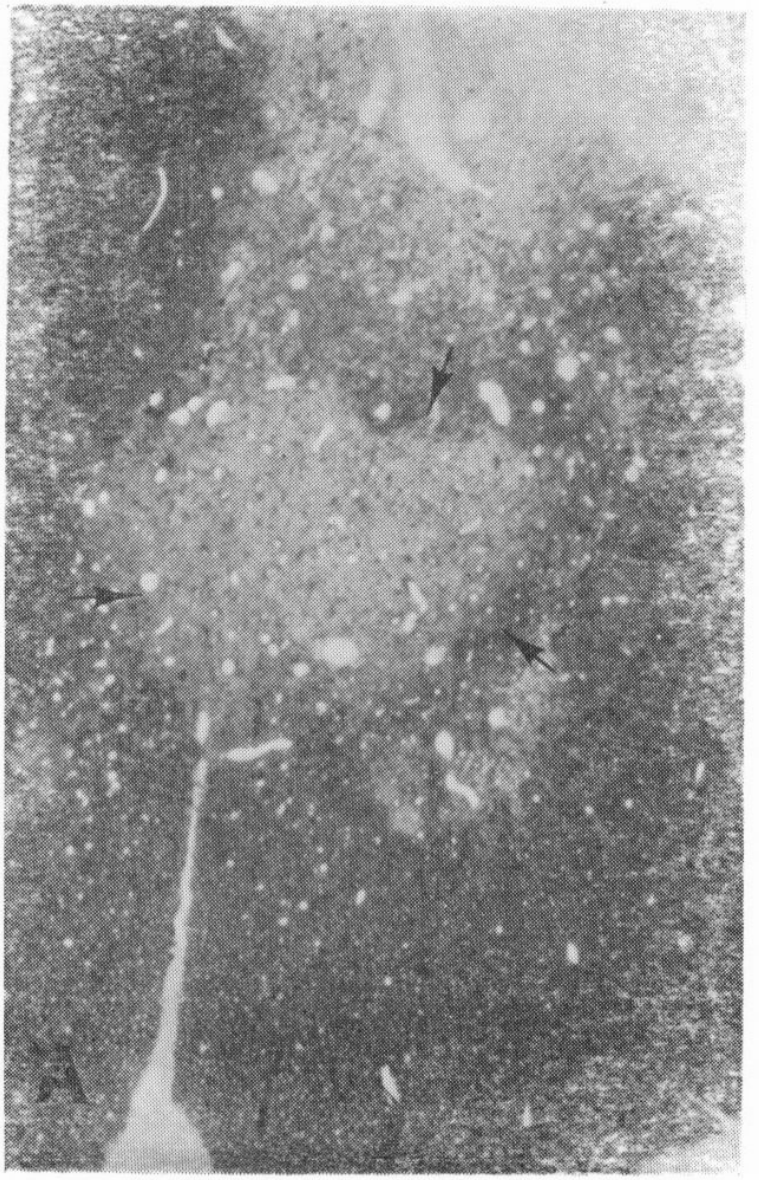

Fig. 2.A. — *An E15 spinal cord implant one month after implantation. The implanted spinal cord (arrows) has invaded the host and formed a knot of neuronal like cells in the spinal grey matter. Toluidine Blue.*

RESULTS

The surgery and damage of the pia alone did not produce any remarkable pathology of the spinal cord in control animals only an occasional thickening of the dura (Fig. 1A). This was confirmed in electron microscopic sections of the dorsal surface of the spinal cord. The embryonic and early postnatal normal spinal cords examined confirmed that properly dissected spinal cord tissue of the appropriate age were used for transplantation.

Within 1-2 weeks post implantation, overlaid fetal spinal cord transplants had begun to incorporate themselves into the host spinal cord (Fig. 1C). At this time it was possible to readily identify cells, usually glial in appearance, labelled with [$_3$H] thymidine within the transplant [Fig. 1B]. At later time intervals the numbers of labelled cells decreased, apparently due to dilution of the label with cell division.

With time, the embryonic transplants were located further inside the host spinal cord, apparently progressing toward the host grey matter. At later time intervals, neuronal elements from grafts (usually E15) were found just above the host central ca-

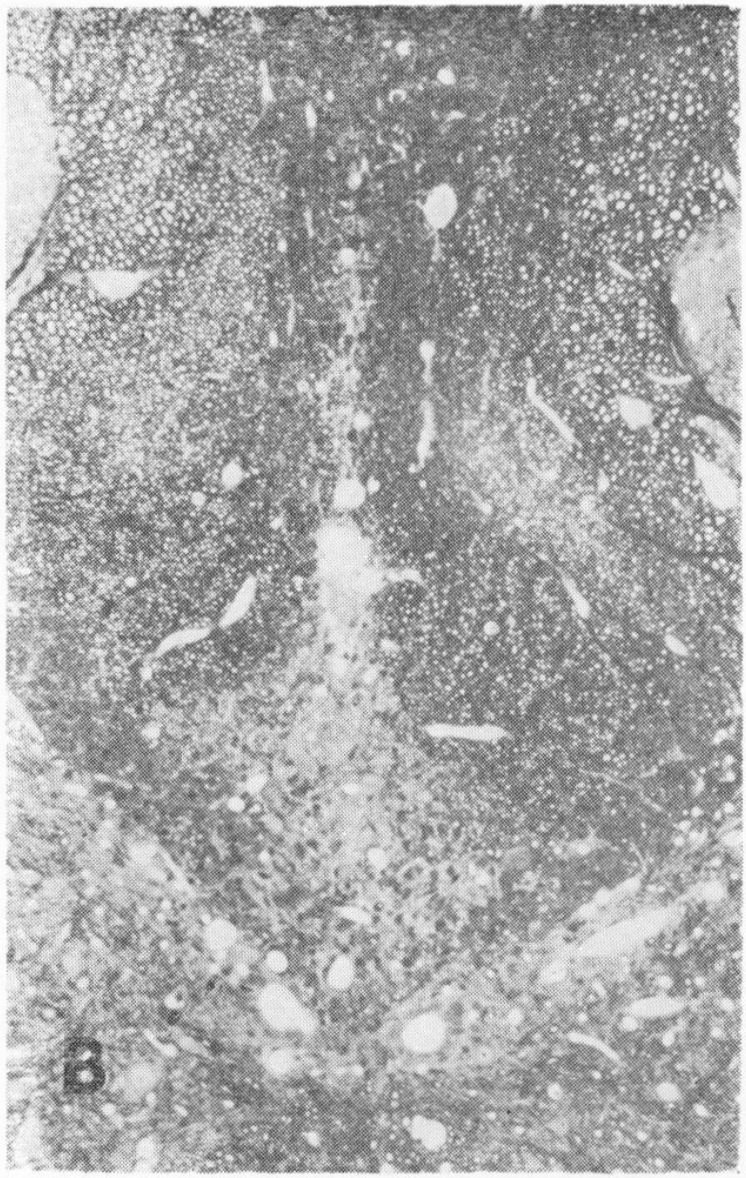

Fig. 2.B. — *An E15 implant which has invaded to a position just above the host spinal grey matter.*

nal. Such transplants could be distinguished as a knot of neuronal cells (Fig. 2 A,B). Typically, the course of invasion of cells from implants was down the midline glial septa. This may have been due to the initial placement of the transplant in this position. However, transplants were also found which apparently had migrated along different pathways, perhaps due to the displacement of the implant soon after the operation. In most instances, a progressive migration toward the center of the host spinal cord was suggested, regardless of a midline or other route of incorporation.

Transplants from E15 often showed morphologically identifiable neurons incorporated into the host spinal cord. At the electron microscopic level, areas containing implants had organized neuropil and neurons with synapses (Fig. 2C). The position of the E15 implant in a cross section could vary within a single host over the rostrocaudal extent of the implant. In one instance, the position of the implant changed from just over the central canal to outside of the dorsal horn within one vertebral level. The maximum rostrocaudal extent of implants were 1-2 cm within the host. In later time intervals (3-6 months) it was often difficult to distinguish neurons of implant origin since many cells within growing implants were not identifiable by

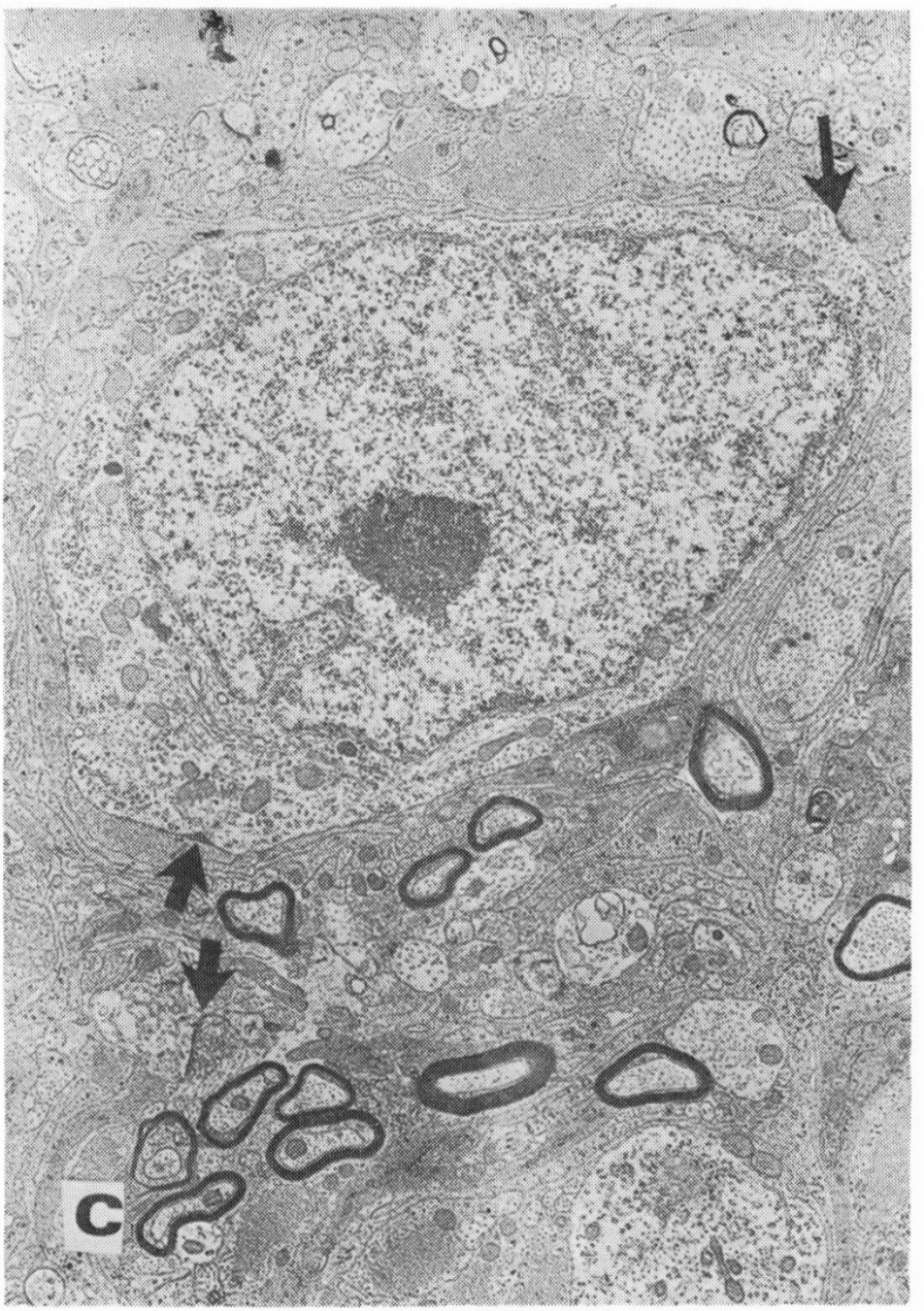

Fig. 2.C. — *Electron micrograph showing a neuron and neuropil from the implant of 2A. Organized neuropil and synapses (arrows) are present. Uranyl acetate and lead citrate.*

[^{3}H] thymidine labeling of nuclei. It was therefore not possible to tell whether the implants were becoming indistinguishable due to implant degeneration or cell migration followed by differentiation at their final destination.

By comparison, E11 implants were less likely to show morphologically differentiated neurons than E15, or congruous masses of tissue. However, E11 implants showed a much more vigorous growth and invasion. The E11 spinal cord overlays also appeared in early time intervals as distinct tissue masses (Fig. 1C), but in later time intervals, a widely dispersed invasion of cells was observed and often a severe pathology of the host spinal cord. This was not observed in the case of any E15 spinal cord implants. Some of the profiles suggested processes of inflammation and/or rejection of

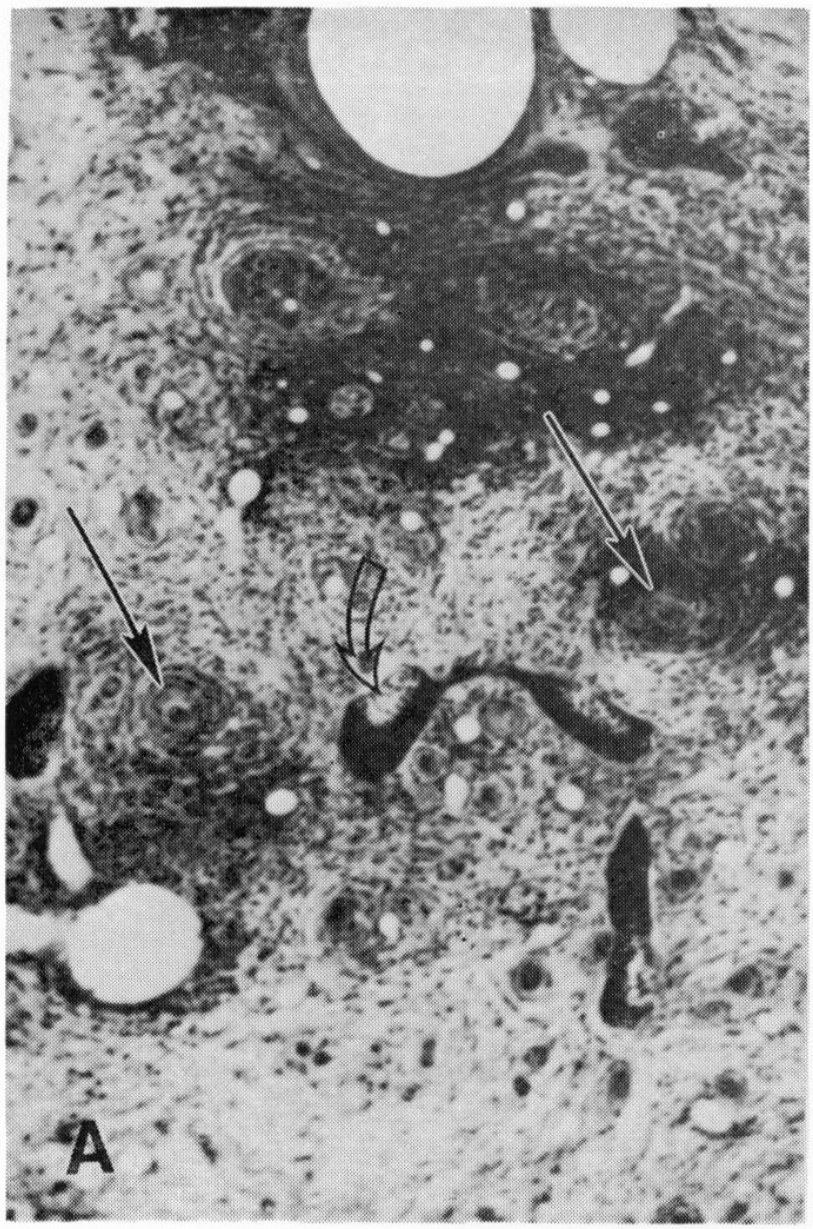

Fig. 3.A. — *An E11 implant 1 month after implantation. The implant has partially dispersed and spread across the host. The circular profiles (arrows) are small bundles of myelinated axons of unknown origin. Membrane bound profiles of small cells are shown by an open arrow.*

the implant. The area of spinal cord over which implants were placed suggested an invasion of cells which could involve an entire cross-section of the host spinal cord (Fig. 3 A,B). The primary cell type of such implants were small dark nucleated cells, some of which were in membranous cavities (Fig. 3B). It was not possible to distinguish if such tissues in the host spinal cord originated from the host or implant. Viable implants or portions of them containing neuronal like cells were also found external to the host spinal cord (Fig. 4A).

While the most severe pathologies were associated with E11 stage implants, some indications were present that implants of both stages could produce pathology in the host. Incorporation of the implant through the dorsal columns was often accompanied by degeneration of what appeared to be host fibers in the dorsal However, axons persisted in these areas and remyelination of axons, usually by Schwann cells, was occurring (Fig. 4B). This degeneration of axons

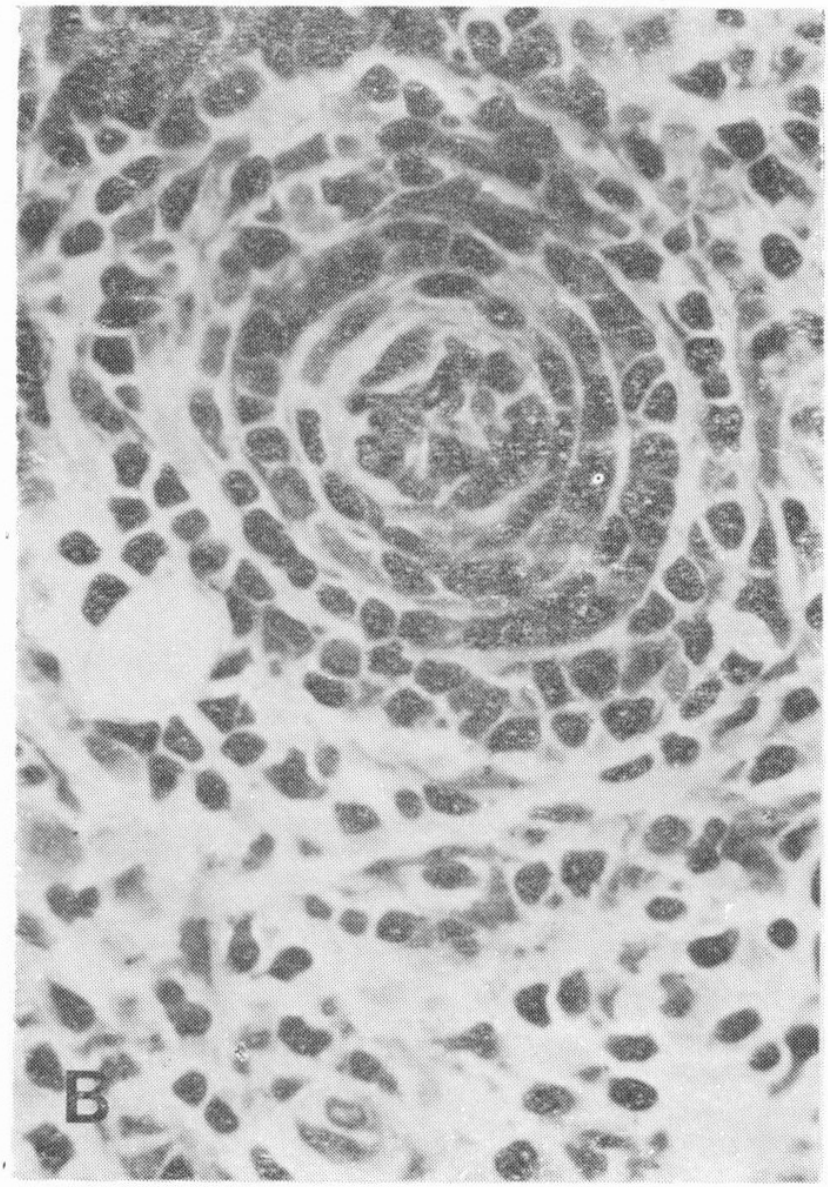

Fig. 3.B. — *A higher magnification detail of 3A showing cell types.*

could be noted rostral and caudal to the main implant mass within the host. At later time interval, the degenerating fibers appeared to have been cleared from these areas to leave a smooth intervening of glial processes and unmyelinated axons. The implant growing from both ages of implant could also produce degeneration of host grey matter, perhaps by compression (Hallas, 1984).

The degeneration of host fibers in some implants was not necessarily an indication that the implanted tissue had an environment incompatable with axonal growth. Little or no scarring occurred in conjunction with nerve fiber degeneration even at the site of implantation. Axons were often seen in areas of implants along with figures of cells suggesting cell division.

DISCUSSION

These data demonstrate that cells from fetal spinal cord from E11 and E15 embryos overlaid on a host spinal cord can incorporate themselves into the host without introduction by traumatic methods. In addition, masses of tissue attributable to the transplant were noted over a rostro-caudal distance considerably larger (1-2 cm) than the initial transplant (1-2 mm) suggesting continued growth in the host (Patel and Bernstein 1983). This observation is in con-

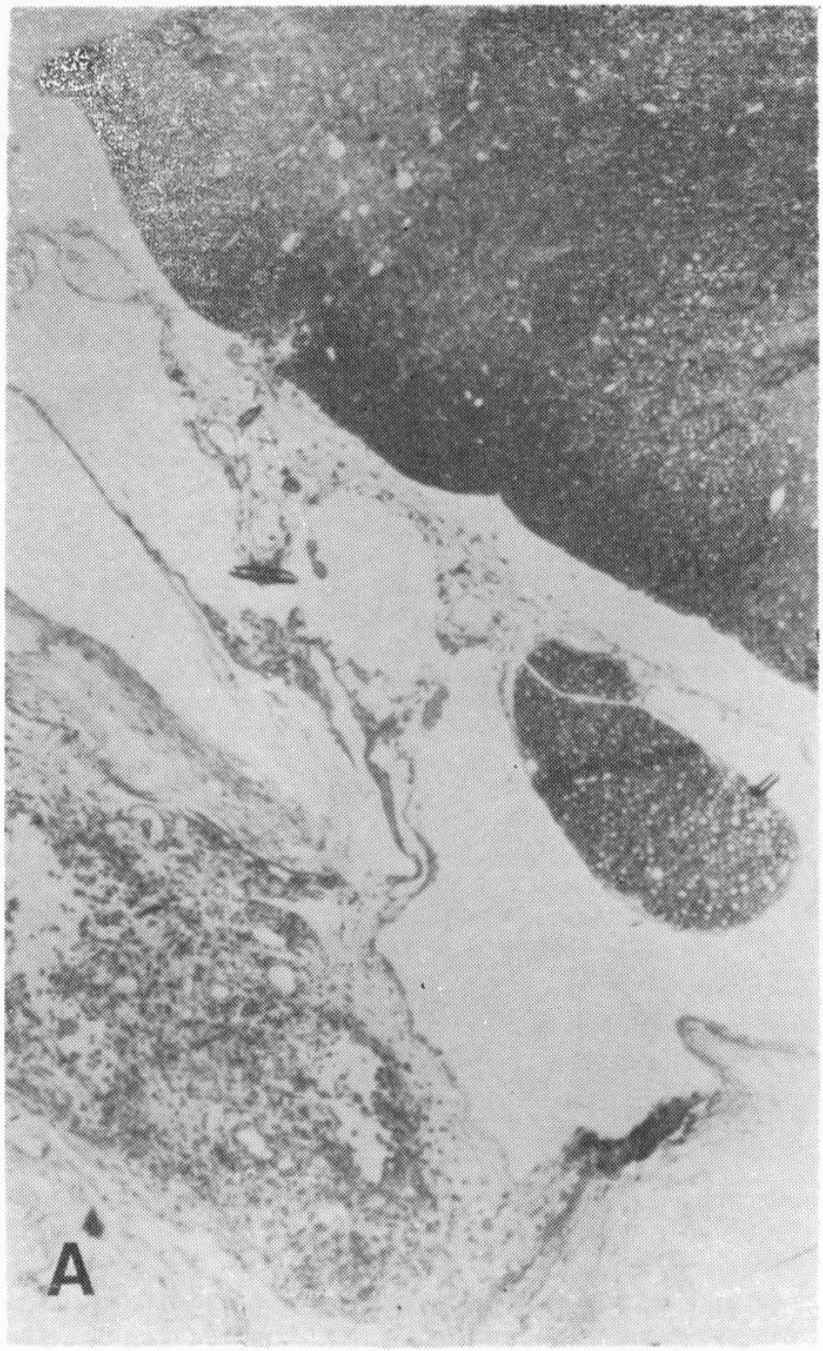

Fig. 4.A. — *An E11 implant mass external to the host spinal cord at two months after implantation. Strands of tissue connect the implant with a point of incorporation into the host spinal cord. Other blocks of this animal showed incorporation of the implant into the host.*

trast to the experiments of Bunge, Johnson, and Thuline (1983) where cultured embryonic spinal cord strips were placed over cavities made in the dorsal columns of adult hosts. Under these conditions survival of the implant was obtained, but no incorporation occurred.

The mechanism for the incorporation of the transplant is not clear. It is certainly possible mechanical factors such as pressure from transplant growth may play a role. However, if this were the only mechanism, a more simple displacement or compression of the host spinal cord by the implant should have been observed. Instead, a progressive incorporation of the implant into the host was observed, suggesting an almost invasive incorporation of the implant often

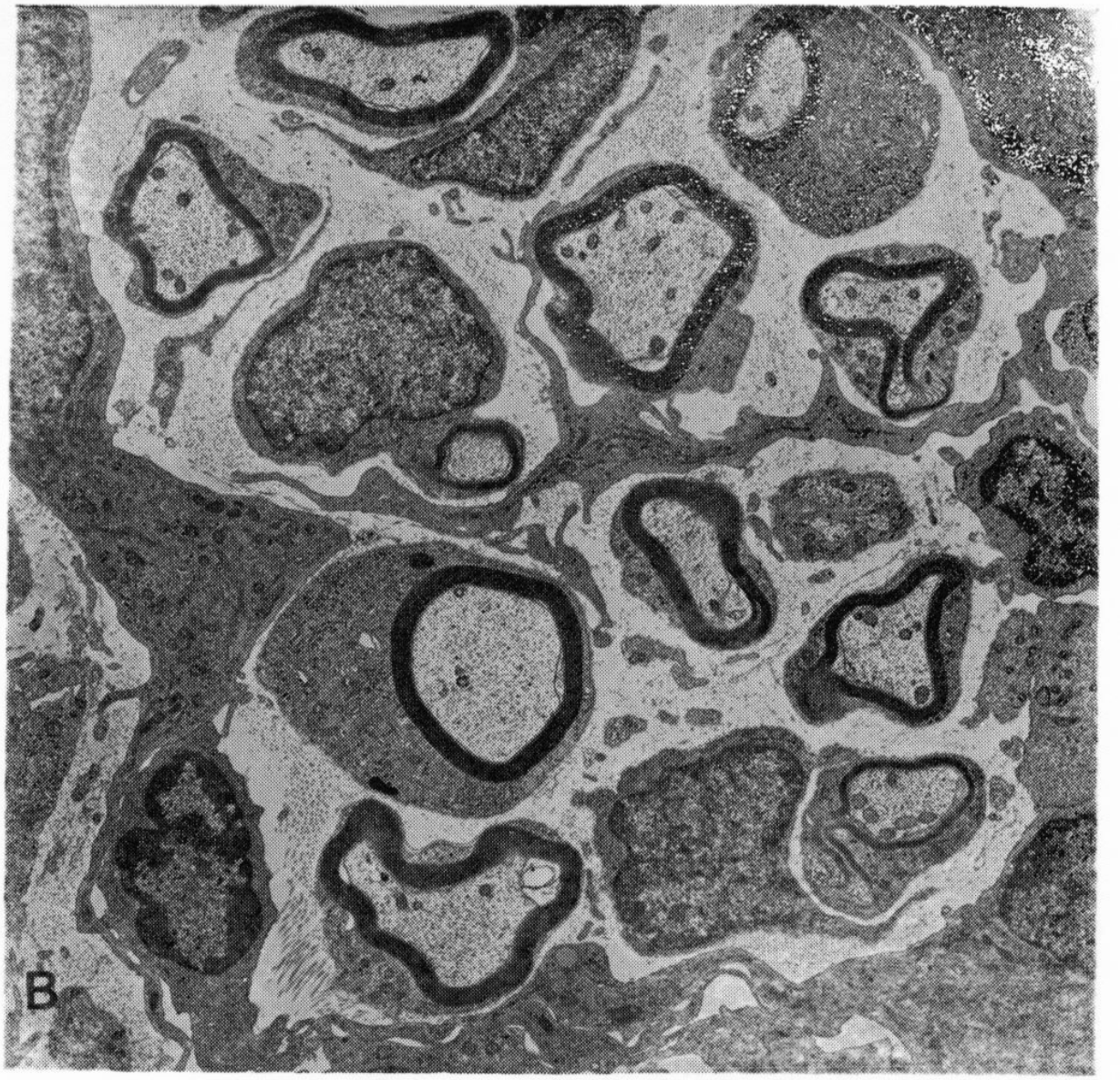

Fig. 4.B. — *An electron micrograph of an area of remyelination in the dorsal columns of an E15 implant 1 month after implantation.*

in the direction of the host spinal grey matter.

Cellular migration and histogenetic differentiation of fetal transplants have been reported in the graft itself, but cellular movement of grafted neurons and neuroglial cells to develop in a different site of host tissue is receiving increased attention (Lindsay and Raisman 1984; Sotelo and Alvarado-Mellart 1986, Privat, Monsour, pavy Geffard, and Sandillon 1986; Goldberg and Bernstein 1987). The migration of glial cells from hippocampal transplants, and their possible role in guiding axonal growth from transplanted neurons into host brain have been described (Lindsay and Raisman 1984; Sotelo and Alvarado-Mallart, 1986). In spinal cord and brain, the migration of astrocytes from fetal cortical implants appears to be quite extensive (Berstein and Goldberg 1987; Goldberg and Bernstein 1988). This further suggests some interaction of implant with the host such that directed cellular migration occurs.

In some preliminary experiments (data not shown), we had concluded that damage of the host pia was necessary for successful entry of the transplant into the host spinal cord. Accordingly, the most common course of the transplanted cells into the spinal cord appeared to follow a midline glial septa at the approximate site of damage to the pia.

However, in the course of the present experiments, it was observed that transplants could enter the host from positions in which the pia would have not been directly damaged. Some profiles suggested that compression from the growing transplant mass could have contributed to this process. Participation of transplanted glial elements was also suggested by an atypical appearance of axonal degeneration often in the dorsal columns. Glial cells in these areas occasionally appeared labelled with (^{3}H) thymidine.

It was clear that E15 implants could form localized masses of tissue in the host spinal cord, while E11 implants generally did not. In both fetal stages, the implant began as a discrete mass, but E11 implants did not remain organized and dispersed to result in extensive disorganization and pathology of the host spinal cord. While this could reflect stage dependent change in the ability of spinal cells to migrate and differentiate, we could not eliminate the possibility that the characteristics of E11 implants could also have been due to the inclusion of peripheral fetal tissue with the transplant. Although this was not indicated by the examination of samples of the tissue which had been transplanted, even small amounts of fetal peripheral tissues included with transplants can proliferate extensively (Das 1983). In either case, the presence of such tissues in the host and their extensive proliferation suggests that this stage of embryo may be less suitable for transplantation than slightly older stages.

In some animals where the invasion of the transplant was only partially successful, a rudimentary implant external to the spinal cord could be seen, occasionally with strands of cells connecting to areas where migration into the host spinal cord had occurred. Some cells of neuronal-like morphology were present in these tissues. This confirmed the observations of others (Das, 1985; Hoovler and Bernstein 1985) that embryonic CNS can survive outside the host central nervous system in site that are not necessarily immunologically privileged.

The potential of the transplant to actually damage the host is an important consideration in the possible use of transplants for spinal cord repair or the induction of regeneration. The ability of the fetal spinal cord to enter the host spinal cord may bear some similarity with the growth of fetal cortical tissue injected into adult spinal cord (Goldberg and Bernstein 1987). In these studies, it was concluded that the transplants growth was preceded by an advancing front of fetal cells which invaded the host spinal cord grey or white matter. The advance of fetal cells resulted ultimately in the necrosis of localized areas of the host spinal pressure of the growing transplant or substances released from it. In the present study, degeneration of host fibers in the area of a transplant which had incorporated itself into the host was a frequent finding, but intact myelinated axons were also present in the transplant. The myelination of some of these axons appeared to be by Schwann cells. Because of the invasive nature of the transplants, it was impossible to be certain whether axons had grown through the implant or were merely surrounded by it. The capability of fetal tissue to invade the host suggests that this latter possibility must be carefully considered in the present and future studies involving spinal injury and transplantation.

Our data suggests that invasive transplantation involving more severe trauma to the host spinal cord is not necessary for the incorporation of embryonic spinal cord may be stage dependent. The potential use of this techniques for transplanting tissues into injured spinal cord is presently under study.

SUMMARY

Rat embryonic spinal cords (E11 or E15) were placed subdurally on the mid-thoracic spinal cord of adult rats and examined at time intervals of two weeks to 12 months after implantation. Implants of both stages invaded the host spinal cord, grew in rostro-caudal length, and cross sectional area.

The incorporation of the implant was usually accompanied by the degeneration of

some host axons. Ell implants grew vigorously, but lacked identifiable neurons in all cases examined. E15 stage embryonic spinal implants invaded the host spinal cord in a more organized manner, forming a more congrous mass of tissue often containing morphologically differentiated neurons, an organized neuropil, and synaptic contacts. The results suggest that the use of fetal grafts in spinal cord repair may not necessarily require invasive implantation for successful incorporation of grafts into the host.

RESUMEN

Médulas espinales de ratas embrionarias (E 11 o E 15) fueron colocadas en el espacio subdural de médulas espinales en sector toráxico mediano, de ratas adultas y examinadas en intervalos de tiempo de dos semanas a doce meses después de la implantación. Implantes de ambos grados invadieron la médula espinal receptora, crecieron en extensión rostro caudal y área transversal. La incorporación del implante fué habitualmente acompañada por la degeneración de algunos axones del anfitrion. Implantes E 11 crecieron vigorosamente, pero en todos los casos examinados se carecían de identificación de neuronas. Implantes grado E 15 embrionarios espinales invadieron la médula espinal receptora en una forma más organizada, dando lugar a una masa más congruente de tejido, a menudo conteniendo neuronas diferenciadas morfológicamente, neuropil organizado y contactos sinápticos. Los resultados sugieren que el uso de injertos fetales en reparo de la médula espinal puede no requerir necesariamente implantación hostil para exitosa incorporación de injertos en el anfitrion.

RÉSUMÉ

Des moelles épiniéres d'embryons de rats (E 11 ou E 15) furent misent dans l'espace sous-dural de moelles épiniéres du secteur toracique moyen de rats adultes. Elles furent éxaminées a des intervales de temp de 2 semaines a 12 mois aprés l'implantation. Les implants ont envahit la moelle réceptrice et ont grandi en longueur et en largeur. L'incorporation de l'implant fut habituellement par la dégénération de quelques axones chez le subjet récepteur.

Les implants E 11 ont grandis vigoureusement, mais dans tous les cas, il fut impossible d'identifier les neurones. Les implants E 15 ont envahi le moelle de manire plus organisée.

Ceci donne une masse plus congruente de tissus qui très souvent continnent des neurones différenciées, neuropiles organisés et des contacts synaptiques. Touch ceci sugère que l'usage d'implants de tissu foetal pour la réparation de la moelle épinière peut ne pas être forcement une implantation hostile, mais être une intervention utile.

ZUSAMMENFASSUNG

Das Rueckenmark von Rattenembrionen (E11 und E15) wurden inden Subduralraum von Rueckenmark, im mittleren Brustabschnitt, von erwachsenen Ratten eingepflanzt und nachgeprueft in Zwischenraeumen von zwei Wochen und zwoelf Monaten nach der Implantation. Implantate bei der Grade wuchsen in das Rueckenmark der Empfannger ein, wuchsen in Richtung rostro-caudal undauch transversal. Die Einverleibung des Implantats war gewoehnlich beglei tet von der Degeneration einiger Axone des Empfaengers. Die Implantate E 11 wuchsen kraeftig, aber in all den unterushten Faellen vermisste man dieldentifikation der Neuronen.

Die Implantate E15 von embryonaeren Rueckemark drangen in das Rueckemmark des Empfaengers mehr organisiert, ein und man beobachtete eine mehrkongruente Masse von Gewebe, oft mit morfologisch diffe-

renzierte Neuronen, organisiertem Neuropil und synaptischen Kontakten. Die Resultate lassen vermutdn, dass die foetalen Implantationen bei der Reparation des Rueckenmarks eventuell tkeine invasive Implantationen erfordert, um eine erfolgre iche Einverleibung des Transplantates im Empfaenger zu erreichen.

REFERENCES

1. *Aguayo, A. J., David, S.,* and *Bray, G. M.*: Influences of the glial environment on the elongation of axons after injury: Transplantation studies in adult rodents. J. Exp. Biol. 95: 231-240. 1981.
2. *Bernstein, J. J., Patel, U., Kelemen, M.,* and *Turtil, S.*: Ultrastructure of fetal spinal cord and cortex implants into adult rat spinal cord. J. Neurosci. Res. 11: 359-372. 1984.
3. *Bjorklund, A.* and *Stenevi, U. eds.*: Neural Grafting in the mammalian CNS. Elsevier, The Netherlands 709 p., 1985.
4. *Bregman, B. S.* and *Reir, P. J.*: Neural tissue transplants rescue axotomized rubrospinal cells from retrograde death. J. Comp. Neurol. 244: 86-95. 1986.
5. *Bunge, R. P., Johnson, M. I.* and *Thuline, D.*: Spinal cord reconstruction using cultured embryonic spinal cord strip. In: Kao C, Bunge R, Reir P, (eds.) Spinal Cord Reconstruction. Raven Press, New York, pp 341-358. 1983.
6. *Commissiong, J. W.*: Fetal locus coeruleus transplanted into the transected spinal cord of the adult rat: Some observations and implications. Neuroscience 12: 839-853. 1984.
7. *Das, G. D.*: Neural transplantation in the spinal cord of adult rats-conditions, survival, cytology and connectivity of the transplants. J. Neurol. Sci. 62: 191-210. 1983.
8. *Das, G. D.*: Intra parenchymal transplantation. In: Neural Grafting in the mammalian CNS. A. Bjorklund and U. Stenevi eds. Elsevier, B. V. p. 23-30. 1985.
9. *David, S.* and *Aguayo, A. J.*: Peripheral nerve transplantation techniques to study axonal regeneration from the CNS of adult mammals. In: Neural Grafting in the Mammalian CNS. A. Bjorklund and U. Stenevi, eds. pp. 61-70, 1985.
10. *Hallas, B. H.*: Transplantation of embryonic rat spinal cord or neocortex into the intact or lesioned adult spinal cord. Appl. Neurophysiol. 47: 43-50. 1984.
11. *Hoovler, D. W.* and *Bernstein, J. J.*: Transplantation of fetal rat cortex into regenerating nerve to the biceps femoris of adult rat. *Exp. Neurol.* 89: 337-347. 1985.
12. *Lawrence, J. M., Huang, S. K.,* and *Raisman, G.*: Vascular and astrocytic reactions during establishment of hippocampal transplants in adult host brain. Neuroscience 12: 745-760. 1984.
13. *Lindsay, R. M.,* and *Raisman, G.* (1984): An autoradiographic study of neuronal development, vascularization and glial cell migration from hippocampal transplants labelled in intermediate explant culture. Neuroscience 12: 513-530.
14. *Lund, R. D., McLoon, L. K., McLoon, S. C., Harvey, A. R.,* and *Jaeger, C. B.*: transplantation of the developing visual system of the rat. In: Seil FJ (ed) Nerve, Organ, and Tissue Regeneration: Research Perspectives. Academic Press, New York. pp. 303-323. 1983.
15. *Nornes, H., Bjorklund, A.,* and *Stenevi, U.*: Transplantation strategies in spinal cord regeneration. In: Sladek JR, Gash DM (eds) Neural Transplants, Development and Function Plenum Press, New York and London. p. 407-421. 1984.
16. *Patel, U.* and *Bernstein, J. J.*: Growth, Differentiation and viability of fetal rat cortical and spinal cord implants into adult rat spinal cord. J. Neurosci. Res. 9: 303-310. 1983.
17. *Privat, A., Monsour, H., Pavy, A., Geffard, M.,* and *Sandillon, F.*: Transplantation of disscoiated foetal serotonin neurons into the transected spinal cord of adult rats. Neurosic. Lett. 66: 61-66. 1986.
18. *Reir, P. J.*: Neural tissue grafts and repair of the injured spinal cord. Neuropathol Appl. Neurobiol. 11: 81-104, 1985.
19. *Reir, P. J., Bregman, B. S.,* and *Wujeck, J. R.*: Intraspinal transplantation of embryonic spinal cord tissue in neonatal and adult rats. J. Comp. neurol. 247: 275-296, 1986.
20. *Ross, D. T.* and *Das, G. D.*: Regenerative growth of retinofugal axons into and through neocortical transplants following transection of the optic tract in adult rats In Das GD, Wallace RB (eds) Neural Transplantation and Regeneration. Springer Verlag, N. Y., pp. 181-227. 1985.
21. *Saldek, J. R.* and *Gash, D. M.*: (eds) Neural Transplants, Development and Function. Plenum Press, New York and London. pp. 1-454, 1984.
22. *Stenevi, U., Bjorklund, A.,* and *Kromer, L. F.*: Use of CNS implants to promote regeneration of central axons across denervating lesions in the adult rat brain. In: Sladek, J. R.. Gash, D. M. (eds) Neural Transplants, Development and Function. Plenum Press, New York and London. pp. 325-339. 1984.
23. *Sotelo, C.* and *Alvarado-Mallart, R. M.*: Growth and Differentiation of cerebellar suspensions transplanted into the adult cerebellum of mice with heredodegenerative ataxia. Proc. Natl. Acad. Sci. U.S.A. 83: 1135-1139.

Assessment of Autologous Adrenal-to-Caudate Grafting for Parkinsonism in man

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INTRODUCTION

Because Parkinson disease (PD) is caused by progressive loss of nigrostriatal dopaminergic neurons, it is rational to consider replacement with new dopamine-producing cells from other souces. The possible sources of tissue that could be transplanted include adrenal medulla, fetal substantia nigra from a donor, and neuroblastoma or pheochromocytoma cells grown in tissue culture. Recent work in nonhuman primates and rodents, using animal models of parkinsonism, has indicated that transplantation of fetal substantia nigra cells and adrenal medullary tissue can reverse the parkinsonian features seen in these animals (Bakay et al., 1987; Redmond et al., 1986; Freed et al., 1981; Stromberg et al., 1985). In the parkinsonian primates that received fetal nigral implants, there was biochemical improvement as well as behavioral improvement, indicated by an increase in the amount of dopamine metabolites in the cerebrospinal fluid (CSF) (Bakay et al., 1987).

When considering the application of this technique to PD patients, the adrenal medulla has the great advantage of being an autograft, i.e., it can be taken from one of the patient's own two adrenal glands. The chromaffin cells of the adrenal medulla are of neural crest origin, and normally they make norepinephrine and epinephrine under the trophic influence of the adrenal cortex. However, because dopamine is the precursor of norepinephrine in the catecholamine synthetic pathway, the biochemical machinery is present in chromaffin cells to make dopamine if removed from the influence of the adrenal cortex and placed in the brain. The first clinical trials were performed by Backlund and colleagues (1985), but they were unsuccessful in obtaining a sustained benefit in two advanced PD patients using stereotaxic implantation of suspensions of small adrenal medullary tissue fragments. In early 1987, Madrazo et al. reported dramatic clinical improvement in two young PD patients following transplantation of autologous adrenal medullary tissue into the head of the right caudate nu-

cleus. Their technique differed from that used by Backlund et al. in that they utilized a transcortical intraventricular approach and transplanted pieces of the entire adrenal medulla into a surgical bed prepared in the caudate, with a portion bathed by the ventricular CSF.

After the reporte of success by the Mexican investigators, United States investigative teams began performing adrenal-caudate transplants in PD patients to determine whether their successful results be replicated. We have performed the adrenal medulla to right caudate nucleus transplantation procedure in twelve patients with moderately severe to severe PD, ranging in age from 36 to 65 years. All had reached a stage where pharmacotherapy was no longer sufficient to control their symptoms, a necessary condition for entry into our study protocol. We have had two major goals from the outset: to determine 1) whether the procedure is *safe* and 2) whether the procedure is *effective* in producing a lasting improvement in the patients' motor performance. Corollary questions are, 1) if improvement is seen, how long does it last? and, 2) what is the mechanism by which the procedure produces improvement? Herein are reported our initial findings on the first ten patients.

PATIENT SELECTION CRITERIA

Because of the potential risks of the procedure, only patients with moderately severe to severe parkinsonism who were not responding well to conventional pharmacotherapy were considered for entry into the study. In addition, further selection criteria included: 1) good general health; 2) no prior history of adrenal insufficiency or current exogenous corticosteroid therapy; 3) non-smokers preferred; 4) prior good response to L-dopa replacement therapy (Sinemet) preferred, 5) no prior history of major neurological disease other than parkinsonism, and 6) mental competence.

PRE-OPERATIVE ASSESSMENT

Before proceeding with the pre-operative evaluation all patients underwent a thorough neurologic evaluation to confirm the diagnosis of idiopathic Parkinson disease. The patients subsequently underwent an initial endocrine evaluation to determine that both adrenal glands were present and functioning normally. All patients had an abdominal CT scan to visualize both adrenal glands and ensure their correct anatomical location. In addition, supine and standing blood pressures were checked, electrolytes evaluated, and a cortrosyn stimulation test performed, all on an outpatient basis. Further testing in the Clinical Research Center (CRC) while off dopamine-altering medications included tests of hypothalamic and pituitary reserve, and tests for renin-aldosterone and catecholamines.

The pre-operative evaluation subsequently included a videotaped examination using a Modified Columbia PD Rating Scale during "OFF" and "ON" states. Patients fasted and were without their antiparkinsonian medications overnight prior to an early morning "OFF" evaluation (without medications 10-12 hours), then they were allowed to take their standard dosage of medications and evaluated while in the "ON" state. Plasma L-dopa levels were drawn during this evaluation, resulting in dose response curves for each patient. To evaluate movement time (physiologic measure of bradykinesia), reaction time (a measure of premovement neural processing), and tremor, patients underwent quantitative electrophysiological motor testing. The patients had a detailed battery of neuropsychological tests to quantitatively assess cognitive function and to screen for depression. (Details of the electrophysiological, neuropsychological and dose response studies are in preparation for separate publication). Monamine metabolites from the CSF were checked after a four day dopaminergic "drug holiday", and a true, steady-state "OFF" modified Columbia PD rating scale examination was done after 72 hours off medications during the "drug holiday". All patients had brain CT and MRI scans. Our protocol calls for this comprehensive evaluation to be performed preoperatively and at 3-6, 7-12, 13-18, and 19-24 months postoperatively.

METHODS

CSF Analysis: In order to determine whether any behavioral improvement that

may be seen is secondary to stimulation of dopamine production by the graft and/or brain following transplantation we measured monamine metabolites in lumbar CSF preoperatively. All patients were admitted to the CRC and a lumbar puncture was performed following a 4 day interval off of all dopamine-altering medications ("drug holiday"). They were maintained on a standard diet consisting of 0.8 grams of protein and 35 kilocalories per kg of body weight, along with 80 meq of sodium daily. The patients were supine and fasting overnight prior to the LP. Routine CSF analysis included cell count, protein, glucose and bacterial culture. In addition, monamine metabolites (HVA and 5-HIAA) were measured using high performance liquid chromatograpy with electrochemical detection (Iuvone et al., 1987). Several ml also were stored at -70ºC for future analysis.

Surgical Procedure: Implantation of autologous adrenal medullary tissue into the caudate nucleus was performed via open craniotomy. The patient was placed in a left semi-lateral decubitus position with the head flexed and turned to the right so that a brow-up head position was achieved. This allowed access to the right frontal lobe and to the right adrenal gland without repositioning of the patient. The operating table was flexed downward underneath the left flank to allow greater exposure of the right retroperitoneal area.

A partial Soutar incision which crossed the midline was used in earlier operations. This allowed for surgical access to both lateral ventricles so that a shunt catheter with an attached Ommaya reservoir could be placed into the left lateral ventricle while the right lateral ventricle was opened to expose the head of the caudate nucleus. In order to avoid violating the left frontal lobe as well as the right, the current technique involves placement of the shunt catheter into the right lateral ventricle following implantation of the graft into the caudate. The purpose of the Ommaya reservoir was to obtain CSF for biochemical studies. The newer approach utilized a right horseshoe incision followed by a high right frontal craniotomy. A U-shaped opening of the dura mater based on the superior sagittal sinus was performed. The middle frontal gyrus was identified. Intraoperative ultrasound was used to aid in cannulating the right lateral ventricle thorugh the middle frontal gyrus. In earlier patients, the BRW stereotactic frame was used for ventricular cannulation but this proved to be more time consuming and no more accurate than intraoperative ultrasound guidance.

After the ventricle was cannulated, the tract around the catheter was enlarged allowing for direct visualization of the interior of the lateral ventricle. Selfretaining retractors were then placed and the operating microscope was brought into position. The head of the caudate nucleus was seen bulging into the lateral inferior portion of the ventricle.

The adrenalectomy was begun after the cranial portion of the procedure had started, and it was timed so that the graft tissue, once obtained, could be placed into the caudate without delay. A right retroperitoneal approach was favored over a transabdominal approach because this technique causes less postoperative ileus and results in better postoperative absorption of oral anti-parkinsonian medications. After the adrenalectomy, the medullary tissue was microscopically dissected in cold Collins solution from the adrenal cortex and cut into pieces approximately 1x1x5 mm.

Just prior to the final preparation of the graft pieces, an incision was made in the head of the caudate nucleus anterior to, or at the level of, the foramen of Monro. Care was taken to avoid injuring any blood vessels on the surface of the caudate. Biopsy micro-forceps were then inserted into the caudate incision and used to create a subependymal pocket. The tissue removed from the caudate during this step was saved for later anatomical and biochemical analysis. All the graft pieces were then placed into the subependymal pocket. The graft site was covered with a gelfoam-vicryl screen which adhered to the caudate with the aid of autologous blood and thrombin. A ventricular catheter was reinserted into the right lateral ventricle via the existing tract and attached to an Ommaya reservoir. Rou-

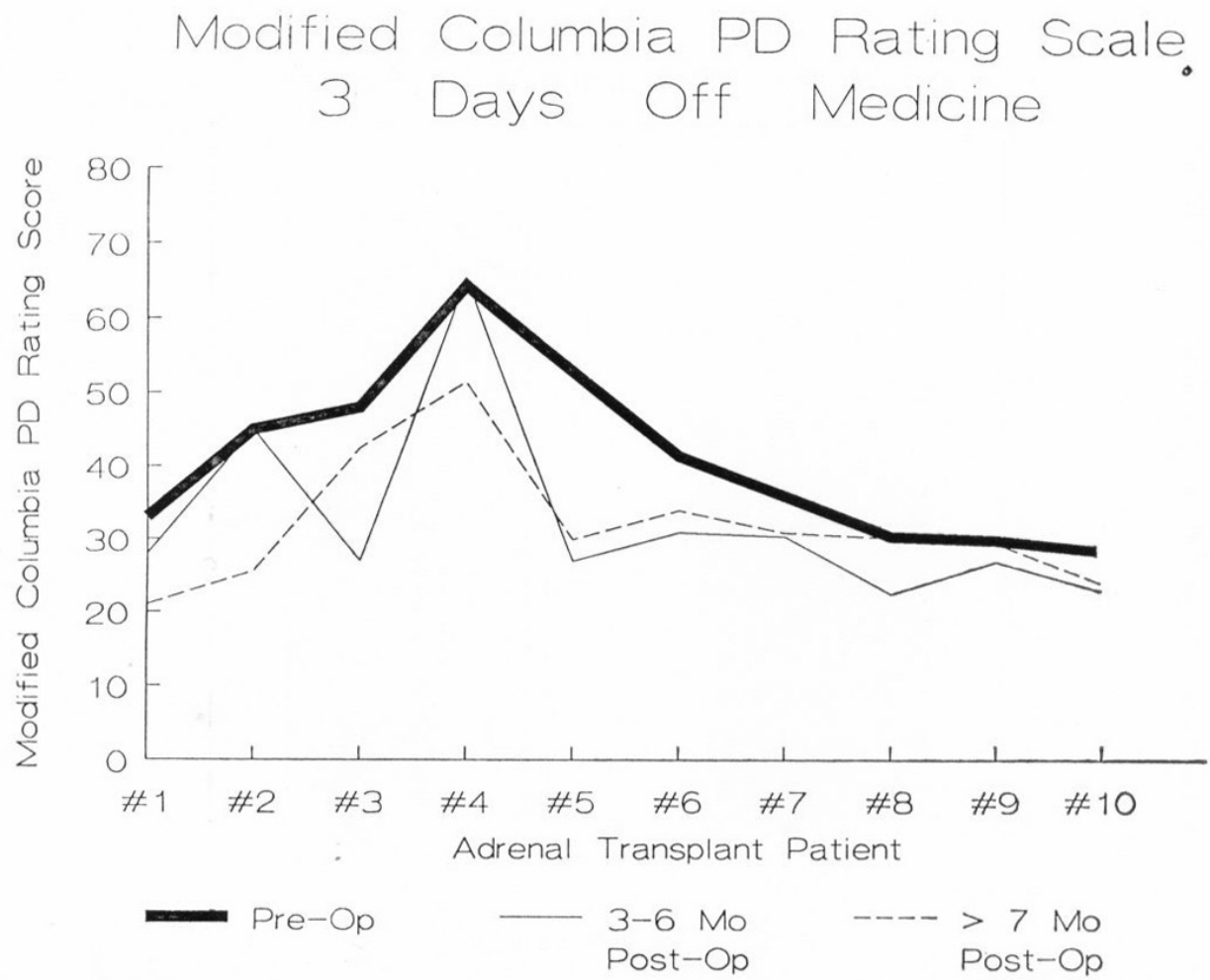

Fig. 1. — *The behavioral performance of our first 10 adrenal medullary grafted patients is illustrated in relation to time. The bold line represents the baseline performance. Nine of the patients demonstrated improvement in the 3-6 months postoperative period (thin line). With longer followup all of the patients demonstrated some degree of improvement. All of these modified Columbia PD ratings were performed 3 days after discontinuing all dopamine-altering medications.*

tine closure was performed and the Ommaya reservoir was incorporated into the craniotomy flap.

RESULTS

Our first ten patients consisted of 8 males and 2 females whose mean age was 53 years. Their mean duration of PD was 9.5 years and their mean Hoehn and Yahr stage off medications was 4.3 (Table 1).

The postoperative course was generally uneventful for most patients. The average postoperative ICU stay was 4 days, with no patient intubated longer than 12 hours postop. The average postoperative hospital stay was 22 days (range 11-46 days). Most patients were hospitalized for 2-3 weeks, however, the average duration was increased because two patients were hospitalized for 44 and 46 days. There were no deaths, surgical complications, or major postoperative medical morbidity (pneumonia, pulmonary embolism, venous thrombosis, myocardial infarction, hemiparesis, or seizures). All patients were gradually restarted on their antiparkinsonian medicines within two days. Seven patients developed urinary tract infections with Foley catheters and required oral antibiotics. Transient hypertension was seen on the first postoperative day, but was easily controlled. Low grade hallucinosis/delusions developed in two patients for several weeks but gradually resolved. Three other patients developed confusion and delirium transiently for several days postoperatively. These alterations in mental state occurred in the older patients.

There were no ill effects endocrinologi-

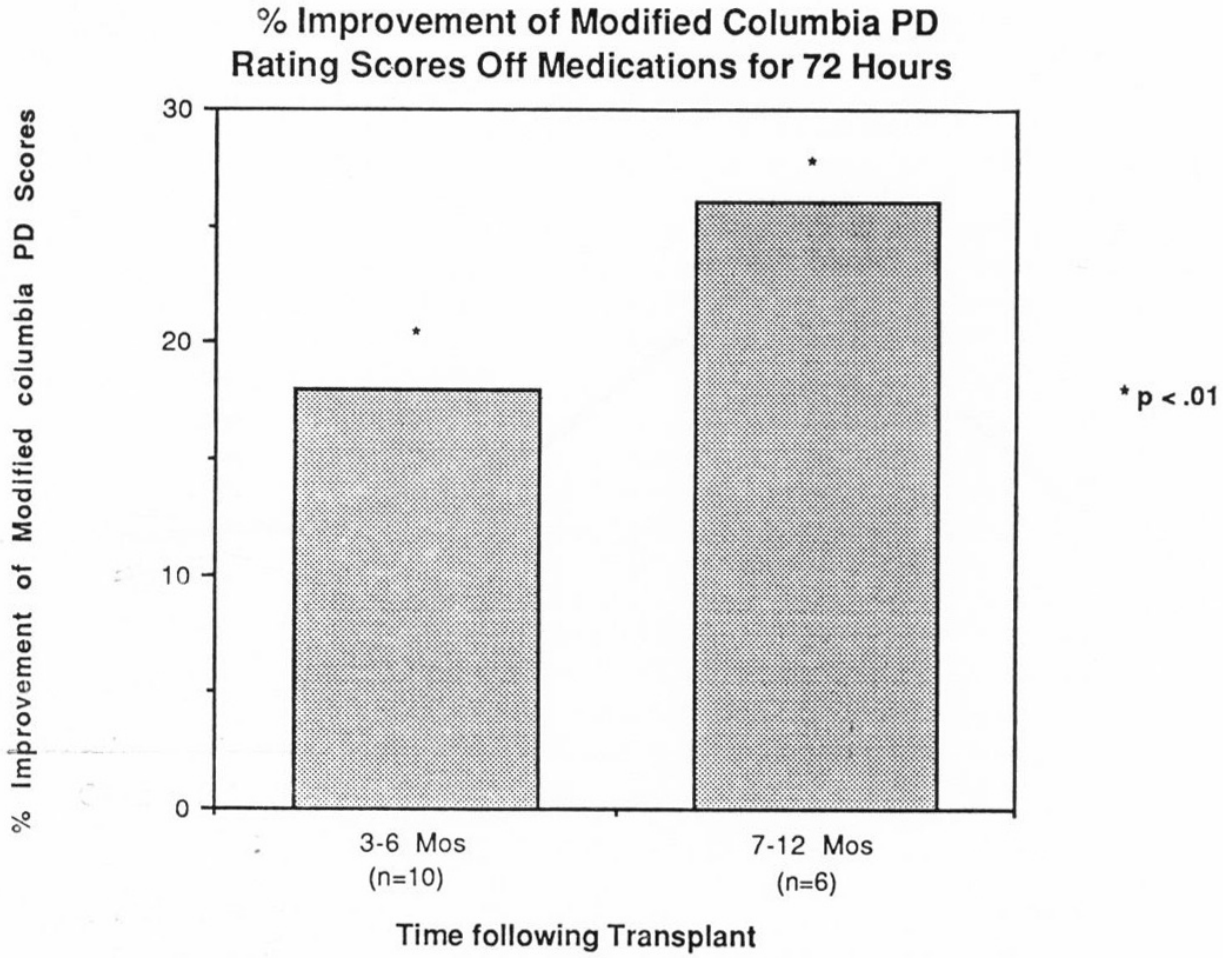

Fig. 2. — *Graphical representation of average percent improvement of modified Columbia PD rating scores off dopamine-altering medication for 72 hours (steady-state "OFF" condition). There was an 18 % improvement of PD disability scores at 3-6 months postoperatively (n=10) and 26 % at 7-12 months (n=6) (p<.01 for both).*

cally, and basal hormone levels were not changed significantly. There were changes in pituitary hormone responses to provocative stimuli, but these changes did not appear to be deleterious (reported in detail separately; Watts et al., 1989).

Neuroimaging revealed that the grafts in all patients were correctly located in the head of the right caudate. Complicating the postoperative evaluation were injuries to patients #2 and #5. Patient #2 developed a subdural hematoma on the left (contralateral to operative site) after a fall 2 months postoperatively. However, this was removed successfully and the patient showed no permanent effects. Patient #5 had a concussion from a motor vehicle accident 2 months postoperatively. He did not require surgery but did not fully recover.

The modified Columbia PD disability score obtained after 72 hours of the drug holiday showed improvement in all patients at some point during the postoperative evaluation (Fig. 1). Analysis shows an average 18 % decrease in disability scores for 10 patients at 3-6 months postoperatively (p<.01) and a 26 % decrease for 6 patients evaluated at 7-12 months postoperatively (p<.01) (Fig. 2). Our most severely affected patient (#4) showed no change of his 3-6 months postoperative disability score, but at 7-12 months he showed a modest improvement (20 %), although he remained severely impaired by his PD.

The lumbar CSF monamine metabolites have been analyzed on 10 patients at 3-6 months postoperatively and on an additional six patients at 7-12 months. The results reported were obtained from the 3rd-4th ml of CSF in all patients. HVA levels showed an average increase to 159 +/— 16 % of preoperative levels (p<.05) at 3-6 months

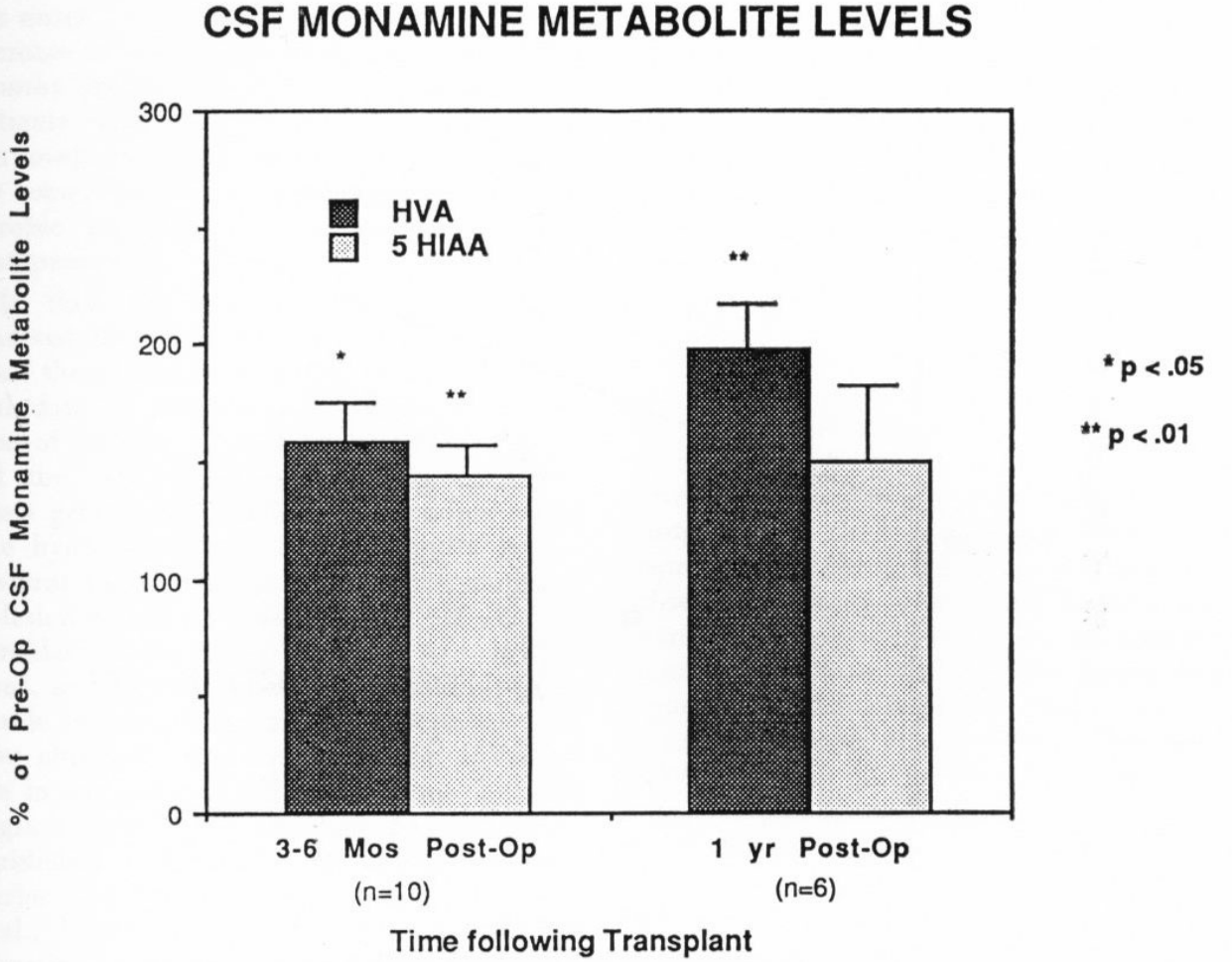

Fig. 3. — *CSF monamine metabolite levels (HVA and 5-HIAA) at 3-6 months and 1 year postoperatively, expressed as percent of preoperative levels.*

and 198 +/— 19 % ($p<.01$) at 7-12 months postoperatively. At 3-6 months postoperatively 5-HIAA levels increased to 144 +/— 13 % of preoperative levels ($p<.01$) and at 7-12 months to 151 +/— 32 % (not significant). All of our patients were being treated preoperatively with levodopa (mean 650 mg; range 150-1550 mg) combined with carbidopa (mean 151 mg; range 37.5-387.5 mg.). Postoperatively all patients were continued on levodopa/carbidopa combination, though several required lower doses due to the development or worsening of dyskinesia. The average postoperative decrease of levodopa was 10 % (mean 585 mg, range 150-1150 mg) and of carbidopa was 23 % (mean 116 mg, range 37.5-287.5 mg) at 3-6 months; at 7-12 months levodopa was decreased 7 % and carbidopa 24 %. Many of the patients required less levodopa /carbidopa tablets or were switched to a less potent strength tablet. Even when on these lower doses, clinical scores from the modified Columbia PD rating scale showed a trend toward improvement.

In order to determine which factors may contribute to the observed improvement, we further analyzed the patients who improved. The percentage of improvement of the modified Columbia PD disability score obtained at 72 hours off of dopamine-altering medications at both 3-6 months and 7-12 months postoperatively was used for the correlations. We did not find a correlation with age of Hoehn and Yahr stage at either of these postoperative times. The preoperative modified Columbia PD disability score did, however, show a correlation with improvement only in the short-term followup period. Those more severely affected appeared to make the greatest improvement ($p<.05$). The percentage change in CSF HVA from baseline appeared to have a modest correlation with the improvement of the PD disability score but did not reach significance (Fig. 4).

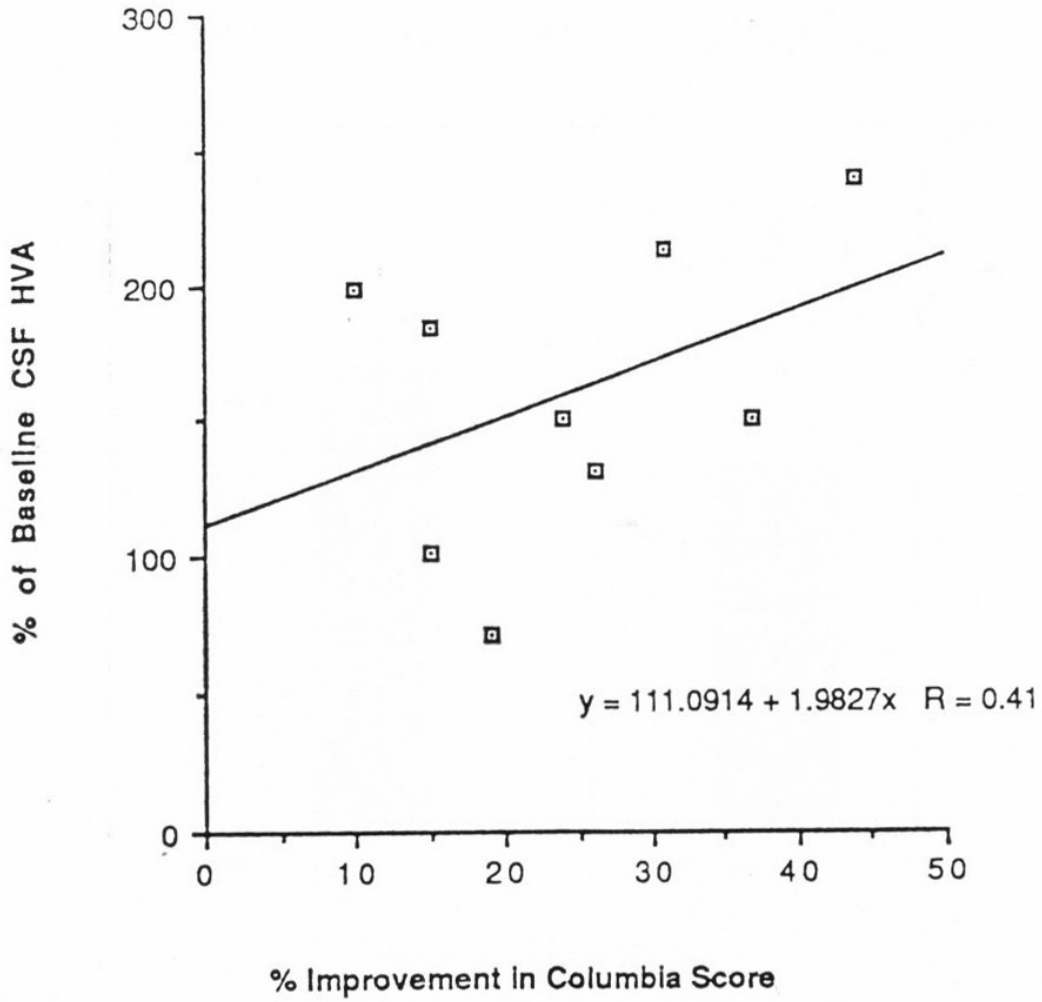

Fig. 4. — *A correlation was attempted between the percentage of improvement in the modified Columbia PD disability score at 3-6 months following adrenal medullary grafting and the percentage change in the CSF HVA concentration from baseline. The modified Columbia PD disability score was obtained 3 days after discontinuing all dopamine-altering medications. The concentration of HVA (ng/ml) was obtained by HPLC measurements as discussed in the text. Although this appeared to demonstrate a strong trend, statistical significance was not achieved.*

DISCUSSION

All of our patients selected for surgery were no longer responding well to antiparkinsonian medications. The patients all had idiopathic Parkinson disease and no patients were considered who had atypical features or Parkinson-plus syndromes. The surgery was uneventful and the postoperative course was free of serious complications. These results may be explained by: 1) our rigid patient selection criteria, 2) a flank incision for the adrenalectomy, which reduced postoperative ileus and allowed the patients to restart their medications quickly, and 3) the surgical approach used by our surgical teams. All patients were in good general health and therefore able to tolerate the anesthesia and the two major surgical procedures involved. In our hands the procedure appears to be safe.

A major issue we addressed concerned the efficacy of the procedure in producing an improvement in the patients' motor performance. Clinical evaluation showed an improvement in most patients. Symptoms of Parkinson disease are felt to occur after 80 % or more of the nigrostriatal dopamine system is lost. It is possible that the transplanted cells stimulate residual dopaminergic neurons. Moreover, most patients showed an initial improvement during the first 1-2 weeks postoperatively ("honeymoon period"). This clinical effect is likely due to lysis of the transplanted adrenal medullary cells, thus causing a temporary surge of catecholamine levels. Several patients noticed

the onset of dyskinesia and others noted an increase in severity of dyskinesia about two months postoperatively. Subsequently, most patients required a decrease in antiparkinson medication, although only temporarily for some. Clinical improvement became noticeable beginning approximately 2 months postoperatively.

Is, then, the improvement that we have observed the result of dopamine production from the grafted medullary cells? The animal data on dopamine production in this type of grafting would suggest that this is not the case. However, grafting of fibroblasts genetically modified to produce tyrosine hydroxylase and L-dopa results in behavioral improvement of 6 hydroxydopamine-lesioned rats, while grafting of control fibroblasts does not (Wolff et al., 1989). Thus, secretion of catecholamines may play a role in neurological improvement. We have observed an increase in CSF HVA levels in our patients off medications, but the origin of the HVA increase has not been established. Alterations in the blood-brain barrier may facilitate improvement (Becker et al., 1988). This facilitation may not necessarily be related to the catecholamines but may be related to the penetration of the dopaminergic medications or carbidopa into the CSF. One may ask whether or not these medullary cells survive and maintain this abnormal blood-brain barrier. The patients who have come to autopsy in other series have demonstrated very little in terms of adrenal medullary graft survival, although none of these has been what would be called an unqualified clinical success; therefore, the importance of cell viability remains undetermined (Hanson et al., 1988, Bohn et al., 1987). Animal studies have yet to directly answer this question but our data suggest that the presence of surviving cells correlates with behavioral improvement (R. L. Watts et al., 1989). These cells may contribute to the behavioral improvement not so much through their production of dopamine but through the stimulation of a neurotrophic effect, as suggested by animal studies (Bakay et al., 1989; Becker et al., 1988; Bohn et al., 1987).

In the laboratory we are examining methods by which to improve survival of adrenal medullary cells grafted into the central nervous system of nonhuman primates and address the issue of the importance of cell viability. More basic science and clinical research will be required before adrenal to caudate transplantation can be viewed as a proven adjuvant to the treatment of parkinsonism.

CONCLUSION:

In summary, we have shown that there is a mild to moderate improvement with this therapeutic approach although it is not yet a "cure". Followup studies of these patients and our subsequent transplant patients will be needed to judge whether the improvement continues and if there is a subset of patients who would benefit most from this procedure. Other investigative teams throughout the United States have reported results which are similar to ours in general (Goetz et al., 1989; Allen et al., 1989; Kelly et al., 1989). This degree of improvement is not as dramatic as was initially expected based on Madrazo's preliminary results, but we believe that we are observing a definite biological phenomenon which produces up to a moderate degree of improvement. It is likely that this technique will be the first step in a series of advancements which will lead to a greater degree of improvement and perhaps an eventual cure of Parkinson disease.

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ADRENAL-CAUDATE TRANSPLANT PATIENT PRE-OPERATIVE CLINICAL DATA

Patient #	Age	Sex	PD (years) Duration of	(Off Drugs) PD Stage Hoehn & Yahr	Carbidopa (mg) Dosage Levodopa/ Pre-Operative
1	48	M	7	4	900/225
2	57	M	11	4-5	750/75
3	56	M	10	4-5	600/150
4	62	M	14	5	1550/387.5
5	65	M	8	4	800/200
6	39	M	5	4-5	500/125
7	36	M	10	4	300/75
8	51	F	8	4	400/100
9	64	M	11	4	150/ 37.5
10	56	F	11	4	550/137.5
MEAN	53.4		9.5	4.3	650/151

SUMMARY

Implantation of catecholaminergic tissue into the striatum is a new experimental treatment approach for parkinsonism. We have grafted autologous adrenal medullary tissue into the right caudate nucleus in 10 patients with advanced Parkinson disease (PD) (8 male, 2 female; mean age 53 yr; mean duration of PD 9.5 yr; mean Hoehn & Fahr stage "off" meds 4.3), using a transcortical technique similar to Madrazo, et al (1987). A right adrenalectomy was performed via a flank approach. There have been no deaths, surgical complications, or serious postoperative morbidity (pneumonia, myocardial infarction, stroke, or seizures). Uncomplicated urinary tract infection occurred in 7 patients; 5 patients experienced transient postoperative confusion/psychosis. Patients were evaluated preoperatively, and 3-6 months and 7-12 months postoperatively. Videotaped modified Columbia PD ratings of patients off all dopaminergic agents for 72 hours revealed 18 % improvement at 3-6 months postoperatively ($p<.01$,$n=10$) and 26 % improvement at 7 12 months ($p<.01$,$n=6$). Dyskinesias necessitated reduction of L-dopa by 10 % and carbidopa by 23 % at 3-6 months postoperatively. Analysis of lumbar CSF monamine metabolites following 4 day "drug holiday" revealed: homovanillic acid (HVA; dopamine metabolite) increased by 59 % ($p<.05$) at 3-6 months postoperatively ($n=10$) and 98 % ($p<.01$) at 7-12 months ($n=6$); 5-hydroxyindole acetic acid (5-HIAA; serotonin metabolite) increased by 44 % ($p<.01$) at 3-6 months ($n=10$) and 51 % (not significant) at 7-12 months ($n=6$). Magnetic resonance and computed tomographic scans demonstrated correct placement of grafts in the right caudate. We have observed a mild to moderate degree of improvement persisting up to 1 year with this therapeutic approach, but further study is needed.

RESUMEN

El implante de tejido catecolaminérgico en el estriado es un nuevo enfoque experimental de tratamiento para el parkinsonismo. Nosotros hemos insertado tejido medular adrenal antólogo, en el núcleo caudado derecho en 10 pacientes con avanzada enfermedad de Parkinson (8 hombres, 2 mujeres, edad intermedia, 53 años, duración media de la enfermedad de Parkinson 9 años y medio; medio estado de Hechn y Yahr fue 4.3.), utilizando una técnica transcortical similar al Madrazo y otros

(1987).

Una adrenalectomía derecha fué realizada. No hubieron muertes, complicaciones quirúrgicas o una morbidez postoperatoria seria (neumonía, infarto miocárdico, accidente vascular cerebral o crisis epilépticas.

No complicada infección del tracto urinaria ocurrió en 7 pacientes; 5 pacientes experimentaron un transitorio estado postoperatorio de confusión psicosis. Los pacientes fueron evaluados preoperativamente y 3-6 meses y 7-12 meses postoperativamente. Grabaciones en cinta de video modificaron clasificación de la Columbia en enfermos de Parkinson privados de agentes dopaminérgicos por 72 horas revelaron 18 % de mejoría en los 3-6 meses postoperatorios y 26 % de mejoría a los 7-12 meses. Diskinesias necesitaron reducción de L dopa en un 10 % y carbidopa en 23 % a los 3-6 meses postoperatorios. Análisis de monoamina metabolitos del líquido cefaloraquideo lumbar siguiendo 4 días "de vacaciones de drogas" revelaron: ácido homovanílico (metabolito de dopamina) aumentados en un 59 % en los 3 a 5 meses postoperatorios hidroxidólico 5 (metabolito de serotonina) aumentó un 44 % en los 3 a 6 meses y un 51 % en los 7 a 12 meses. Estudios de resonancia magnética y de tomografía computarizada demostraron correcto emplazamiento de injertos en el candado derecho. Nosotros hemos observado un leve a moderado grado de mejoría que persistió hasta 1 año con este enfoque terapéutico; pero es necesario continuar el estudio.

RÉSUMÉ

Les implants de tissus cathécolaminergiques dans les corps strié est une neuvelle perspective experimentale de traitment du Parkinsonisme. Nous avons implanté un tissu médulaire adrenal anthologue dans le noyau caudé droit, aur 10 patients en Parkinson avancé (8 hommes, 2 femmes, âge moyen 53 ans, duré moyenne de la maladie 9.5. ans; état moyen de Hechn et Yahr 4.3), technique transcorticale similaire de la Madrazo et col. (1987).

Une adrenalectomie fut réalisée-morbidité =0 % complications chirurgicales, postoperatoires serieuses 0 % (pneumonie, infarctus, A.V.E. crise epileptique).

Infection uninaire simple dans 7 cas, 5 cas de confusion ou psycose postoperatoire transitoire.

Il fut faite un évaluation préopératoire et 3-6 mois et 7-12 mois aprés l'opération.

Les enregistement vidéos out modifié la classification de Columbia de Parkinsoniens privés de Dopaminergiques pendant 72 h. et ont révélés un 18 % d'amelioration hez les 3-6, m postoperatoires et 26 % chez les 7-12 m. Des Diskynesies ont ohligés á une réduction de la L.dopa en 10 % et une diminution de la carbidopa de 25 % chez les 3-6m. Une analyse de la monoamine (metabolite du liquide cephalo-rachidien lombaire aprés 4 jours de yeune de drogue ont montré de l'acide homovanilique (métabolite de la dopamine) augmenté de 59 % chez les 3-6 m hidroxidocolico 5 (metabolite de la serotimine) augmente 44 % chez les 3-6m et 51/ chez les 7-12m. Des études de résonance magnétique et Tom. Comp ont montrés une situation correcte des implants. Nous avons observé une amelioration moderée qui persistait un an plus tard avec cette solution thérapeutique.

It est nécessaire de poursuivre les études.

ZUSAMMENFASSUNG

Die Implantation von catecolaminergischem Gewebe in das Striatum iat sihzt eine Behandlung im experimentallen Stadium fuer Parkinsonkranke. Wir ueberpflanzten autologes adrenomedullaeres Gewebe in den rechten Nuclees Caudatus von zehn Patientdn mit fortgeschrittener Parkinsons cher Krankheit (8 Maenner, 2 Frauen); mittleres Alter 53 Jahre; mittlere Dauer der Krakheit 9,5 Jahre, mitt lerer Hoehn und Jahr Status "off" 4.3; mit transcorticaler Technk aehnlich der von Madrasso. Rechte Adrenalektomie durch Flankenschnitt. Keine Fodesdaelle, keine chirurgischen Komplikationene, keine ernste postoperative Erkrankung (Pneumonie, Myocardinfarkt, Schlaganfall oder Epileptische Anfaelle) Eine nichtkomplizierte Harntraktinfektion

bei sieben Patienten; 5 Patiente litten an voruebergehender postoperativer Confusion oder Psychose. Die postoperative Auswertung nach 3 bis 6 Monaten und 7 bis 12 Monaten. Alle Patienten wurden vor der Operation ausgewertet. Modifizierte Video-Band-Aufnahmen mit der Columbia-Klassifizierung bei allen Kranken ohne Dopaminergische Medikation waehrend 72 Studen ergaben 18 % Besuerung 3 bis 6 Monate nach der Operation (p.0,1,n=10) und 20 % Bessering 7 bis 12 Monaten nach der Operation (p.0,1,n=6) Bei Faellen von Dyskinesie war notwendig eine Verminderung von L-Dopa un 10 %, und von Carbidona um 23 % 3 bis 6 Monaten achder Operation. Die Analyse der lumbaren Rueckenmarkfluessigkeit aut Monaminabbaustoffe nach 4 taegiger Drogenenthaltung ergab: Homovallininsaeure, ein Donamiin-Abbaustoff, war vermehrt um 59 % (p 0,5) 3 bis 6 Monate nach der Operation (n=10) und um 98 % (p 0,1) nach 7 bis 12 Monaten (n=6) Die 5-Oxyindolessignaeure, ein Serotonin-abbaustoff, war um 44 % (p 0,1) nach 3 bis 6 Monaten vermehrt (n=10) und um 51 % (kein signifikanter Wert) nach 7 bis 12 Monaten (n=6). Untersuchungen mit Magnetischer Resonanz und Computer-tomographie zeigten, dass die Implantate im rechten Nucleus Caudatus korrekt eingepflanzt waren. Wir beobachteten einen leichten bis mittleren Grad der Besserung bis zu einem Jahr mit dieser Operation; aber es ist noetig, diese Arbeiten fortzusetzen.

REFERENCES

1. *Allen, G. S., Burns, S., Tulipan, N. B., et al.*: Adrenal medullary transplantation to the caudate nucleus in Parkinson's disease: Initial clinical results in 18 patients. Arch. Neurol. 46: 487-491, 1989.
2. *Bakay, R. A. E., Barrow, D. L., Fiandaca, M. S., et al.*: Biochemical and behavioral correction of MPTP parkinson-like syndrome by fetal cell transplantation. Ann. N. Y. Acad. Sci. 495: 623, 1987.
3. *Becker, J. B., Freed, W. J.*: Neurochemical correlates of behavioral changes following intraventricular microdialysis in freely moving rats, In: Gash. D. M., Sladek, Jr., J. R. (eds): Transplantation into the mammalian CNS. Prog. Brain Res., Elsevier. Amsterdam 78: 527:533, 1988.
4. *Backlund, E. O., Granberg, P. O., Hamberger, B., et al.*: Transplantation of adrenal medullary tissue to striatum in parkinsonism: First clinical trials. J. Neurosurg. 62: 169, 1985.
5. *Bohn, M. C., Cupit, L., Marciano, F., et al.*: Adrenal medulla grafts enhance recovery of striatal dopaminergic fibers. Science 237: 913-916, 1987.
6. *Freed, W. J., Murihisa, J. M., Spoor, E., et al.*: Transplanted adrenal chromaffin cells in rat brain reduce lesion-induced rotational behavior. Nature 292: 351, 1981.
7. *Goetz, C. G., Olanow, C. W., Koller, W. C., et al.*: Multicenter study of autologous adrenal medullary transplantation to the corpus striatum in patients with advanced Parkinson's disease. N. Eng. J. Med. 320: 337-341, 1989.
8. *Iuvone, P. M., Boatright, J. H., Bloom, M.* M.s Dopamine mediates the light-evoked suppression of serotonin N-acetyltransferase activity in retina. Brain Res. 418: 314-324, 1987.
9. *Kelly, P. J., Ahlskog, J. E., Van Heerden, J. A., et al.*: Adrenal medullary autograft transplantation into the striatum of patients with Parkinson's disease. Mayo Clni. Proc 64: 282-290, 1989.
10. *Madrazo, M., Drucker-Colin, R., Dirr, V., et al.*: Open microsurgical autograft of adrenal medulla to the right caudate nucleus in two patients with intractable Parkinson's disease. N. E. J. M. 316: 831-834, 1987.
11. *Redmond, D. E., Roth, R. H., Elsworth, J. D., et al.*: Fetal neuronal grafts in monkeys given MPTP. Lancet 1: 1125, 1986.
12. *Stromberg, I., Herrera-Marschetz, M., Unyestedt, U., et al.*: Chronic implants of chromaffin tissue into the dopamine-denervated sriatum: effects of NGF on graft survival, fiber growth and rotational behavior. Exp. Brain Res. 60: 335, 1985.
13. *Watts, N. B., Clark, R. V., Watts, R. L. et al.*: Changes in endocrine function after adrenal medullary transplantation to the central nervous system. Submitted for publication. 1989.
14. *Watts, R. L., Bakay, R. A. E., Herring, C. J., et al.*: Preliminary report on adrenal medullary grafting and cografting with sural nerve in the treatment of hemiparkinson monkeys. Prog. Brain Res. (in press).
15. *Wolff, J. A., Fisher, L. J., Xu, L., et al.*: Grafting fibroblasts genetically modified to produce L-dopa in a rat model of Parkinson disease. Proc. Natl. Acad. Sci. USA (in press; 1989).

Neural Grafting In The Mammalian Hypothalamus:

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INTRODUCTION

Attention has recently been focused upon neural transplantation as a novel method to restore function(s) lost due to a variety of neuropathies. Despite advances in our fund of practical knowledge dealing with host-graft interactions, important caveats still exist with respect to the use of neural grafting in clinical practice. Neural grafting is by no means a new technique and historically dates back to the first decade of the 20th Century when Dunn, (1917) described one of the earliest attempts to graft neural tissue in the adult brain. In the last ¾th of a century, hundreds of published reports have described various aspects of neural transplantation in a number of vertebrate species under a wide range of physiological and pharmacological paradigms: the bulk of these publications have emerged since 1970. For a review see Sladek and Gash (1984). During this time there has been a significant increase in the number of published reports and symposium proceedings that deal with this intriguing area of neurobiology. Over the last several years, research from this laboratory has focused primarily upon the ultrastructural correlates of neural grafting in the endocrine hypothalamus. We employ the Brattleboro strain of Long-Evans rat with autosomal homozygous diabetes insipides (DI). This strain of rat has served as a useful animal model to assess whether or not neural transplants alter the physiological status of these animals, namely whether neural grafts reduce their normally profound polyuria and polydipsia.

The present report is one in a series of ongoing experiments designed to examine the morphological correlates and physiological alterations that transpire when hypothalamic tissue from normal 15 day post-coitus (PC) Long-Evans fetal rats is stereotaxically positioned into the third cerebral ventricle of DI host rats. The focus of the present investigation is directed toward revealing the neurovascular and neuroanatomical interactions which occur between the transplant and the host together with the intrinsic neuroanatomical organization and biochemical neuroanatomy of the parenchyma of neural grafts.

MATERIALS AND METHODS

Timed, pregnant Long-Evans dams from Blue Spruce farms were killed at 15 days post-coitus. Fetuses were removed and placed in a container with ice cold Eagle's medium. Brains were then immediately excised. Under a dissecting microscope, the

rostral (anterior) hypothalamus was dissected from the surrounding brain. This block of tissue contains the primordia of normal magnocellular supraoptic and paraventricular neurons with portions of the adjacent third cerebral ventricular wall and numerous parvicellular catecholaminergic neurons, glia and ependymal elements. The block of dissected tissue, kept moist in Eagle's medium, was quartered, minced and drawn into the lumen of a 18 gauge spinal needle affixed to a Kopf stereotaxic instrument, following the techniques described by Gash and Sladek (1981) in earlier investigations.

Eighty-seven adult male Brattleboro rats of the Long-Evans strain acted as the recipients for these embryonic, hypothalamic transplants. In addition, 24 adult male Long-Evans rats which had undergone recent hypophysectomy were also employed as recipients for neural grafting. Adult DI host rats were anesthetized with sodium pentobarbital, a burr hole was drilled in the calvaria. A spinal needle was lowered stereotaxically through the cortex and the corpus callosum into the lumen of the third cerebral ventricle. Indwelling fragments of minced embryonic fetal donor hypothalami were extruded from the spinal needle with a stylette into the lumen of the third cerebral ventricle of the DI and/or hypophysectomized rats. Control rats underwent the same stereotaxic surgery, but in these cases either no embryonic tissue was transplanted and the spinal needle with Eagle's medium was introduced, or other portions of the fetal brain, such as the occipital cortex, were substituted. Recipients were maintained on a 12/12 hour light/dark cycle with food and water ad libitum. Water consumption, urine volume and specific gravity were monitored daily, ten days prior to the surgical implantation and until the termination of the experiment. This was done in order to determine changes in their physiological parameters of urine concentration and water consumption.

TRANSMISSION ELECTRON MICROSCOPY

DI host rats with viable indwelling neural grafts were anesthetized with sodium pentobarbital, as were controls. Following deep anesthesia, animals were heparinized with 1 ml of 5000:1 lipoheparin, a thoracotomy performed, heart exposed, the pericardium resected and the left cardiac ventricle was cannulated. The descending aorta was cross-clamped and the right atrium was opened. A perfusion of warm heparinized saline at 37ºC was initiated at 100 mmHg for 1 minute. Following this, the perfusate was switched to buffered Karnovski's aldehyde fixative (pH 7.3) and the cerebral vasculature was perfused for approximately 20 minutes at 100 mmHg perfusion pressure. Following perfusion fixation, brains were dissected intact from the surrounding calvaria and appropriate regions of the ventricular wall containing hypothalamic transplants were then prepared for correlative scanning electron microscopy following the techniques of Scott and Sherman (1984). The results from scanning microscopy have been reported in earlier publications (Scott, D.E., 1985; Scott and Sherman, 1984).

MICROANGIOGRAPHY

Rats were anesthetized and heparanized with 5000:1 lipoheparine. The heart was exposed, the ascending aorta cannulated, the descending aorta clamped, and the right atrium opened. The aorta and cerebral vasculature were perfused first with warm heparinized saline until the effluent was clear of blood. Following this procedure, the perfusate was changed to 300 ml of Karnovsky's aldehyde fixative. The perfusate was then switched to orange microfil (Canton Biochemicals, Boulder, CO), a polymerizing silastic which reaches capillary levels. Perfusion of the silastic was maintained until return flow was noted from the right atrium and both retinae assumed a bright orange hue. Rat brains were dissected intact from the skull, fixed overnight and 50u sections were cut on a Lancer Vibratome. Certain sections were dehydrated in ascending acetone and cleared in methyl salycilate prior to mounting on glass slides. Other sections were reacted with antisera against arginine vasopressin (AVP) and tyrosine hydroxilase (TH) for immunocytochemistry (vide infra).

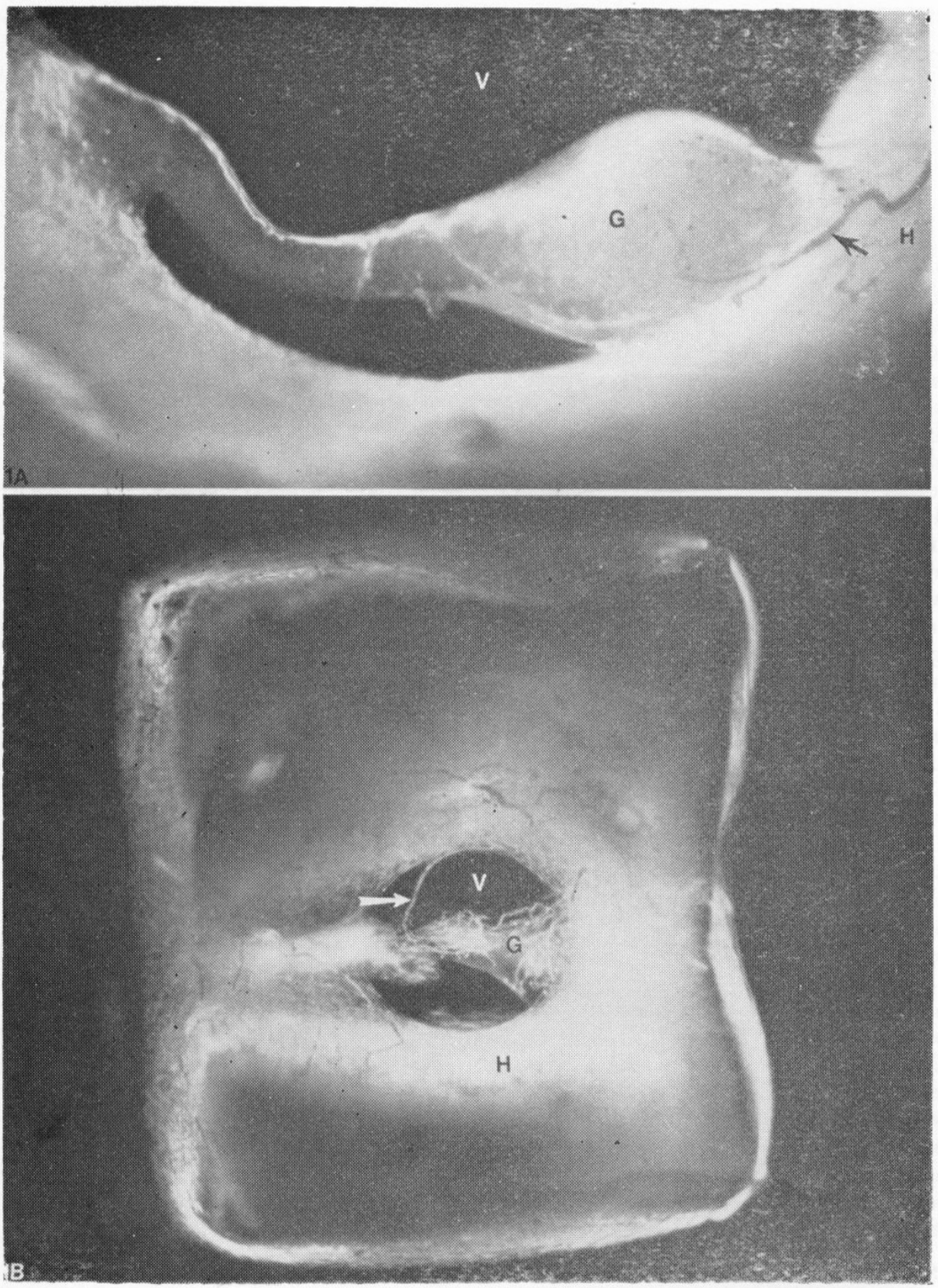

Fig. 1a. — *Coronal view of a whole mount preparation of a neural graft (G) that spans the lumen of the third cerebral ventricle (V) of a DI host rat. Noteable here is the presence of a distinct blood vessel (arrow) that appears to arise from the host ventricular eall (H) and penetrates the parenchyma of the graft. X80. 1b. Dorsal view of whole mount preparation of endocrine hypothalamus (H0 of DI host rat euthanized 20 days post-surgery demonstrating a neural graft (G) that extends rostro-caudally across the lumen of the third cerebral ventricle (V). An arteriole (arrow) can be observed to extend across the ventricular lumen and penetrate the substance of the graft. X48.*

IMMUNOCYTOCHEMISTRY

The immunocytochemical techniques for the localization of AVP and TH were similar to those described previously by Sladek and Gash (1984) and Joseph and Piekut (1986).

Certain rats were anesthesized with sodium pentobarbital and perfused with 200 ml of physiological saline followed by 300 ml of fixative containing 4 % paraformaldehyde, 0.2 % picric acid and 0.5 % glutaraldehyde in phosphate buffered saline at a Ph. of 7.4. The cerebral vasculature was then infused with 9 ml of microfill to delineate the cerebral vascular system relevant to the areas of interest. Brains were removed intact from the calvaria, blocked and returned to the fixative overnight. Fifty microm sections were cut on a Lancer vibratome and tissue sections were collected in wells containing phosphate buffered saline and washed for 2 to 4 hours. Free floating sections were incubated in primary antisera, diluted in PBS containing 1 % BSA and 0.2 % triton-X-100 for 48 hours at 4°C. Sections were continuously agitated during incubation. Dilution of antisera for AVP was 1:6000; for TH, 1:2000. Sections were rinsed in PBS three times, ten minutes each and then incubated in Biotinylated goat anti-rabbit immunoglobulin G (using the Vectastain ABC Kit) for 40 minutes at room temperature. Following this, the sections were incubated in ABC reagents of the Vectastain Kit for 60 minutes at room temperature. Sections were then rinsed and incubated in 0.05 % DAB and 0.03 % H_2O for 3 to 10 minutes or until a brown reaction product was observed. Sections were rinsed in distilled water and mounted on glass slides and dried overnight, and cover slipped. Alternate sections were flat embedded in Epon and prepared for routine electron microscopy.

OBSERVATIONS

Between 1 and 90 days following neural grafting, delicate networks of blood vessels were observed to arise from various regions of the periventricular neuropil of the host rats and were observed to invade the parenchyma of fetal neurografts (Figures 1a,b, and 2a,b). Fetal neurografts were opportunistic and derived their blood supply from the preoptic region as well as other areas of the periventricular stratum, paraventricular nucleus, and the underlying median eminence of the host hypothalamus. Early neuroanatomical patterns of vascular development set the stage for the long-term survival of neural grafts. In this series of 111 experimental animals, no areas of focal necrosis were observed in graft parenchyma nor was there any evidence of rejection by surrounding host tissue as had been observed in earlier investigations from this laboratory (Scott and Sherman, 1984). Blood vessels arising from the underlying median eminence of the host (presumably portal in origin) were at first observed to be fenestrated at the interface between the host and the neurograft. However, these fenestrated vessels were generally restricted to the ventral zones of neural grafts and to the interface between the graft and the underlying apendymal surface of the median eminence (Figure 5c). Limited numbers of neurovascular regions, the so called neurohemal zones (Hofer, 1958), were apparent. These neurovascular zones exhibited large perivascular spaces with luminal and abluminal basal laminae. Commonly, those neurites that terminated on these vessels at the interface between host and the neural graft, as well as in ventral areas of the graft, harbored numerous dense core vesicles, clear microvesicles, mitochondria and they also appeared to exhibit the ultrastructural correlates of active exocitosis (Figure 5c). However, as these vessels continued their distalward growth into the parenchyma of the graft, they appeared to lose their surrounding perivascular spaces and became unfenestrated (Figures 5a, 5b). The transition from a fenestrated to an unfenestrated organization was relatively abrupt. At no time were fenestrated vessels observed deep within the substance of the neural grafts regardless of the age of the graft. Unfenestrated vessels exhibited distinct tight junctions and clearly defined basal laminae. It appeared that the distribution of bona fide neurovascular zones was exclusively restricted to the ventral zone of transplants as well as interfaces between the median eminence of

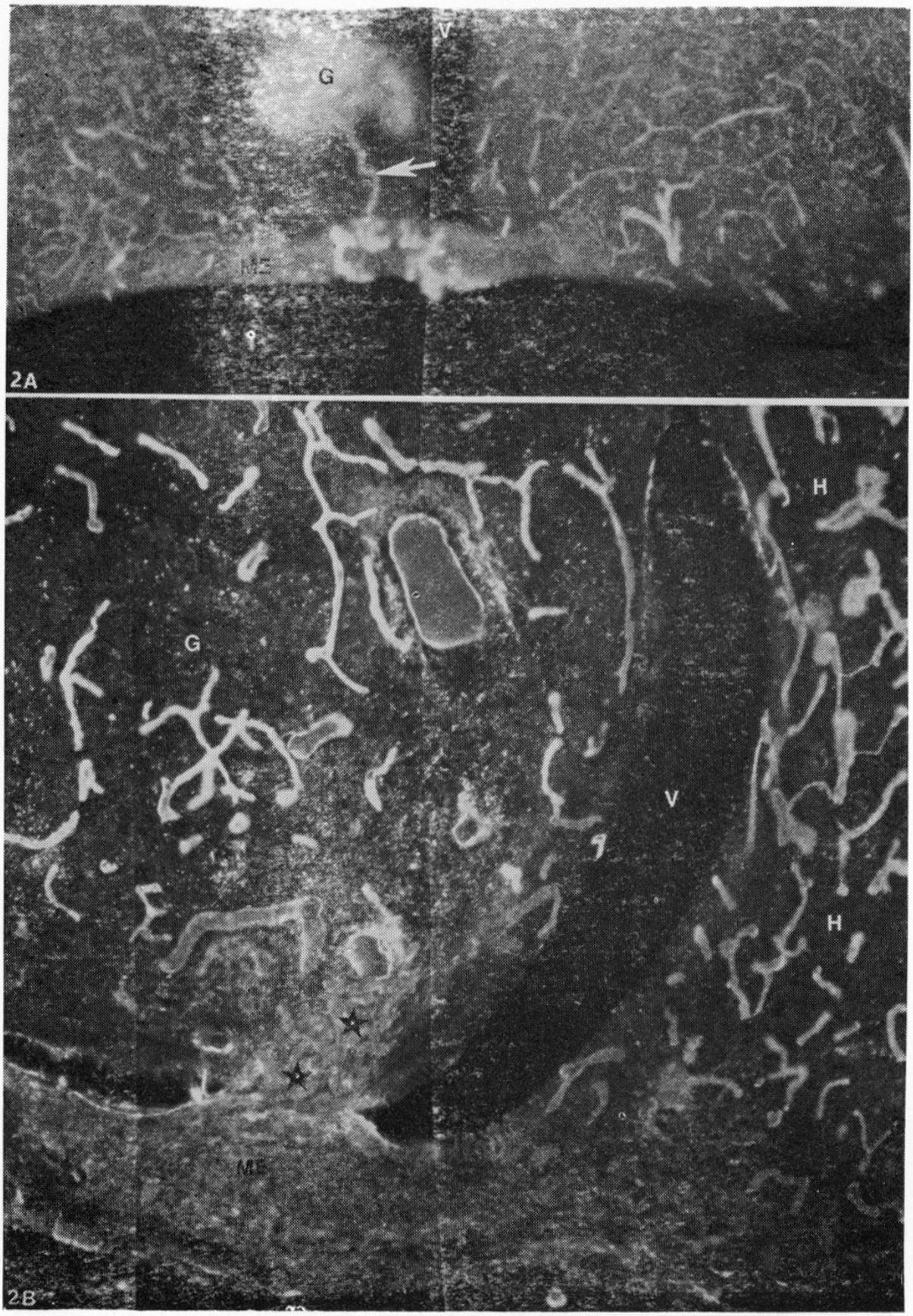

Fig. 2a. — *100 microm thick vibratome section of thebasomedial hypothalamus (H) of ahost D1 rat euthanized 2 weeks following stereotaxic placement of a normal fetal neurograft. Following fixation of tissue, the host rat was perfused with microfil to delinate the vascular organization of a discrete graft (G) at the base of the third cerebral ventricle (V). Arising from the hypophyseal portal plexus of the underlying median eminence (ME) is a distinct blood vessel (arrow) that is observed to penetrate the neural graft. Such grafts are rapidly vascularized within 24 to 48 hours. X98.*

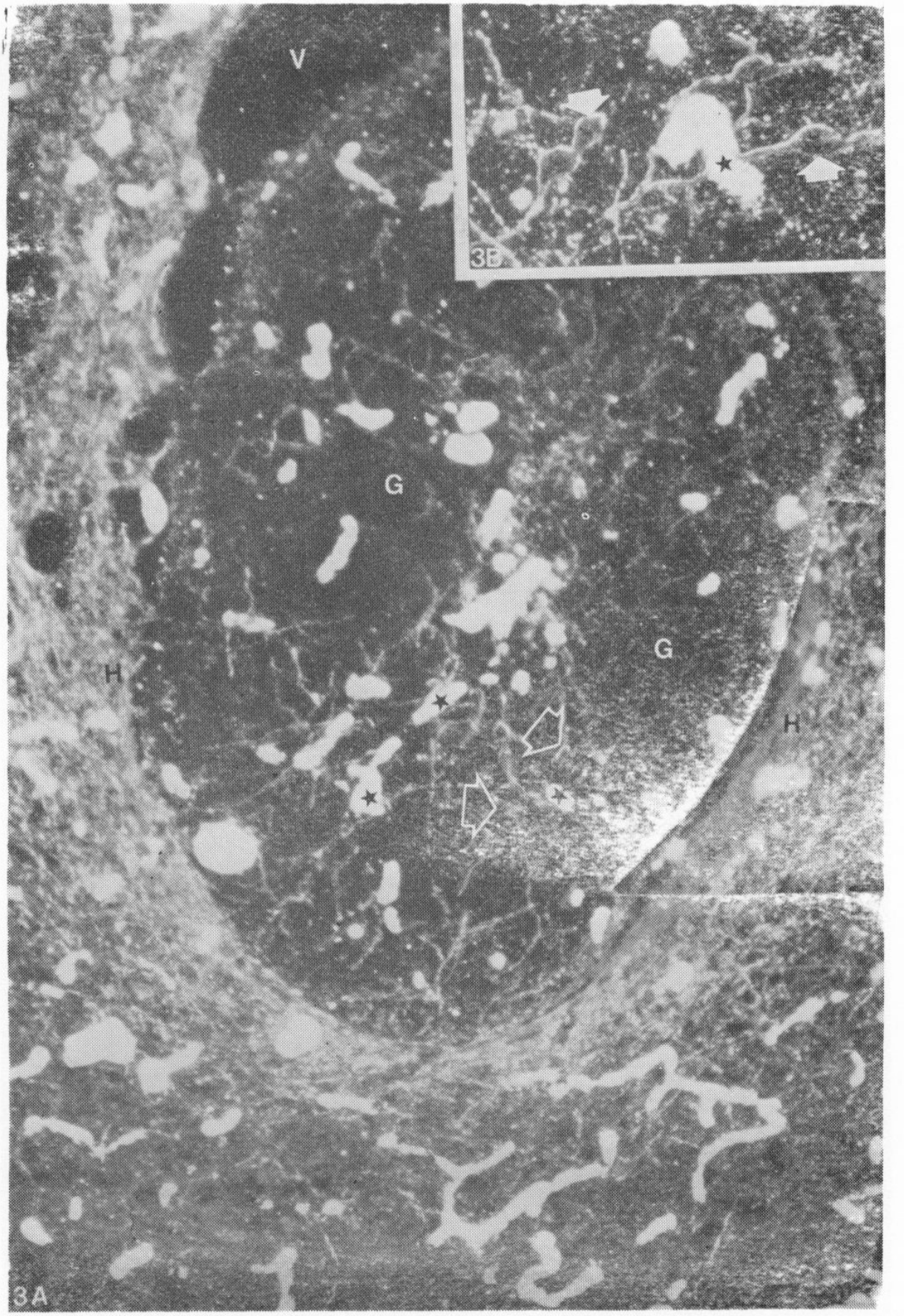

Fig. 3a. — *Fifty microm vibratome section of the basomedial hypothalamus of a DI rat killed 30 days following stereotaxic placement of a normal fetal long-Evans graft. This section was reacted with antisera against tyrosine hydroxylase. Dominating the field is a large neural graft (G) which occupies virtually the entire lumen of the third cerebral ventricle (V). Numerous TH positive catecholaminergic neurons (arrows) are visible within the parenchyma of the graft. The neurites of these cells are often in close anatomical proximity to graft blood vessels (*) which appear white in this photomicrograph (H, host ventricular wall). X500. 3b. Insert. High magnification photomicrograph of TH positive neurons (arrows) whose processes appear to course in close anatomical proximity to intrinsic blood vessel of the neural graft. X900.*

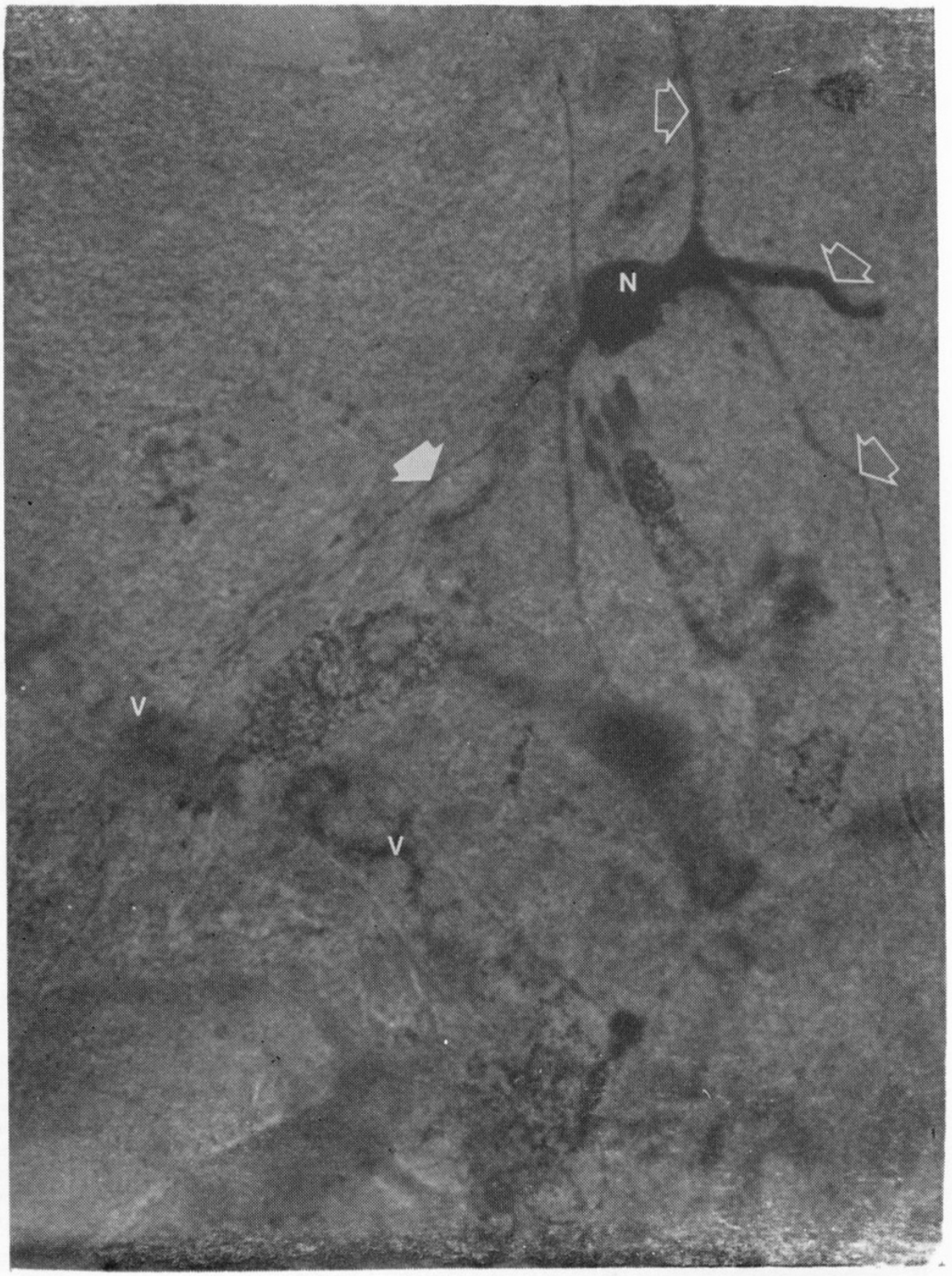

Fig. 4. — *High magnification photomicrograph of a fifty microm thick vibratome section from the central region of a normal transplant in the third cerebral ventricle of a DI rat. The host rat was killed 30 days following stereotaxic placement of the fetal graft. This section was reacted with antisera against arginine vasopressin. Noteable here is an intensely staining magnocellular neuron (N) with several large dendritic processes (open arrows). The axon (white arrow) from this neuron can be observed to traverse the field diagonally and its varicosities are seen in close anatomical juxtaposition to local blood vessels (V) of the graft. X1200.*

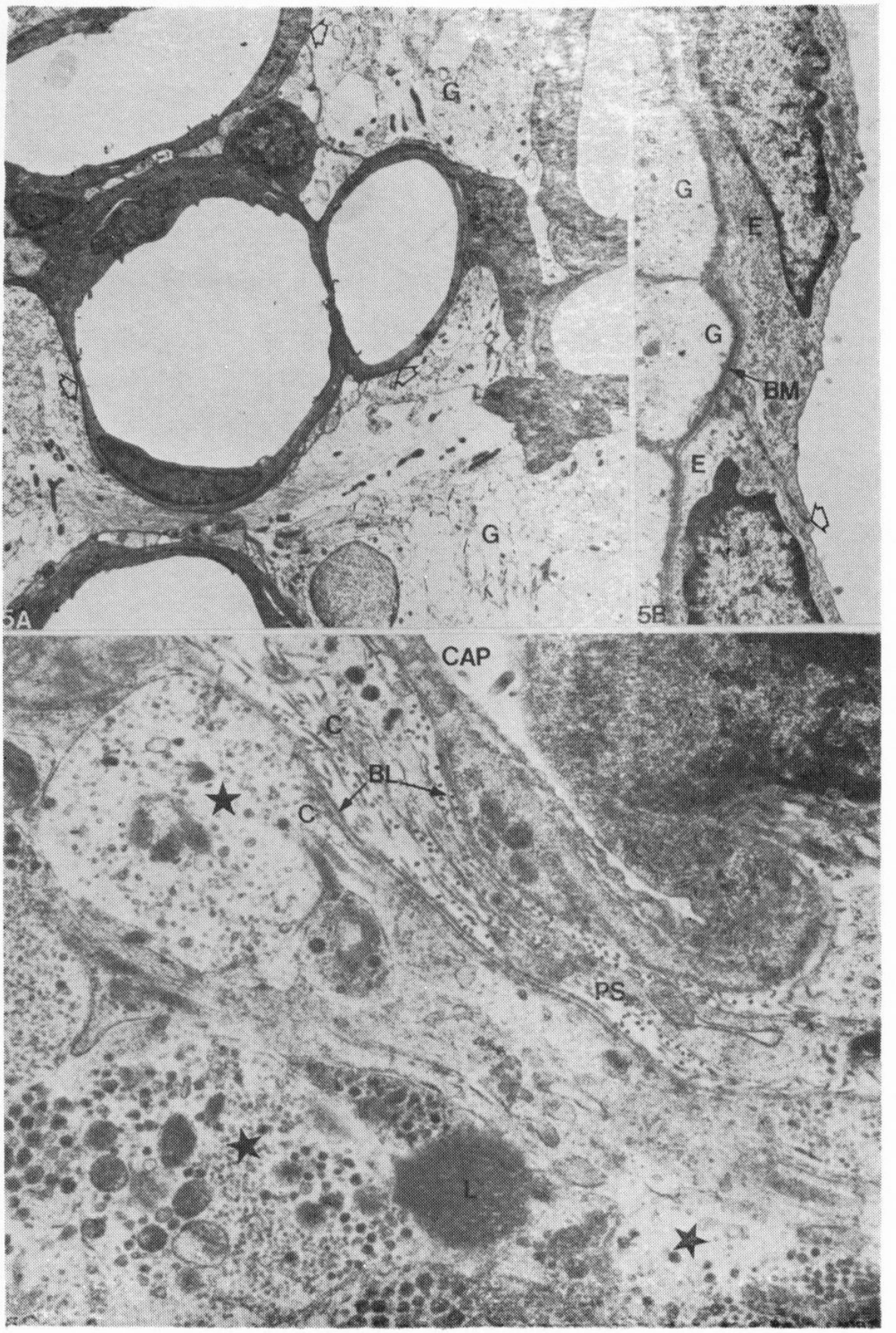

Fig. 5a. — *Low magnification transmission electron microgram of a cluster of blood vessels (arrows) that have arisen from the median eminence of the host brain and have penetrated the ventral region of an adjacent neural graft (G). The host rat was killed 20 days following*

the host and the dorsally positioned neural graft. Upon occasion, perivascular spaces were observed to surround nonfenestrated capillaries within the parenchyma of neural grafts (Figure 7b). This unique vascular organization has not been demonstrated in earlier reports from this or other laboratories. The anatomical reorganization of fenestrated capillaries derived from the hypophyseal portal bed of the median eminence into non-fenestrated vessels was a consistent finding in all grafted DI host and hypophysectomized rats. In addition, intrinsic neurites from neuronal pools within grafts were never observed to grow into the subjacent median eminence of DI host rats.

Employing antisera against tyrosine hydroxylase (TH) and argine vaspressin (AVP), immunocytochemical techniques revealed the presence of large numbers of catecholaminergic neurons in contrast to relatively small populations of AVP positive neurons in viable grafts (Figures 3a, b and 4). Alternate sections used for transmission electron microscopy (TEM) demonstrated that surviving magnocellular neurons (Figure 7a), although few in number, harbored a significant array of organelles and inclusions that characterized active protein syntheses and an apparent heightened metabolic activity. In addition, these neurons exhibited numerous axosomatic and axodendritic synapses (Figure 7a). Catecholaminergic neurons appeared smaller but the frequency of synaptic contact was as great as with the large magnocellular variety. Commonly the neurites of both types of neurons were anatomically juxtaposed to the blood vessels of the graft (Figures 3a, b, and 4). Graft neurons were often seen in close anatomical proximity to intrinsic unfenestrated vessels which occasionally exhibited large distinct perivascular space (Figure 7b).

DISCUSSION

The DI rat has become a useful model in assesing fine structural correlates that may characterize pathophysiological mechanisms that impact upon the mammalian neurohypophyseal system (Marciano et al., 1985a, 1985b; Scott and Sherman, 1984). This laboratory was the first to describe the ultrastructural correlates of this model system over twenty years ago (Scott, 1968). The present investigation focuses upon the ultrastructural correlates of recovery of neuroendocrine function, neurovascular reorganization and neuronal plasticity. A wealth of new literature has emerged concerning the transplantation of various homologous and heterolygous neuroendocrine tissues into the mammalian central nervous system (Brightman et al., 1985; Gash and Sladek, 1980; Gash and Scott, 1980; Kruger et al., 1982; 1985; Marciano et al., 1985b; Scott, et al., 1982; Scott, D. E., 1984). Until recently, the mammalian central nervous system was conceptualized as an immunologically privileged site (Kreiger, et al., 1982). However, recent observations (Scott and Sherman, 1982) have suggested that despi-

stereotaxic implantation of the graft. Vessels generally arising from the underlying median eminence are portal in origin and for the most part are fenestrated in appearance and lack the barrier properties of bona fide cerebral capillaries. However, commonly, these penetrating vessels will. upon distalward growth into graft parenchyma, alter their phenotype and become unfenestrated in their ultrastructural appearance. Since growth of vessels into graft parenchyma may involve the fusion of extrinsic vessels from the host with intrinsic endothelial primordia of the graft, the possibility exists for the fusion of phenotypically different tissue types and hence a reason for altered endothelial expression. X11.000.

Fig. 5b. — *The endothelial cells (E) that constitute the walls of deep penetrating capillaries in fetal neurografts exhibit apparent tight junctions (arrows) and thick basement membranes (BM). Glial end-feet (G) terminate upon this basement mtmbrane in a fashion similar to normal continuous cerebral capillaries. X26.000. 5c. Transmission electron microgram of a neurovascular (neurohemal) zone at the interface between graft and host. Here a fenestrated vessel (Cap) witht a distinct perivascular space (PS) demarcated by luminal as well as abluminal basal laminae (BL) can be observed. Axon terminals (asterisks) with numerous dense core vesicles and lucent microvesicles can be seen to terminate upon or in close proximity to these perivascular spaces. Fenestrated capillaries and perivascular spaces are restricted to the interface, entry points, and the ventral regions of adjacent neural grafts. C. collagen, L. lipid; X21.800.*

te rigid controls and aseptic conditions, cross-strain transplantation often triggers apparent cell mediated immune responses. These responses are characterized by an acute inflammatory reaction involving the active invasion of neural grafts by histiocytes coupled with lymphocytic-fibroblastic infiltration and the deposition of collagen at the interface between the neural graft and the adjacent parenchyma of the host. Hence, the concept of immune privilege should be acknowledged but must be measured in degrees rather than in absolute terms. Over prolonged periods of time following neural grafting, antigens can clearly leak into the systemic circulation of the host. Once the immune system of the host "sees" these antigenic proteins, the brain will respond vigorously and rejection will ensue.

The rapid vascularization of fetal grafts is a well established phenomenon and underscores the importance of angiogenesis factors synthesized and sequestered in transplanted fetal tissue. One such factor has been recently synthesized and characterized as a small protein, angiogenin *Folkman, 1982, Feh, et al., 1985). The process of early neurovascularization of the fetal grafts is complex and may involve the invasion and fusion of extrinsic invading mesenchymal elements from the host with endothelial primordia of the fetal donor tissue which are phenotypically quite different and diverse. To date, little is known about the mechanisms underlying these events. However, recent investigations have established that the type of ground substance, the so called biomolecular matrix, upon which vascular primordia grow and proliferate, have much to do with their behavioral characteristics and properties of growth and development (Milici, et al., 1985). The molecular patterning of ground substances, whether they are mucopolysaccharides, proteoglycans or simple proteins such as laminin or fibronectin, may function as the template for the basic expression of primordial endothelial growth during neurovascularization of fetal hypothalamic transplants. Evidence is also mounting that glial cells, especially astrocytes, may also be involved in this inductive process. However, above and beyond crude guidance mechanisms, their precise participation in this complex process is not yet understood (Brightman et al., 1985; Paull and Scott, 1975). Recent observations by Jansen and Raff (1987) have demonstrated that astrocytes can induce phenotypic alteration in endothelial cells *in vivo*. They have shown that endothelial cells will form putative tight junctions (zonulae occudentes) and become "non-leaky" in the presence of astrocyte aggregates in the anterior chamber of the eye. However, endothelial cells that proliferated in the presence of meningeal cell aggregates will remain "leaky" to the movement of protein dyes and do not exhibit blood-brain barrier properties *in vivo*. By confronting the issue of how blood vessels are formed in neural transplants, we can begin to address fundamental questions such as, what criteria dictate their patterns of growth during normal development? what mechanisms may serve to inhibit further uncontrolled growth that might be injurious to the host?, and what are the fine structural criteria that characterize successful recovery of function in DI hosts following neural grafting versus the failure to reestablish normal physiological parameters of urine concentration and drinking behavior?

Primary neurovascular events that occur within the first few hours following neural transplantation provide an opportunity to focus upon mechanisms of growth and survival of neural grafts in a variety of CNS regions. Recently it has been demonstrated by Redmond et al. (1985) that in African green monkeys rendered Parkinsonian by the injection of MPTP (methylphenyltetrahydropyridine), there is a remarkable remission in rigidity and tremor which characterizes this syndrome following the stereotaxic placement of normal fetal nigral neurons into the corpora striata of recipient hosts. Subsequent elegant follow-up investigations of this phenomenon by Sladek et al. (1987) have described the cell biological correlates of recovery at the light microscopic level. Employing antisera against tyrosine hydroxylase (for dopaminergic neurons), they were able to demonstrate extensive neuritic outgrowth of fibers from the intrinsic fetal neurons of grafts into the surrounding neuropil of the host. Of equal

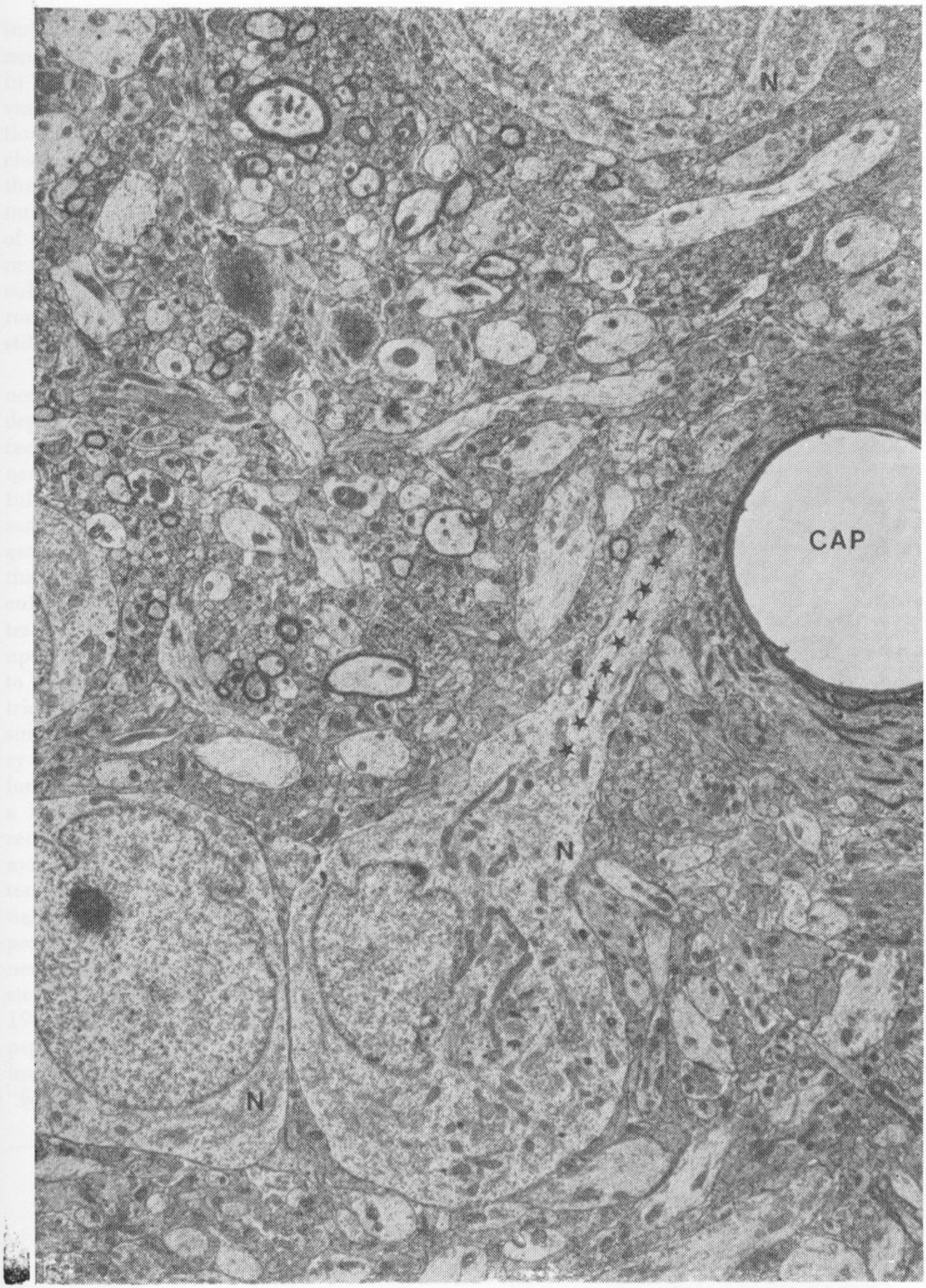

Fig. 6. — *Low magnification TEM of central region of a neural graft in a DI host rat killed 30 days after stereotaxic placement. Several neurons (N) are evident, one of which exhibits a neurite (*) which is anatomically juxtaposed to an unfenestrated capillary (CAP). Compare with figure 5c. X9.500.*

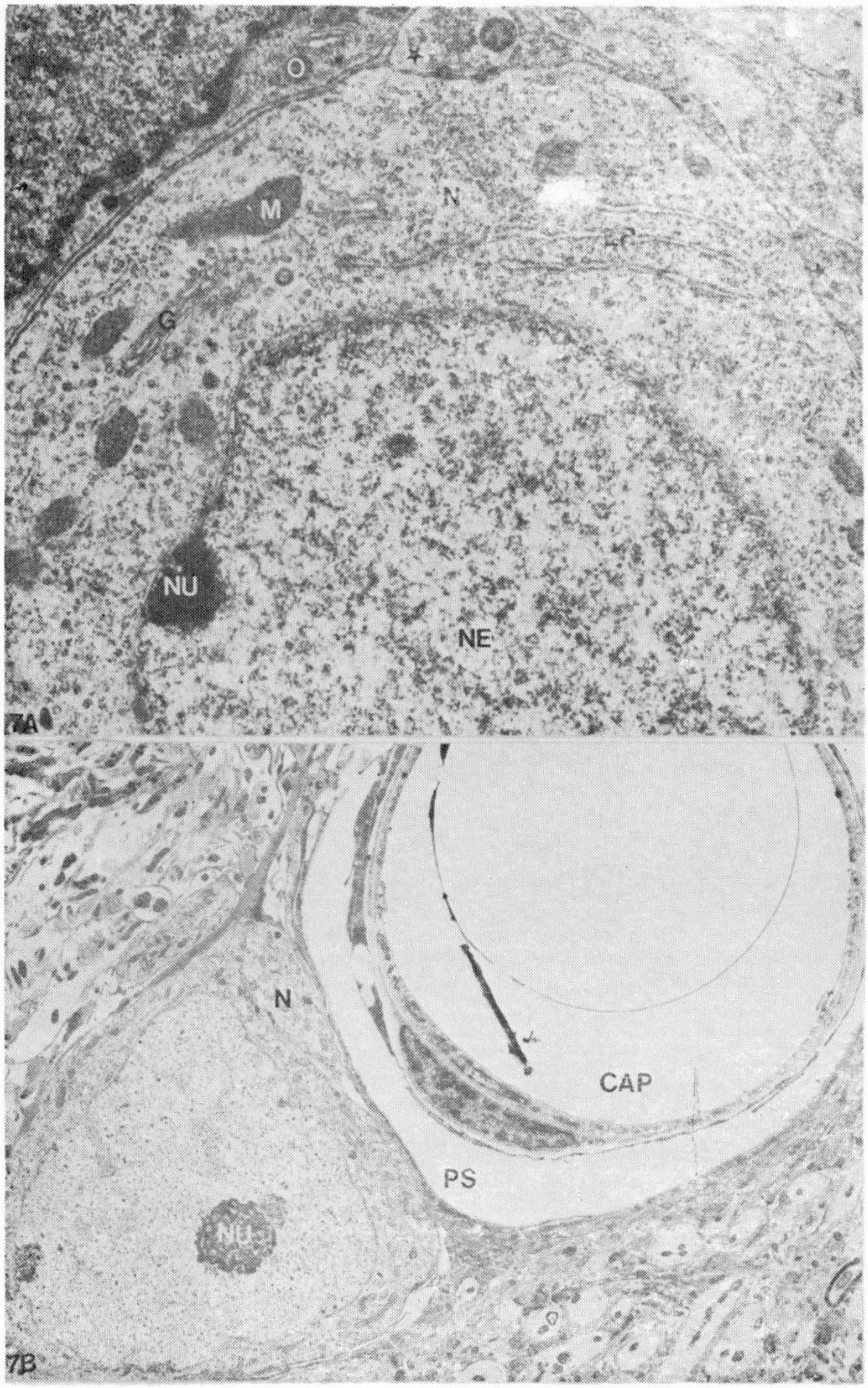

Fig. 7a. — *Mid-range TEM of neuron (N) and a satellite oligodendrocyte (O) in a graft transplanted into the third cerebral ventricle of a DI host rat for 30 days. No-*

importance was their observation that these neural grafts of fetal dopaminergic neurons in adult monkey host brains were richly vascularized. The implications and applications of these current investigations seem clear and may serve as practical approaches thay may be of future clinical value to human patients who suffer with a variety of congenital, post-traumatic or degenerative neuropathies. However, the complex molecular interactions between normal fetal neurons and the surrounding host environment still remain to be elucidated.

The relatively low survival rate for magnocellular neurons in neural grafts may be dependent upon a number of contributing factors. The large size of this type of hybrid nerve cell may mitigate against its successful survival in a dynamic biochemical environment within the graft. This is especially germaine with respect to the time period that it takes for these grafts to become vascularized. During the early phases post-transplantation, graft survival is predicated upon circulating levels of cerebrospinal fluid to provide adequate oxygenation and nutrients. A further contributing factor to low survivability of magnocellular neurosecretory neurons above and beyond competition forblood supply by other cell pools, may be a lack of adequate catecholaminergic afferent input to them. It has been amply demonstrattd that the neuroanatomical patterns of normal development and differentiation of hypothalamic magnocellular components is directly related to catacholaminergic input from other regions of the brain stem and hypothalamus (Sladek, et al., 1984). Furthermore, the central delivery of peptide hormones such as hypothalamic releasing factors, vasopressin, oxytocin, etc. in "bona fide" neurovascular zones of the neurohypophyseal system is dependent upon the physical presence of adjacent excitatory or inhibitory aminergic neurites that occupy the same neurohemal region (McNeill, et al., 1980). Recent investigations in our laboratory have demonstrated with immunocytochemistry that catecholaminergic afferent fields terminate upon AVP positive neurons of neural grafts at different ages (Scott, et al., 1987). At the ultrastructural level, neurons of the graft exhibit numerous axosomatic and axodendritic synapses. Both these phenomena become evident only after 14 days of growth following transplantation. Despite an apparent catecholamine innervation of magnocellular neurons in grafted animals, coupled with the presence of catecholamineaminergic neurites in and around graft vessels, the critical mass of efferent aminergic input to these areas may be insufficient to effectively influence normal patterns of development, differentiation, and survival of grafted magnocellular neurons and their neurovascular terminals. Hence, the functional repetoire of these hybrid neurons may be altered with respect to their neurosecretory capabilities. Despite this caveat, a significant proportion of grafted DI rats and/or hypophysectomized rats exhibited altered drinking behavior and urine concentration. The questions that remain unanswered at this juncture are 1) what are the critical mass of magnocellular (AVP positive) neurons that are necessary to orchestrate changes in the physiological status and responsiveness of these experimental animals? 2) what are the neuroanatomical interactions that transpire between grafted magnocellular neurons and their afferent catecholamine aminergic input?

Recent preliminary clinical approaches have employed the use of adrenal medulla-

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teable here axosomatic synapses (*) filled with dense core vesicles and microvesicles. It is noteable that synaptic patterning is not observed until approximately two weeks after neural grafting. E. R., rough endoplasmic reticulum; G., Golgi cisterns; M., mitochondria; N. E., nucleus; N. U. nucleolus. X2300. 7b. Low magnification TEM of central region of a normal hypothalamic graft 30 days following stereotaxic transplantation into the third cerebral ventricle of a DI host rat. A neuron (N) can be observed in close physical proximity to a distinct pervascular space (PS) surrounding an apparently unfenestrated continuous capillary (cap). Direct termination of peptidergic neurons in fetal hypothalamic grafts upon bona fide perivascular spaces may represent a mechanism for the direct secretion and transport of centrally acting neural hormones in grafted brains. NU, nucleolus. X9800.

Table 1

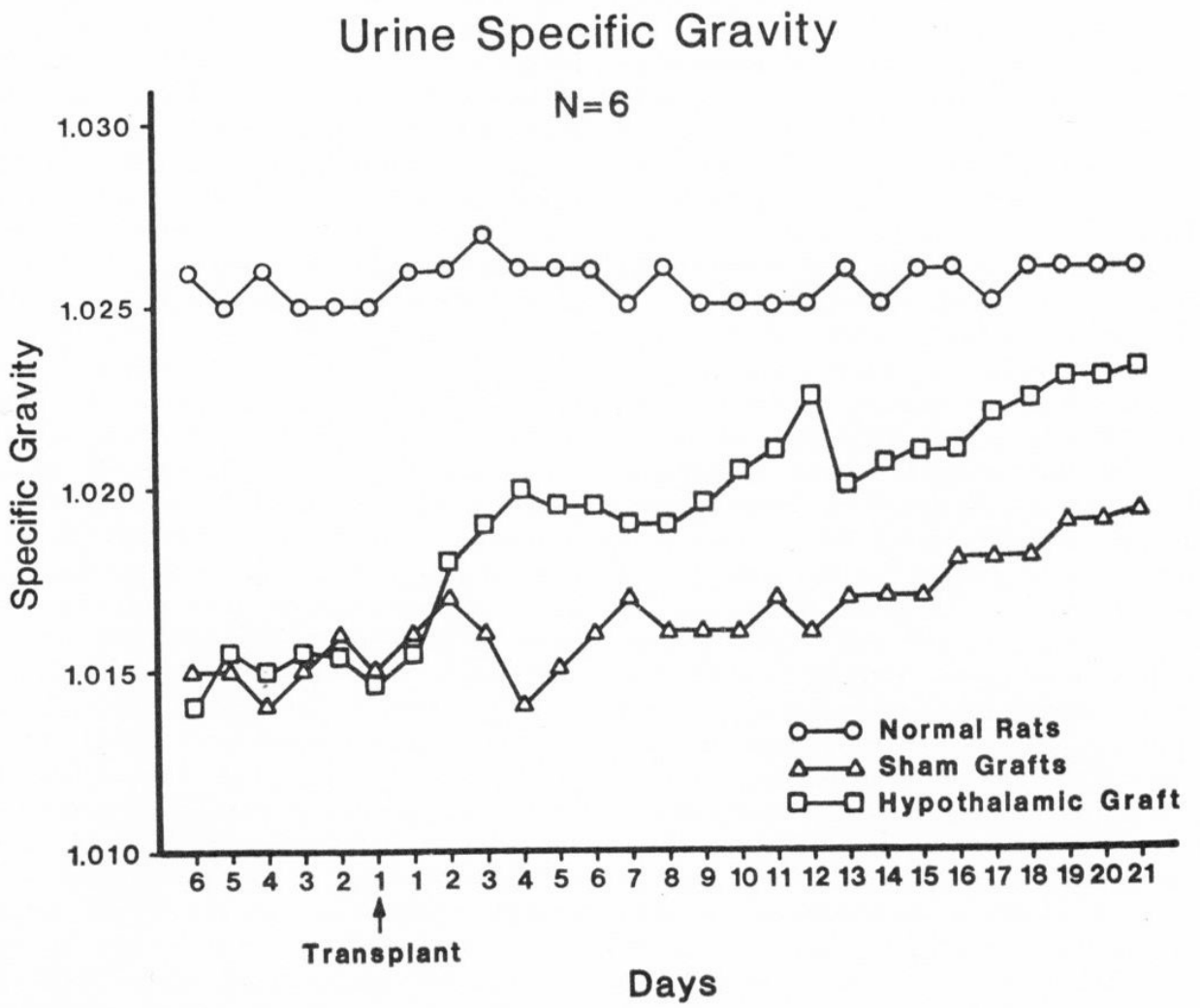

Table 1. — *This table demonstrates that in a selected population of recipient host rats that there is a significant increase in urine specific gravity commencing two days following stereotaxic placement of the fetal neurograft. Despite an improvement in the capacity to produce a more concentrated urine, these parameters never fully achieved normal control valves.*

ry transplantation techniques in human patients with Parkinsons disease. Using a technology first reported upon by Backlund et al. (1985), a number of neurosurgical groups have reported variable results following the stereotaxic placement of adrenal medullary autografts into the caudate nucleus of Parkinsonian patients (Allen, et al., 1987; Jiao, et al., 1987; Drucken-Colin, et al., 1987). In a number of cases these investigations have reported amelioration of many of the constellation of symptons and signs of Parkinsonism. However, little data is yet available regarding the basic anatomy of graft-host interactions, which is the necessary correlate of a successful clinical course of recovery. To date, there is still a significant lack of information dealing with the fine structural and molecular biological correlates of interaction between neural grafts and surrounding host parenchyma. Furthermore, little is understood concerning the biochemical basis of neuronal connectivity between cellular elements of the donor transplant and the host. Growth promoting factors that orchestrate

patterns of graft proliferation as well as intrinsic synaptogenesis within neural grafts are simply not well understood. Coupled with this there is a paucity of data dealing with neurovascularization of grafts, which is the most critical and primary factor in survival and ultimate recovery of function. Finally, the long term impact on graft survival and the immunological consequences of graft aging are yet to be defined.

Table 2

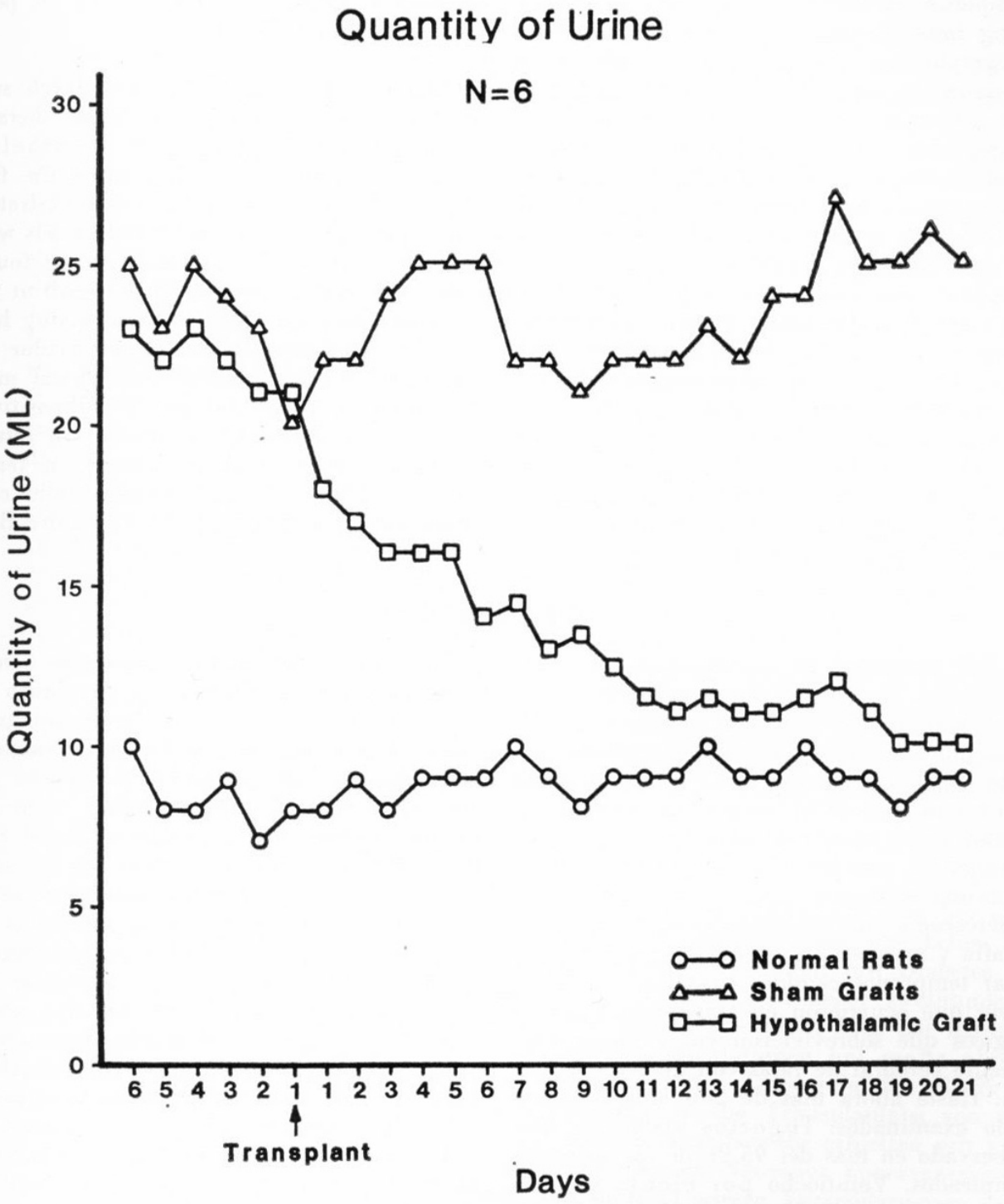

Table 2. — *Within 24 hours following neural grafting with fragments of normal fetal anterior hypothalamus, urine output fell and by 20 days post surgery it approached normal limits.*

SUMMARY

This program of research has focused upon the concepts and mechanisms of recovery of function following the stereotaxic transplantation of normal fetal hypothalamic fragments into the third cerebral ventricle of Brattleboro rats that suffer with congenital autosomal homozygous diabetes insipidus. A series of experiments employing immunoelectron microscopy, microangiography, and electron microscopy were designed to assess early vascular and inductive changes that occurred in neurological transplants that survived in the third cerebral ventricle of DI host rats. To date over 200 animals have been examined. Viable heterografts have been found in over 95 % of host animals. Twenty-eight percent of the host population receiving normal fetal explants that harbored vasopressinergic (supraoptic paraventricular) neurons exhibited changes in the physiological parameters of urine osmolarity and water consumption. Immuno-electron microscopy has revealed that relatively small populations of AVP-positive neurons (the so-called magnocellular variety) survive in heterografts. The most dominant neuronal population as observed with immunocytochemistry are catecholeaminergic neurons that are seen to richly innervate surviving the vasopressinergic variety. Surviving fetal neural grafts are opportunistic in developing a blood supply which can arise from underlying fenestrated vessels of the median eminence or from uniformly unfenestrated vessels of the periventricular neuropil.

The results of the current research support the concept that graft tissue dictates the phenotypic expression of endothelial cells inhabiting the graft. Despite the fact that most graft vessels are unfenestrated, small populations of fenestrated vessels with large perivascular spaces can be found along the ventral portion of the graft at the interface between graft and underlying host median eminence. These neurovascular zones coupled with a small but critical mass of surviving peptidergic (AVP-positive) neurons are sufficient to promote a return of normal functional parameters in terms of urine osmolarity and reduced water consumption. Supported by NSF Grant BNS 8709687.

RESUMEN

Este programa de investigación ha tenido en cuenta los conceptos y mecanismos de recuperación funcional, a continuación del transplante estereotáxico de fragmentos fetales hipotalámico normales, en el tercer ventrículo cerebral normal de ratas Brattlesboro que sufren de diabetes insípida homosigótica autosomal congénita. Se realizaron una series de experimentos empleando microscopía inmunoelectrónica, microangiografía y microscopía electrónica para evaluar tempranos cambios vasculares e inductivos que ocurrieron en transplantes neurológicos que sobrevivieron en el tercer ventrículo cerebral de ratas con diabetes insípida. Hasta ahora más de 200 animales han sido examinados. Ingertos viables se han observado en más del 95 % de los animales empleados. Veintiocho por ciento de esta población que recibieron implantes fetales normales que albergaron neuronas vasopresinérgicas (paraventricular supróoptica) exivieron cambios en los parámetros fisiológicos de orina osmolaridad y consumo de agua, microscopía inmunoelectrónica puso en evidencia que poblaciones relativamente pequeñas de neuronas AVP-positivos (la llamada variedad magnocelular) sobrevive en los injertos. La población neuronal más dominante tal como observado con inmunocitoquímica, son neuronas catecolaminérgicas que son vistas ricamente inervadas sobrevivientes la variedad vasopresinérgica. Injertos sobrevivientes fetales neurales tienen oportunidad en desarrollar una provisión de la sangre la cual puede originarse de vasos subyacentes abiertos de la eminencia mediana o de vasos uniformemente imperforados de la red nerviosa periventricular.

Los resultados de la investigación corriente soportan el concepto que tejido insertado dieta la expresión fenotípica de células endoteliales existentes en el injerto. A pesar del hecho de que la mayoría de los vasos son

imperforados, pequeñas poblaciones de vasos abiertos con amplios espacios perivasculares pueden ser hallados junto con la porción ventral del injerto en el espacio entre injerto y eminencia mediana subyacente del receptor. Estas zonas neurovasculares acoplados con una pequeña pero crítica masa de neuronas sobrevivientes peptidérgicas, son suficientes para promover una vuelta de parametros funcionales normales en términos de orina osmolaridad y reducido consumo de agua.

RÉSUMÉ

Ce program d'investigation a pris en consideration des concepts et mécanisme du des changements des paramétres physiologi-recuperation fonctionelle posterieur à un transplant stereotaxique de fragments d'hypotalamus fétal normal, dans le 3e ventricule normal de rats Brattleshose qui souffre de diabete insipide homozygodique autosomal congenital. Il a été réalizé une serie d'expériences en employant la microscopie inmunoelectronique, microangiographie el la microscopie électronique pour évaluer de très home heurr des changments vasculaires et inductifs qui se produisent au niveau des transplants neurologiques qui survivent dans le 3e ventricule cérébral de rats diabétiques. Jusque a maintenant plus de 200 animaux ont été éxaminés. Des implants viahles on éte observés dans le 95 % des animaux employés de 28 % qui reçurent des implants de foetus normaux qui portaitent des neurones vasopresinquergiques (para ventriculaires suprooptiques) out montré des changements des paramétres physioloqiques de l'urine, l'osmopolarité et consomation d'eau. La microscopie immunoelectronique a montré que des quantités relativement restreintes de neurones AVP + (appelée variété magnocelulaire) survivent dans les implants. Les ensambles dominants de neurones observé comme tels avec des immunocitochimiques, sont des neurones catecolaminergiques richement inervées, survivant seulement la variété vasopresinengique.

Les implants ont la possibilité de développer un aprovisionement de sang laquel peu provenir de vaissaux subyacents "ouvert" de l'eminence mayenne on de vaisseaux imperferés des tissues nerveux periventriculaire.

Des résultats de l'investigation courante provien le concept que des tissus implantés provien l'expression fenotipique des célules endoleliales existents de la implant. Malgrés que la mayorité des vaisseaux sont imperforés une certaine de vaisseau "ouverts" avec de grands capaces périvasculaires pouvent etre tronvés sur la portion ventrole de l'implant.

Ces zones neurovasculaires acouplés a une masse critique du neurones peptidergiques sont suffisantes pour modifier les paramétres fonctionels normaux in terme d'urine, osmoralité et pouvre consomation d'eau.

ZUSAMMENFASSUNG

Bei diesen Forschungsprogramm wurden in Betracht gezogen die Konzepte und Mechanismen der funktionellen Wiederherstellung nach der stereotaktischen Ueberpflanzung von foetalen Fragmenten von normalem Hypothalamus in den Dritten normal en Hirnventrikel von Ratten Brettlesboro, die an angdborenem Diabetes insipidus autosomal homozigot leiden. Es wurden eine Reihe von Experimenten durchgefuehrt, wobei man benutzte die immunoelektronisch Mikroskopie, Mikroangiographie und elektronische Mikroskopie, um, auszuwerten die fruehzeitigen vaskulaeren und induktiven Veraenderungen, die sich bei neurologischen Ueberílanzungen ereigneten, die bei ueberkebenden Ratten mit Diabetes Insipidus. Bis jetzt sind ueber zweihundert Ratten geprueft worden. Man hat lebensfaehige Transplantationen bei mehr als 95 % der operierten Tiere gefunden. 28 % dieser Population, die Transplantabe von normalem Foetalen Gewebe erhielten mit vasopresinergikehen Neuronen (paraventricular supraoptica) wiesen Veraenderungen der physiclogischen Parameter der Urin-Csmolaritaet und des Wasserkonsums auf. Die Immunoelektoonische Mikroskopie zeigte, dass

relativ geringe Populationen von AVP-positiven Neuronen (die sogenannte magnozellulaere Varietaet) bei Heterotransplanten ueberleben. Die am meisten dominierende neuronale Population, die mittels der Immunozytochemischen Methode studiert wurde, sind katecholaminoergische Neuronen, wobei die ueberlebende vasopresinergische Varietaet reichlich innerviert war.

Die ueberlebenden foetalen neuralen Transplantate haben die Gelegenheit die Blitversorgung zu entwickeln, die von den unterliegenden perforierten Gefaessen der eminencia media oder von den gleichfoermig nicht-perforierten Gefaessen des neuronalen periventri culaeren Natzes stammt.

Die Ergdbnisse der laufenden Studien bestaetigen die Annahme, das die ueberpelanzten Gewebe den phaenotypischen Ausdmuck der endothelialen Zellen, die das Transplantat beherfergt diktieren. Trotz der Tatsache dass die meisten ueberpflanzten Gefaesse nicht perforiert sind, kann man kleine Populationen von perforierten Gefaessen mit weiten perivasculaeren Raeumen in der ventralen Portion des Transplantates, im Zwischenraum zwischen Transplantatund der darunterliegenden Eminencia media des Empfaengers finden. Diese Neurovasculaeren Zonen zusammen mit einer kleinen aber kritischen Masse von ueberlebenden peptidergischen (AVP-Positiv) Neuronen sind genug, um die Wiederherstellung normeler funktioneller Parameter bezueglich der Urinosmolaritaet und dem verminderten Wasserkunsum, zu foerdern.

REFERENCES

1. *Allen G., Burns R. S., Tulipan N. B.,* (1987): Human adrenal autografts as potential therapy for Parkinsons disease IN transplantation into the mammalian CNS Proc. 2nd Schmitt Neurological Sciences Symposium. JR Sladek Jr. and DM Gash. Eds. Elsevier IN Press.
2. *Backlund E. O., Gronberg P. O., Homburger B., Sedvall G., Serger* and *Olsen L.,* (1985): Transplantation of adrenal medullary tissue to situation in Parkinsonism. IN. Neural grafting in the mammalian CNS. A Bjorklund and U Steneui, eds., Elseiver. 551-558, 1985.
3. *Brightman M., Markey, Klein D.,* (1985): Co-transplants of pineal and superior-cervical ganglion of the IV ventricle. In Neural Grafting in the Mammalian CNS. A Bjorklund and U Stenevi, eds., Elsevier, 235-242.
4. *Drucker-Colin R., Madrazo I., Shkurovich M., Ostiosky-Solis F.,* and *Tones C.,* (1987): Open microsurgical autograft of adrenal medulla to caudate nucleus of patients with Parkinsons disease. IN Transplantation into the mammalion CNS. JR Sladek, Jr. and DM Gash, eds., Proc. 2nd Schmitt Neurological Sciences Symposium. Elsevier IN Press.
5. *Dunn E. H.,* (1971): Primary and secondary findings in a series of attempts to transplant cerebral cortex in the albino rat. J. Comp. Neurol. 27, 575.
6. *Feh J., Strydom R. M., Lobb D. J., Alderman R. R., Rividan J. L.,* and *Vallee B. L.,* (1985): Isolation and characterization of angiogenin. An angiogenic protein from human carcinoma cells. Biochem, 5480-5499.
7. *Folkman J.,* (1982): Angiogensis: Initiation and Control. Ann NY Acad Sci, 401: 212-227.
8. *Gash D. M., Sladek J. R., Jr.* (1980): Vasopressin neurons grafted into Brattleboro rats: Viability and activity. Peptides, 1: 11-14.
9. *Gash D. M., Scott D. E.,* (1980): Fetal hypothalamic transplants in the third cerebral ventricle of the adult rat brain. Cell Tiss. Res., 211: 191-206.
10. *Hofer H.,* (1158): Zur Morphologie der Circumventricularen organe des Zwiscenhirns der Saugetiere. Vorh, Deutch, Zool Ges. Frankfort 202-251.
11. *Jansen R. C.,* and *Raff M. C.,* (1987): Astrocytes induce blood brain barrier properties in endothelial cells. Nature, 325: 253-257.
12. *Joseph S.A.,* and *Piekut D. T.,* (1986): Dual immunostaining procedure demonstrating neurotransmitter and neuropeptide codistribution in same brain secion. Amer. J. Anatomy 175, 331-342.
13. *Kreiger D., Gibson M. J., Perlow M. J., Davies T., Perlow M., Ferin M., Charlton H. M.,* (1985): Correction of genetic gonadotropin-releasing (GNRH) deficiency by grafts of fetal preoptic area (POA) tissue. In transplantation in the Mammalian CNS. A Bjorklund and U Stenevi, eds., Elsevier.
14. *Kreiger D. T., Perlow M. J., Davies T. F., Zimmerman E. A., Ferin M., Charlton H. M.* (1982): Brain grafts reverse hypogonadism of gonadotropic releasing hormone deficiency. Nature, 298: 468-471.
15. *Marciano F. F., Gash D. M., Wiegand S. J.,*

(1985): Neuroanatomical, neurovascular and functional relationships of transplanted vasopressin neurons, Proc. Soc. Neuroscience, 606.

16. *Marciano F., Sladek J. R., Jr., Gash D. M.* (1985): Transplanted vasopressin neurons: Structural and functional correlates. In Transplantation in the Mammalian CNS. A Bjorklund and U Stenevi, eds., Elsevier, 617-628.

17. *McNeill T. H., Scott D. E.* and *Sladek J. R. Jr.* (1980): Simultaneous monoamine histofluorescence and neuropeptide immunocytochemistry: I Localization of catecholamines and gonadotropin-releasing hormone in the rat median eminence. Peptides 1: 59-68.

18. *Milici A. J., Furie M. B., Carley W. W.*, (1985): The formation of fenestrations and channels by capillary endothelium in vitro. Proc. Nat. Acad. Sci., 82: 6181-6185.

19. *Paull W. K., Scott D. E.*, (1976): Perinatal development of the mammalian neurohypophyseal system. In Sexual Maurity: Phisiological and Clinical Parameters. EEE Hafez and J. J. Peluso. Ann Arbor Sci., 3-36.

20. *Redmond D. E., Roth R. H., Elseworth J. D., Sladek J. R., Jr., Collier T. J., Deutch A. Y.*, (1985): Fetal neural grafts in mon keys given methylphenyltetrahydropyridine. Lancet, 1125-1127.

21. *Scott D. E.*, (1968): Fine structural features of the neural lobe of the hypophysis of the rat with homozygous diabetes insipidus Brattleboro strain). Neuroendocrinol 3: 156-176.

22. *Scott D. E.*, (1984): Fetal hypothalamic transplants: Neuronal and neurovascular in. terrelationships. Neuroscience Letters, 51: 93-98.

23. *Scott D. E.*, (1985): Scanning electron microscopy of fetal hypothalamic transplants. Neuroscience Letters, 61: 159-164.

24. *Scott D. E., Gash D. M., Sladek J. R., Jr., et al* (1982): Organization of the mammalian cerebral ventricular system: Ultrastructural correlates of CSF-Neuropeptide secretion in Frontiers of Hormone Research, TJB, Van Wirnersma, Gredanius, Ed., Darger, Basel, 15-35.

25. *Scott D. E., Krobisch-Dudley G., Weindl A., Joynt R. J.*, (1973): An electron microscopic autoradiographic analysis of hypothalamic magnocellular neurons. Zellforsch, 138: 421-438.

26. *Scott D. E., Sherman D.*, (1984): Neuronal and neurovascular integration following transplantation of the fetal hypothalamus into the third cerebral ventricle of Brattleboro rats. Neurological Transplants I. Brain Res. Bull 12: 453-467.

27. *Sladek J. R., Jr.* and *Gash D. M.*, (1984): Neural transplants development and function. Plenum Press, New York.

28. *Sladek J. R., Jr., Collier T. J., Haber S. N., Roth R. H.*, and *Redmond D. E., Jr.*, (1987): Survival and growth of fetal catecholamine neurons transplanted into primate brain. Brain Res. Bull 17: 908-918.

29. *Sladek J. R., Jr., Fields J., Phelps C.*, and *Khachaturian H.*, (1984): Development of catecholamine innervation of the supraoptic nucleus in the Brattleboro rat. Peptides VI, Supp. I. 151-155.

30. *Stenevi U., Bjorklund A., Kromer L. F., Paden C. M., Gerlach J. L., McEwen B. S., Silverman A. J.*, (1980): Differentiation of embryonic hypothalamic transplants cultured on the choroidal pia of the brains of adult rats. Cell Tiss Res., 205: 217-228.

31. *Jiao S. L., Zhong W. G., Ding M. C.*, and *Sun J. B.*, (1987): The clinical study of adrenal medullary tissue transplantation to striatum in Parkinsonism. IN. Transplantation into the mammalian CNS. JR Sladek Jr. and DM Gash, eds., Proc. 2nd Schmitt Neurological Sciences Symposium, Elsevier, IN Press.

32. *Scott D. E.*: Unpublished data.

The Neurobiology of Non-fetal Implants into the Dopamine-deprived Neostriatum, with Special Reference to Sympathetic Ganglia

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ABBREVIATIONS

A	Adrenaline, epinephrine
BBB	Blood-brain barrier
CNS	Central nervous system
CP	Caudate-putamen
CSF	Cerebrospinal fluid
DA	Dopamine
DBH	Dopamine beta hydroxylase
DOPAC	Dihydroxyphenylacetic acid
ENK	Enkephaline
5-HIAA	5-Hydroxyindoleacetic acid
5-HT	5-hydroxytriptamine, serotonin
HIS	Histamine
HRP	Horseradish peroxidase
HVA	Homovanillic acid
MHPG	Metoxy-hydroxyphenyglycol
MOPEG	Methoxy-hydroxyphenylethylene glycol
MPTP	Methyl-phenyl-tetrahydropyridine
NA	Noradrenaline, norepinephrine
NGF	Nerve growth factor
NPY	Neuropeptide Y
PD	Parkinson's Disease
PNMT	Phenylethanolamine-N-methyl transferase
6-OHDA	6-hydroxydopamine
SCG	Superior cervical ganglion
SP	Substance P
SS	Somatostatin
TH	Tyrosine hydroxylase
VIP	Vasoactive intestinal polypeptide

The many attempts to ameliorate the behavioral alterations resulting from deficits in the dopaminergic innervation of the neostriatum by means of brain implants have led to the conclusion that the most promising source is the embryonic or fetal substantia nigra (for review see Lindvall, 1989). The advantages of this material include the experimentally obtained improvement in spontaneous behavior as opposed to just the reduction of deficits evoked by special tests, the long term survival of the grafts, and the synaptic contacts established between grafted cells and host neurons. The application of this approach to PD patients, however, is fraught with numerous ethical and legal considerations derived from the intended use of human fetal donor material.

As a consequence of these concerns, the U.S. Department of Health and Human Services instituted a moratorium, presently in its third year, on the Department funded research "utilizing human fetal tissue, obtained from induced abortions, for therapeutic transplantations" (for review of these issues see Hellerstein, 1988). The need for alternative sources and/or methodologies have become imperative. The present survey scrutinizes the various possibilities tested to date with particular emphasis on the use of sympathetic ganglia tissue. In the past, the research has focused on the applicability of other neural crest derivatives, certain cell lines maintained in culture, chronic delivery systems, and very early attempts at genetic engineering. These approaches will be reviewed first.

IMPLANTS OF ADRENAL MEDULLARY TISSUE

NON-PRIMATE EXPERIMENTS

Most of the studies use the rat with unilateral destruction of the dopaminergic nigrostriatal system by 6-OHDA as the recipient of rat adrenal medulla, starting with the original demonstration that fragments of such a tissue grafted into the lateral ventricle significantly decrease the apomorphine-induced rotations in these preparations (Freed et al., 1981). The rationale for these experiments is provided by the capacity of chromaffin cells to change their phenotype in the absence of cortical adrenal tissue and become neuron-like. A further assumption is that these transformed cells produce the dopamine needed to reverse the effect of striatal denervation and reconstruct the synaptic circuitry by integration with the host. At least four variables and three outcomes can be culled from these studies. The variables include the site of the graft: either intraventricular (i.v.) or intraparenchymal (i. p.); the age of the donor: newborn, young or old; the type of graft: solid fragments or cell suspensions; and the local treatment with neurotrophic factors. The outcomes examined are the reduction in drug-induced rotations, biochemical changes, and the morphology of the grafts after various survival periods.

Regarding the behavioral improvement, it is apparent that the best results are reported with i.v. solid grafts derived from young rats. Thus apomorphine-induced rotations are decreased by 40 %-50 % and amphetamine-evoked rotations by 27 % (Freed et al., 1981; Becker and Freed, 1986; Pezzoli et al., 1988). In contrast, similar grafts within the CP give either a reduction of only 30 % in postapomorphine rotations for 3 months, the improvement no longer being present at 6 months (Strömberg et al., 1985), or no significant effects at all (Freed et al., 1986; Pezzoli et al., 1988). It is of interest that spontaneous contralateral rotations occur after adrenal medullary implants, with maximum effect at about 2 minutes, and continuing for 4-5 additional hours (Herrera-Marschitz et al., 1984). This behavior is "dose dependent", being stronger when more tissue is implanted. In addition, the effect is most marked with central placement within the CP as opposed to rostral or caudal locations, and is blocked by DA receptor antagonists. Only partial successes are reported when using cell suspensions instead of solid grafts, with decreases of 60 % of postamphetamine rotations after one month (Bing et al., 1988), and positive results in only one third of the cases (Nishino et al., 1988). Finally, no significant changes occur when the tissue derives from old rats, even in i.v. implants (Freed, 1983; Pezzoli, 1988).

The morphology supports only partially the hypotheses behind the original rationale. Observations with histofluorescence techniques reveal that in i.v. grafts of young material, there are surviving chromaffin cells, only 25 % of which emit processes which remain within the graft, and a halo of fluorescence in the neighboring striatum (Freed et al., 1981, 1983; Freed, 1983). However, no TH-positive cells are seen at 11 weeks (Pezzoli, 1988). More information is available on i.p. solid grafts. The number of fluorescent cells and the extent of the halo decrease dramatically from 2 minutes to 6-7 hours postoperatively (Strömberg et al., 1984). Moreover, only few neuronlike cells with no fiber outgrowth are seen at 2-3 months (Ström-

berg et al., 1985; Freed et al., 1986a), and only 45 % of the implants survive at all by the end of the second month (Korfali et al., 1988). Lastly, no TH-positive cells are found in the graft (Pezzoli et al., 1988). The results are quite different when using cell suspensions instead of solid grafts, but they are also conflicting to some extent. It has been reported that cell suspensions of adrenal medullary tissue implanted into the CP of *normal* rats start sprouting processes as early as one hour after implantation, continuing for at least 4 weeks (Patel-Vaidya et al., 1985). In the 6-OHDA-lesioned model, some authors report only few TH-positive cells present, with immunoreactive fibers apparently derived from the host, and no synapses (Bing et al, 1988), whereas others observe TH-positive neuron-like cells giving off fibers which invade the host (Nishino et al., 1988). The latter study also offers electron microscopic results: the TH-positive cells contain no chromaffin granules and are postsynaptic at their somata and dendrities to TH-negative boutons. In turn, TH-positive terminals synapse with TH-negative somata and dendrites. These findings are suggestive of graft-host integration, and will greatly benefit from independent confirmation.

Biochemical results show a high content of DA in 5-month old i.v. grafts with levels 3-4 times higher than in the normal substantia nigra, and 20 times that of the denervated striatum (Freed et al., 1983), as well as elevated DA in plasma (Becker and Freed, 1986). However, *in vivo* dialysis gives undetectable levels of DA in the CSF with increases in DOPAC (Becker and Freed, 1986a). Solid i.p. grafts initially contain high levels of DA, NA and A but they drop rapidly within 6-7 hours after implantation (Strömberg et al., 1984), and TH activity remains unchanged (Korfali et al., 1988). In the behaviorally and morphologically successful cases of cell suspensions grafts, *in vivo* dialysis shows elevations of 50 % in the levels of DA, DOPAC and HVA with undetectable NA and A (Nishino et al., 1988).

Of particular interest is the effect of neurotrophic factors on graft survival and functioning. Both i.v. and i.p. implants benefit from NGF treatment. The maximal effect occurs with continuous infusion for 4 weeks by way of implanted osmotic minipumps, resulting in early reductions of apomorphine-induced rotations of 70 %, and still of 50 % by one year (Strömberg et al ., 1985; Pezzoli et al., 1988). The effect is less marked with the administration of NGF during the grafting procedure or later in intermittent doses (Strömberg et al., 1985). The degree of behavioral improvement is paralleled by the morphologic findings. Four times as many fluorescent cells are present with than without NGF treatment. Most of them are neuron-like with abundant processes in the case of continuous infusion, and more are of the chromaffin type with little fiber outgrowth after intermittent administration. Fluorescent fibers persist after removal of the SCG, indicating that they are not sproutings from the innervation of blood vessels (Strömberg et al., 1985). Graft survival of 100 % also occurs when coimplanted with fragments of a gelatin sponge previously placed for 10 days within a cavity in the cerebral cortex of young rats, presumably rich in neurotrophic factors. In this experiment, chromaffin cells are observed to emit sprouts but fail to invade the recipient, in spite of the good contact between graft and host, and the presence of newly formed capillaries in both (Korfali et al., 1988). It is noteworthy that NGF treatment does not overcome the negative results of implanted tissue derived from aged donors (Freed, 1983; Pezzoli et al., 1988).

The conclusion from the studies discussed above is that homografts or allografts of adrenal medullary tissue produce DA, which most probably reaches the host through newly formed capillaries connecting the vascular systems of graft and host, that is through an altered BBB. This view is further supported by studies showing that adrenal implants prevent the reconstitution of the BBB which normally heals from surgical trauma in 2-3 weeks. For at least 9 months chromaffin cells from an i.v. implant take up [^{3}H]DA injected intravenously, and similarly administered HRP diffuse into the host tissue from the implant

site (Rosenstein, 1987). In addition, it appears that the graft produces neurotrophic factors which enhance the recovery of DA neurons from the host as shown in a mouse model where the nigrostriatal system is damaged by MPTP treatment (Bohn et al., 1987). Two to 3 weeks after the implantation, there is a dense plexus of TH-positive fibers in the striatum which are interpreted as originating from the host, not from the graft. It should be noted, however, that the neurotrophic effect may derive from trauma to the host. In fact, major reductions in apomorphine-induced rotations are obtained in the rat model after i.v. implantation of adipose tissue or sciatic nerve together with an infusion of NGF in all similar to those seen after adrenal medullary implants. NGF alone gives no such effects (Pezzoli et al., 1988).

NON-HUMAN PRIMATE EXPERIMENTS

The possible application of adrenal medullary implants to PD patients requires preceding studies in non-human primates. These are indeed very limited to date. In one investigation, unilateral destruction of dopaminergic nigral neurons is presumably accomplished by 6-OHDA injected stereotaxically in 4 monkeys (*Macaca mulatta*) (Morihisa et al., 1984). After an unspecified period, and with no reported behavioral observations, autografts are made in the head and body of the ipsilateral caudate nucleus, either sterotaxically or by direct visualization, in which case pieces are also left in the lateral ventricle. Following a 3-8-month period, again with no behavioral results given, histofluorescence shows many more chromaffin cells when the implant is made under direct exposure. Most of these cells are round but some are polygonal and with processes remaining within the graft. These results suggest that the greater surgical trauma to the host involved in the direct visualization method increases the chances for graft survival, probably through the production of neurotrophic factors by the damaged tissue.

Another study involves only one monkey (*Cebus apella*) with a "moderately severe parkinsonian behavior" described as bradykinesia, hyperigidity and stooped posture, produced by chronic protracted MPTP treatment administered intravenously over 8 months (Fiandaca et al., 1988). One month later, an i.p. autograft is stereotaxically driven with a tissue carrier into one caudate head extending into the internal capsule and the globus pallidus. After another month, with no reported behavioral observations, the animal is sacrificed and shows modestly reduced TH immunoreactive cells in the substantia nigra, and fibers in the striatum. There is, however, an enhancement of TH-positive fibers up to 5 mm from the implant site. The graft itself is necrotic, with extensive macrophage infiltrates, also recognized in electron microscopic examinations which in addition reveal only few chromaffin cells, non-fenestrated capillaries, and degenerative changes in the neighboring host neuropil (Hansen et al., 1988b). A significant finding is that a similar TH-positive fiber enhancement is observed in a control animal that received only the carrier with no adrenal tissue. The inescapable conclusion, albeit the very small sample, is that the possible reinnervation derives from the host under the influence of some trophic factor induced by the surgical trauma.

The preceding viewpoint is amply supported by the only other experiment carried out in 4 monkeys (*Macaca mulatta*) rendered hemiparkinsonian by intracarotid injection of MPTP (Bankiewicz et al., 1988). This report contains detailed behavioral testing after MPTP, and again after allografts of adrenal medullary tissue. The implants are made in preformed cavities within the heads of both caudate nuclei. At 3 months, there is significant increase in the use of the arm contralateral to the intracarotid injection, and a 70 % reduction in apomorphine-induced rotations. By 6 months, however, the use of the arm diminishes considerably, but rotations to apomorphine are still 40 % reduced. Immunofluorescent studies in 2 of the animals show no TH-positive cells in the implants, but a streaming of immunoreactive fibers from the ventral striatum toward the implant site. Very significantly, a similar pattern of reinnervation is seen in 3 other MPTP treated mon-

keys that received implants of adrenal *cortex* or fat tissue, or remained with only the preimplant cavity. These animals also show a modest behavioral improvement at 3 months and maintain the same level at 6 months.

Taken together the extensive results with the rat model and the meager information from the monkey model, it appears that adrenal medullary implants into the dopamine-deprived striatum may produce transient recovery of function. The most probable mechanism is a partial reinnervation process from remaining dopaminergic neurons in the recipient under the influence of trophic factors derived from the trauma to the host tissue.

REPORTS ON PARKINSON DISEASE PATIENTS

In spite of the rather discouraging results of animal experiments, approximately 75 parkinsonian patients have been reported to date as receiving autografts of adrenal medullary tissue, in at least 6 neurosurgical units. The number of variables are higher than in the animal studies and include: the age, sex and length of history, the time elapsed between removal of the gland and implanation of medullary tissue, the site and amount of the graft, and the type of surgical procedure. The evaluated outcomes are the changes in the clinical condition, the requirement of antiparkinsonian medication, the length of the follow-up and, in some cases, biochemical and/or pathological descriptions.

The first attempts were made in 1982-1983, in a 55-year old man and a 46-year old woman who were implanted in one or two sites, within the center of the right caudate nucleus head, using a stereotaxic approach, with or without the aid of a spiral metal carrier (Backlund et al., 1985). The amount of tissue grafted and the time elapsed between adrenalectomy and implantation were approximately 30 mm^3 and about 2 hours, respectively. Results were disappointing. Only the woman showed some improvement in motor function of arms and hands lasting for more than 2 weeks, and requiring 20 % less medication than preoperatively. The outcome was similar in two other cases (46 and 63 years old) implanted with similar techniques in the right putamen (Lindvall et al., 1987) although the interval was reduced to one hour. In one of these cases, the transient improvement was more marked on the side contralateral to the implant. In contrast, more significant recoveries were reported in 4 patients (43-57 years old) implanted with the same methodology in the head of the right caudate nucleus, but with somewhat larger amounts, up to 80 mm^3, and shorter intervals of 5-15 minutes (Jiao et al., 1988). Although the postoperative course was fluctuating, there was an overall improvement of 50 % or more in 3 of the cases. All patients, however, required the same medication as before surgery.

Following the initial report of dramatic improvement in two young patients implanted with a different surgical approach (Madrazo et al., 1987a), the majority of the remaining published cases were operated with this same procedure in the attempt to replicate the findings. The original technique consisted in grafting a total of 0.8-1.0 g of adrenal medullary tissue by direct visualization within a cavity made in the ventricular surface of the head of the right caudate nucleus, so that the graft remained in contact with both, the parenchyma and the CSF. The same surgical team reported on additional 20 patients, most of them 33-56-year old men (10 in Madrazo et al., 1987b; additional 10 quoted in Lindvall, 1989), 60 % of whom showed important bilateral reductions in tremor, rigidity, akinesia, as well as in the required medication (two of the patients with no drug treatment at all), for up to 27 months postoperatively. The results were considerably less dramatic in three better documented studies involving 19 (Goetz et al., 1989, 1990; also in Penn et al., 1988), 18 (Allen et al., 1989) and 7 (Kelly et al., 1989) cases, respectively, which were followed for 6-12 months. These cohorts, comprising a total of 36 men and 8 women, ranging in age between 35 and 69 years, showed either a prolongation of the mean "on" time from 48 % to 75 %, and an increase in the "on" time without dyskinesia from 29 % to 59 % (Goetz et

al., 1989, 1990); or a slow improvement in dexterity, stability and speech, and none in rigidity and tremor, in 22 % of the cases, with no effect or even further deterioration in the remaining patients (Allen et al., 1989); or a moderate improvement in the early morning parkinsonian evaluation score in only one of 7 cases (14 %) (Kelly et al., 1989). One to 4 months after surgery, all of these patients required the same doses of antiparkinsonian agents as before the implant, except for 4 who tolerated a reduction in medication of 12 % to 30 % (Allen et al., 1989). It should be noted that even the patients showing the above mentioned improvements remained in the same severity stage of their parkinsonian syndrome.

A general observation in all of these studies was that older patients (> 60 years old) tended to do poorer than younger cases. The mortality rate of the procedure was rather high: 4 of 22 patients died (Madrazo et al., 1988, quoted in Lindvall, 1989), plus one additional case (see below Hurtig et al., 1989), resulting in an 8 % of all reported cases. Morbidity was also considerable. The most frequent adverse effects were psychiatric disturbances in 68 % of the cases, pneumonia and other infections (58 %), sleep alterations (30 %), and orthostatic hypotension (12 %).

Two variables, not considered in detail before, are noteworthy. One is the size of the graft: in the original studies (Madrazo et al., 1987a, and probably 1987b as well) 0.8-1.0 g of tissue was implanted, whereas in the other reports, a variable amount, not exceeding 100 mm^3 (Goetz et al., 1989; Tyce et al., 1989 for the patients of Kelly et al., 1989) and as little as 7-30 mm^3 (Allen et al., 1989) was grafted. These volumes represent 1/10 or less of that of the initial report since the specific gravity of brain tissue is close to 1. The other factor is the time elapsed between adrenalectomy and implantation. This datum is missing in some reports (Madrazo et al., 1987a and b; Goetz et al., 1989), and is given as between 2 and 20 minutes in the others (Allen et al., 1989; Kelly et al., 1989).

It is difficult to assess the efficacy of adrenal medullary implants for the amelioration of parkinsonian symptoms given the variety of conditions of the various reports, despite of the attempts to replicate some aspects such as surgical techniques. The optimistic results of the Mexican (Madrazo et al., 1987a,b; 1988, quoted in Lindvall, 1989) and Chinese (Jiao et al., 1988) groups stand in contrast to all other remaining reports. Although the possibility is small that factors such as the amount of tissue grafted and the time elapsed between adrenalectomy and implantation play a significant role in the success of the procedure, it is clear that the Mexican surgeons implanted large amounts of tissue, perhaps 10 times as much as the other studies, in contact with both the caudate parenchyma and the CSF; and the Chinese workers used a stereotaxic intraparenchymal graft with a short interstage lapse. These two conditions have not been met by any of the other studies, and should be carefully considered in any future attempts.

Several of the clinical investigations discussed above contain biochemical data of interest for the understanding of the mechanisms operating in the rather meager beneficial effects, or the failures of the procedure. Thus, the lumbar CSF of 3 patients with significant clinical improvement showed progressive increases in DA levels over the first 3 postoperative months, reaching 3-4 times the preoperative values, and declining thereafter (Jiao et al., 1988). More modest increments of about 55 % were recorded at 6 weeks, dropping to 30 % by 26 weeks after surgery, with no significant correlations with the clinical picture and/or the amount of L-dopa treatment (Tyce et al., 1989). In the latter cases, the DA concentration in plasma did not change after implantation. Positron emission tomography with a radioactive D_2 receptor ligand, revealed no remarkable changes in D_2 receptor density (Lindvall et al., 1987). The levels of HVA dropped in most of the cases measured, (Backlund et al., 1985; Lindvall et al., 1987; Tyce et al., 1989), but one patient showed consistent increases reaching 350 % of preoperative values at 6 months (Backlund et al., 1985). Other catecholamines and their metabolites were minimally affected. NA levels either fluctuated

over a 20-week period (Jiao et al., 1988) or did not change at all (Tyce et al., 1989). Similar results were obtained regarding the concentrations of MOPEG (Backlund et al., 1985; Lindvall et al., 1987) or MHPG (Tyce et al., 1989). Levels of A remained unchanged as well (Tyce et al., 1989). Finally, 5-HIAA concentration was initially elevated but returned to preoperative values by 6 months (Backlund et al., 1985). Taken together, these results indicate that the graft stopped producing important amounts of NA and A, and that by some unknown mechanism a limited amount of additional DA became available to the brain.

It is of interest that a neuron-specific antibody present in the ventricular CSF of 6 of 7 PD patients, labeled cells of the rat substantia nigra, and progressively disappeared over a period of 1-6 months after adrenal medullary implants (McRae-Degueurce et al., 1988). The antibody was absent in non-PD controls. Whether the removal of the antibody from the CSF is effected by the implant itself or by the possible action of factors released by the CNS as a result of the procedure remains an open question.

Additional information on the mechanism of action of the implants derived from studies of alterations in the BBB (Ahlskog et al., 1989). By simultaneous measures of albumin and IgG in plasma and CSF, it was found that only 1 of 7 patients with implan sthad a persistent passage of these substances through an altered BBB. In all others, the barrier became adequately reconstituted 3-6 months after surgery. In contrast, exogenously given carbidopa, which does not cross the BBB in the normal, resulted in significantly increased levels of this compound in the CSF during the 6-month period of study in 4 of 5 such cases. These findings raise the possibility that postoperative carbidopa treatment may contribute to the poor clinical results of the implants by blockage of dopa decarboxylation in the CNS.

Since all clinical investigations involved autologous implants, it is of paramount importance to recognize the condition of the grafted tissue prior to implantation. It is known that human adrenal medulla from non-neurological patients contains a variety of neuroactive substances in addition to catecholamines (Winkler and Westhead, 1980), as well as numerous cellular elements other than chromaffin cells. The latter include neurons, nerve fibers and terminals, Schwann cells, fenestrated capillaries, pericytes, fibroblasts and smooth muscle fibers (Hansen et al., 1988c). Of particular interest is the presence of scattered islands of adrenal cortical cells which precludes the preparation of 100 % cortex-free fragments of medullary tissue for implantation. This contamination has been noted in surplus remnants of the tissue in some of the clinical series (Penn et al., 1988; Jiao et al., 1988) and may indeed interfere with the phenotypic transform of chromaffin into neuron-like cells. Of particular significance are studies comparing postmortem specimens of adrenal medulla from non-parkinsonian and parkinsonian patients. The results are somewhat controversial, some investigators reporting small, non-significant declines of A and NA levels as well as in TH activity in the parkinsonian cases (Cervera et al., 1988), whereas others specify major decreases in A (74 %), NA (40 %) and DA (75 %) (Stoddard et al., 1989b). The latter authors suggest that the differences in results could be due to their use of considerably shorter postmortem delays in harvesting the adrenals (1.5-15 hr as opposed to 6.5-30 hr). They also argue that the reduction might be a reflection of the pathologic process, or that it might be induced by the prolonged antiparkinsonian medication. It is also noteworthy that the adrenals of PD patients show abnormal cytoplasmic inclusion bodies containing sphingomyelin, free fatty acids and polysaccharides (den Hartog Jager, 1970).

Several reports on adrenal medullary implants include biochemical and/or histologic data on surplus samples of the tissue to be implanted. Some mention the presence of intensely fluorescent chromaffin cells, indicative of large amounts of catecholaminesnes (Backlund et al., 1985; Lindvall et al., 1987; Jiao et al., 1988). Others report relatively low concentrations of A (4.3-8.8 mg/g tissue), NA (0.5-2.3 mg/g) and DA (0.007-0.015 mg/g) (Lindvall et al., 1987;

Tyce et al., 1989). In fact, these levels may represent an 81 % decrease of those found in adrenals of non-parkinsonian nefrectomy patients (Stoddard et al., 1989a). These results question the adequacy of autologous grafts of adrenal medullary tissue in PD patients.

There are two postmortem examinations of patients receiving adrenal medullary implants (Peterson et al., 1989; Hurtig et al., 1989). Both benefited little from the procedure and died 4 months after surgery from postictal aspiration, or from a cervical epidural abscess. In one, the implant showed some immunoreactivity to a chromogranin A antibody, indicating its original chromaffin nature, but the tissue was necrotic, with no viable cells and no TH immunostained elements. The focus was surrounded by macrophages and neutrophils, and it was separated from the host brain by a loose gliotic layer (Peterson et al., 1989). The other exhibited some surviving cells with chromaffin properties such as immunostaining with antibodies against chromogranin A, or neurofilament proteins, but no TH immunoreactivity, and no neurites extending between graft and host. In addition, there were increases in the density of DA uptake sites, evaluated by autoradiography after [^{3}H]mazindol binding, as well as decreases in DA receptors densities, but only at the level of the implant, suggesting that the graft provided a trophic stimulus for the growth of dopaminergic fibers from the host, leading to receptors downregulation (Hurtig et al., 1989).

In summary, the results of both animal experiments and clinical reports strongly suggest that adrenal medullary implants into the DA-deprived neostriatum have a limited viability. They may initially produce DA which diffuses into the host probably through shunts between the host and graft vascular systems. The modest beneficial effect appears to be more attributable to the production of trophic factor(s) by the graft or the host than to the purported change of chromaffin cells into neuron-like phenotypes. Possibilities for better outcomes include an increase in the amount of tissue grafted, a reduction of the lapse between adrenalectomy and implantation, and the use of adrenals from non-parkinsonian cadaveric organ donors instead of autologous grafts.

IMPLANTS OF CAROTID BODY TISSUE

The rationale for using carotid body tissue as a source of dopaminergic cells derives from the high DA content of this organ in the rat, cat and rabbit (Altes et al., 1977; Hansen and Christie, 1981) and the possibility that DA synthesis occurs in its main cellular components, the so-called glomus cells (McDonald and Mitchell, 1975). It is also of interest that these cells are immunoreactive for ENK, but not for other neuropeptides (SP, VIP, NPY) which are present only in nerve fibers and terminals of this organ (Lundberg et al., 1979; Jacobowitz and Helke, 1980; Wharton et al., 1980; Kondo et al., 1986). Experiments were made using the 6-OHDA lesioned rat model, by stereotaxic injections of cell suspensions obtained from carotid bodies of donor rats in two sites of the CP of the lesion side (Bing et al., 1988). It was further reported that the number of cells implanted was adjusted to 20,000 per site (Hansen et al., 1988a). This procedure resulted in a significant 50 % mean reduction in amphetamine-induced rotations when tested 10 and 24 days after implantation, an improvement similar to the one obtained after adrenal grafts (see above). Morphologic findings at 30 days postgrafting included the presence of 25-100 TH-positive cells at each implant site, round or oval in shape, with processes extending 10-20 μm. TH-positive fibers were also present in the host tissue close to the implant but they were of lower density than that observed in adrenal grafts. At the ultrastructural level, glomus cells showed less number of densecore vesicles than in the normal, an apparent sign of dedifferentiation, and although they were seen extending processes toward the host neuropil, no synaptic contacts were ever observed. These results raise a similar question to that of adrenal implants in the same animal model, namely whether the observed behavioral improvement is due to the functioning of the implanted cells, or to trophic factors which elicit dopaminergic reinner-

vation from host tissues. The reduced number of surviving cells, the lack of neuron-like transformation with synapse formation, and the unknown effects of implants longer than one month survival, do not provide more optimistic prospects than those of the adrenal grafts discussed above.

IMPLANTS OF CULTURED CELL LINES

Two tumor cell lines maintained in culture are known to produce catecholamines: the PC12 pheochromocytoma and the B16/C3 melanoma, and both have been used as sources for implants into the DA-deprived neostriatum. In addition, implantation of genetically engineered cells has also been attempted but with no relation to brain dopamine deficits as yet.

PC12 CELLS

This cell line derives from a rat pheochromocytoma (Greene and Tischler, 1976), and produces large amounts of DA and lesser quantities of NA (Greene and Rein, 1977). In initial studies either cell suspensions or pellets were placed in the CP and other brain structures of *normal* young rats (Jaeger, 1985, 1987). Two to 8 weeks later, immunocytochemistry showed survival and proliferation of cells, with tumor formation, and expressing TH, DBH but not PNMT reactivity, indicating that they had the enzymatic machinery to produce DA, NA, but not A. There were signs of host-graft interaction such as migration of astroglia and endothelial, capillary-forming cells from the host, as well as short processes originating in the grafted cells. Ultrastructural observations, however, provided no evidence for synapses formed by PC12 elements. In a concurrent experiment, PC12 cell suspensions were injected into the CP in the 6-OHDA-lesioned rat model (Hefti et al., 1985). When two sites were implanted, there was a significant 27 % reduction in apomorphine-induced rotations during the first 2 postoperative weeks. Histochemistry showed aggregates of numerous highly fluorescent PC12 cells after one week but with no signs of interaction with the host. By 2 weeks, however, only the periphery of the aggregates survived, and beyond that period there were no fluorescent cells, and only degenerated material was present. The results were similar when injecting PC12 cells grown in the presence of NGF, although they had acquired a neuron-like appearance. In a further study, approximately 10,000 PC12 cells were injected into the CP of the rat model, and the animals were sacrificed one day to 20 weeks later (Freed et al., 1986b). Tissues were examined with histofluorescence or immunofluorescence with antibodies against TH or PC12-surface antigens. All positive cells were found within 1 mm of the implant site. In some animals, the number of cells increased in the first 2 weeks but most of them disappeared in 7-10 weeks. A small number of cells survived, however, for up to 20 weeks in which cases they developed long, fine processes giving them a neuron-like appearance. Finally, using a similar model, 20,000 PC12 cells which had been labeled with [^{3}H]thymidine were injected in each of two sites of the CP (Bing et al., 1988), with one half of the animals immunosuppressed by Cyclosporin A treatment. Amphetamine-induced rotations were unchanged at 10 and 24 days postoperatively in both groups, i.e. with and without immunosuppression. At 30 days, TH immunocytochemistry demonstrated a rather poor survival of only 15-50 thymidine-labeled PC12 cells per site in 4 of 6 suppressed animals and in only 1 of 6 non-suppressed rats. These cells were elongated and gave off processes of 80-100 μm. Two of the suppressed animals developed tumors with frequent occurrence of mitotic figures.

In summary, although PC12 cells may be a source of dopamine, it is essential to control their proliferation and consequent tumor formation. NGF apparently inhibits their division and contributes to their acquiring neuron-like characteristics (Greene and Tischler, 1976), leading perhaps to the few positive results (Hefti et al., 1985; Bing et al., 1988). The need for immunosuppression to prolong the survival, however, may interfere with this effect, resulting in tumor formation (Bing et al., 1988). In any event, the behavioral improvement is either short lived (Hefti et al., 1985), or non-existent (Bing et al., 1988), making PC12 cells poor candidates for pro-

viding brain implants of DA-producing neurons.

B16/C3 MELANOMA CELLS

A spontaneously occurring mice melanoma (Hu and Lesney, 1964), which produces L-DOPA and melanin (Burnett, 1971) is known to synthesize also catecholamines including DA in culture (Laskin and Piccinini, 1986). Suspensions of these cells, some of which had been labeled with a nuclear fluorescent vital dye, were injected in the amount of 10,000-15,000, into the CP of the 6-OHDA lesioned rat as well as in *normal* rats and mice (Freed et al., 1989). Mice developed pigmented tumors which, at 12 days, showed no catecholamines by histofluorescence and TH immunocytochemistry, and killed the animal within 7 weeks with generalized metastases. In contrast, the implant sites of rats, both lesioned and normals, showed melanoma cells which continued to proliferate for at least 6 weeks, stabilized by 11-14 weeks with no signs of tumor spread or metastases, and the aggregate became delineated by a glial capsule. The cells accumulated pigment from the second week, expressed transiently tyrosinase and TH immunoreactivities as well as catecholamine histofluorescence, and were surrounded by host cells that immunostained with an antibody against the Ia antigen, indicating the initiation of an immune response to the graft. Amphetamine- and apomorphine-induced rotations remained the same as preoperatively in the lesioned rats.

Although B16/C3 cells may be useful to investigate immune processes triggered by intracerebral tumors as well as regulation of the production of melanin and DOPA, their transient synthesis of catecholamines does not warrant their use as a source of dopamine when implanted into the DA-deprived striatum.

GENETICALLY ENGINEERED CELLS

There have been very preliminary attempts to utilize genetically engineered cells for implantation into the striatum of the *normal* rat (Gage et al., 1988). The transgenes were inserted by way of murine retrovirus vectors into rat fibroblasts and perinatal astrocytes, since the nonreplicating nature of neurons precluded their use. The genes, however, were selected only to develop a model system and were not related to the cause or mechanisms resulting in a DA-deprived neostriatum. The experiment succeeded to the extent that the engineered cells implanted into the *normal* rat CP survived for at least 7 weeks and, when recultured, continue to express the inserted genes. It is apparent that the use of non-neuronal cells can not lead to the reconstruction of functional circuits in a lesioned host. Nonetheless, this approach deserves further efforts in the case of the DA-deprived striatum since, as discussed in previous sections, some functional recovery is obtained with grafts of DA-producing cells which do not establish synaptic connections with the host.

IMPLANTS OF CHRONIC DELIVERY SYSTEMS

The fact that functional restoration, albeit partial, might depend upon diffusion of DA into the host striatum without necessarily involving synaptogenesis, provided the rationale for the development of artificial continuous delivery systems of the neuroactive substance. Thus, osmotic minipumps (Sendelbeck and Urquhart, 1985) containing DA were implanted and connected to a dialysis fiber placed in the CP of the 6-OHDA-lesioned rat model (Strömberg et al., 1985). These animals spontaneously exhibited strong acute contralateral circling, and the apomorphine-induced rotations were markedly reduced after 2 weeks. Fluorescence histochemistry showed extensive halos surrounding the dialysis fibers. This preliminary report did not give the rate or duration of the mininfusion. A more detailed investigation used the same animal model and a similar system, except that a steel cannula was used instead of the dialysis fiber. The minipump delivered DA at the rate of 0.5 or 5.0 μg/hr during 2 weeks (Hargraves and Freed, 1987). The high dose elicited strong contralateral spontaneous circling for 1-2 days only. Over 50 % significant reductions in apomorphine-induced rotations were obtained with either dose, during

the infusion period but not later. The use of tracer amounts of [^{3}H]DA allowed to estimate the spread of the infused compound and its metabolites by radioactivity measurements. The distribution was similar at 1,3 and 7 days of infusion and the radioactivity, which occupied a total volume of approximately 200 mm^3, decreased 10-fold for each 3 mm away from the infusion site.

A different delivery system was utilized in the same animal model. It consisted of stereotaxic injections of microencapsulated DA into 2 sites of the CP (McRae-Degueurce et al., 1988). DA was encapsulated within a biodegradable polymer (poly [lactide-co-glycolide]) similar to that used in resorbable suture material. The capsules sizes were 5-45 μm and DA represented approximately 40 % of their weight. A total of 900 μg of the encapsulated product, i.e. about 360 μg of DA, was injected in each site. Circling behavior was recorded for 2-4 hr starting 30-45 minutes after the injections. Strong contralateral spontaneous rotation occurred in all animals lasting 3 hr except in one which continued for several more hours. Three weeks later, CP sections were immunostained with an anti-DA antibody and showed capsules without and some with remaining DA, with no macrophages or other signs of tissue reaction to the infused material. Although this biocompatible system is capable of delivering DA *in situ*, its value is limited by the duration of the effect. In fact, as stated by the authors and assuming the slowest release in the best of conditions, the biodegradable polymer carrier should be totally resorbed within 6 weeks.

Another chronic DA delivery system was tested first *in vitro* (Freese et al., 1989) and then in *normal* rats (During et al., 1989), and consisted of 30 % by weight DA incorporated into a biocompatible, but apparently non-degradable, copolymer (ethylene-vinyl acetate) matrix. A specially designed disc of this material, 4 mm in diameter and 1 mm thick, was implanted on the surface of the CP after removal of the overlaying cortex and white matter. In vivo dialysis over 65 days showed slow constant >200-fold increases of the extracellular DA concentrations, as well as >4-fold increments of DA metabolites, with a 5-fold raise in the DOPA/HVA ratio. Histologic examination at 70 days revealed only a gradient of moderate gliosis for about 250 μm adjacent to the implant. The authors suggested a number of possible modifications of the device to allow intraparenchymal injections of microspheres which could release DA in more physiologic amounts and for periods of several years.

An earlier report described a redox delivery system for the sustained release of DA in the brain (Bodor and Simpkins, 1983. In this instance, DA was coupled to a dihydropyridine carrier which could cross the BBB. Oxidative and hydrolytic processes transformed the complex into a DA precursor which was cleared from the organism except from the brain where it remained locked-in, thus providing an intracerebral source of slow and sustained DA release. The success of such a system was shown by intravenous administration to *normal* rats with a resulting marked drop in serum prolactin levels. Apparently, the technique has not been applied as yet to the DA-deprived neostriatum.

Finally, it is worth noting that intraventricular administration of DA through a chronically implanted reservoir in a patient with severe PD resulted in mild decreases of the bradykinesia after each single dose of 8-16 mg (Venna et al., 1984). A similar result occurred on the second week of a regimen comprising 4 mg daily for 4 consecutive days in each of 2 weeks. The improvement, however, was transient and accompanied by a confusional state, hallucinations and dyskinesias.

IMPLANTS OF SYMPATHETIC GANGLIA

RATIONALE

A clear rationale for the use of sympathetic ganglia as a source for brain implants emerges from the findings that they contain a mixed population of short axon neurons, in addition to the long axon principal efferent cells, hereinafter designated as P-cells, most of which are classically considered noradrenergic. The interneurons are represented by the SIF cells, thus named be-

cause they are small and intensely fluorescent, many of which are dopaminergic. Excision of the ganglionar chain severs both the incoming cholinergic axons as well as the axons of the P-cells, presumably leaving the interneurons intact, thereby maximizing their chances of survival. The information to be reviewed derives from studies on the rat SCG, unless stated otherwise.

MORPHOLOGY

It has long been known that the organization of sympathetic ganglia is considerably more complex than that of a simple relay between a cholinergic afferent fiber and a noradrenergic efferent neuron. The discovery of distinct small, intensely yellow, fluorescent cells (SIF cells), as opposed to the green fluorescence of variable magnitude which characterizes the P-cells (Eränkö and Härkönen, 1963) is the origin of the concept of more than one cellular component in these ganglia. The SIF cells are considered a new variety of non-chromaffin, amine-containing elements different from P-, mast and chromaffin cells (Eränkö and Härkönen, 1965). The neuronal nature of SIF cells is supported by the electron microscopic observations of small neurons with 140 nm dense core vesicles which can be followed into presynaptic boutons. It is therefore possible that they represent a type of ganglionar interneuron (Williams, 1967). SIF cells are 6-12 μm in diameter, do not show Nissl bodies and are usually arranged in clusters of 2-12 cells, as opposed to P-cells which are 15-40 μm, with prominent Nissl masses and isolated from each other (Matthews and Raisman, 1969; Williams and Palay; 1969). Over 30 such clusters can be recognized in a single ganglion. They have a non-uniform distribution, are surrounded by an incomplete sheath of satellite cells, and are usually in apposition to a capillary. The somata and short processes are postsynaptic to boutons of preganglionic fibers. The longer processes form synapses with dendrites probably belonging to P-cells. Somata are frequently observed forming somatodendritic synapses with probable P-cell dendrites (Matthews and Raisman, 1969). It should be noted that SIF cells with these features are apparently absent in the cat SCG (Williams et al., 1975). A recent count based on size differences of histofluorescent cells of the rat SCG gives a total of 14,850 large, presumably P-cells, and 860 small, probably SIF cells (Itakura et al., 1988).

CHEMISTRY

SIF Cells. Much attention has been paid to the neuroactive substances contained in sympathetic neurons in addition to the classic noradrenergic nature of the P-cells. Thus, SIF cells were found to contain DA by microspectrofluorimetric analysis in the cat and pig sympathetic chain (Björklund et al., 1970) as well as in the rabbit and rat SCG (Libet and Owman, 1974; Baker et al., 1977), and various DA concentrations were measured in biochemical assays of laser-dissected SIF cell clusters (Gerold et al., 1982). Moreover, SIF cells took up exogenous DA, and activation of muscarinic receptors by cholinergic preganglionic fibers resulted in DA release from these cells, which in turn induced slow inhibitory postsynaptic potentials in P-cells (Libet and Owman, 1974). These results supported the concept that dopaminergic SIF cells acted as inhibitory interneurons.

A minor proportion of SIF cells (5 %-10 %) was shown to contain NA, either by the actual presence of the compound determined by x-ray microanalysis after aldehyde and dichromate treatment (Lever et al., 1977), and in assays of dissected cluters (Gerold et al., 1982), as well as their immunoreactivity for DBH (König and Heym, 1978; but see Baker et al., 1977 for contrary results) and NA (Verhofstad et al., 1981). In the latter study, however, the crossreactivity of the antibody against NA with DA could not be excluded. At some variance with the preceding reports are the results of immunofluorescence studies with anti-TH and anti-DBH antibodies in contiguous sections, showing that all SIF cells, particularly those of the inferior mesenteric and coeliac ganglia of the guinea pig, are positive to both, indicating their noradrenergic nature (Elfvin et al., 1975; Hökfelt et al., 1977b; Schultzberg et al., 1979). Finally, A could also be present in some

SIF cells as revealed in biochemical assays (Gerold et al., 1982) and by immunoreactivity to PNMT antibodies in the guinea pig inferior mesenteric ganglion (Elfvin et al., 1975).

In addition to their well established catecholamine content, some SIF cells, regionally localized in the cranial and caudal poles of the ganglion, were shown to store 5-HT by immunofluorescence (Verhofstad et al., 1981), a possibility already entertained in the original description of this cell class (Eränkö and Harkönen, 1965). Observations on contiguous sections stained with an antibody against NA, led to the conclusion that serotoninergic and noradrenergic SIF cells formed two separate populations. A later study utilizing TH antibodies, however, suggested colocalization of the two compounds in clusters of SIF cells (Päivarintä et al., 1987). Immunofluorescence also demonstrated that the SIF cells contained HIS (Häppölä et al., 1985) as well as histidine decarboxylase (Päivärinta et al., 1987). These neuroactive substances coexisted with both 5-HT and a catecholamine. Finally, few met-ENK immunoreactive SIF cells were observed in the guinea pig, but not in the rat, mesenteric ganglion and SCG with some suggestion of colocalization of this neuropeptide with NA (Schultzberg et al., 1979).

P-cells. The classically considered noradrenergic nature of these cells was repeatedly verified either by microspectrofluorometry in the cat and pig sympathetic chain (Björklud et al., 1970), by their immunoreactivity for NA (Verhofstad et al., 1981), or by being DBH-positive in the guinea pig (Elfvin et al., 1975; Hökfelt et al., 1977a, b,c) and cat coeliac ganglia (Lundberg et al., 1985). Some P-cells of the guinea pig SCG, however, were found to be TH-positive but DBH-negative, which suggested their dopaminergic nature (Elfvin et al., 1975). DA-containing P-cells were particularly prominent in the lower thoracic and upper sacral sympathetic ganglia of the dog that had a higher DA:NA ratio than the rest of the chain (Bell and McLachlan, 1982). These cells were found to innervate the kidney and hind paw by retrograde transport of HRP, were able to bind DA after reserpine-induced depletion and, although they exhibited histofluorescence, they were DBH-negative (Bell and Muller, 1982).

Antibodies recognizing a series of neuropeptides stained a number of P-cells. Few were reactive to anti-met-ENK, and there was some indication of colocalization of this compound with NA in both the guinea pig and rat (Schultzberg et al., 1979; Ariano and Tress, 1983). A similar number of P-cells immunostained for SS and DBH, suggesting the coexistence of yet another peptide with NA (Hökfelt et al., 1977a). A large number of P-cells in the cat coeliac ganglion were NPY-positive and DBH-positive as well (Lundberg et al., 1985). More controversial are the issues concerning SP and VIP. Some investigators have found few SP-positive (Ariano and Tress, 1983) and VIP-positive P-cells (Lundberg et al., 1985) whereas others have observed only fibers, probably of extrinsic origin, exhibiting these immunoreactivities (Hökfelt et al., 1977b,c). Finally, examination of human surgical specimens of lumbar sympathetic ganglia revealed only fibers immunostained for SP, met-ENK and bombesin (Helen et al, 1984).

In summary, the neuronal composition of sympathetic ganglia comprises a mixed population of short-axoned SIF cells, many of which contain DA. Smaller proportions of these cells store NA, A, 5-HT, HIS, and ENK with some suggestion of colocalization of catecholamines with 5-HT and HIS, as well as ENK with NA. The majority of the long-axoned efferent P-cells is noradrenergic, but some contain DA and/or a variety of neuropeptides such as ENK, SS, NPY, and perhaps also SP and VIP. This rich composition, and particularly the existence of dopaminergic short-axoned neurons, make these structures promising sources for implantation into the DA-deprived neostriatum.

IMPLANTS

Long survival of transplanted sympathetic neurons was obtained first in cats with autografts of upper lumbar ganglia into intercostal muscles, some of the cells enduring

up to 287 days under these conditions (Ward, 1936). Nissl bodies recovered from the initial trauma by the first week, and became somewhat irregular in size and distribution by 10 weeks. There were occasional neuronal processes crossing the scar tissue and invading the host.

Forty years passed before transplants of sympathetic ganglia were made into the *normal* CNS, in this instance to explore various requirements for survival (Stenevi et al., 1976). Slices of the ganglion with its capsule from newborn or adult rats were inserted into the thalamus or CP, or placed on the pial vascular bed of the choroidal fissure in contact with the hippocampus, after removal of the overlying cortex. The best conditions for survival were the use of adult donors, and the intimate contact with highly vascular tissue. In these cases, about 150 neurons representing about 2 % of the total implanted, remained by one month and survived for 6 months. Most of them had morphologic features of SIF cells, and gave rise to an extensive fiber outgrowth particularly along and around blood vessels which had been previously denervated by sympathectomy. Some of the processes invaded the adjoining host tissue and persisted for at least 6 months with patterns suggestive of contacts within the recipient dentate gyrus and hippocampus. Intraparenchymal grafts had a poor survival rate which did not improved with preincubation of the tissue with NGF. More recently, an entire ganglion divided into 3 pieces was placed in a cavity made in the cerebral cortex (Itakura et al., 1988). Although 17 of 20 grafts survived with recognizable P-cells and SIF cells, their number decreased progressively and only 8 % remained by 2 weeks. Abundant fluorescent fibers arouse from these cells but none penetrated the host tissue.

Several studies addressed the issues of graft-host interactions and alterations in the BBB. The model system consisted in implanting a fragment of rat SCG onto the floor of the IV ventricle or on the surface of the medulla, as well as on the cerebral cortex, with no disruption of the blood supply, within 15 minutes from the excision of the ganglion (Rosenstein and Brightman, 1979). Allografts and autografts were made in newborn and young rats, respectively. Good survival occurred only when the donor was at leaast 3-4 weeks old. In these instances, variuos sign of interaction were apparent at the electron microscopic level from 1 hr to 6 months. They included vascularization of the implant by fenestrated and non-fenestrated capillaires, extension of ependymal processes toward the implant, formation of astrocytic laminae in contact with the implant, and sharing of basal laminae between astrocytes from the host and Schwann cells from the ganglion. There was also penetration of the glial layer by neurites of the ganglion cells, but terminations of the latter processes could not be traced. As shown earlier (Purves, 1975), excision of the ganglion involving the axotomy of P-cells, resulted in the death of many of its neurons, with degeneration of most boutons within the first few days. The small number of surviving neurons, which could still be observed at 6 months, developed neurites with growth cones and, by 2-6 weeks, formed new synapses between processes of regenerating cells within the graft. Contrary to the normal morphology of the ganglion, many of the nerve fibers in the implant acquired a myelin sheath provided by hyperactive Schwann cells. It is puzzling that none of the surviving neurons or their boutons had the ultrastructural characteristics of SIF cells or their terminals. These findings may be due in part to phenotypical changes occurring as a result of new environmental factors, or to the lack of appropriate target cells (Dibner at al., 1977). Fragments in contact with the cerebellar surface survived for up to 2 years and induced changes in the host structure so that the cerebellar cortex either remained arrested in development or invaded the graft (Rosenstein and Brightman, 1981). In the latter case, astroglial bridges grew into the graft first, and then granule cells migrated along them. However, no connections were established between cerebellar and ganglion cells. The possibility was entertained that neurotropic and gliotropic and gliotropic factors of graft origin were responsible for these outcomes.

The same model system was used to in-

vestigate the effect of the transplant on the BBB since the normal ganglion lacks a corresponding barrier (Jacobs, 1977). It was found that HRP, injected systemically and let circulate 1-60 minutes before sacrifice, filled the graft within 1-2 minutes, and spread into the host brain within 10-15 minutes for a distance equal to the width of the graft. The spread into the brain was reduced from 1000 μm to 200 μm when the implant was intraparenchymal instead of intraventricular (Rosenstein and Brightman, 1986). The implant appeared reperfused after 15-24 hr. By labeling newly formed vessels with tritiated thymidine, it was shown that sprouts of brain capillaries anastomosed with surviving graft vessels only at the interface region (Krum and Rosenstein, 1987). It was concluded that the HRP left the graft through fenestrated capillaries and entered the brain through interconnected extracellular spaces of graft and host. This opening of the BBB persisted as long as the graft was viable. Similar experiments demonstrated that the graft vessels indeed survived and remained fenestrated. Since in these cases no neurons were present in the graft, it appeared that the capillary phenotype was more determined by interactions of fibroblasts with Schwann cells than with neurons (Wakai et al., 1986). The revascularization pattern, however, was somewhat different when a whole decapsulated ganglion was implanted in the choroidal fissure or the septal nuclei (Zhou et al., 1986a). In this experiment, host capillaries, labeled with India ink, were observed to penetrate the graft reaching its center within one week. The number of surviving neurons decreased progressively and only few remained after 9 weeks. Glial fibrillary acidic protein immunocytochemistry revealed the additional invasion of the graft by host astrocytes along the blood vessels and Schwann cell fascicles attaining a maximum at one month and changing little by 3 months (Zhou et al., 1986b).

Very scanty information is available on the fate of sympathetic ganglia implanted into catecholamine-deprived brain structures. In initial studies, autografts of one half ganglion were made into a preformed cavity in the dorsal entorhinal cortex (Björklund et al., 1976) or the ventral septohippocampal junction (Björklund and Stenevi, 1977) of rats with previous intraventricular injections of 6-OHDA that presumably removed the noradrenergic innervation. Close to 100 % of the grafts survived with the presence of up to 250 neurons by 3 months and fiber outgrowth into the corresponding target field of the damaged pathway. These cells were considered noradrenergic on the basis of their histofluorescence. Aggregates of SIF cells were also noted among the surviving elements. In a similar experiment, rats with 6-OHDA lesions of the parietal cortex and implants into the damaged area were examined 1-4 weeks later (Itakura et al., 1988). Thick, strongly fluorescent catecholamine fibers were present in the graft, some of which grew into the host tissue in contrast with implants into non-denervated cortex that did not show such an outgrowth (see above). In the same study, 5 monkeys (*Macaca fuscata*), rendered parkinsonian by 6 doses of MPTP (0.5 mg/kg) given in a 12-day period, received decapsulated fragments, 1-2 mm in diameter, of autologous SCG implanted stereotaxically into the head of the caudate nucleus. After two weeks, fluorescent cells were found in the implant with fibers growing into the caudate nucleus. The same investigative group reported behavioral observations with telemetric measurements of motor activity in 4 monkeys with MPTP-induced parkinsonism (Nakai et al., 1990). Three of the animals received bilateral implants into both caudate heads, two of which showed full recovery by 4 weeks, and the third reverted to the preimplant status after an incomplete recovery. The non-implanted animal continued to show akinesia and rigidity up to 5 weeks after the MPTP treatment.

The use of adult human sympathetic ganglia as a source of brain implants was first explored in our laboratories (Pasik et al., 1988). Two adolescent monkeys (*Macaca fascicularis*) were rendered parkinsonian by MPTP treatment. Two paravertebral sympathetic ganglia were surgically removed from a patient for the treatment of severe Reynaud's syndrome. After a pa-

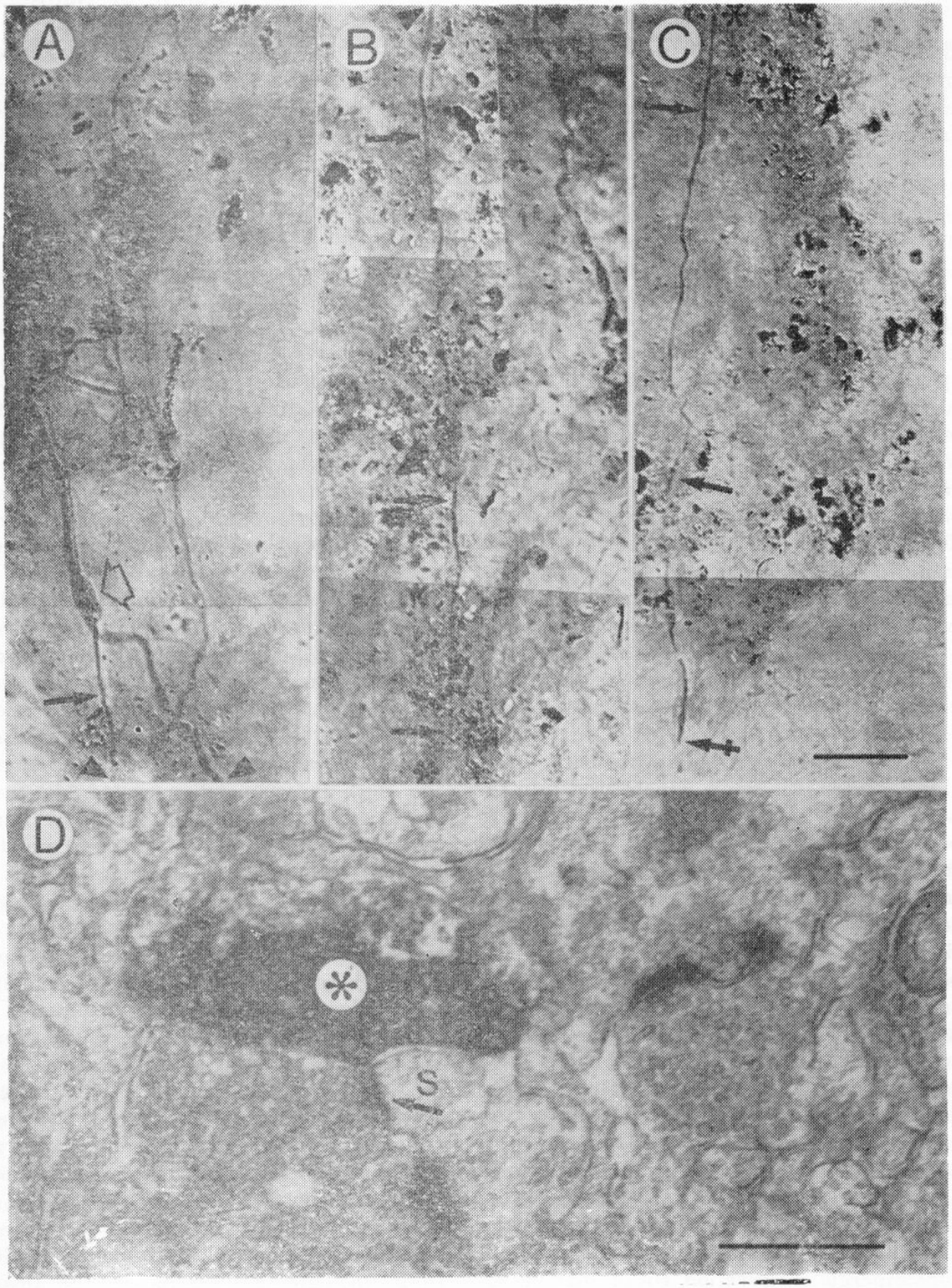

Fig. 1. A, B. C. — *Photomontage of a small neuron, located close to the border of an implant of sympathetic ganglia into the dopamine-deprived putamen, is strongly immunoreactive for tyrosine hydroxylase. The same symbols at sequential bottoms and tops indicate continuity. Note the fusiform soma* ▵*open arrow in A*Q*, bipolar dendritic distribution. and an axon (solid arrows) which invades the host tissue (crossed arrow in C). TH antibody dilution 1:1000 (PAP method. Nomarski optics. Scale bar: 20* μ*m. D. Electron micrograph of labeled axonal bouton, filled with synaptic vesicles. observed approximately 2 mm from the margin of the implant within the host neostriatal neuropil. The bouton (asterisk) forms a symmetric contact with an unlabeled spine (s) which is also postsynaptic to a converging input from a TH-negative terminal establishing an asymmetric contact (arrow). Same immunocytochemical technique as in A,B,C. Scale bar: 0.5* μ*m.*

thologist verified the nature of the specimen, the remainder was dissected free of connectvie tissue and divided into small fragments which were deposited stereotaxically into 2-3 loci of the right putamen, after producing a bed by aspiration of putaminal tissue and injecting 10 μl of 0.01 % NGF. Although the delay was approximately two hours, confidence was gained from the evidence that similar tissue, obtained 4-6 hours postmortem, survived and regenerated in culture, and showed both spontaneous electrical activity and normal ultrastructure (Kim et al., 1979). Starting at one week following the transplant, the monkeys showed progressive improvement, remaining with minimal hypokinesia by 3-4 weeks, and appearing normal by 6-7 weeks. They were sacrificed at 6 months, and TH immunocytochemistry showed at the light microscopy level a space at the end of the needle track, partly filled with cells, some of which had neuronal characteristics, lightly stained globular somata, and broad processes. Deeply immunoreactive neurons could be recognized as well, exhibiting eiter bipolar or multipolar dendritic branching, some with axons penetrating into the host tissue, which also contained many TH-positive fibers in the region surrounding the implant (Fig. 1A,B,C,). The remainder of the neostriatum, contrary to the n. accumbens septi, exhibited only few labeled fibers. The substantia nigra had few TH-positive neurons, but the ventral tegmental area, hypothalamus and locus ceruleus contained numerous labeled cells. Electron microscopy showed immunostained varicose fibers within the characteristic striatal neuropil. Some of the labeled boutons formed synaptic contacts with spines which were also postsynaptic to other unlabeled profiles (Fig. 1D). It appeared therefore that grafts of human sympathetic ganglia into the putamen of monkeys deprived of dopaminergic innervation could survive for long periods. Some of the grafted neurons retained the capacity to express tyrosine hydroxylase, and processes of these cells invaded the host tissue, where they formed synaptic connections. We are presently investigating whether these neurons are dopaminergic and/or noradrenergic, as well as the use of human ganglia from cadaveric organ donors, the possibility of cryopreservation of this material, and the influence of various growth factors on their survival when implanted into the monkey DA-deprived striatum.

GENERAL CONCLUSIONS

The use of non-fetal material as a source of DA for brain implants has met with limited success. Three neurobiologic mechanisms can be delineated as playing a role in the mediation of possible beneficial effects. (1) Simple diffusion of DA from the implant into the DA-deprived recipient tissue. This process would not require the actual reinnervation of the host with consequent reconstruction of neuronal circuits. The most pertinent examples are offered by artificial chronic delivery systems, and perhaps by genetically engineered cells of non-neuronal nature. These methods, however, have been tested only in short term experiments or not at all, in models of DA-deprived neostriatum. (2) Partial reinnervation of the DA-deprived structure by sprouting of remaining dopaminergic fibers of the host induced by neurotrophic factors produced by the grafting procedure and/or by local exogenous NGF treatment. It appears that most of the results with implants of adrenal medullary tissue or carotid body cells fall within this category, particularly in view of the similar outcomes obtained in control experiments involving the implantation of peripheral nerve or adipose tissue, as well as the mere limited ablation (cavitation) of host tissue. (3) Reinnervation of the recipient by processes of implanted neurons invading the neuropil and establishing synaptic connections with host nerve cells. This mechanism appears to be the most promising in terms of the attempt to reconstruct neuronal circuits. It has been obtained with implants of tissue from sympathetic ganglia in the primate model, and in some adrenal medullary implanrts in the rat model.

In any event, it is clear from the preceding survey that although considerable amount of information has been gathered in the last 10 years on the use of brain implants as a source of DA, a number of neu-

robiologic data have yet to be provided to gain a more complete understanding of, not only what makes these implants survive but most importantly, how do they function if at all. The basic research issues must be dealt with in full before further attempts are made to apply these procedurers to PD patients.

SUMMARY

The present survey covers the investigations into the use of brain implants of non-fetal origin as sources of dopamine for neural tissues deprived of dopaminergic innervation. Experiments were made with (1) grafts of adrenal medullary tissue in animal models and Parkinson's disease patients, and exclusively in animals with (2) implants of carotid body cells, (3) certain cultured lines of dopamine-producing cells or genetically engineered non-neuronal cells, (4) artificial chronic delivery systems, and finally with (5) sympathetic ganglia tissue. Although many of the studies show some beneficial effect in ameliorating the behavioral deficits produced by the lack of dopamine, the extent of the functional recovery is rather limited, and uncertainty remains about the neurobiologic processes responsible for these effects. Simple diffusion of dopamine from the implanted source into the host tissue applies to chronic delivery systems, and probably also to the future use of appropriate genetically engineered non-neuronal cells. Reinnervation by sprouting of remaining dopaminergic fibers in the host, triggered by the grafting procedure through the release of neurotrophic factors, appears to occur in the case of adrenal tissue or carotid body cells. Grafts of cultured cell lines has thus far been essentially unsuccessful. Finally, there is evidence that sympathetic ganglia tissue may provide catecholaminergic neurons which integrate synaptically with the host neuropil. In this case the most likely candidates within the cellular and neurochemical complexity of sympathetic ganglia are the SIF cells, the majority of which appear to be dopaminergic.

RESUMEN

La presente revista abarca las investigaciones realizadas sobre el uso de implantaciones cerebrales de origen no-fetal, como fuentes de dopamina para tejidos nerviosos desprovistos de inervación dopaminérgica. Los experimentos han sido realizados con (1) injertos de médula suprarrenal en modelos animales y en enfermos con enfermedad de Parkinson, y exclusivamente en animales con (2) células del glomus carotídeo, (3) cultivos de ciertas líneas de células productoras de dopamina, o de células no-neuronales modificadas genéticamente, (4) sistemas de administración crónica, y finalmente con (5) tejidos de ganglios simpáticos. Aunque muchos de estos estudios muestran un efecto benéfico en mejorar las anormalidades producidas por la falta de inervación dopaminérgica, el grado de recuperación funcional es más bien limitado, y los procesos neurobiológicos responsables de tales efectos siguen siendo inciertos. La simple difusión de dopamina de la fuente implantada al tejido del recibidor se aplica a los sistemas de administración crónica y probablemente también al uso futuro de células apropiadas que hayan sido modificadas genéticamente. La reinervación por brotes de fibras dopaminérgicas aun presentes en el tejido recibidor, por la acción de factores neurotróficos cuya producción se daba al procedimiento quirúrgico, parece ocurrir en los casos de tejido adrenal o de células de glomus carotídeo. Los injertos de líneas celulares no han tenido esencialmente éxito hasta ahora. Finalmente, hay evidencia que implantaciones de tejido de ganglios simpáticos pueden ofrecer neuronas catecolaminérgicas que se integran sinápticamente con el neuropilo del recibidor. En este caso, los candidatos más posibles, dentro de la complejidad celular y neuroquímica de los ganglios simpáticos, son las células SIF cuya mayoría parece ser dopaminérgica.

RÉSUMÉ

Le sujet de la présent revue est l'investigation de l'usage des implantations cérébrales, d'origine non-foetal, comme source de dopamine pour les tissus nerveux déprivés d'innervation dopaminergique. Les expériences ont étaient avec (1) des implantations de médullaire surrénale dans des modèles animaux et dans les patients atteints de la maladie de Parkinson, et exclusivement dans les animaux avec (2) des cellules de glomus carotidien, (3) certaines lignées cellulaires produisant de la dopamine ou des cellules non-neuronales produites de façon génétique, (4) des systèmes de distribution chronique artificielle, et finalement avec (5) des tissus des ganglions sympathiques. Bien que plusieurs travaux rapportent des effets bénéfiques en améliorant les déficits comportementaux résultant de l'absence de dopamine, le degré du rétablissment fonctionel est limité, et des doutes existent concernant les méchanismes neurobiologiques responsables de ces résultats. Une simple diffusion de dopamine à partir de la source implantée dans le tissue de l'hôte s'applique au système de distribution chronique et artificielle et probablement aussi à l'usage futur de lignées cellulaires non-neuronales produites génétiquement. Une réinnervation par germination de fibres dopaminergique subsistant dans l'hôte, promue par la procédure de greffage en relâchant le facteur neurotrophique, se produit apparemment dans le cas du tissu surrénalien ou de cellules de glomus carotidien. Les greffes de lignées cellulaires non pas été couronnés de succès à ce jour.

Finalement, il y existe des indiquations que les ganglions sympathiques pourraient fournir des cellules nerveuses catecholaminergiques qui s'intègreraint de façon synaptique avec le neuropile de l'hôte. Dans ce cas, les cellules SIF, en majorité dopaminergique, sont les candidates les plus probables parmi la complexe organisation cellullaire et neurochimique des ganglions sympathiques.

ZUSAMMENFASSUNG

Die gegenwäertige Untersuchung betrifft die Verwendung von Gehirn Einpflanzungen von nicht foetal stammenden Quellen von Dopamine für Nervengewebe die der dopaminergische Innervation beraupt sind. Es wurden Experimente mit (1) Einpflanzung vom adrenalen Mark in Tier-Modellen und Patienten mit Parkinson krankheit gemacht, und exclusiv in Tieren mit (2) Zellen vom glomus caroticum, (3) gewisse kultivierte Linien von Dopamine produzierenden Zellen, oder non-neuralen genetischerzeugten Zellen, (4) küntslich, chronisch Auslieferungssystem, und endlich mit (5) sympathische Ganglien Gewebe. Obwohl mehizere Studien einigermassen günstige Besserung der Symptomen, die zeigen vom Dopamine Mangel entstanden sind, der Umfange der funktionellen Regenerierung ist ziemlich begrenzt, und Ungewissenheit verbleibt bezüglich des verantwortliches neurobiologischen Prozesses. Einfache Ausbreitung von Dopamine von eingepflanzter Quelle in das Gast-Gewebe bezieht sich auf chronisch Auslieferungssysteme und vielleicht auf zukünftigen Gebrauch von genetisch-erzeugten non-neuralen Zellen. Nerven Belebung durch ausspeinen von verbleibenden dopaminergisch Fasern, ausgelöst durch die Einpflanzungsprozedure, Auslösung eines neurotrophischen Faktors erscheint im Falle der adrenalen Gewebe oder glomus caroticum Zellen zu erfolgen.

Einpflanzungen von Zellkulturen waren bisher im Prinzip erfolglos. Schliesslich, es gibt Beweis, dass sympathische ganglien möglicherweise catecholaminergische Nervenzellen produzieren, welche synaptisch mit der Gast-neuropil sich eingliedern. In diesem Falle sind die SIF Zellen die am meisten dopaminergisch erscheinen, die wahrscheinliche Kandidaten innerhalb der sympathischen ganglien Zellen und Neurochemischen Verwicklung.

REFERENCES

1. *Ahlskog, J. E., Tyce, G. M., Kelly, P. J., van Heerden, J. A., Stoddard, S. L. and Carmichael, S. W.*: Cerebrospinal fluid indices of blood-brain barrier permeability following adrenal-brain transplantation in patients with Parkinson's disease. Exp. Neurol. 105: 152-161, 1989.
2. *Allen, G. S., Burns, S., Tulipan, N.B. and Parker, R.A.*: Adrenal medullary transplantation to the caudate nucleus in Parkinson's Disease. Initial clinical results in 18 patients. Arch. Neurol. 46: 487-491, 1989.
3. *Altes, H., Kindler, J., Knoche, H., Matthiessen, D., Mollmann, H. and Pagnucco, R.*: The biogenic amines in the carotid body. Proc. Histochem. Cytochem. 10: 1-69, 1977.
4. *Ariano, M. A. and Tress, E. L.*: Co-localization of cyclic GMP in superior cervical ganglion with peptide neurotransmitters. Brain Res. 289: 362-365, 1983.
5. *Backlund, E.-O., Grandberg, P.-O., Hamberger, B., Knutsson, E., Martensson, A., Sedvall, G. and Seiger, A.*: Transplantation of adrenal medullary tissue to striatum in parkinsonism. J. Neurosurg. 62: 169-173, 1985.
6. *Baker, H. A., Burke, J. P., Bhatnagar, R. K., Van Orden, V. E., Van Orden III, L. S. and Hartman, B. K.*: Histochemical and biochemical characterization of the rat paracervical ganglion. Brain Res. 132: 393-405, 1977.
7. *Bankiewicz, K. S., Plunkett, R. J., Kopin, I. J., Jacobowitz, D. M., London, W. T. and Oldfield, E. H.*: Transient behavioral recovery in hemiparkinsonian primates after adrenal medullary allografts. In: Gash, D. M. and Sladek, J. R. (eds.). Transplantation into the mammalian CNS, Progr. Brain Res., Pol. 78: 543-549, Elsevier: Amsterdam, 1988.
8. *Becker, J. and Freed, W. J.*: Adrenal medulla grafts enhance functional activity of the striatal dopamine system following substantia nigra lesions. Brain Res. 462: 401-406, 1988.
9. *Bell, C. and McLachlan, E. M.*: Dopaminergic neurons in sympathetic ganglia of the dog. Proc. R. Soc. Lond. 215: 175-190, 1982.
10. *Bell, C. and Muller, B. D.*: Absence of dopamine-beta-hydroxylase in some catecholamine-containing sympathetic ganglion cells of the dog: evidence for dopaminergic autonomic neurones. Neurosci. Lett. 31: 31-35, 1982.
11. *Bing, G., Notter, M.F.D., Hansen, J. T. and Gash, D. M.*: Comparison of adrenal medullary, carotid body and PC12 cells grafts in 6-OHDA lesioned rats. Brain Res. Bull. 20: 399-406, 1988.
12. *Björklund, A., Cegrell, L., Falck, B., Ritzén, M. and Rosengren, E.*: Dopamine-containing cells in sympathetic ganglia. Acta Physiol. Scand. 78: 334-338, 1970.
13. *Björklund, A., Stenevi, U. and Svengaard, N.-A.*: Growth of transplanted monoaminergic neurones into the adult hippocampus along the perforant path. Nature 262: 787-790, 1976.
14. *Björklund, A. and Stenevi, U.*: Experimental reinnervation of the rat hippocampus by grafted sympathetic ganglia I. axonal regeneration along the hippocampal fimbria. Brain Res. 138: 259-270, 1977.
15. *Bodor, N. and Simpkins, J. W.*: Redox delivery system for brain-specific, sustained release of dopamine. Science 221: 65-67, 1983.
16. *Bohn, M. C., Cupit, L. and Marciano, F.*: Adrenal medulla grafts enhance recovery of striatal dopaminergic fibers. Science 237: 913-916, 1987.
17. *Burnett, J. B.*: The tyrosinase of mouse melanoma. 1971 246: 3079-3091, J. Biol. Chem.
18. *Cervera, P., Rascol, O., Ploska, A., Gaillard, G., Raisman, R., Duyckaerts, C., Hauw, J. J. et al.*: Noradrenaline and tyrosine hydroxylase in adrenal medulla from Parkinsonian patients. J. Neurol. Neurosurg. Pschiatr. 51: 1104-1105, 1988.
19. *Dibner, M. D., Mytilineou, C. and Black, I. B.*: Target organ regulation of sympathetic neuron development. Brain Res. 123: 301-310, 1977.
20. *During, M. J., Freese, A., Sabel, B. A., Saltzman, W. M., Deutch, A., Roth, R. H. and Langer, R.*: Controlled release of dopamine from a polymeric brain implant: In vivo characterization. Ann. Neurol. 25: 351-355, 1989.
21. *Elfin, L. G., Hökfelt, T. and Goldstein, M.*: Fluorescence microscopical, immunohistochemical and ultrastructural studies on sympathetic ganglia of the guinea pig, with special reference to the SIF cells and their catecholamine content. J. Ultrastr. Res. 51: 377-396, 1975.
22. *Erankö, O. and Harkonen, M.*: Histochemical demonstration of fluorogenic amines in the cytoplasm of sympathetic ganglion cells of the rat. Acta Physiol. Scand. 58: 285-286, 1963.
23. *Erankö, O. and Harkonen, M.*: Monoamine-containing small cells in the superior cervical ganglion of the rat and an organ composed of them. Acta. Physiol Scand. 63: 511-512, 1965.

24. *Fiandaca, M. S., Kordower, J. H., Hansen, J. T., Jiao, S.-S. and Gash, D. M.*: Adrenal medullary autografts into the basal ganglia of cebus monkeys: injury-induced regeneration. Exp. Neurol. 102: 76-91, 1988.

25. *Freed, W. J.*: Functional brain tissue transplantation: reversal of lesion-induced rotation by intraventricular substantia nigra and adrenal medulla grafts, with a note on intracranial retinal grafts. Biol. Psychiat. 18: 1205-1267, 1983.

26. *Freed, W. J., Adinolfi, A. M., Laskin, J. D. and Geller, H. M.*: Transplantation of B16/C3 melanoma cells into the brains of rats and mice. Brain Res. 485: 349-362, 1989.

27. *Freed, W. J., Cannon-Spoor, H. E. and Krauthamer, E.*: Intrastriatal adrenal medulla grafts in rats. Long-term survival and behavioral effects. J. Neurosurg. 65: 664-670, 1986a.

28. *Freed, W. J., Karoum, F., Spoor, H. E., Morihisa, J. M., Olson, L. and Wyatt, R. J.*: Catecholamine content of intracranial adrenal medulla grafts. Brain Res. 269: 184-189, 1983.

29. *Freed, W. J., Morihisa, J. M., Spoor, E., Hoffer, B. J., Olson, L., Seiger, A. and Wyatt, R. J.*: Transplanted adrenal chromaffin cells in rat brain reduce lesion-induced rotational behavior. Nature 292: 351-352, 1981.

30. *Freed, W. J., Patel-Vaidya, U. and Geller H. M.*: Properties of PC12 pheochromocytoma cells transplanted to the adult rat brain. Exp. Brain Res. 63: 557-566, 1986b.

31. *Freese, A., Sabel, B. A., Saltzman, W. M., During, M. J. and Langer, R.*: Controlled release of dopamine from a polymeric brain implant: in vitro characterization. Exp. Neurol. 103: 234-238, 1987.

32. *Gage, F. H., Wolff, J. A., Rosenberg, M. B., Xu, L., Yee, J.-K., Shults, C. and Friedmann, T.*: Implantation of genetically engineered cells to the brain. In: Gash, D. M. and Sladek. J. R. (eds.). Transplantation into the mammalian CNS, Progr. Brain Res. Vol. 78: 651-658, Elsevier: Amsterdam, 1888.

33. *Gerold, N., Enz, A., Schröder, H. and Heym, Ch.*: Biochemical analysis of catecholamines in small intensely fluorescent (SIF) cell clusters of the rat superior cervical ganglion. J. Neurosci. Meth. 6: 287-292, 1982.

34. *Goetz, C. G., Olanow, C. W., Koller, W. C., Penn, R. D., Cahill, D., Morantz, R. and Stebbins, G.*: Multicenter study of autologous adrenal medullary transplantation to the corpus striatum in patients with advanced Parkinson's disease. New Engl. J. Med. 320: 137-141, 1989.

35. *Goetz, C. G., Tanner, C. M., Penn, R. D., Stebbins III, G.T., Gilley, D. W., Shannon, K. M., Klawans, H. L. et al.*: Adrenal medullary transplant to the striatum of patients with advanced Parkinson's disease: 1-year motor and psychomotor data. Neurology 40: 273-276, 1990.

36. *Greene, L. A. and Tischler, A. S.*: Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. Proc. Natl. Acad. Sci. USA 73: 2424-2428, 1976.

37. *Greene, L. A. and Rein, G.*: Release, storage and uptake of catecholamines by a clonal cell line of nerve growth factor (NGF)-responsive pheochromocytoma cells. Brain Res. 129: 247-263, 1977.

38. *Hansen, J. T., Bing, G., Notter, M. F. D. and Gash, D. M.*: Paraneuronal grafts in unliateral 6-hydroxydopamine-lesioned rats: morphological aspects of adrenal chromaffin and carotid body glomus cell implants. In: Gash, D. M. and Sladek, J. R. (eds.). Transplantation into the mammalian CNS, Prog. Brain Res., Vol. 78: 507-511, Elsevier: Amsterdam, 1988a.

39. *Hansen, J. T., Kordower, J. H., Fiandaca, M. S., Jiao, S.-S., Notter, M. F. D. and Gash, D. M.*: Adrenal medullary autografts into the basal ganglia of cebus monkeys: graft viability and fine structure. Exp. Neurol. 102: 65-75, 1988b.

40. *Hansen, J. T. and Christie, D. S.*: Rat carotid body catecholamines determined by high performance liquid chromatography with electrochemical detection. Life Sci. 29: 1791-1785, 1981.

41. *Hansen, J. T., Notter, M. F. D., Okawara, S.-H. and Gash, D. M.*: Organization, fine structure, and viability of the human adrenal medulla: considerations for neuronal transplantation. Ann. Neurol. 24: 599-609, 1988c.

42. *Happöla, O., Soinila, S., Paivarinta, H., Panula, P. and Erankö, O.*: Histamine-immunoreactive cells in the superior cervical ganglion and in the coeliac-superior mesenteric ganglion complex of the rat. Histochemistry 82: 1-3, 1985.

43. *Hargraves, R. and Freed, W. J.*: Chronic intrastriatal dopamine infusions in rats with unilateral lesions of the substantia nigra. Life Sci. 40: 959-966, 1987.

44. *Hartog Jager, W. A. den*: Histochemistry of adrenal bodies in Parkinson's disease. Arch Neurol. 23: 528-533, 1971.

45. *Hefti, F., Hartikka, J. and Schlumpf, M.*: Implantation of PC12 cells into the corpus striatum of rats with lesions of the dopaminergic nigrostriatal neurons. Brain Res. 348: 283-288, 1985.

46. *Helen, P., Panula, P., Yang, H.-Y. T., Her-*

vonen, A. and Rapoport, S. I.: Location of substance P-, bombesin-gastrin-releasing peptide, [Met^5]enkephalin- and [Met^5]enkephalin- Arg^6-Phe^7-like immunoreactivities in adult human sympathetic ganglia. Neuroscience 12: 907-916, 1984.

47. *Hellerstein, A. W.*: Fetal research and fetal-tissue research: Policy debate but no resolution. FASEB J. 2: 3041-3042, 1988.

48. *Herrera-Marschitz, M., Strömberg, I., Olsson, D., Ungerstedt, U. and Olson, L.*: Adrenal medullary implants in the dopamine-denervated rat striatum. II. Acute behavior as a function of graft amount and location and its modulation by neuroleptics. Brain Res. 297: 53-61, 1984.

49. *Hökfelt, T., Elfvin, L. G., Elde, R. Schultzberg, M., Goldstein, M. and Luft, R.*: Occurrence of somatostatin-like immunoreactivity in some peripheral sympathic noradrenergic neurons. Proc. Natl. Acad. Sci. 74: 3587-3591, 1977a.

50. *Hökfelt, T., Elfvin, L.-G., Schultzberg, M., Fuxe, K., Said, S. I., Mutt, V. and Goldstein, M.*: Immunohistochemical evidence of vasoactive intestinal polypeptide-containing neurons and nerve fibers in sympathetic ganglia. Neuroscience 2: 885-896, 1977b.

51. *Hökfelt, T., Elfvin, L.-G., Schultzberg, M., Goldstein, M. and Oilsson, G.*: On the occurrence of substance P-containing fibers in sympathetic ganglia immunohistochemical evidence. Brain Res. 132: 29-41, 1977c.

52. *Hu, F. and Lesney, P. F.*: The isolation and cytology of two pigment cell strains from B16 mouse melanomas. Cancer Res. 24: 1634-1643, 1964.

53. *Hurtig, H., Joyce, J., Sladek, J. R. and Trojanowski, J. Q.*: Postmortem analysis of adrenal-medulla-to-caudate autograft in a patient with Parkinson's disease. Ann. Neurol. 25: 610-614, 1989.

54. *Itakura, T., Kamei, I., Kakai, K., Kaha, Y., Nakakita, K., Imai, H. and Komai, N.*: Autotransplantation of the superior cervical ganglion into he brain. A possible therapy for Parkinson's disease. J. Neurosurg. 68: 955-959, 1988.

55. *Jacobowitz, D. M. and Helke, C. J.*: Localization of substance P immunoreactive nerves in the carotid body. Brain Res. Bull. 5: 195-197, 1980.

56. *Jacobs, J. M.*: Penetration of systemically injected horseradish peroxidase into ganglia and nerves of the autonomic nervous system. J. Neurocytol. 6: 607-618, 1971.

57. *Jaeger, C. B.*: Immunocytochemical study of PC12 cells grafted to the brain of immature rats. Exp. Brain Res. 59: 615-624, 1985.

58. *Jaeger, C. B.*: Morphological and immunocytochemical characteristics of PC12 cell grafts in rat brain. In: Azmitia, E. C. and Björklund, A. (eds.). Cell and tissue transplantation into the adult brain, Ann. New York Acad. Sci., Vol. 495: 334-349, 1987.

59. *Jiao, S., Zhang, W., Cao, J., Zhang, Z., Wang, H., Ding, M. and Zhang, Z.*: Study of adrenal medullary tissue transplantation to striatum in parkinsonism. In: Gash, D. M. and Sladek, J. R. (eds.). Transplantation into the mammalian CNS, Prog. Brain. Res., Vol. 78: 575-580, Elsevier: Amsterdam, 1988.

60. *Kelley, P. J., Ahlskog, J. E., van Heerden, J. A., Carmichael, S. W., Stoddard, S. L. and Bell, G. N.*: Adrenal medullary autograft transplantation into the striatum of patients with Parkinson's disease. Mayo Clin. Proc. 64: 282-290, 1989.

61. *Kim, S. U., Warren, K. G. and Kalia, M.*: Tissue culture of adult human neurons. Neurosci. Lett. 11: 137-141, 1979.

62. *Kondo, H., Kuramoto, H. and Fujita, T.*: Neuropeptide tyrosine-like immunoreactive nerve fibers in the carotid body chemoreceptor of rats. Brain Res. 372: 353-356, 1986.

63. *König, R. and Heym, C.*: Immunofluorescent localization of dopamine-beta-hydroxylase in small intensely fluorescent cells of the rat superior cervical ganglion. Neurosci. Lett. 10: 187-191, 1978.

64. *Korfali, E., Doygun, M., Ulus, I. H., Rakunt, C. and Aksoy, K.*: Effects of Neuronotrophic factors on adrenal medulla grafts implanted into adult rat brains. Neurosurgery 22: 994-998, 1988.

65. *Krum, J. M. and Rosenstein, J. M.*: Patterns of angiogenesis in neural transplant models: I. Autonomic tissue transplants. J. Comp. Neurol. 258: 120-134, 1987.

66. *Laskin, J. D. and Piccinini, L.*: Tyrosinase isozyme heterogeneity in differentiating B16/C3 melanoma. J. Biol. Chem. 261: 16626-16635, 1986.

67. *Lever, J. D., Santer, R. M., Lu, K. S. and Presley, R.*: Electron probe x-ray microanalysis of small granulated cells in rat sympathetic ganglia after sequential aldehyde and dichromate treatment. J. Histochem. Cytochem. 25: 275-279, 1977.

68. *Libet, B. and Owman, C.*: Concomitant changes in formalehyde-induced fluorescence of dopamine interneurones and in slow inhibitory post-synaptic potentials of the rabbit superior cervical ganglion, induced by stimulation of the preganglionic nerve or by a muscarinic agent. J. Physiol. 237: 635-662, 1974.

69. *Lindvall, O., Backlund, E.-O., Farde, L., Sedvall, G., Freedman, R., Hoffer, B. and Nobin, A.*: Transplantation in Parkinson's disease: Two cases of adrenal medullary

grafts to the putamen. Ann. Neurol. 22: 457-468, 1987.

70. *Lindvall, O.*: Transplantation into the human brain: present status and future possibilities. J. Neurol. Neurosurg. Psychiat. Special supplement: 39-54, 1989.

71. *Lundberg, J. M., Hökfelt, T., Fahrenkrug, J., Nilsson, G. and Terenius, L.*: Peptides in the cat carotid body (glomus caroticum): VIP-, enkephalin- and substance P-like immunoreactivity. Acta Physiol. Scand. 107: 279-281, 1979.

72. *Lundberg, J. M., Änggard, A., Pernow, J. and Hökfelt, T.*: Neuropeptide Y-, substance P-, and VIP-immunoreactive nerves in cat spleen in relation to autonomic vascular and volume control. Cell Tissue Res. 239: 9-18, 1985.

73. *Madrazo, I., Drucker-Colín, R., Díaz, V., Martínez-Mata, J., Torres, C. and Becerril, J. J.*: Open microsurgical autograft of adrenal medulla to the right caudate nucleus in two patients with intractable Parkinson's disease. New Engl. J. Med. 316: 831-834, 1987a.

74. *Madrazo, I., Drucker-Colín, R., León, V. and Torres, C.*: Adrenal medulla transplanted to caudate nucleus for treatment of Parkinson's disease: Report of 10 cases. Surg. Forum 38: 510-512, 1987b.

75. *Matthews, M. R. and Raisman, G.*: The ultrastructure and somatic efferent synapses of small granule-containing cells in the superior cervical ganglion. J. Anat. 105: 255-282, 1969.

76. *McDonald, D. and Mitchell, R. A.*: The innervation of glomus cells, ganglion cells and blood vessels in the rat carotid body: a quantitative ultrastructural analysis. J. Neurocytol. 4: 177-230, 1975.

77. *McRae-Degueurce, A., Hjorth, S., Dillon, D. L., Mason, D. W. and Tice, T. R.*: Implantable microencapsulated dopamine (DA): a new approach for slow-release DA delivery into brain tissue. Neurosci. Lett. 92: 303-309, 1988.

78. *McCrae-Degueurce, A., Klawans, H. L., Penn, R.D., Dahlstrom, A., Tanner, C. M., Goetz, C. G. and Carvey, P. M.*: An antibody in the CSF of Parkinson's disease patients disappears following adrenal medulla transplantation. Neurosci. Lett. 94: 192-197, 1988.

79. *Morihisa, J. M., Kakamura, R. K., Freed, W. J., Mishkin, M. and Wyatt, R. J.*: Adrenal medulla grafts survive and exhibit catecholamine-specific fluorescence in the primate brain. Exp. Neurol. 84: 643-653, 1984.

80. *Nakai, M., Itakura, T., Kamai, I., Kakai, K., Naka, Y., Imai, H. and Komai, N.*: Autologous transplantation of the superior cervical ganglion into the brain of parkinsonian monkeys. J. Neurosurg. 73: 91-95, 1990.

81. *Nishino, H., Ono, T., Shibata, R., Kawamata, S., Watanabe, H., Shiosaka, S. and Tohyama, M.*: Adrenal medullary cells transmute into dopaminergic neurons in dopaminedepleted rat caudate and ameliorate motor disturbances. Brain Res. 445: 325-337, 1988.

82. *Paivarinta, H., Happöla, O., Joh, H., Steinbusch, H., Watanabe, T. and Panula, P.*: Immunocytochemical colocalization of histamine, histidine decarboxylase, 5-hydroxytryptamine and tyrosine hydroxylase in the superior cervical ganglion of the rat. Histochem. J. 19: 319-326, 1987.

83. *Pasik, P., Martínez, J. P., Yahr, M. D., Pasik, T., Holstein, G. R. and Schanzer, H.*: Grafting of human sympathetic ganglia into the brain of MPTP-treated monkeys. Soc. Neurosci. Abstr. 13: 4, 1988.

84. *Patel-Vaidya, U., Wells, M. R. and Freed, W. J.*: Survival of dissociated adrenal chromaffin cells of rat and monkey transplanted into rat brain. Cell Tissue Res. 240: 281-285, 1985.

85. *Penn, R. D., Goetz, C. G., Tanner, C. M., Klawans, H. L., Shannon, K. M., Comella, C. L. and Witt, T. R.*: The adrenal medullary transplant operation for Parkinson's disease: clinical observations in five patients. Neurosurgery 22: 999-1004, 1988.

86. *Peterson, D. I., Price, L. and Small, C. S.*: Autopsy findings in a patient who had an adrenal-to-brain transplant for Parkinson's disease. Neurology 39: 235-238, 1989.

87. *Pezzoli, G., Fahn, S., Dwork, A., Truong, D. D., Yebenes, J. G., Jackson-Lewis, V., Herbert, J. et al.*: Non-chromaffin tissue plus nerve growth factor reduces experimental parkinsonism in aged rats. Brain Res. 459: 398-403, 1988.

88. *Purves, D.*: Functional and structural changes in mammalian sympathetic neurones following interruption of their axons. J. Physiol. 252: 429-463, 1975.

89. *Rosenstein, J. M.*: Adrenal medulla grafts produce blood-brain barrier dysfunction. Brain Res. 414: 192-196, 1987.

90. *Rosenstein, J. M. and Brightman, M. W.*: Regeneration and myelination in autonomic ganglia transplanted to intact brain surfaces. J. Neurocytol. 8: 359-379, 1979.

91. *Rosenstein, J. M. and Brightman, M.*: Anamalous migration of central nervous tissue to transplanted autonomic ganglia. J. Neurocytol. 10: 387-409, 1981.

92. *Rosenstein, J. M. and Brightman, M. W.*: Alterations of the blood-brain barrier after transplantation of autonomic ganglia into the mammalian central nervous system. J. Comp. Neurol. 250: 339-351, 1986.

93. *Schultzberg, M., Hökfelt, T., Terenius, L., Elfvin, L.-G., Lundberg, J. M., Brandt, J. and Elde, R. P.*: Enkephalin immunoreactive nerve fibres and cell bodies in sympathetic ganglia of the guinea-pig and rat. Neuroscience 4: 249-270, 1979.

94. *Sendelbek, S. L. and Urquhart, J.*: Spatial distribution of dopamine, methotrexate and antipyrine during continuous intracerebral microperfusion. Brain Res. 328: 251-258, 1985.

95. *Stenevi, U., Björklund, A. and Svendgaard, N-A.*: Transplantation of central and peripheral monoamine neurons to the adult rat brain: Techniques and conditions for survival. Brain Res. 114: 1-20, 1976.

96. *Stoddard, S. L., Ahlskog, J. E., Kelly, P. J., Tyce, G. M., van Heerden, J. A., Zinsmeister, A. R. and Carmichael, S. W.*: Decreased adrenal medullary catecholamines in adrenal transplanted parkinsonian patients compared to nephrectomy patients. Exp. Neurol. 104: 218-222, 1989a.

97. *Stoddard, S. L., Tyce, G. M., Ahlskog, J. E., Zinsmeister, A. R. and Carmichael, S. W.*: Decreased catecholamine content in Parkinsonian adrenal medullae. Exp. Neurol. 104: 22-27, 1989b.

98. *Strömberg, I., Herrera-Marschitz, M., Hultgren, L., Ungerstedt, U. and Olson, L.*: Adrenal medullary implants in the dopamine denervated rat striatum. I. Acute catecholamine levels in grafts and host caudate as determined by HPLC-electrochemistry and fluorescence histochemical image analysis. Brain Res. 297: 41-54, 1984.

99. *Strömberg, I., Herrera-Marschitz, M., Ungerstedt, U., Ebendal, T. D. and Olson, L.*: Chronic implants of chromaffin tissue into the dopamine-denervated striatum. Effects of NGF on graft survival, fiber growth and rotational behavior. Exp. Brain Res. 60: 335-349, 1985.

100. *Tyce, G. M., Ahlskog, J. E., Carmichael, S. W., Chritton, S. L., Stoddard, S. L., van Heerden, J. A. and Yaksh, T. L.*: Catecholamines in CSF, plasma, and tissue after autologous transplantation of adrenal medulla to the brain in patients with Parkinson's disease. J. Lab. Clin. Med. 114: 185-192, 1989.

101. *Venna, N., Sabin, T. D., Ordia, J. O. and Mark, V. H.*: Treatment of severe Parkinson's disease by intraventricular injection of dopamine. Appl. Neurophysiol. 47: 62-64, 1984.

102. *Verfofstad, A. A. J., Steinbusch, H. W. M., Penke, B., Varga, J. and Joosten, H. J. J.*: Serotonin-immunoreactive cells in the superior cervical ganglion of the rat. Evidence for the existence of separate serotonin- and catecholamine-containing small ganglionic cells. Brain Res. 212: 39-49, 1981.

103. *Wakai, S., Meiselman, S. E. and Brightman, M. W.*: Focal circumvention of blood-brain barrier with grafts of muscle, skin and autonomic ganglia. Brain Res. 386: 209-222, 1986.

104. *Ward, J. W.*: A histological study of transplanted sympathetic ganglia. Am. J. Anat. 58: 147-177, 1936.

105. *Wharton, J., Polack, J. M., Pearse, A. G. E., McGregor, G. P., Bryant, M. G., Bloom, S. R., Emson, P. C. et al.*: Enkephalin-, VIP- and substance P-like immunoreactivity in the carotid body. Nature 284: 269-271, 1980.

106. *Williams, T. H. W.*: Electron microscopic evidence for an autonomic interneuron. Nature 214: 309-310, 1967.

107. *Williams, T. H., Black, A. C., Chiba, T. and Bhalla, R. C.*: Morphology and biochemistry of small, intensely fluorescent cells of sympathetic ganglia. Nature 256: 315-317, 1975.

108. *Williams, T. H. and Palay, S. L.*: Ultrastructure of the small neurons in the superior cervical ganglion. Brain Res. 15: 17-34, 1969.

109. *Winkler, H. and Westhead, E.*: The molecular organization of adrenal chromaffin granules. Neuroscience 5: 1803-1823, 1980.

110. *Zhou, C.-F., Lawrence, R. J., Morris, R. J. and Raisman, G.*: Migration of host astrocytes into superior cervical sympathetic ganglia autografted into the septal nuclei or chroid fissure of adult rats. Neuroscience 3: 815-827, 1986.

111. *Zhau, C.-F., Lindsay, R. M., Lawrence, J. M. and Raisman, G.*: Extent of survival and vascularization of adult superior cervical sympathetic or modose ganglia transplanted into the septal nuclei or choroid fissure of adult rats. Neuroscience 3: 803-813, 1986.

History and Current Status of the Functional Effects of Cholinergic Brain Implants[1]

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ABSTRACT

Several studies involving implantation of cholinergic-rich fetal brain grafts in rodents have been carried out. Host sites in the brain include the septohippocampal pathway following aspiration lesion of the fimbria fornix, and the neocortex, following neurotoxic lesions of the basal forebrain cholinergic system. Cholinergic-rich grafts have also been placed in the hippocampal formation of aged rats. Several laboratories have reported partial restorative effects of such grafts on lesion-induced or age-related deficits. For example, fimbria-fornix lesions produce deficits in rewarded alternation and maze learning. These deficits are ameliorated by cholinergic-rich grafts. In some studies, pharmacological manipulations support the conclusion that recovery is based on cholinergic mechanisms. Cholinergic-rich grafts placed in the neocortex have had more limited effects, but partial restorative effects have been seen in the retention of a passive avoidance task, and in the use of a spatial strategy in a water maze in rats with basal forebrain lesions. Little effects has been noted on lesion-induced hyperactivity. Mixed effects have been noted on various sensorimotor tasks in these animals.

HISTORY AND CURRENT STATUS OF THE FUNCTIONAL EFFECTS OF CHOLINERGIC BRAIN IMPLANTS D. P. KIMBLE

Within the last two decades, methods for the transplantation of fetal brain tissue into the CNS of adult host animals have been developed to the point where such procedures can now be considered to be well-established in neuroscience. Transplants of fetal brain tissue can be characterized not only by their site of origin in the fetal brain, but by the predominant neurotransmitter present within the graft. Thus, although such characterization is a simplification (as other neurotransmitters are present even in small fragments from the CNS), tissue from the fetal substantia nigra has been characterized as primarily *dopaminergic*, tissue from the fetal locus coeruleus has been described as primarily *noradrenergic*, and tissue from the septal nucleus/diagonal band region has been described as primarily *cholinergic*.

Cholinergic systems in the mammalian brain. The identification of clusters of neuronal cell bodies that contain acetylcholine (ACh) has led to the construction of ACh maps for different species. There is no current method for accurately localizing ACh itself, but there is considerable agreement on the localization of cholinergic neurons based on the use of monoclonal antibodies for the ACh synthesizing enzyme, choline acetyltransferase (ChAT). Histochemical localization of the enzyme that degrades ACh, acetylcholinesterase (AChE), is also widely used to localize cholinergic neurons. This enzyme is not limited to cholinergic neurons, although in some regions, such as the basal forebrain, it is a very reliable marker of cholinergic neurons (Mesulam, Mufson, Wainer, and Levey, 1983b).

Mesulam, Mufson, Levey and Wainer

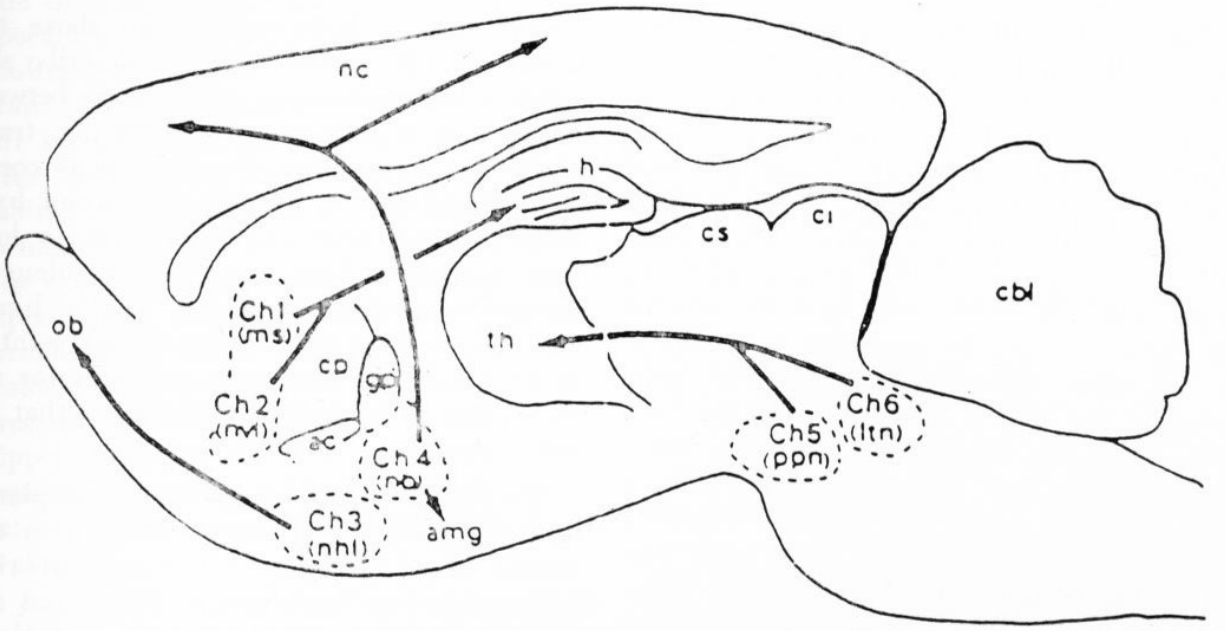

Fig. 1. — *Shematic represntation of some ascending cholinergic pathways. The traditional nuclear groups which most closely correspond to the Ch subdivisions are indicated in parentheses. Abbreviations: ac, anterior commissure; amg, amygdala; cbl, cerebellum; ci, inferior colliculus; cp, caudate-putamen; cs, superior colliculus; gp, globus pallidus; h, hippocampus; ltn, laterodorsal tegmental nucleus; ms, medial septal nucleus; nb, nucleus basalis; nc, neocortex; nhl, nucleus of the horizontal limb complex; nvl, nucleus of the vertical limb complex; ob, olfactory bulb; ppn, pedunculopontine tegmental nucleus; th, thalamus (from Wainer et al., 1984).*

(1983a) have provided a useful nomenclature and map (Wainer, Levey, Mufson, and Mesulam, 1984) of cholinergic systems in the rat brain based on their anatomical findings using both ChAT monoclonal staining techniques and AChE histochemistry. These results are directly relevant to this review, as virtually all of the research involving transplantation of cholinergic fetal brain tissue has involved rodents, usually the rat. Six major sectors of cholinergic cell bodies, representing sources of projection pathways to various areas of the brain, are shown in Figure 1.

Sectors Chl and Ch2 refer to cholinergic cell bodies in the medial septal nuclei and vertical limb of the diagonal band of Broca. These neurons project to the hippocampal formation and provide this structure with most of its cholinergic afferent input. It should be noted, however, that somewhat over half of the projection fibers from neurons in the medial septal nuclei and vertical limb of the diagonal band are non-cholinergic (Wainer, Levey, Rye, Mesulam, and Mufson, 1985). Sector Ch3 is located primarily in the lateral portion of the horizontal limb nucleus of the diagonal band, and projects primarily to the olfactory bulb. Ch4 sector refers to cholinergic neurons in the nucleus basalis and nearby regions of the basal forebrain and projects to widespread areas of the neocortex as well as the amygdala. In the human, the homologous nuclei that comprise this sector include the nucleus basalis of Meynert and nuclei such as nucleus substantia innominata and the nucleus of the ansa peduncularis (Wainer et al., 1984). Degeneration of this system in humans has been implicated in the cognitive deficits associated with Alzheimer's disease (Bartus, Dean, Beer, and Lippa, 1982; Coyle, Price, and DeLong, 1983).

Sectors Ch5 and Ch6 refer to those neurons contained within the pedunculopontine nucleus of the pontomesencephalic reticular formation (Ch5) and the laterodorsal tegmental gray of the periventricular area (Ch6). These neurons provide the major cholinergic input to the thalamus and some afferents to the neocortex. General agreement with this distribution of cholinergic neurons in the rat is to be found in other research reports, although some minor dis-

crepancies do still exist (Sofroniew, Campbell, Cuello, and Eckenstein, 1985).

Fetal brain transplants involving cholinergic systems. Experiments involving either the implantation of cholinergic fetal brain tissue or implantation of fetal tissue into one of the above cholinergic sectors will be discussed in this review. In some cases, both the fetal tissue and the site of the transplant can be classified as cholinergic; in other experiments either the graft tissue or the site is cholinergic, but not both. All of the experiments involving brain grafts to date have been confined to sectors Ch1, Ch2, and Ch4. To this reviewer's knowledge, no tissue grafts have been placed within sectors Ch3, Ch5 or Ch6. Also, as noted above, in all reports where functional aspects have been examined, the subjects have been rodents.

Terminology. Some of the terms used in transplantation research are listed below (Freed, 1983). These terms are useful in describing the genetic relationship between donor and host, or the anatomical relationship between the location of the graft and host tissue.

Allograft. Allografts are grafts made between different individuals from the same species, but known to be genetically dissimilar, such as between rats of the same strain obtained from the same supplier. A similar term, *homograft*, is used for grafts between individuals presumed, but not known with certainty to be genetically dissimilar.

Heterograft. These are grafts between individuals of different species, such as mouse tissue grafted into rat CNS. As a general rule, the closer the degree of genetic similarity, the greater the degree of survival of the graft tissue; but successful cross-species transplants have been reported (Björklund, Stenevi, Dunnett, and Gage, 1982; Daniloff, Bodony, Low, and Wells, 1985; Sollars and Kimble, 1988).

Homotopic graft. This term refers to a placement of donor graft tissue into the same or very similar location as its site of origin in the fetal brain.

Heterotopic graft. Tissue transplanted into a region different from its site of origin is termed heterotopic. The above two terms are often insufficient to describe adequately the anatomical relationship between donor and host tissue. For example, transplants of tissue from the fetal locus coeruleus placed into a cavity produced by a fimbria-fornix lesion is heterotopic in location, but axons from the locus coeruleus do normally terminate in the nearby hippocampus, so that from a functional point of view, this translation site provides for the possibility of graft-host connections that are not unusual.

Technical details regarding transplantation procedures will not be covered in this article. Good reviews of transplantation techniques can be found in Björklund and Stenevi (1985), and Sladek and Gash (1984).

Cholinergic tissue grafts and the septohippocampal syndrome. When the neural connections between the hippocampus and the septal nuclei are severed, a syndrome consisting of maze learning deficits, reduction of alternation behavior, and other behavioral changes is produced in rats. These changes can be considered to constitute a rodent "septohippocampal syndrome". Reviews of these behavioral effects have been published (O'Keefe and Nadel, 1978; Olton, Becker, and Handelmann, 1979). A similar behavioral syndrome has been observed with lesions to the anterior hippocampal formation itself (Kimble, 1975).

The lesion that produces the septohippocampal syndrome in rats also provides a suitable transplantation site for exogenous tissue. An aspiration lesion of the fimbria-fornix, along with supracallosal stria, produces a cavity that exposes a rich vasculature bed on the dorsal surface of the thalamus that provides a particularly good location for the survival of fetal brain tissue grafts (Gage, Björklund, Stenevi, and Dunnett, 1985). Thus, production of a recognizable behavioral syndrome and a suitable transplantation site can be accomplished with the same surgical procedure.

The fimbria-fornix includes cholinergic axons from neurons in the medial septal nucleus and the vertical limb of the diagonal band (MS/VDB). These axons consti-

tute the main cholinergic input into the hippocampal formation. There is also a ventral cholinergic pathway from the basal forebrain to the hippocampal formation that is left intact following fimbria-fornix lesions. This ventral path supplies 10-15 % of the cholinergic innervation of the hippocampal formation (Gage and Björklund, 1986a). The extent of cholinergic innervation of the hippocampal formation following fimbria-fornix lesions usually has been evaluated by histochemical procedures that measure the presence and distribution of AChE in the hippocampal formation. In some cases, measurements of ChAT have been carried out, sometimes in conjunction with AChE evaluation.

Possible mechanisms by which brain grafts could affect function of the host.

At present, there are no mechanisms that can be definitely identified as being responsible for observed functional effects. Several possibilities exist. For example, implantation of brain tissue could reduce the degree of necrosis of host tissue, or improve the recovery of damaged tissue, perhaps by providing various neurotrophic substances. For example, rat fetal hippocampal tissue contains nerve growth factor (NGF) (Thoenen, Bandtlow, and Heumann, 1987). NGF has been reported to increase the survival and regeneration of cholinergic neurons from the septal nuclei, diagonal band area and basal forebrain following fimbria-fornix lesions (Hefti, 1986; Williams et al., 1986; Kromer, 1987).

Second, fetal brain tissue (and indeed, other types of tissue as well) can serve as a suitable matrix or substrate for the regeneration of host axons transected by lesions. Such "tissue bridges" have been observed with fetal hippocampal tissue implanted into the cavity produced by fimbria-fornix lesions, as have other tissues such as amnionic membrane (Davis, et al., 1987).

Third, implanted brain tissue could serve as a source of neurotransmitter substances and/or other neuroregulators that influence host neurons without the development of any synaptic contacts between the graft and host tissue. Such organic "minipumps" could be self-regulated, or (if some synaptic connections are formed from host to graft tissue) in part influenced by the host CNS (Björklund et al., 1987).

Fourth, the graft could provide novel but functional synaptic input to nearby host neurons. Some evidence exists that cholinergic grafts can form functional reciprocal synapses with host brain neurons. Whether or not such synaptogenesis can duplicate normal circuitry is not known. These possibilities do not exhaust the list of potential mechanisms of action of brain implants, but they provide some framework in which to view the current results. It should be emphasized that this area of research is new; results are, for the most part, preliminary, and in no case can the mechanism of action be identified with certainty.

Amelioration of the septohippocampal syndrome with fetal cholinergic-rich transplants. In several different experiments transplants of fetal septal tissue have been placed into the CNS cavity produced by a fimbria-fornix lesion. Partial amelioration of the behavioral changes produced by the lesion have been noted in several situations. Dunnett, Low, Iversen, Stenevi, and Björklud (1982) examined the behavior of rats with bilateral fimbria-fornix lesions after they had been implanted with fetal tissue grafts that were either cholinergic (from septal area) or noradrenergic (from locus coeruleus). Partial recovery of rewarded alternation in a T-maze was observed in those rats receiving septal tissue, but not in those rats implanted with locus coeruleus tissue. In 11/14 rats receiving septal transplants, the level of performance approached 90 % correct, not significantly different from shamoperated controls. The other three septal-grafted rats showed no behavioral improvement. Lesion-induced increases in levels of spontaneous activity in an open field and in home cages was found to be unchanged by any grafts. Histological results revealed that there was a significant positive correlation between the degree of recovery seen in the alternation task and the degree of cholinergic ingrowth into the hippocampus from the septal grafts.

In related experiments, Dunnett, Gage, Björklund, Stenevi, Low, and Iversen

(1982) reported improvement in fimbria-fornix lesion-induced deficits in the learning of a radial-arm maze and a water-maze when rats with septal grafts were injected with the cholinergic-potentiating agent physostigmine. Rats with grafts of tissue from the fetal locus coeruleus showed no change in maze performance, with or without physostigmine injections. Interestingly, in this study fetal locus coeruleus grafts did significantly normalize the lesion-induced hyperactivity observed in home cages, suggesting that alternation behavior may be more dependent upon cholinergic innervation of the hippocampal formation, and under some circumstances at least, normalized activity levels may be dependent upon noradrenergic innervation of the hippocampal formation.

In both of these studies, the cholinergic ingrowth into the hippocampal formation in the rats with septal grafts was derived primarily from neurons in the graft tissue, although some ingrowth, particularly in the ventral hippocampus, appears to have come from the sprouting of intact ventral cholinergic pathways from the basal forebrain not severed by the lesion. The spatial distribution of the cholinergic reinnervation was approximately normal, and the ingrowing axons seem to be specifically directed toward terminal zones appropriate for septal neurons, although the course taken by ingrowing axons did not follow any anatomically recognizable path. Grafts from the implanted locus coeruleus tissue showed a similar specificity of reinnervation, growing primarily into terminal zones in the hippocampal formation normally innervated by noradrenergic axons.

More recent experiments by Nilsson, Shapiro, Gage, Olton, and Björklund (1987) have examined the effects of the implantation of fetal septal neurons following fimbria-fornix transections on performance in the Morris water maze. Rats with bilateral septohippocampal pathway transections were implanted with fetal septal tissue in the lesion cavity, and their performance in the water maze examined. Grafted rats showed significant recovery in using spatial strategies in finding the platform hidden just below the surface of the water. Atropine injections abolished this recovery.

Implants of fetal septal tissue apparently can produce viable synapses betwen graft neurons and host hippocampal neurons. Using electrophysiological techniques, Segal, Björklund, and Gage (1985), found that slices from grafthost tissue that included septal and hippocampal components could be investigated in tissue culture. Responses recorded from the host hippocampal tissue could be found when the graft tissue was stimulated electrically. These responses were blocked by locally applied atropine and potentiated by physostigmine, as would be anticipated if the responses were being mediated by cholinergic synapses.

Still other studies have examined the functional effects produced by placing fetal hippocampal tissue into cavities produced by aspiration lesions of the anterior hippocampus itself. Kimble, BreMiller, and Stickrod (1986) found that such implants significantly improved spatial maze learning in lesioned animals. Functional recovery was not complete, but those animals in which clear fusion of graft and host tissue was seen showed better behavioral recovery than in those animals in which the graft tissue was associated only with restricted regions of the host hippocampal formation. In a similarly designed study, Woodruff, Baisden, Whittington, and Benson (1987) placed grafts of fetal hippocampal tissue into the cavity produced by dorsal hippocampal lesions in rats. They found that such implants partially corrected a lesion-induced deficit in DRL (differential reinforcement of low rats) in an operant situation. Transplants of fetal tissue from the brainstem placed into similar locations in other rats survived, but did not produce any substantial improvement in the DRL task, although some other behavioral effects were observed in these animals.

Just how such tissue implants effect behavioral change in the above two studies is not known. One possibility is that the implanted tissue was able to provide a matrix or "tissue bridge" such that regenerating host neurons that had been axotomized but not killed by the lesion could reinnervate

portions of the host hippocampal formation. The most likely candidates for such regeneration are the neurons located in the medial septal nucleus and vertical diagonal band (MS/VDB). It has been established that similar implants can serve as tissue bridges for the regeneration of MS/VDB neurons in the rat brain (Kromer, Björklund, and Stenevi, 1981). No behavioral observations were made in these animals, but Segal, Stenevi, and Björklund (1981) found that in animals with similar bridging grafts excitatory atropine-sensitive connections were formed not only in the grafted hippocampal tissue, but in the dorsal region of the host hippocampus. These observations demonstrate that such implants can support functional regeneration across a substantial lesion within the CNS of mammals. Whether or not noncholinergic axons from the MS/VDB also regenerated is not at present known.

Another possible explanation for the Kimble et al. (1986) and the Woodruff et al. (1987) findings is that the implanted fetal tissue contains neurotrophic factors that increase the survival of axotomized septal neurons, and provide support for the regeneration of these axons and their reinnervation of target neurons in the hippocampal formation, independent or in conjunction with any bridging function the implanted tissue might serve. The best characterized neurotrophic factor in hippocampal tissue is nerve growth factor (NGF). Kimble, BreMiller, Stickrod, and Matthews (in preparation) have investigated the capacity of NGF to improve maze learning ability in rats following fimbria-fornix lesions. Rats were implanted in the cavity produced by a bilateral fimbria-fornix lesions with sub-maxillary tissue from male mice, a rich source of NGF. Other fimbria-fornix lesioned rats were implanted with sub-maxillary tissue and, in addition, two to three weeks later, with co-grafts of either fetal hippocampal tissue or rat amnionic membrane attached to nitrocellulose paper. Both of these latter materials were designed to provide possible substrates for regenerating MS/VDB axons. Male mouse submaxillary tissue has been demonstrated to increase the survival of axotomized medial septal neurons in the rat when placed in the lateral ventricle adjacent to the septal area in rats with fimbria-fornix lesions (Springer, Collier, Sladek, and Loy, in press).

In the Kimble, BreMiller, Stickrod, and Matthews study all three groups of implanted rats showed initial maze learning deficits comparable to that of animals given only fimbria-fornix lesions. In tests made at four, five and six months postoperatively, however, all implanted groups showed significant recovery of maze learning capacity (See Figure 2). No significant differences appeared among the two co-grafted groups and the group that received submaxillary tissue only, suggesting that the presence of male mouse sub-maxillary tissue itself is sufficient for significant behavioral recovery. It is possible this tissue provided NGF as well as a suitable tissue matrix for neuronal regeneration. Histology to determine the extent of septal neuronal survival and degree of reinnervation of host hippocampal formation is now in progress.

Cholinergic brain implants in rats with basal forebrain lesions: An animal model of Alzheimer's disease? A major cholinergic system in the rat brain is the Ch4 sector associated with neuronal cell bodies in the basal forebrain known in the ventral forebrain nucleus basalis magnocellularis (NBM) (homologous to human nucleus basalis of Meynert). Histological examination of the brains of Alzheimer's patients has revealed marked decreases in the markers for acetylcholine in the neocortex and hippocampal formation, and evidence for degeneration and atrophy in these basal forebrain cholinergic neurons. While abnormalities in other neurotransmitter systems are also found in Alzheimer's patients, the decrease in cholinergic innervation of the neocortex and hippocampal formation has been considered to be the primary defect in this condition (Bartus et al., 1982; Coyle et al., 1983). The possible relationship between degeneration of the basal forebrain cholinergic system and Alzheimer's disease (and possibly other dementias) has led to attempts to develop an animal model of Alzheimer's disease based on interruptions of

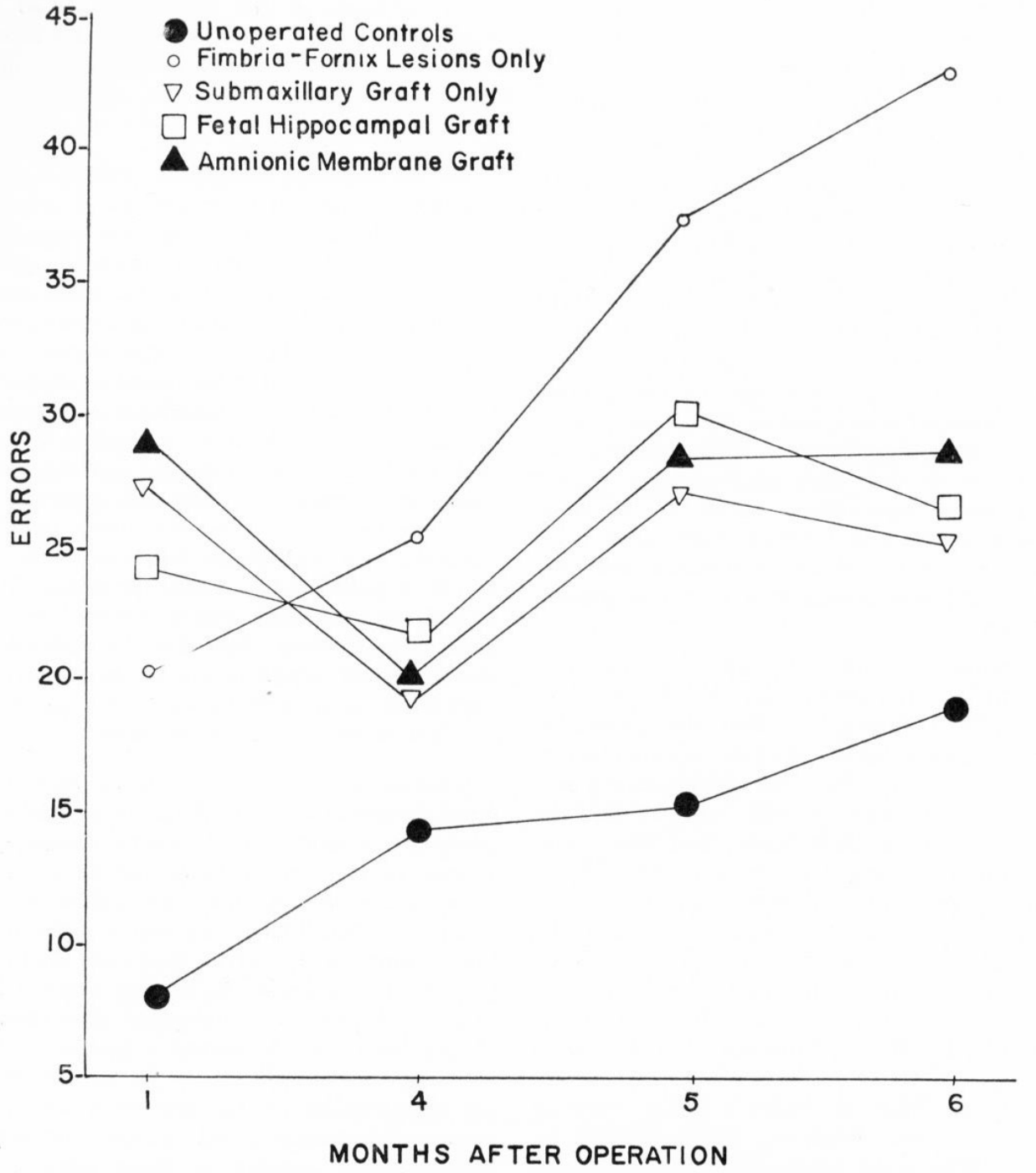

Fig. 2. — *Maze performance on four problems of increasing difficulty, at various postoperative times (Kimble, BreMiller, Stickrod and Matthews [in preparation]).*

this system. Administration of AF64A, a toxin structurally similar to choline, destroys cholinergic nerve terminals and interferes with cholinergic function (Smith, 1988), but to date no transplant studies using this technique have come to the attention of this reviewer.

Relatively selective lesions of cell bodies (sparing axons of passage) in the basal forebrain have been attempted using other neurotoxins such as ibotenic acid. Such treatment has been reported to produce a 40-75 % reduction in the levels of cholinergic innervation of the ipsilateral neocortex as evaluated by measurements of choline acetyltransferase (Whishaw, O'Conner, and Dunnett, 1985; Fine, Dunnett, Björklund, Clarke, and Iversen, 1985). Such disruptions of the cholinergic innervation of the neocortex produces a variety of long-lasting

behavioral deficits. These include delayed alternation, water-maze performance, impaired passive avoidance learning, and a locomotor hyperactivity related to reduced within-trial habituation. More transient abnormalities include decreases in food and water consumption, sensory neglect and changes in several different sensorimotor coordination tasks (Whishaw et al., 1985; Dunnett, Toniolo, Fine, Ryan, Björklund, and Iversen, 1985).

Several reports have appeared in which the cholinergic input to the neocortex has been partially restored by transplantation of cholinergic-rich fetal brain tissue into the neocortex of rats with ibotenic lesions of the NBM (e.g., Fine et al., 1985; Dunnett, et al., 1985). In these experiments NBM lesions were performed unilaterally. These lesions resulted in a 60 % decrease in cholinergic markers in the ipsilateral dorsolateral frontal and frontoparietal cortex. The fetal brain tissue used for grafts was taken from the region of the ventral forebrain. These grafts presumably contained at least some of the cholinergic neurons that normally innervate the forebrain. When placed on the surface of the host frontal cortex these grafts sent fibers 3 mm or more into the host brain, resulting in a restoration of ChAT activity to approximately 60 % of normal. Other putative neurotransmitters were identified in the graft tissue, but with the exception of some neuropeptide. Y cells, no other identified neurons were observed to penetrate the host-graft interface. As a control measure, fetal hippocampal tissue (which contains few, if any, cholinergic cell bodies) was also placed on the surface of the cortex in some NBM-lesioned rats.

Both acquisition and retention measures were made in the passive avoidance task. Cholinergic-rich ventral forebrain grafts did ameliorate the lesion-induced *retention* deficit but had no effect on the *acquisition* of this same task. In the water-maze no improvement was seen in the cholinergic grafted rats in learning to escape by finding the location of the hidden platforms, using escape latencies as a measure. However, when a special "spatial probe" test was used on the last day of the test (in which the hidden escape platform is removed and the animals' paths evaluated), the animals with ventral forebrain grafts displayed much more normal goal directed swimming than did animals with control grafts or lesion-only controls. No changes were seen in lesion-induced hyperactivity, but some amelioration was observed in the sensory neglect and in some of the sensorimotor coordination tasks. Throughout these experiments no differences were seen in lesion-only controls and those animals receiving cholinergic-poor tissue taken from the hippocampal formation. Because of the limited penetration by cholinergic graft neurons into the host brain, and the abnormal location of the graft tissue, it is unlikely that normal circuitry could have been restored in these animals. For example, the graft neurons would have been unlikely to receive the afferents that basal forebrain neurons would normally receive. It is possible that the grafts acted as mini-pumps for the secretion of acetylcholine and/or other substances that tended to normalize the function of the neocortical neurons denervated by the basal forebrain lesions. While some synaptic contacts may have been formed by graft neurons, it is also possible that a more diffuse or *paracrine* effect of graft-released substances may have been responsible for the behavioral effects. On the other hand, analysis of the ingrowth from the grafts was not random, but tended to follow the typical laminar pattern normally provided by the basal forebrain cholinergic system. This result is not totally compatible with a paracrine explanation, but does not rule it out either.

Cholinergic implants in aged rats. Cholinergic implant experiments have also been conducted with intact aged rats. For example, Gage, Björklund, Stenevi, Dunnett, and Kelly (1984) found that grafts of fetal septal cells into the ventral hippocampus improved the performance of aged (22-23 months old) rats. Only about 25-33 % of rats of this age show any impairment on the spatial learning of the water-maze task used in these studies. Old, impaired rats not given any transplant tissue were used as controls for old impaired, implanted rats. Two to three months after surgery rats with cholinergic grafts improved in their maze

performance, but old impaired, non-implanted rats did not. The brains of the implanted rats showed extensive AChE innervation of the host hippocampal formation (intrinsic septohippocampal innervation was removed by fimbria-fornix lesions a week before the animals were sacrificed). Follow-up studies (Gage and Björklund, 1986b) demonstrated that the behavioral improvement was indeed due to some cholinergic influence as it was completely abolished by atropine.

SUMMARY AND CONCLUSIONS

A growing body of evidence suggests that implants of fetal brain tissue, as well as other types of tissue, are accepted by host brain tissue and can influence the behavior of the host via interactions with the host brain. In the cholinergic systems of the brain, such graft procedures have been shown to influence cognitive behaviors in animals with fimbria-fornix lesions, aged animals, and animals with neurotoxic lesions of the basal forebrain. In all cases, some improvement in cognitive behaviors such as maze learning have been observed. The mechanisms by which such tissue implants remain to be determined, although several possibilities have been suggested, including the hypothesis that such tissue implants provide important neurotrophic substances that support survival of axotomized host neurons and the reinnervation of normal target neurons. Another possibility is that tissue implants can serve as a source of neurotransmitter substances that can partially ameliorate deficits in the functioning of the host brain.

RESUMEN

Un creciente número de evidencias sugieren que implantes de tejido nervioso fetal, lo mismo que de otros tipos de tejidos, son aceptados por el tejido nervioso receptor; estos implantes pueden influir en la conducta del receptor por interacción con su sistema nervioso. En lo que concierne al sistema colinérgico cerebral, se ha demostrado que tales injertos pueden influir la conducta cognitiva de los animales portadores de lesiones del fimbria-fornix, de animales mayores y de animales que han sufrido lesiones neuroquímicas de la base del encéfalo. En cada caso mejorías de conductas cognitivas, tales como el aprendizaje laberíntico han sido observadas. Los mecanismos por los cuales estos injertos de tejido afectan el funcionamiento del cerebro y la conducta del receptor quedan por determinar. Varias posibilidades se han adelantado como particular la hipótesis que el injerto suministra substancias neurotróficas importantes que permiten la sobrevida de neuronas axotomizadas del receptor y la reinervación de neuronas blancos normales. Otra posibilidad es que el tejido implantado constituye una fuente de neurotransmisor que puede mejorar parcialmente las deficiencias de funcionamiento del cerebro receptor.

RÉSUMÉ ET CONCLUSIONS

Un nombre croissant d'évidences suggère que des implants de tissu nerveux foetal, de même que d'autres types de tissu, sont acceptés par le tissu nerveux du receveur; ces implants peuvent influencer le comportement de l'hôte en interagissant avec son système nerveux. En ce qui concerne le système cholinergique cérébral, il a été démontré que de telles greffes peuvent influencer le comportement cognitif d'animaux porteurs de lésions du fimbria-fornix, d'animaux âgés, et d'animaux ayant subi des lésions neurochimiques de la base de l'encéphale. Dans chaque cas, des ameliorations de comportements cognitifs tels que l'apprentissage de labyrinthe, ont été observées. Les mécanismes par lesquels ces greffes de tissu affectent le fonctionnement du cerveau et le comportement du receveur restent à déterminer. Plusieurs possibilités ont été avancées, comme en particulier l'hypothèse que le greffon fournisse des substances neurotrophiques importantes permettant la survie de neurones axotomisés du

receveur et la réinnervation de neurones cibles normaux. Une autre possibilité est que le tissu implanté constitue une source de neurotransmetteur pouvant améliorer partiellement les déficiences de fonctionnement du cerveau du receveur.

ZUSAMMENFASSUNG UND SCHLUBFOLGERUNGEN

In zunehmendem Maβe deuten Befunde darauf hin, daβ Implantate fotalen Gehirngewebes wie auch anderer Gewebearten, vom Gehirngewebe des Wirts angenommen werden und das Verhalten des Wirts durch Interaktion mit dem Gehirn des Wirts beeinflussen konnen. Im cholinergen System des Gehirns wurde gezeigt, daβ solche Transplantationen bei Tieren mit Fimbria-Fornix Lasionen, bei alten Tieren und bei Tieren mit neurotoxischen Lasionen des basalen Endhirns das cognitive Verhalten beeinflussen. In allen Fallen wurde eine Verbesserung des cognitiven Verhaltens wie Lernen im Labyrinth beobachtet. Die Mechanismen, mit denen solche Gewebstransplantate auf die Funktion des Gehirns und des Verhaltens des Wirts einwirken, bleiben noch zu untersuchen. Es wurden mehrere Moglichkeiten vorgeschlagen, darunter die Hypothese, daβ solche Gewebstransplantate wichtige neurotrophische Substanzen liefern konnten, die das Uberleben der axotomierten Neurone des Wirts und die Reinnervierung des normalen Zielneurons unterstutzen. Andererseits besteht die Moglichkeit, daβ Gewebsimplantate selbst Neurotransmittersubstanzen produzieren, die teilweise die Defizite in der Funktionsfahigkeit des Wirtsgehirns vermindern.

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REFERENCES

1. *Bartus, R. T., Dean, R. L., Beer, B.,* and *Lippa, A. S.* (1982). The cholinergic hypothesis of geriatric memory dysfunction. *Science*, 217, 408-417.
2. *Björklund, A., Lindvall, O., Isacson, O., Brundin, P., Wictorin, K., Strecker, R. E., Clarke, D. J.,* and *Dunnett, S. B.* (1987). Mechanisms of action of intra-cerebral neural implants: studies on nigral and striatal grafts to the lesioned striatum. *Trends in Neurosciences*, 10, 509-516.
3. *Björklund, A.,* and *Stenevi, U.* (1977). Reformation of the severed septohippocampal pathway in the adult rat by transplanted septal neurones. *Cell Tissue Research*, 185, 289-302.
4. *Björklund, A.,* and *Stenevi, U., Eds.* (1985). *Neural grafting in the mammalian CNS.* Amsterdam: Elsevier.
5. *Björklund, A., Stenevi, U., Dunnett, S. B.,* and *Gage, F. H.* (1982). Cross-species neural grafting in a rat model of Parkinson's disease. *Nature (London)*, 298, 652-654.
6. *Coyle, J. T., Price, D. L.,* and *DeLong, M. R.* 1983). Alzheimer's disease: a disorder of cortical cholinergic innervation. *Science*, 219, 1184-1190.
7. *Daniloff, J. K., Bodony, R. P., Low, W. C.,* and *Wells, J.* (1985). Cross-species embryonic septal transplants: restoration of conditioned learning behavior. *Brain Research*, 346, 176-180.
8. *Davis, G. E., Blaker, S. N., Engvall, E., Varon, S., Manthorpe, M., and Gage, F. H.* (1987). Human amnion membrane serves as a substratum for growing axons *in vitro* and *in vivo*. *Science*, 236, 1106-1109.
9. *Dunnett, S. B., Gage, F. H., Björklund, A., Stenevi, U., Low, W. C.,* and *Iversen, S. D.* (1982). Hippocampal deafferentation: transplant-derived reinnervation and functional recovery. *Scandinavian Journal of Psychology, Supplement*, 1, 104-111.
10. *Dunnett, S. B., Low, W. C., Iversen, S. D., Stenevi, U.,* and *Björklund, A.* (1982). Septal transplants restore maze learning in rats with fornix-fimbria lesions. *Brain Research*, 251, 335-348.
11. *Fine, A., Dunnett, S. B., Björklund, A.,* and *Iversen, S. D.* (1985). Cholinergic ventral forebrain grafts into the neocortex improve passive avoidance memory in a rat model of Alzheimer disease. *Proceedings of the National Academy of Sciences, U.S.A.*, 82, 5229-5230.
12. *Fine, A., Dunnett, S. B., Björklund, A., Clarke, D.,* and *Iversen, S. D.* (1985). Trans-

plantation of embryonic ventral forebrain neurons to the neocortex of rats with lesions of nucleus basalis magnocellularis-I. Biochemical and anatomical observations. *Neuroscience*, 16, 769-786.

13. *Freed, W. J.* (1983). Functional brain tissue transplantation: reversal of lesion-induced rotation by intraventricular substantia nigra and adrenal medulla grafts, with a note on intracranial retinal grafts. *Biological Psychiatry*, 18, 1205-1267.

14. *Gage, F. H., Armstrong, D. M., Williams, L. R.*, and *Varon, S.* (1988). Morphological response of axotomized septal neurons to nerve growth factor. *Journal of Comparative Neurology*, 2, 147-155.

15. *Gage, F. H.*, and *Björklund, A.* (1986a). Compensatory collateral sprouting of aminergic systems in the hippocampal formation following partial deafferentation. In R. L. Isaacson and K. H. Pribram (Eds.), *The Hippocampus* (Vol. 3, pp. 33-63). New York: Plenum.

16. *Gage, F. H.*, and *Björklund, A.* (1986b). Cholinergic septal grafts into the hippocampal formation improve spatial learning and memory in aged rats by an atropine-sensitive mechanism. *Journal of Neuroscience*, 6, 2837-2847.

17. *Gage, F. H., Björklund, A., Stenevi, U., Dunnett, S. B., and Kelly, P.A.T.* (1984). Intrahippocampal septal grafts ameliorate learning impairments in aged rats. *Science*, 225, 533-536.

18. *Gage, F. H., Björklund, A., Stenevi, U.*, and *Dunnett, S. B.* (1985). Grafting of embryonic CNS tissue to the damaged adult hippocampal formation. In A. Björklund and U. Stenevi (Eds.), *Neural Grafting in the Mammalian CNS* (pp. 559-573). Amsterdam: Elsevier.

19. *Hefti, F.* (1986). Nerve growth factor promotes survival of septal cholinergic neurons after fimbrial transections. *Journal of Neuroscience*, 6, 2155-2162.

20. *Kimble, D. P.* (1975). Choice behavior in rats with hippocampal lesions. In R. L. Isaacson and K.H. Pribram Eds.), *The hippocampus, a comprehensive treatise* (Vol. 2, pp. 309-326). New York: Plenum.

21. *Kimble, D. P., BreMiller, R.*, and *Stickrod, G.* (1986). Fetal brain implants improve maze performance in hippocampal-lesioned rats. *Brain Research*, 363, 358-363.

22. *Kimble, D. P., BreMiller, R.*, and *Stickrod, G., and Matthews, M.* (in preparation). Effects of sub-maxillary tissue, fetal hippocampal tissue, and amnionic membrane grafts on the behavior of rats with fimbria-fornix lesions.

23. *Kromer, L. F.* (1987). Nerve growth factor treatment after brain injury prevents neuronal death. *Science*, 235, 214-217.

24. *Kromer, L. F., Björklund, A.*, and *Stenevi, U.* (1981). Regeneration of the septohippocampal pathways in adult rats is promoted by utilizing embryonic hippocampal implants as bridges. *Brain Research*, 210, 173-200.

25. *Mesulam, M-M., Mufson, E. J., Wainer, B. H.*, and *Levey, A. I.* (1983a). Central cholinergic pathways in the rat: An overview based on an alternative nomenclature (Ch1-Ch6). *Neuroscience*, 10, 1185-1201.

26. *Mesulam, M-M., Mufson, E. J., Levey, A. I.*, and *Wainer, B. H.* (1983b). Cholinergic innervation of cortex by the basal forebrain: cytochemistry and cortical connections of the septal area, diagonal band nuclei, nucleus basalis (substantia innominata) and hypothalamus in the rhesus monkey. *Journal of Comparative Neurology*, 214, 170-197.

27. *Nilsson, O. G., Shapiro, M. L., Gage, F. H., Olton, D. S.*, and *Björklund, A.* (1987). Spatial learning and memory following fimbria-fornix transection and grafting of fetal septal neurons to the hippocampus. *Experimental Brain Research*. 67, 195-215.

28. *O'Keefe, J.*, and *Nadel, L.* (1978). *The hippocampus as a cognitive map*. Oxford: Clarendon.

29. *Olton, D. S., Becker, J. T.*, and *Handelmann, G. E.* (1979). Hippocampus, space and memory. *Behavioral Brain Science*, 2, 313-365.

30. *Segal, M., Björklund, A.*, and *Gage, F. H.* (1985). Transplanted septal neurons make viable cholinergic synapses with a host hippocampus. *Brain Research*, 336, 302-307.

31. *Sladek, J. R., Jr.* and *Gash, D. M.* (1984). *Neural transplantss Development and function.* New York: Plenum.

32. *Smith, G.* (1988). Animal models of Alzheimer's disease: experimental cholinergic denervation. *Brain Research Reviews*, 13, 103-118.

33. *Sofroniew, M. V., Campbell, P. E., Cuello, A. C.*, and *Eckenstein, F.* (1985). Central cholinergic neurons visualized by immunohistochemical detection of choline acetyltransferase. In G. Paxions and C. Watson (Eds.), *The rat nervous system* app. 471-517). New York: Academic.

34. *Sollars, P. J.* and *Kimble, D. P.* (1988). Cross-species transplantation of fetal hypothalamic tissue restores circadian locomotor rhythm to SCN-lesioned hosts. *Neuroscience Abstracts*, 14, 49.

35. *Thoenen, H., Bandtlow, C.*, and *Heumann, R.* (1987). The physiological function of nerve growth factor in the central nervous system: comparison with the periphery. *Reviews of Physiology, Biochemistry and Pharmacology*, 109, 145-178.

36. *Wainer, B. H., Levey, A. I., Mufson, E. J.,* and *Mesulam, M.-M.* (1984). Cholinergic systems in mammalian brain identified with antibodies against choline acetyltransferase. *Neurochemistry International*, 6, 163-182.

37. *Wainer, B. H., Levey, A. I., Rye, D. B., Mesulam, M.-M.,* and *Mufson, E. J.* (1985). Cholinergic and non-cholinergic septohippocampal pathways. *Neuroscience Letters*, 54, 45-52.

38. *Whishaw, I. Q., O'Connor, W. T.,* and *Dunnett, S. B.* (1985). Disruption of central cholinergic systems in the rat by basal forebrain lesions or atropine: effects on feeding, sensorimotor behaviour, locomotor activity and spatial navigation. *Behavioural Brain Research*, 17, 103-115.

39. *Williams, L. R., Varon, S., Peterson, G. M., Wictorin, K., Fischer, W., Björklund, A.,* and *Gage, F. H.* (1986). Continuous infusion of nerve growth factor prevents basal forebrain neuronal death after fimbria-fornix transection. *Proceedings of the National Academy of Sciences, U.S.A.*, 83, 9231-9235.

40. *Woodruff, M. L., Baisden, R. H., Whittington, D. L., and Benson, A. E.* (1987). Embryonic hippocampal grafts ameliorate the deficit in DRL acquisition produced by hippocampectomy. *Brain Research*, 408, 97-117.

Human Adrenal and Fetal Brain Grafting in Parkinson's Disease. Clinical Experience

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Key words: Autoadrenal brain grafting. Fetal brain homotransplantation. Surgical treatment of Parkinson's disease.

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INTRODUCTION

Our reports first in 1987 and then in 1988 on the amelioration of Parkinson's disease (PD) signs in patients with autologous adrenal medullary (AM) grafts (Madrazo et al. (1987a)) and fetal ventral mesencephalon (VM) and fetal adrenal gland (A) homotransplants (Madrazo et al. (1988c)) to the caudate nucleus (CD), prompted many groups worldwide to attempt these procedures for the treatment of PD (Fig. 1). As a result of these studies and our own ongoing experience (Madrazo et al. (1987b), (1988a), (1989a,b,c), (1990), Drucker-Colin et al. (1987)), the general consensus that is evolving is that AM transplantations and fetal, especially VM, homografts can significantly reduce the disabling symptoms of PD, increase the patients' response to L-dopa medication, which in turn reduces the drug's side effects, and possibly decrease the rate of progression of the disease. For many transplanted parkinsonians, these improvements have meant an enhanced quality of life.

The purpose of the present communication is not to give a detailed account of our brain transplantation studies, which we have chosen only to summarize, but rather to describe and comment on selected methodological, brain graft imaging, clinical, neuropsychological, and biochemical aspects, we have noted during the development of our brain grafting procedures, and on the pre and postoperative evaluations of our transplanted parkinsonians, which have revealed a complex set of responses to them, possibly in accordance with the heterogeneous nature of PD. The analysis of this information has given us a better insight into the effectiveness of these new therapies in the treatment of PD. For a comprehensive description of the progress of our work, we refer the reader to our publications cited above, as well as to Franco-Bourland and Madrazo (1988), Franco-Bourland et al.

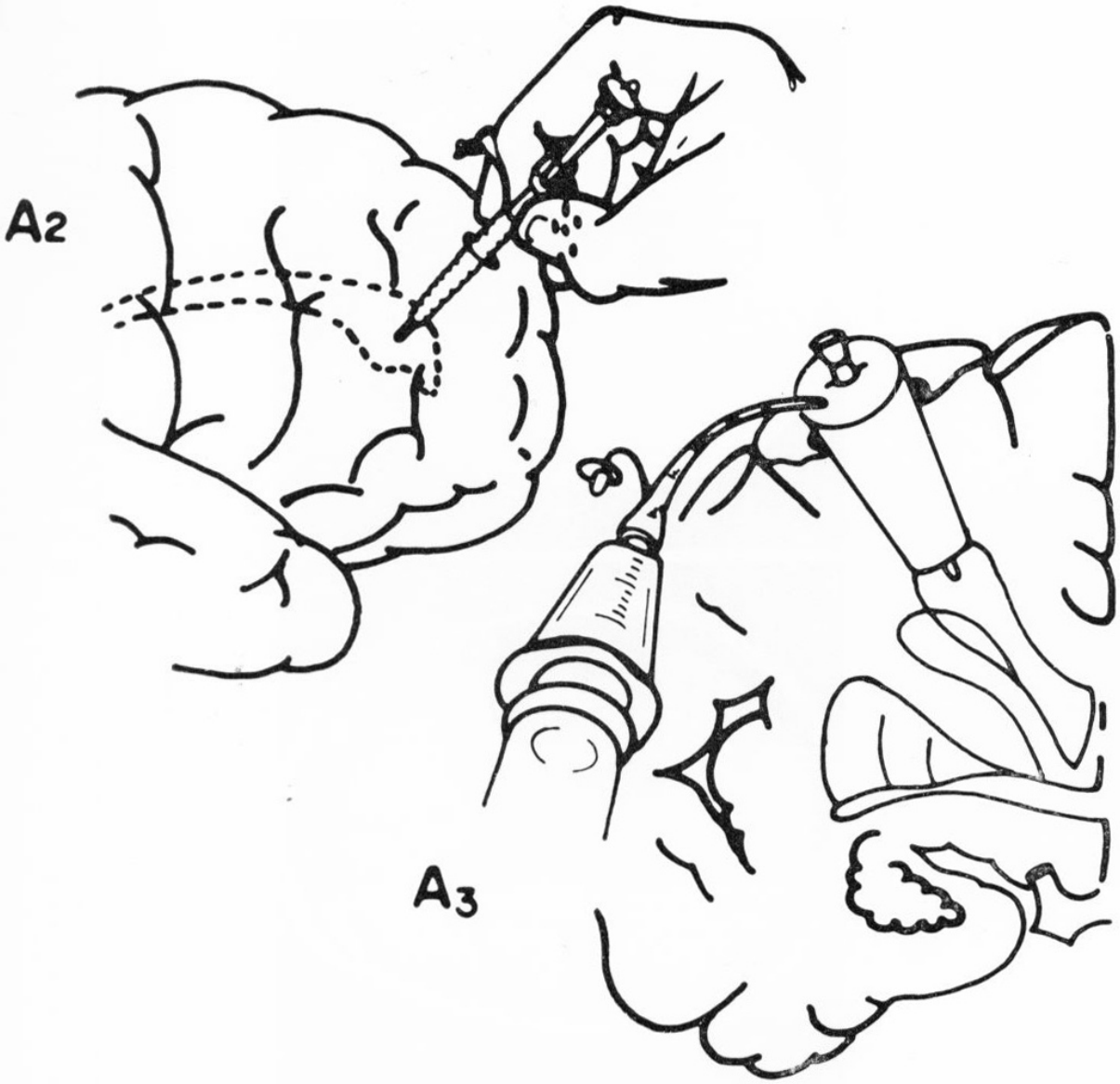


Fig. 1. — *Balloon dissecting catheter for transcortical ventricular approach.*

(1988), Graef et al. (1988), Madrazo et al. (1988b), and Ostrosky-Solis et al. (1988), (1989 a,b,c).

AUTOLOGOUS ADRENAL MEDULLARY BRAIN TRANSPLANTATIONS

Methodological aspects.

An open microsurgical technique was selected initially, for the paraependymal ventricular placement of the graft, apparently crucial for the success of the procedure (Freed et al. (1981)), thereby assuring the nutritional support of the graft by the CSF, and allowing it to be bathed by the neurotrophic factors released by the cavity in the CN, and also permitting the proper difusion of factors released from it through the CSF. Open microsurgery also allowed us to make an adequate niche, and by direct viewing, make the proper, rapid (less than 15 min), and bloodless placement and attachment of the very gently handled blocks of tissue in the cavity. For this purpose, we designed an "atraumatic" transcortical approach into the ventricle, with a balloon dissecting catheter (Fig. 2), as well as the microsurgical fragmentation of the adrenal tissue into blocks (preserving their stroma), rather than cell suspensions (Backlund et al. (1985)). Neuroscientists are presently in the process of unraveling the mechanisms underlying the beneficial effects of this open technique, which in itself may be a contributing factor.

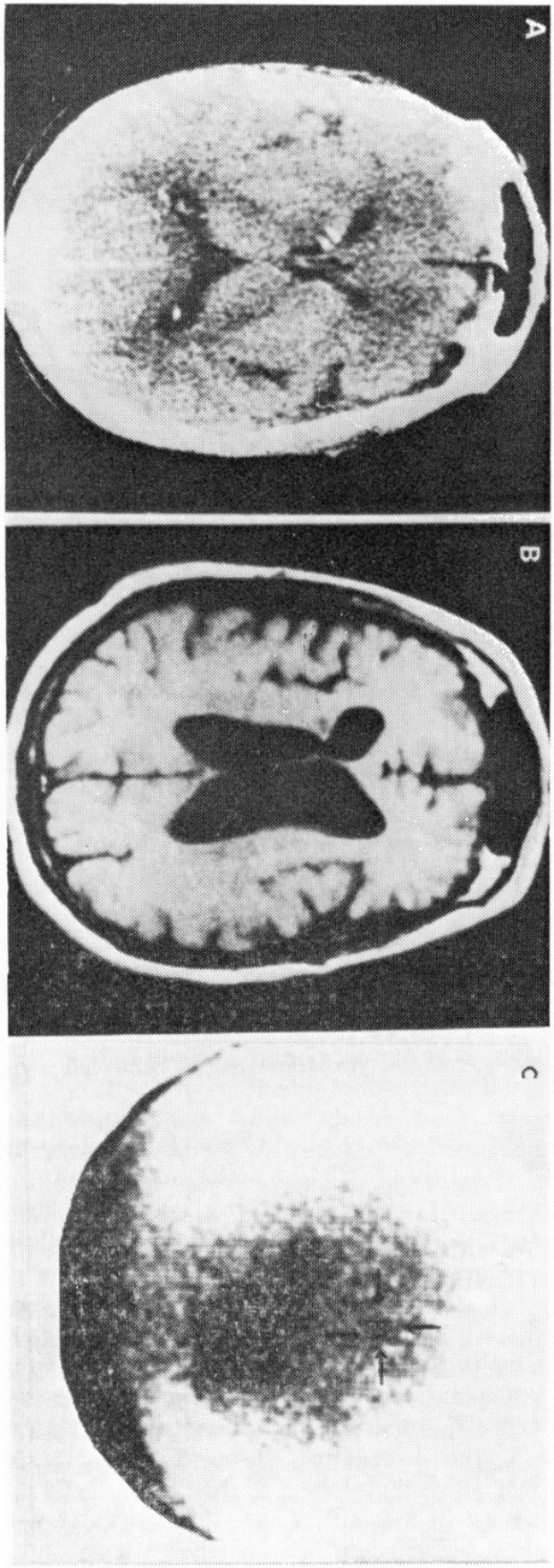


Fig. 2. — *Brain graft imagings. A: CT; B: NMR; C: scintigraphy using 131-I-MIBG.*

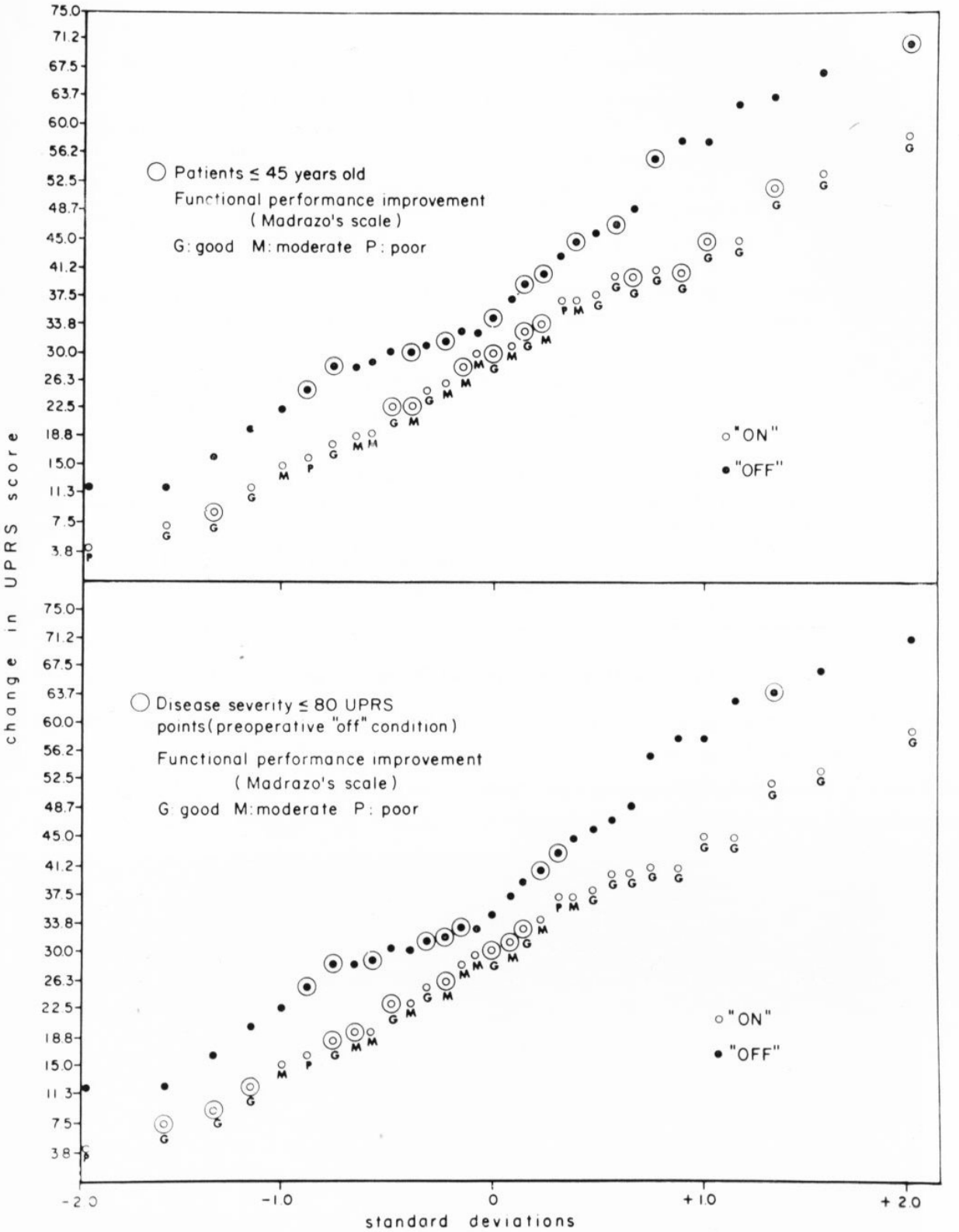


Fig. 3. — *Normal distribution curve for parkinsonians' response to auto-AM transplantation (difference between UPRS pre and postoperative scores), in relation to age (A), severity of disease (B), and functional performance improvements (A and B).*

Undoubtedly another important aspect of the successfull response to the technique has been the design of a surgical system for the rapid sequence of steps for the removal, dissecting, and implantation of the AM tissue. involving the unprecedent simultaneous performance of 2 major operations, namely, a craniotomy and a laparotomy.

TABLE I

Parkinsonians' response to auto-AM transplantation.

A. Patients' preoperative condition

Patients evaluated	:	34 out of 42 consecutive cases. (4 lost out of control; 4 died).
Age	:	47.7 ± 9.3 years *
Evaluation of PD	:	8.9 ± 3.3 years *
Severity of PD (global UPRS scoring)	:	"on", 61.2 ± 4.4 **
		"off", 88.4 ± 4.6 **
Functional disability (global scoring on Madrazo's scale)	:	
		"on", 2.7 ± 0.17 **
		"off", 2.1 ± 0.14 **
Preoperative L-dopa dose	:	1269 ± 115 mg/day **
Duration of L-dopa treatment	:	87.4 ± 68.5 months *
Drug side effects	:	dyskinesias, 57%
		"on-off" and "wearing-off phenomena, 54%
		Hallucinations and delusions, 7%

B. Patients' postoperative condition 12-38 months post-transplant

Recovery from PD (global UPRS scoring) : "on", 37.5 ± 6.0
"off", 55.7 ± 6.0

Individual response to surgery (UPRS) :

"on",		"off",	
44%	- good	59%	- good
20%	- moderate	20%	- moderate
18%	- poor	12%	- poor
9%	- no response	9%	- worse
9%	- worse		

Functional performande improvement (global scoring on Madrazo's scale) :

"on", 3.8 ± 0.26
"off", 3.6 ± 0.21

(53% show distinct improvements in the quality of their lives).

Bilaterally and symmetrically most improved PD signs:

rigidity	postural imbalance
bradykinesia	gait disturbances

Postoperative L-dopa dose: 500 ± 143 mg/day **
(6 discontinued medication,
3 with "gastric" intolerance became tolerant)

a, patients were also evaluated on the Hoehn & Yahr and the Schwab & England scales; * mean ± SD, ** mean ± SEM.

TABLE II

Surgical incidents, morbidity, and mortality in auto-AM transplantations.

I Surgical incidents

Difficulty in approaching the ventricle - 2[b]
Fornical lesion - 1
Placement of the graft in the corpus callosum - 1

II Postoperative complications [a]

Neurological reversible
Depression of consciousness - 7
Hallucinations & delusions - 13
Mental confusion - 9
Subdural hygromas - 2

Neurological non-reversible
Cerebral venous thrombosis - 1
Psychotic states - 2

III Postoperative complications [a]

Non neurological-reversible
Bronchoaspiration - 11
Pulmonary infection - 13
Respiratory restriction due to rigidity - 6
Subphrenic abscess - 2
Pancreatitis - 2
Pulmonary thromboembolism - 1

IV Causes of death [c]

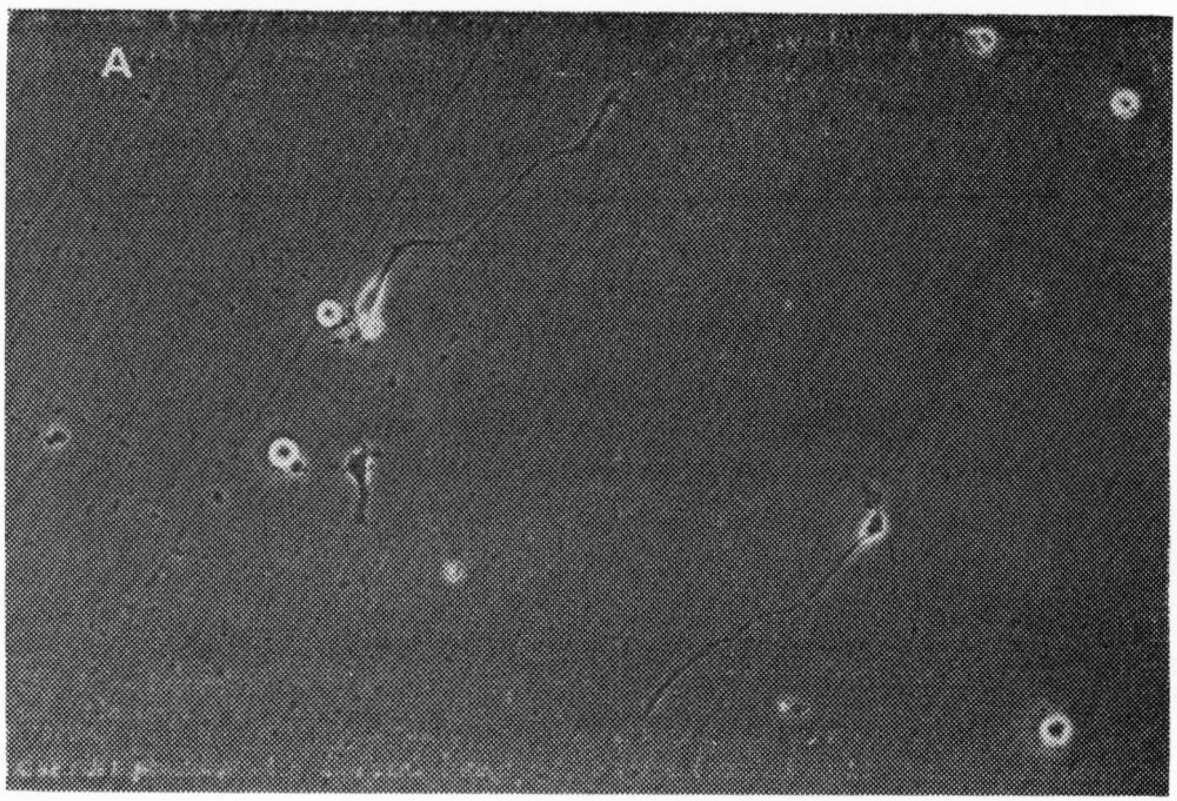
Neurological
Cerebral venous thrombosis - 1

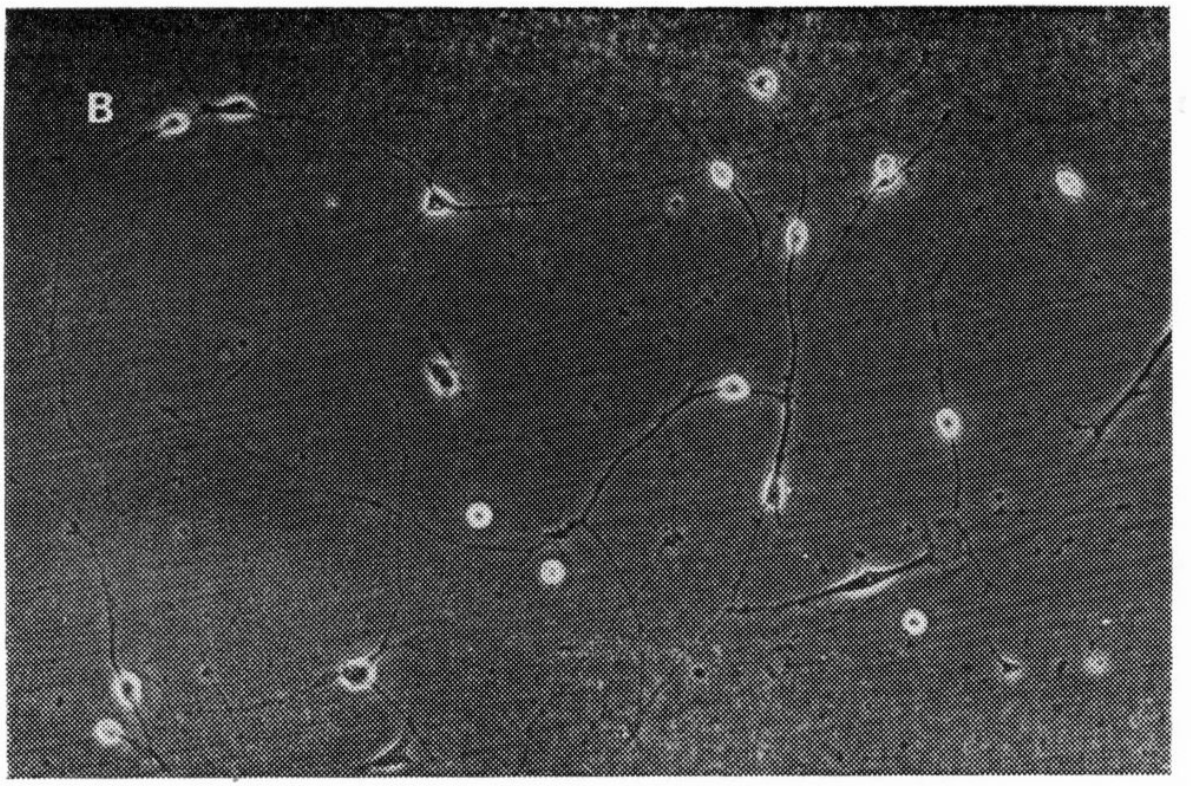
Non-neurological
Acute necrotic pancreatitis - 1
Massive pulmonary thrombosis - 1
Heart stroke (5 months after surgery) - 1

a, some patients had more than one complication; c, a patient lost out of control died 1 year after surgery of bronchoaspiration; case 48 died of asceptic meningitis.

Brain graft imagings.

For the visualization of the grafts after surgery, we have been able to use computed tomography (CT) (Fig. 3A) and nuclear magnetic resonance (NMR) (Fig. 3 B), taking advantage of the use of titanium clips for tissue attachment. We assessed the viability of the AM fragments after implan-

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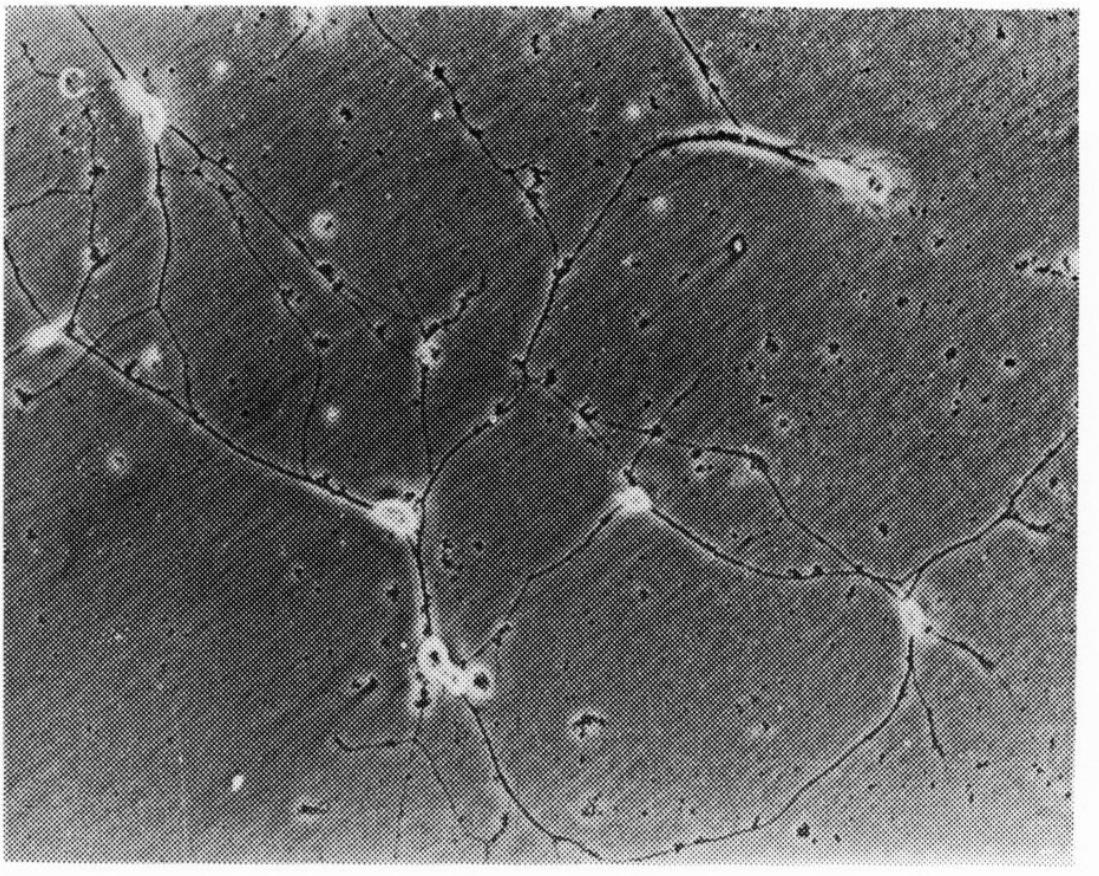

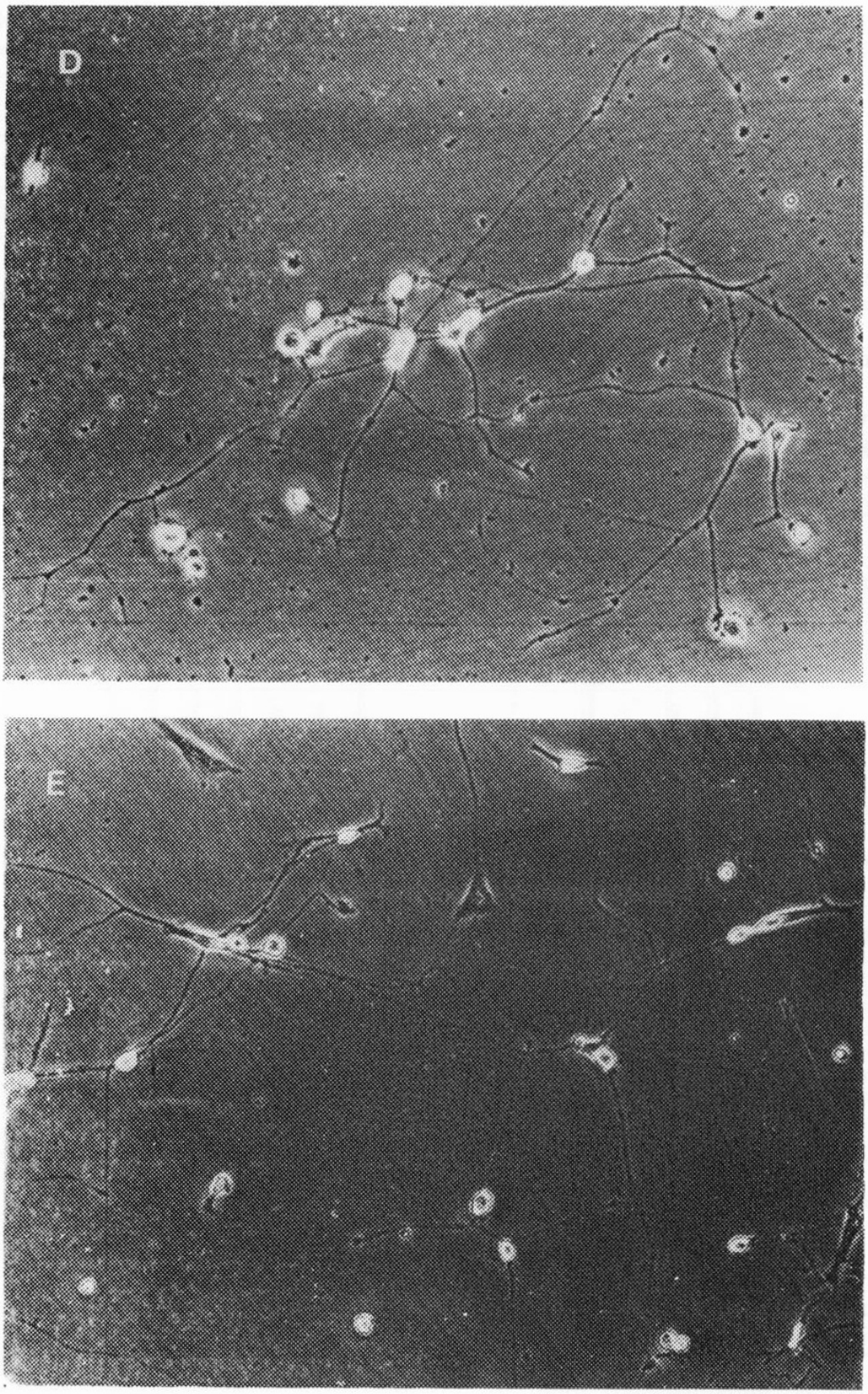

Fig. 4. — *Identification of NOPA in parkinsonians CSF. L and V pre and postsurgical CSF were bioassayed for NOPA by Frank Longo (UCSF, San Francisco, CA, USA), according to Mirraruelo et al. (1988). A: control; B: laminin; C: presurgical L CSF; D: postsurgical L CSF; E: postsurgical V CSF.*

tation (lacking positron emission tomography (PET) facilities), by brain scintigraphy (Fig. 3C), using 131-1-meta-iodo-benzylguanidine (131-1-MIBG), a radiopharmaceutical usually employed in the diagnosis of pheochromocytomas.

Clinical Observations.

Table 1 summarizes the global response of 34 parkinsonians to autologous AM brain transplantations, 12-38 months after surgery.

Table II shows the surgical incidents, morbidity, and mortality rates of this AM grafted group.

Figure 4 illustrates the relation of their age (Fig. 4A), severity of the disease (Fig. 4B), and their degree of functional performance improvements (Fig. 4A and 4B), on the normal distribution curve of their UPRS pre and postsurgical score changes, in "on" and "off".

The normal distribution curves show that patients over 45 years of age and with a severe case of PD, like young severely ill individuals, can respond well to surgery, showing large changes between their UPRS scorings before and after surgery, in both their "on" and "off" conditions. The impact of the neurological improvements of these severely ill parkinsonians on the quality of their lives depends however, on the severity of their disease, and thus, some severely afflicted parkinsonians with a good response to surgery, show only moderate

Table III

Pre and postoperative biochemical assessments in lumbar and ventricular CSF.

Biochemical parameter	Lumbar			p value 1-pre-on vs post 2-pre-off vs post	Ventricular		p value S vs post
	pre-on L-dopa, n	pre-off L-dopa, n	post, n		S, n	post, n	
HVA, ng/ml	53.6 ± 39.4, 5	51.8 ± 34.8, 6	88.5 ± 33.2, 2	a	188.8 ± 66.7, 5	145, 1	a
			57.3 ± 51.6, 6	1-NS[b]		172.4 ± 84.2, 4	
			29.2 ± 19.6, 6	2-NS[b]		199.4 ± 79.6, 5	NS[b]
			87.0 ± 37.7, 3			332.0 ± 101.1, 2	
5-HIAA, ng/ml	26.5 ± 7.3, 5	39.3 ± 18.3, 6	45.8 ± 8.1, 2	a	91.8 ± 30.1, 5	85, 1	a
			35.7 ± 12.4, 6	1-NS[b]		87.8 ± 18.0, 3	
			28.8 ± 7.0, 6	2-NS[b]		84.3 ± 19.7, 4	NS[b]
			31.3 ± 4.2, 3			92.0 ± 9.2, 2	
MHPG, ng/ml	13.4 ± 4.5, 5	13.0 ± 3.9, 6	11.6 ± 4.3, 2	a	7.4 ± 2.3, 6	15, 1	a
			14.4 ± 11.7, 6	1-NS[b]		16.0 ± 17.4, 4	
			9.7 ± 4.6, 6	2-NS[b]		9.7 ± 11.1, 4	NS[b]
			13.3 ± 4.2, 3			16.8 ± 8.1, 2	
True AChE, U/mg protein	172.5 ± 86.7, 11	235.0 ± 82.4, 11	139.1 ± 48.4, 9	c	28.3 ± 16.8, 3	39.4 ± 23.6, 5	d
			152.8 ± 41.9, 9	1-NS[b]		60.4 ± 26.3, 9	
			162.8 ± 56.5, 5	2- < 0.01[b]		64.4 ± 32.9, 7	NS[b]
						56.0 ± 28.3, 5	
						52.8 ± 34.3, 4	

HVA, homovanillic acid; 5-HIAA, 5-hydroxy-indoleacetic acid; MHPG, 3-methoxy - 4-hydroxy - phenyl - ethyleneglycol; AChE, acetylcholinesterase. a, 1-10, 11-30, 31-50, 51-70 days postsurgery; b, NOVA; c, 11-30, 31-60, 61-90 days postsurgery; d, 11-30, 31-60, 61-90, 91-120, 121-150 days postsurgery. n, number of cases; S, surgery.

HVA, 5-HIAA, and MHPG were measured by high performance liquid chromatograpy and electrochemical detection by Paul Carvey, Rush Presbyterian, Chicago, IL, USA; true AChE was measured according to Ellman et al., 1961 by Dalila Martínez-Muñoz and Laura González, CINVESTAV - IPN, México, D.F., MEXICO.

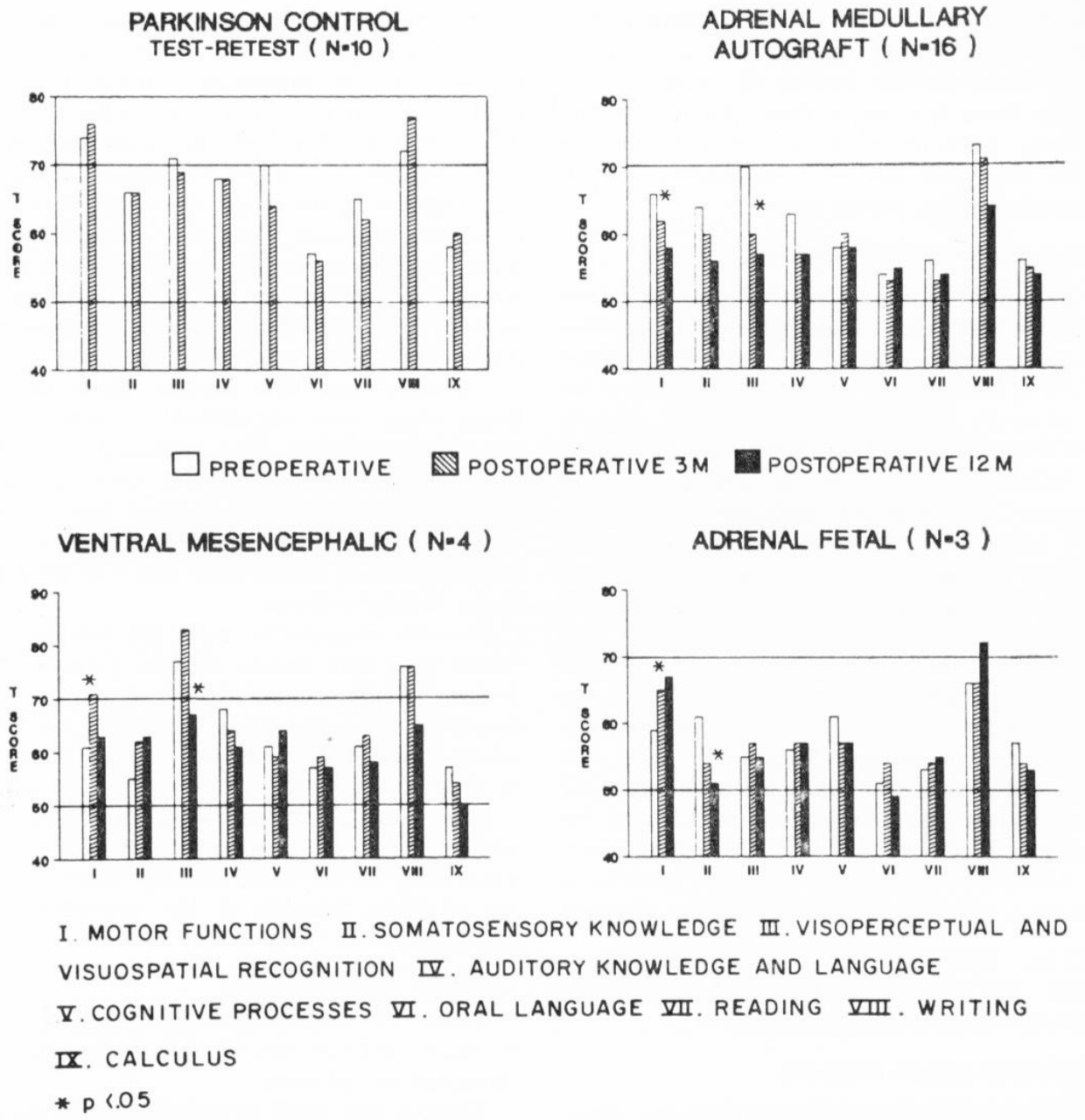

Fig. 5. — *Pre and postoperative mean scores on the neuropsychological diagnostic squeme of auto-AM (n=16), fetal VM (n=4), and fetal A (n=3) transplanted parkinsonians, compared to a control unoperated PD group (n=10) in thye test-retest condition. Dark lines show the mean and the limits of 2 SD.*

improvements in their daily living activities. On the other hand, midly afflicted parkinsonians, regardless of their age, with only a small response to surgery, can experience significant improvements in the quality of their lives.

A group of AM transplanted parkinsonians mainly elderly and severely afflicted individuals, did not respond well, possibly because of the double surgery and/or because of other factors we have not yet been able to determine, which in some cases could be related to the quality of their AM tissue. Some (Stoddard et al. (1989)), but not others (Cervera et al. (1988)), have found AM tissue to be altered in elderly parkinsonians.

These results and the possibility that AM transplantations may reduce the rate of progression of the disease, stress the great importance of an adequate selection of patients for brain transplantation, at the best time for them after the onset of their disease, in order to be able to offer them the best chance of functional recovery with the least surgical risk. We thus believe now, that youn-

ger PD patients with a milder form of the disease may benefit from AM brain graftings early in the course of their disease, before there is a major damage to the brain. Elderly patients with severe PD, on the other hand, are not good candidates for this transplantation procedure.

Biochemical Observations.

Table III summarizes the pre and postoperative biochemical assessments in lumbar (L) and ventricular (V) cerebrospinal fluid (CSF), and Fig. 5 depicts the bioassay for a neurite outgrowth promoting activity (NOPA) identified in these same samples.

AChE appears to be an L-dopa-sensitive enzyme, and thus it's unchanged postoperative levels in patients receiving a decreased L-dopa treatment may indicate an improved cholinergic control in the basal ganglia (Rubert et al. (1986)).

A particularly outstanding finding has been the identification of NOPA in the preoperative parkinsonian CSF, which does not appear to change significantly after surgery. It's presence in parkinsonian CSF may reflect an endogenous mechanism for neuron "regeneration" of the diseased brain. It remains to be seen if such an activity is common to other neurodegenerative diseases.

FETAL VENTRAL MESENCEPHALON AND FETAL ADRENAL GLAND BRAIN HOMOTRANSPLANTATIONS

Methodological aspects.

This neurosurgical procedure was designed with the same criteria as AM transplantations, that is of a rapid transplantation of gently handled blocks of tissue. In compliance with Mexican Law, our only source of human fetuses are spontaneous abortions.

Accordingly, our fetal transplantation surgery must be carried out as an emergency procedure, initiating the craniotomy at the time the fetus is en route to the neurosurgical unit.

The fetuses we used for brain homotransplantation are of a gestational age between 12-14 weeks, approximately 8,5-12.0 cm long, and fetal tissue is implanted within the first 4 hr after fetal dealth diagnosis.

Gently handled blocks of fetal VM tissue, were obtained by handsfree dissection. The dissecting procedure was performed on ice, and under the dissecting microscope. The skull was coronally cut open with scissors. The cerebral hemispheres were removed, carefully preserving the thalamus. After retracting the brain stem, the tentorium was bilaterally severed, and the cranial nerves were cut. Upon visualizing the formen magnum, the junction at the medulla oblongata and spinal cord was severed, freeing the entire brain stem and thalamus. The cephalic flexure and the various parts of the brain stem were identified, to dissect out the mesencephalon. The various parts of the mesencephalon were likewise identified, and peduncles, tectum, and medial aspect were removed. The remaining untouched dissected 2 blocks of tissue were the VM used for brain transplantation.

Because chromaffin cells are not concentrated in a core in the adrenal gland of the fetuses of these gestational ages, but are dispersed troughout the gland, we used whole gland fragments for implantation (6 -8 of 2x2x2 mm).

Like auto-AM transplantations, fetal VM and A grafts are implanted into the ventricular wall of the CN, usually omitting the use of clips, because of the consistency of the tissue.

Clinical observations.

Table IV summarizes the pre and postoperative evaluations of our 7 fetal homotransplanted patients.

Though the fetal A and fetal VM homotransplanted patients show similar improvements on the UPRS, the quality of life (assessed during clinical observations and through family testimony), was distinctly better for the VM transplanted patients. Under identical circumstances of transplantation, the better response seen for the VM group, strongly suggests that neural tissue (VM) is a better material for implantation than A at this gestational age.

VM cases 3 and 5 had transitory postoperative complications, with no sequelae. VM-3 developed an infection of the bone flap and a brain abscess, and VM-5 a deep thrombophlebitis of the right lower limb. There were no deaths. The A group showed no morbidity or mortality.

TABLE IV

Parkinsonian's response to fetal homotransplantation.

A Patients' preoperative condition

Case/Sex	Age (years)	Evolution Of PD (years)	Severity Of PD (UPRS) "on"	Severity Of PD (UPRS) "off"	Functional disability (Madrazo's scale) "on"	Functional disability (Madrazo's scale) "off"	Preoperative L-dopa dose mg/day
VM-1/M	50	9	59	79	3	2	1000
VM-2/M	45	16	110	129	2	2	2000[a]
VM-3/M	52	13	100	136	3	2	375
VM-4/M	47	9	31	108	5	3	1000[a]
A-1/F	35	5	71	88	4	3	500
A-2/M	54	8	99	148	3	1	1000[a]
A-3/M	56	12	50	92	4	3	750

B. Patients' postoperative condition

Case/months postsurgery	Recovery from PD (UPRS) "on"	Recovery from PD (UPRS) "off"	Functional performance improvement (Madrazo's scale) "on"	Functional performance improvement (Madrazo's scale) "off"	Global response to surgery	Postoperative L-dopa dose mg/day
VM-1/19	6	15[b]	5	4	very good	500
VM-2/10	29	59	4	3	good	285
VM-3/9	43	67	4	3	moderate	150
VM-4/6	5	24	5	4	good	700
A-1/19	22	26[c]	5	4	good	375
A-2/10	25	81	4	1	moderate	500
A-3/6	11	31	5	4	good	150

[a] Drug side effects, dystonias and dyskinesias; [b] Most improved PD signs: rigidity, bradykinesia, postural imbalance, gait disturbance, and facial expression; and [c] rigidity and bradykinesia. Improvements were bilateral and symmetric. For surgery patients were immunosupressed with cyclosporine A and prednisone.

The benefit obtained with VM transplants, and the benign postsurgical course after this procedure, suggest that this technique may be useful also in the treatment of patients that are not candidates for AM transplantations.

Neuropsychological observations.

Preoperative neuropsychological evaluations revealed specific cognitive deficits of varying degree. Patients showed frontal lobe type deficits with alterations in behavioral programming, leading to difficulties in the organization of motor sequences and alternating programs. They also showed memory disorders, visuospatial and visuoperceptual deficiencies, and somatosensory alterations.

In the postoperative evaluations (Fig. 6) carried out at 3 and 12 months after surgery, the autoadrenal graft group showed an amelioration of the frontal lobe type symptoms, of the visuospatial deficits, and improved somatosensory exploration. At 3 months, fetal VM and fetal A homotransplanted patients, showed an opposite pattern of performance, with a deterioration of the frontal lobe type symptoms, of somatosensory exploration, of visuospatial deficits, and of their quality of writing. At the 12-month evaluation, the frontal lobe type symptoms of the VM grafted group, returned to their preoperative level of performance, and patients showed a significant improvement in their visuospatial deficits and in the quality of their writing. The A

grafted patients showed only a significant improvement in somatosensory exploration, but remained affected in their frontal lobe type symptoms and the quality of their writing.

Concluding remarks.

Worldwide experience in brain transplantation as expressed throughout this book, acknowledges the effectiveness of these procedures in the treatment of selected cases of PD. The widening of alternatives for these neurosurgical therapies is giving the patients more options, and increasing the population of candidates for surgery, but at the same time complicating the analyses of the information as it produces at times conflicting results among the various research groups. In the immediate future, the outlook seems even more complicated with the incorporation of still other brain grafting alternatives, such as the use of cell lines, genetically engineered cells, preserved tissues, xenografts, co-grafts, and the use of neuronotrophic factors with or without grafts, as well as the development of new surgical techniques. However encouraging our results to date have been, we must still proceed with caution regardless of our surgical approach.

SUMMARY

Thirty four parkinsonians with auto adrenal medullary (AM) brain transplantations, and 7 with fetal brain homotransplantations were evaluated 12-38 months, and 6-19 months after surgery, respectively. Measured as the difference between the pre and postoperative UPRS scores, the response to AM autografting in "on" was good in 44 % of the cases, moderate in 20 %. and poor in 18 %; 9 % of the patients showed no response, and 9 % became worse due to postoperative complications. In "off", response was good in 59 % of the cases, moderate in 20 %, and poor in 12 %; 9 % of the patients became worse. Fifty three percent of these patients showed distinct improvements in the quality of their lives (Madrazo's performance scale).

Though their level of recovery from Parkinson's disease on the UPRS was similar, fetal ventral mesencephalon homotransplanted parkinsonians showed greater functional improvements than the adrenal gland group.

RESUMEN

Treinta y cuatro parkinsonianos con transplante cerebral auto adrenal medular (AM) y siete con homotransplantes cerebral fetal fueron avaluados 12-38 meses y 6-19 meses después de la cirugía respectivamente. Medidos en cuanto a las diferencias entre pre y post operatorios UPRS pruebas, la respuesta al AM auto injerto en "on" fue buena en 44 % de los casos, moderada en 20 %, y pobre en 18 %, 9 % de los pacientes no mostraron respuesta y 9 % empeoraron debido a complicaciones postoperatorias. En "off" la respuesta fué buena en 59 % de los casos, moderada en 20 % y pobre en 12 %, 9 % de los pacientes se pusieron peor. Cincuenta y tres por ciento de estos pacientes mostraron distintas mejorías en la cualidad de sus vidas (graduación funcional de Madrazo).

Aunque su nivel de recuperación de enfermedad de Parkinson fue similar en el UPRS Homotransplador fetal ventral mes en cefalo Parkinsonianos, mostraron mayor funcional majoría que el grupo de la glándula adrenal.

RÉSUMÉ

Trente quatre parkinsoniens avec des implants cérebraux auto-adrénal-médulaire (A/M), et sept avec des homotransplants cérébraux de foetus, sont étudiés 12-38 mois et 6-19 mois après la cirugie-comparisons entre le pré et postopératoire. La réponse al AM auto Implant en "on" a été positive dans le 44 % des cas, moderée dans le 20 % et pauvre dans le 12 %, 9 % ont empiré et 3 % ont montré differentes formes d'ame-

lioration de leur qualité de vie (gradation fonctionelle de Madrazo).

Malgrés que le niveau de recuperation de la maladies de Parkinson a été similaire, chez le UPRS Homadransplantens foetal ventral mesencephal Parkinsonieu, a montrés une amélioration fonctionelle plus important que dans le groupe de la glande adrénale.

ZUSAMMENFASSUNG

Vierunddreissig Parkinsonkranke mit autotransplantaten von adrenomedblärem Gewebe ins Gehirnu. sieben mit homotransplantaten von foetalen Gehirngewebe wurden 12 bis 38 Monate u. 6 bis 19 Monate nach der Operation ausgewertet. Massnahmen bezueglich der Unterschiede zwischen prae u. postoperativen UPRS Proben, die Reaktion auf alam Auto-transplantate ebei "on" Patienten war gut in 44 % der Faelle, mittelmaessig bei 20 %, u. gering bei 18 %. 9 % der Patienten zeigten ueberhaupt keine Reaktion, u. 9 % verschlechterten sich wegen postoperativer Komplikationene. Bei "off" Patienten war die Reaktion gut in 59 % der Faelle, mittelmaessig bei 20 % u. gering bei 12 %. 9 % der Patienten verschlechterten sich. 53 % dieser Patiententatten verschiedene Grade der Besserung bezueglich der Lebensqualitaet. (Funktionelle Gradeinteilung nach Madrasso).

Obwohl das Niveau der Rekuperation von der Parkinsonschen Krankheit ungefaehr gleich war in den UPRS toetalen Homotransplantaten, zeigten sie eine groessere Besserung bei der meduloadrenalen Gruppe.

REFERENCES

1. *Allen, G. S., Burns, S., Tulipan, N. B.* and *Parker, R. A.*: Adrenal medullary transplantation to the caudate nucleus in Parkinson's disease. Initial clinical results in 18 patients. Arch. Neurol., 46: 487-491, 1989.
2. *Backlund, E. O., Granberg, P. O., Hamberger, B., Knutsson, E., Martensson, A., Sedvall, G., Seiger, A.* and *Olson, L.*: Transplantation of adrenal medullary tissue to striatum in parkinsonism. First clinical trials. J. Neurosurg., 62: 169-173, 1985.
3. *Bunyaratavej, S., Puapradit, F., Kanjanapitak, A., Pausawasdi, S.* and *Theerapancharoen, V.*: The adrenal medulla-brain transplant in the treatment of intractable Parkinson's disease. 7th Congress of the Asian Surgical Association, Panang, Malaysia, 1989, p. 329.
4. *Cervera, P., Rascon, O., Ploska, A., Gaillard, G., Raisman, R., Duyckaerts, C., Hauw, J. J., Scherman, S., Monstastruc, J. L., Javoy-Agid, F.* and *Agid, Y.*: Noradrenaline, adrenaline and tyrosine hydroxylase in adrenal medulla from Parkinsonian patients. J. Neurol. Neurosurg. Psychiat., 51: 1104, 1988.
5. *Ding, Y. J.*: Function improvement by auto-adrenal medulla caudate transplantation in patients with Parkinson's disease. Abstracts of the International Evaluation Seminar on Auto-adrenal Medulla Grafting on Parkinson's Disease, Beijing, 1988.
6. *Drucker-Colin, R., Madrazo, I., Ostrosky-Solis, F., Shkurovich, M., Franco, R.* and *Torres, C.*: Adrenal medullary tissue transplants in the caudate nucleus of Parkinson's patients. In: Gash, D. M. and Sladek, J. R., Jr. (Eds.), Transplantation into the Mammalian CNS: Preclinical and Clinical Studies, Progress in Brain Res, Vol. 78, Elsevier, Amsterdam, pp. 567-574.
7. *Ellman, G. L., Courtney, D. D., Andres, V.* and *Featherytone, R. M.*: A new and rapid determination of acetylcholinesterase activity. Biochem. Pharmacol., 1: 88-93, 1961.
8. *Franco-Bourland, R. E.* and *Madrazo, I.*s Transplantes al cerebro. Aspectos bioquímicos. Bol. Syntex Infor. Med., 14: 5-6, 1988.
9. *Franco-Bourland, R. E., Madrazo, I., Drucker-Colin, R., Torres, C., Alvarez, F., Carvey, P., O'Connor, D., Sierra, R.* and *Tamayo, J.*: (Spon: J. Villarreal), Biochemical analyses of lumbar and ventricular CSF from parkinsonians before and after adrenomedullary autotransplantation to the caudate nucleus. Soc. Neurosci. Abstr. 14: 4, 1988.
10. *Freed, W. J., Morihisa, J. M., Spoor, E., Hoffer, J., Olson, L., Seiger, A.* and *Wyatt, R. J.*: Transplanted adrenal chromaffin cells in rat brain reduce lesion-induced rotational behaviour. Nature, 292: 351-352, 1981.
11. *Gimeno-Alava, A.* and *Riva-Meana, C.*: Tratamientos actuales de la enfermedad de Parkinson: autoinjertos cerebrales. Rev. Esp. Neurol., 4: 165-177, 1989.
12. *Goetz, C., Olanov, W., Koller, W., Penn, R., Cahill, D., Morantz, R., Stebbins, G., Tanner, C., Klawans, H., Shannon, K., Comella, C., Witt, T., Cox, C., Waxman, M.* and

Gauger, L.: Multicenter study of autologous adrenal medullary transplantation to the corpus striatum in patients with advanced Parkinson's. N. Engl. J. Med., 320: 337-341, 1989.

13. *Graef, A., Franco-Bourland, R. E., Marquez, A., Madrazo, I., Drucker-Colin, R., Torres, C., Alvarez, F.* and *Manzano, E.*: (Spon: V. Aleman), 131-I-meta-iodobenzylguanidine scintigrams of adrenomedullary autotransplants to the caudate nucleus of parkinsonians. Soc. Neurosci. Abstr., 14: 4, 1988.

14. *Hitchcock, E. R., Clough, C., Hughes, R.* and *Kenny, B.*: Embryos and Parkinson's disease. Lancet, 1: 1274, 1988.

15. *Kelly, P. J., Ahlskog, J. E., van Heerden, J. A., Carmichael, S. W., Stoddard, S. L.* and *Bell, G. N.*: Adrenal medullary autograft transplantation into the striatum of patients with Parkinson's disease. Mayo Clin. Proc., 64: 282-290, 1989.

16. *Lieberman, A., Ransohoff, J. Berczeller, F., Brous, F., Eng, K. Goldstein, M., Kaufman, B.* and *Koslow, M.*: Neural and adrenal medullary transplants as a treatment for Parkinson's disease and other neurodegenerative disorders. Trends Clin. Neurol., 4: 1-15, 1988.

17. *Lindvall, O., Rehncrona, S., Brundin, P., Gustavii, B., Astedt, B., Widner, H. Lindholm, T., Bjorklund, A., Leenders, K. L., Rothwell, J. C., Frackowiak, R., Marsden, C. D., Johnels, B., Steg, G., Freedman, R., Hoffer, B. J., Seiger, A., Bygdeman, M., Stromberg, I.* and *Olson, L.*: Human fetal dopamine neurons grafted into the striatum in two patients with severe Parkinson's disease. A detailed account of methodology and a 6-month followup. Arch. Neurol., 46: 615-631, 1989.

18. *Lopez-Lozano, J. J., Bravo, G., Abascal, J., C. P. H.*: Neural Transplantation Grp., Implants of human ventral mesencephalic neurons and perfused adrenal medullary tissue into the caudate nucleus of Parkinson's patients. Preliminary results. Soc. Neurosci. Abstr., 14: 5, 1988.

19. *Madrazo, I., Drucker-Colin, R., Diaz, V., Martinez-Mata, J., Torres, C.* and *Becerril, J. J.*: Open microsurgical autografts of adrenal medulla to the right caudate nucleus in two patients with intractable Parkinson's disease. N. Engl. J. Med., 316: 831-834, 1987.

20. *Madrazo, I., Drucker-Colin, R., Leon, V.* and *Torres, C.*: Adrenal medulla transplanted to caudate nucleus for treatment of Parkinson's disease: report of 10 cases. Surg. Forum, 38: 510-512, 1987.

21. *Madrazo, I. Drucker-Colin, R., Franco-Bourland, R. E., Ostrosky, F., Aguilera, M. C.* and *Torres, C.*: Human fetal substantia nigra and adrenal medullary tissue homografts to the caudate nucleus of parkinsonians. Soc. Neurosci. Abstr. 14: 5, 1988.

22. *Madrazo, I., Drucker-Colin, R., Madrazo, M., Zarate, A., Leon, V., Torres, C.* and *Reyes, P.*: Técnica quirúrgica del injerto autólogo de médula suprarrenal al nucleo caudado para tratamiento de la enfermedad de Parkinson. Gac. Med. Mex. 124: 365-369, 1988.

23. *Madrazo, I., Leon, V., Torres, C., Aguilera, M., Varela, G., Alvarez, F., Fraga, A., Drucker-Colin, R., Ostrosky, F., Skurovich, M.* and *Franco, R.*: Transplantation of fetal substantia nigra and adrenal medulla to the caudate nucleus in two patients with Parkinson's disease. N. Engl. J. Med., 318: 51, 1988.

24. *Madrazo, I., Drucker-Colin, R., Torres, C., Ostrosky, F., Franco-Bourland, R. E., Aguilera, M., Garcia, L., Alvarez, F., Candelas, J.* and *Quintanar, L.*: Long-term (more than one year) evolution of patients with adrenal medullary autografts to the caudate nucleus for the treatment of Parkinson's disease. In: Bunney, W.E. Jr. Hippius, H., Laakmann, G. and Schmauss, M. (Eds.), Neuropharmacology. Proceedings of the XVIth CINF Congress, Springer, Berlin, 1989.

25. *Madrazo, I., Franco-Bourland, R. E., Ostrosky, F., Aguilera, M.* and *Candelas, J. A.*: Human brain transplantation of adrenal medullary and fetal brain tissue for functional restoration in diseased brain (clinical experience in Parkinson's disease). In: Hatase, O. (Ed.), Proceedings of the International Symposium on Information, Transduction, and Processing in Biological Systems. From Cell to Whole Body, Elsevier, Amsterdam, 1989, in press.

26. *Madrazo, I., Ostrosky, F., Franco-Bourland, R., Drucker-Colin, R., Garcia, L., Torres, C., Aguilera, M., Reyes, F., Candelas, J. A.*, and *Meneses, S.*: Initial neurotransplants in humans. In: Koller, W.C. and Paulson, A. G. (Eds.), Therapeutic Approaches to Parkinson Disease. Marcel Dekker, New York, 1989, in press.

27. *Madrazo, I., Franco-Bourland, R., Ostrosky-Solis, F., Aguilera, M., Cuevas, C., Alvarez, F., Magallon, E., Zamorano, C.* and *Morelos, A.*: Neural transplantation (auto-adrenal, fetal nigral, and fetal adrenal) in Parkinson's disease - The Mexican experience. In: Dunnett, S. and Richards, S.J. (Eds.), Neural Transplantation, from Molecular Bases to Clinical Application, Progress in Brain Research, Elsevier, Amsterdam, 1990, in press.

28. *Mendez, J., Court, J., Martinez, P.*: Experiencia con transplante de médula suprarrenal en 23 parkinsonianos. Simposium Internacional sobre Transplantes Neurales en la Enfermedad de Parkinson, Madrid, 1989.

29. *Millaruelo, A. I., Nieto-Sampedro, M.* and *Cotman, C. W.*: Cooperation between nerve

growth factor and laminin of fibronectin in promoting sensory neuron survival and neurite outgrowth. Dev. Brain Res., 38: 219-228, 1988.

30. *Molina, H., Galarraga, J., Quinones, R., Figueredo, R., Estrada, R., Alvarez, L., Hernandez, R., Rachid, M., Oduardo, M., Coutin, P., Diaz, G., Duque, A. M., Hernandez, N.* and *Basco, E.*: El neurotransplante en la enfermedad de Parkinson, experiencia cubana. Reporte preliminar. Bol. Centro Transplante Regeneración Sist. Nerv., Habana, 1988.

31. *Ostrosky-Solis, F., Quintanar, L., Madrazo, I., Drucker-Colin, R., Franco-Bourland, R.* and *Leon-Meza, V.*: Neuropsychological effects of brain autograft of adrenal medullary tissue for the treatment of Parkinson's disease. Neurology, 38: 1442-1450, 1988.

32. *Ostrosky-Solis, F., Madrazo, I.* and *Ardila, A.*: Autotransplante cerebral para el tratamiento de la enfermedad de Parkinson: efectos neuropsicológicos. Gac. Med. Mex., 1989, in press.

33. *Ostrosky-Solis, F., Madrazo, I., Drucker-Colin, R.* and *Quintanar, L.*: Cognitive effects of adrenal autografting in Parkinson's disease. In: Ardila, A. and Ostrosky-Solis, F. (Eds.) Brain Organization of Language and Cognitive Processes Critical Issues in Neuropsychiatry. Plenum, New York, 1989, pp. 197-218.

34. *Ostrosky-Solis, F., Meneses, S., Ruiz, S., Rodriguez, J., Madrazo, I.* and *Drucker-Colin, R.*: Cognitive and electrophysiological effects of intracereral grafts of autoadrenal medullary and human fetal tissue in patients with Parkinson's disease. In: Hatase, O. (Ed.), Proceedings of the International Symposium on Information, Transduction, and Processing in Biological Systems. From Cell to While Body, Elsevier, Amsterdam, 1989, in press.

35. *Penn, R. D., Goetz, C. G., Tanner, C. M., Klawans, H. L., Shannon, K. M., Comella, C. L.* and *Witt, T. R.*: The adrenal medullary transplant operation for Parkinson's disease: clinical observaions in five patients. Neurosurgery, 22: 999-1004, 1988.

36. *Ruberg, M., Rieger, F., Villageois, A., Bonnet, A. M.* and *Agid, Y.*: Acetylcholinesterase and butyrylcholinesterase in frontal cortex and cerebrospinal fluid of demented and non-demented patients with Parkinson's disease. Brain Res., 362: 83-91, 1986.

37. *Stoddard, S. L., Tyce, G. M., Ahlskog, J. E., Zinsmeister, A. R.*, and *Carmichael, S. W.*: Decreased catecholamine content in parkinsonian adrenal medullae. Exp. Neurol., 104: 22-27, 1989.

38. *Watts, R. L., Bakay, R. A. E., Iuvone, P. M., Watts, N.* and *Graham, S.*: Autologous adrenal-caudate transplantation in patients with Parkinson's disease (PD). Neurology, 38: Suppl 1: 143, 1988.

Adrenal Medullary Inplants as Treatment For Parkinson's Disease

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INTRODUCTION

In PD, of all the disorders, there is the clearest relationship among the pathology, the loss of catecholamine containing pigmented neurons in the substantia nigra pars compacta, the neurochemistry, the loss of striatal dopamine (DA) the pathophysiology, the disinhibition of the striatum, and the clinical symptoms. Moreover, the loss of tissue in PD is small and restricted to the nigra. It was thus reasonable to expect that if a transplant could substitute for the lost neurons in patients with PD, the chances would be good that symptoms might improve.

In PD, symptoms appear after the number of pigmented neurons is reduced by 80 %. Compensation for the resulting striatal DA deficiency is achieved by administering levodopa which is converted to DA by the remaining nigral neurons.

The main problem in PD is the large number of patients who, after initially responding to levodopa, become more symptomatic. Worsening symptoms are usually accompanied by diurnal fluctuations. These are mainly "wearing-off" phenomena or "on-off" phenomena. Because most of the symptoms of PD result from the loss of nigral neurons and because of the limitations of levodopa, investigators began to consider replacing the lost tissue.

Animal studies indicate that fetal cells can survive and reverse symptoms when they are transplanted into the striata of parkinsonian animals. (Bjorklund 1980, Olson 1986) However, because the use of fetal tissue raises complex social issues, investigators turned to the adrenal medulla which is embryologically related to the catecholamine containing cells in the nigra. Animal studies revealed that adrenal medullary cells may be transplanted into the striatum (caudate nucleus or putamen) and temporarily reverse the symptoms of PD (Freed 1981). Placement of an inplant especially

adrenal medullary cells cause fiber growth from intact DA neurons (Bankiewicz 1988, Bohn 1987, Fiandaca 1988, Hansen 1988). In these transplanted animals enhancement of DA neural sprouting is observed despite minimal survival of adrenal medullary cells. It was suggested that the improvement might result from the neural sprouting.

The observation that autografts of adrenal medullary cells may reverse parkinsonian symptoms, led to the present interest in transplantation. Although adult adrenal autografts do not reverse parkinsonian symptoms as well, or survive as long as fetal nigral grafts, they offer certain advantages and bypass the social issues involved in fetal transplantation.

Investigators in Sweden originally transplanted suspensions of autologous adrenal medullary tissue into four patients with advanced PD (Backlund 1985, Lindvall 1987). Two implants were placed in the caudate nucleus and two in the putamen. Only two patients transiently improved.

Undeterred by these results, physicians in Mexico modified the procedure and reported marked improvement in two patients (Madrazo 1987). The Mexican and Swedish studies differed in that the Mexicans used more adrenal tissue, they used an open rather than a stereotaxic approach, they did not make the tissue into a suspension, they implanted the tissue directly into the caudate, and anchored the tissue in such a way that some of the adrenal cells remained in contact with the ventricular fluid. Because of the improvement reported in their first two patients, we and others began clinical studies to determine the efficacy of autologous adrenal medullary transplantation.

METHODS

Between July 8, 1987 and April 28, 1988, 12 autologous adrenal medullary to caudate nucleus transplants were performed at the New York University (NYU) Medical Center. Information on the patients is reported as of December 31, 1989. Patients selected for inplantation had advanced PD and were no longer responding satisfactorily to antiparkinson medication. The patients and their relatives consented to the surgery by signing a detailed explanation of the procedure approved by the NYU Institutional Review Board.

The mean age of the patients was 55.1 years (range 37-65 years), the mean duration of their PD was 11.7 years (range 4-40 years). Eleven of the patients were on levodopa/carbidopa (Sinemet). Ten of the patients had diurnal response fluctuations consisting of "wearing off" phenomena and "on-off" phenomena.

Prior to surgery all 12 patients were assessed using the Unified Parkinson Disease Rating Scale (UPDRS). All patients were assessed on the UPDRS both in an "on" and an "off" period. Diurnal response fluctuations were further evaluated using questions from a subset of the UPDRS. All patients were also rated on the Hoehn and Yahr Scale in both an "on" and an "off" period. Assessments were made at baseline, at twice weekly intervals in the hospital and at four monthly intervals outside the hospital.

Preoperatively, 11 of the patients underwent magnetic resonance imaging (MRI) of the brain, and one underwent computed tomography (CT). In all patients, CT scans of the abdomen demonstrated two adrenal glands. All of the patients were evaluated preoperatively by an internist. All patients underwent neuro-psychological testing and quantitative evaluations of tremor and postural stability.

The first six patients were operated through a right frontal craniotomy. When the caudate nucleus was identified an incision was made into it and the adrenal medullary tissue was placed into a single bed. In the next six patients the lateral ventricle was entered through a transcallosal approach. Three separate beds were made in the caudate allowing for a wider dispersal of the adrenal medullary tissue. In all patients, the left adrenal gland was removed via an abdominal approach. Between 3 to 15 fragments of adrenal tissue were removed, the fragments weighing in total up to 1 gram. The adrenal medullary and caudate tissue were assayed for tyrosine hydroxylase (TH) activity, and the caudate tissue

was assayed for DA, homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) (Goldstein 1988).

Changes in patient functioning from baseline to December 31, 1989 were analyzed using non-parametric methods. The primary mode of evaluation was the Wilcoxon matched-pair signed-rank test. Both one tailed and two tailed tests were performed. Level of significance was $p < 0.05$.

RESULTS

The results of the surgery are summarized in table (1). The main findings were a 35 % reduction in levodopa post-operatively ($p < 0.01$), a 13 % reduction in the patient's stage in the "off" period ($p < 0.05$), and a 30 % reduction in fluctuations ($p < 0.05$).

Three patients had excellent responses. Patient Nº 3, a 58-year-old man experienced his first symptoms four years before surgery as difficulty turning while skiing. Two years later, he could not ski, one year later he could not walk, and six months later, he could not speak. Before surgery, he was confined to a wheelchair. Although he could stand, he fell spontaneously. He was a Stage 5 parkinsonian and was "off" all of the time. He was on levodopa 1200 mg/day, carbidopa 300 mg/day and Deprenyl 10 mg/day. Improvement began two weeks after surgery. One month later he was able to walk unaided and speak. One year later, he improved further. Now, 28 months later, he is able to go outside by himself, drive a car, and ski. He is on levodopa 600 mg/day and carbidopa 150 mg/day.

Patient Nº 7, is a 56-year-old man with a 21 year history of PD. He had diurnal response fluctuations. In his "on" period he was a Stage 3 and in his "off" period he was a Stage 5. His "on" periods lasted less than 25 % of the day. He was on levodopa 1750 mg/day and carbidopa 750 mg/day. He underwent the surgery and one month later was moderately improved.

Four months later, he was back to his baseline state. At this time, without any change in his medication, he again began to improve. Twenty-two months after surgery the patient is Stage 2 in his "on" periods and Stage 3 in his "off" periods. He is "on" for up to 75 % of the day and can work unaided. He is on levodopa 1000 mg/day and carbidopa 100 mg/day.

Patient Nº 10, a 46-year-old man, had a 9 year history of PD. He responded to levodopa, but developed diurnal response fluctuations. In his "on" period he was Stage 3 and in his "off" periods he was Stage 5. His "on" periods occupied less than 25 % of his day. He was on levodopa 500 mg/day and carbidopa 50 mg/day. The patient underwent surgery and began to improve two weeks later. Two months later he was a Stage 2 in his "on" periods and Stage 3 in his "off" periods. He was "on" for up to 75 % of the day. He was now able to walk up and down the six flights of stairs from his apartment. He was on the same dose of levodopa/carbidopa. At this time the patient died suddenly and an autopsy failed to reveal the cause of death. There were no surviving adrenal medullary cells in the caudate nucleus. This patient with typical PD, and a typical response to levodopa had few pigmented cells in the nigra, but no Lewy bodies.

Three other patients improved moderately. Patient Nº 6, a 37-year-old female with a 7 year history of PD was able to go for 8 hours between her last dose of levodopa at night and her first dose in the morning. Now 26 months after surgery, she is able to go 18 hours between doses of levodopa. Patient Nº 8, is a 60-year-old man with a 12 year history of PD. Before surgery he was "on" for less than 50 % of the day and would rapidly fluctuate between his "on" and "off" periods. Two months after surgery he was "on" for up to 75 % of the waking day and his fluctuations were less abrupt. However, he did not maintain his improvement. Patient Nº 9, is a 65-year-old woman with a 12 year history of PD. Before surgery she was "on" less than 50 % of the time. Her "off" periods were abrupt and unpredictable. She began to improve one month after the surgery. She is now, 21 months after surgery, "on" for more than 50 % of the day and her "off" periods are predictable.

Two patients did not improve. Patient Nº 4, a 52-year-old physician, was severely affected by PD and died 4 months after the surgery of unrelated causes (spinal epidural abscess). Post mortem examination revealed few pigmented neurons in the nigra and the presence of Lewy bodies. Examination of the caudate nucleus revealed clusters of surviving adrenal medullary cells (Hurtig 1988).

Four patients had major complications, Patient Nº 1, a 58-year-old woman with a 40-year history of PD had a cardiac arrest one week after surgery related to a vagal response to suctioning. She was promptly resuscitated. Twenty-nine months after surgery, her parkinsonism is improved, but she has mental impairment.

Patient Nº 5, a 60-year-old man with a 4-year history of PD suffered a right cerebral infraction (transfrontal approach) resulting in a left hemiplegia. He died 23 months later.

Patient Nº 11, a 59-year-old woman with a 9-year history of PD suffered a right frontal venous hemorrhage (transcallosal approach). The patient slowly recovered and 20 months later she is at her preoperative level of functioning.

Patient Nº 12, had a right frontal venous infarction after a transcallosal approach. He subsequently developed thrombocytopenia and a disseminated intravascular coagulopathy (related to an antibiotic). The patient then hemorrhaged into the right frontal infarction. He was eventually discharged from the hospital but died 5 months after surgery. Adverse effects are summarized in Table 2.

RESULTS

Neural transplantation is an experimental treatment for a debilitating illness. Three of our patients improved with changes that could not be achieved with medication. The improvement in two patients was biphasic suggesting two separate mechanisms. An initial mechanism, perhaps related to release of trophic factors from the striatum or from inflammatory cells, and a second mechanism, perhaps related to reinnervation of striatal neurons by collateral sprouting from the remaining nigral neurons. The improvement is not related to the survival of the grafted cells. (Bankiewicz 1988, Bohn 1987, Fiandaca 1988, Hurtig 1988, Jankovic 1989, Lieberman-patient 4, 10, Peterson 1989).

Three other patients improved moderately with an increased time spent in their "on" periods and a decreased disability in their "off" periods. This improvement, in 50 % of patients, is similar to the improvement reported by others (Table 3). Moreover, improvement is often seen in symptoms such as postural instability symptoms which usually do not respond to antiparkinson drugs. So striking is this improvement (Patient Nº 3) that others have performed adrenal to caudate inplants in patients with progressive supranuclear palsy where symptoms of postural instability are prominent (1989).

In view of the improvement noted in our patients and in others it is relevant to ask why, 3 years after Madrazo's report, there is disillusionment about the efficacy of the procedure. Unfortunately, the surgery is not uniformly beneficial and poses substantial risks. Madrazo reports that his best responses occurred in patients in their 30's and 40's. In general, with some exceptions, the best responses in the U.S. have also been in younger patients. These results are similar to those in animals where the best results are obtained by transplanting juvenile adrenal medullary tissue (Allen 1989, Backlund 1985-Lindvall 1987 combined, Goetz 1989, Jankovic 1989, Jiao 1988, Madrazo 1990). This suggests that, after a certain age, autologous adrenal medullary tissue is less suitable for transplantation, either because of the loss of adrenal medullary tissue that occurs with aging or as an effect of the disease on the adrenal tissue (Carmichael 1988).

In order for the surgery to become accepted the mortality and morbidity of the operation must decrease. The high morbidity is related to performing two operations on debilitated patient. One way of decreasing morbidity is to use a stereotaxic rather than an open approach. Efficacy may be impro-

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Patient Nº 12, had a right frontal venous infarction after a transcallosal approach. He subsequently developed thrombocytopenia and a disseminated intravascular coagulopathy (related to an antibiotic). The patient then hemorrhaged into the right frontal infarction. He was eventually discharged from the hospital but died 5 months after surgery. Adverse effects are summarized in Table 2.

RESULTS

Neural transplantation is an experimental treatment for a debilitating illness. Three of our patients improved with changes that could not be achieved with medication. The improvement in two patients was biphasic suggesting two separate mechanisms. An initial mechanism, perhaps related to release of trophic factors from the striatum or from inflammatory cells, and a second mechanism, perhaps related to reinnervation of striatal neurons by collateral sprouting from the remaining nigral neurons. The improvement is not related to the survival of the grafted cells. (Bankiewicz 1988, Bohn 1987, Fiandaca 1988, Hurtig 1988, Jankovic 1989, Lieberman-patient 4, 10, Peterson 1989).

Three other patients improved moderately with an increased time spent in their "on" periods and a decreased disability in their "off" periods. This improvement, in 50 % of patients, is similar to the improvement reported by others (Table 3). Moreover, improvement is often seen in symptoms such as postural instability symptoms which usually do not respond to antiparkinson drugs. So striking is this improvement (Patient Nº 3) that others have performed adrenal to caudate inplants in patients with progressive supranuclear palsy where symptoms of postural instability are prominent (1989).

In view of the improvement noted in our patients and in others it is relevant to ask why, 3 years after Madrazo's report, there is disillusionment about the efficacy of the procedure. Unfortunately, the surgery is not uniformly beneficial and poses substantial risks. Madrazo reports that his best responses occurred in patients in their 30's and 40's. In general, with some exceptions, the best responses in the U.S. have also been in younger patients. These results are similar to those in animals where the best results are obtained by transplanting juvenile adrenal medullary tissue (Allen 1989, Backlund 1985-Lindvall 1987 combined, Goetz 1989, Jankovic 1989, Jiao 1988, Madrazo 1990). This suggests that, after a certain age, autologous adrenal medullary tissue is less suitable for transplantation, either because of the loss of adrenal medullary tissue that occurs with aging or as an effect of the disease on the adrenal tissue (Carmichael 1988).

In order for the surgery to become accepted the mortality and morbidity of the operation must decrease. The high morbidity is related to performing two operations on debilitated patient. One way of decreasing morbidity is to use a stereotaxic rather than an open approach. Efficacy may be impro-

ved by staging the operation. Thus, precavitation of the striatum may release trophic factors which may make the striatum more hospitable to later graft placement.

Another means of improving the surgery may be to use adrenal medullary tissue from organ donors. This approach bypasses the need for an adrenalectomy and allows the harvesting of at least two adrenal glands. This approach coupled with methods to preserve and culture adrenal medullary tissue may enable the surgery to be done safely, even on patients with advanced disease.

TABLE 1

RESULTS ADRENAL MEDULLARY INPLANTATION

PTS	Levodopa	Hoehn Yahr STAGE "ON"	Hoehn Yahr STAGE "OFF"	UPDRS "ON"	UPDRS "OFF"	FLUCT
				(160)	(160)	(20)
PRE						
12 Mean	1437	3.3	4.8	58	88	8.5
S.D.	± 1037	± 0.83	± 0.45	± 15.8	± 11.1	± 3.1
POST						
12 Mean	929	2.6	4.2	39	78	5.9
S.D.	± 892	± 1.1	± 0.83	± 23.2	± 19.8	± 2.6
CHANGE	— 35%	— 21%	— 13%	— 32%	— 12%	— 30%
	p<0.01	NS	**p<0.05**	NS	NS	**p<0.05**

TABLE 2

ADVERSE EFFECTS	Number of Patients with Permanent Disability	Number of Patients with Transient Disability
Central Nervous System	0	8
Cerebrovascular	3	1
Cardiovascular	1	2
Pulmonary	1	8
Gastrointestinal	0	1
Genitourinary	0	3
Hematological	0	1

TABLE 3

ADRENAL MEDULLARY INPLANTATION

Series		PTS	Age	Duration PD	PTS Improved	Adverse Effects
Lindvall	87	4	53	10.3	1	0
Jiao	88	4	50	8.5	4	0
Allen	89	18	49	10.8	4	0
Goetz	89	19	54	12.9	7	2
Jankovic	89	3	51	16.0	1	0
Kelley	89	8	55	14.5	1	0
Lieberman	90	12	55	11.7	6	4
Madrazo	90	42	48	8.9	29	7
		110	**M 50.8 S.D. ±5.7**	**10.8 ±1.2**	**53**	**13**

SUMMARY

Neural transplants have opened a new era. The transplants are forcing neurologists to rethink their ideas on how the brain is organized and to play a more active role in treatment. Although there have been benefits to some patients the results are inconsistent and the morbidity is high.

It is clear that before the procedure can be used in the management of PD, additional studies will have to be performed in animals. Issues to be resolved include (1) the relative efficacy and viability of fetal versus adult transplants, (2) autologous versus homologous transplants, (3) mechanisms by which the grafts have a beneficial effect.

RESUMEN

Los transplantes nerviosos han iniciado una nueva era. Los transplantes están impulsando a los neurólogos a meditar acerca de como el cerebro está organizado y desplegar un rol más activo en el tratamiento.

Aunque algunos pacientes han recibido beneficios, los resultados son inconsistentes y el estado mórbido es alto.

Es claro que antes de que este procedimiento pueda ser usado en el tratamiento de la Enfermedad de Parkinson, estudios adicionales deben ser realizados en animales. También a ser resueltos incluyen (1) la relativa eficacia y viabilidad de transplantes fetales frente a adultos (2) transplantes, antólogos frente a homólogos, (3) mecanismos por los cuales los injertos tienen un efecto benéfico.

RÉSUMÉ

Les grèfes nerveuses ont iniciés une ère nouvelle. Les grèfes ont poussé les neurologues a penser quelle peut être l'organization du cerveau, et a avoir un rôle plus actif dans le traitment.

Quelques patients en ont reçu bénéfice; mais les résultats sont inconsistants et la morbilité est grande.

Il est clair qu'avant que ces procédes puissent être utilisés pour le traitment de la maladie de Parkinson, d'autres études sur l'animal doiveut se poursuivre. On doit aussi trouver une solution á l'éficacité relative, et a la viabilité de la grèfe foetale sur un adulte. Grèfe étérologue comparé a la grèfe homologue, mécanimes qui interviennent dans l'obtention d'un résultat bénefique.

ZUSAMMENFASSUNG

Die neurologischen Transplantationen haben ein neues Zeit alter begonnen. Diese Transplantatiien zwingen die Neurologen, darueber nachzudenken wie das Behirn organisiert ist undeine aktivere Rolle bei der Behandlung zu spielen.

Obwohl einige Patienten sich gebessert haben, sind doch die Ergebnisseunbestaen dig und der krankhafte Zustand bleibt ernst.

Es istklar, bevor diese Operation bei der Behandlung der Prkinsonschen Krankheit angewa ndt werden kann, weitere Untersuchungen in Tierexperimenten durchgefuehrt werden muessen. Es muessen weiter ge klaert werden 1) die reltive Wirkungfaehigkeit und Lebensfaehigke it der foetalen Transplantate im Gegensatz zu Transplantaten von Erwachsenemgewebe 2) Autologe gegenueber homologen Transplantaten, 3) die Mechanismen, durch die die Ueberpfla nzungen eine vorteilhafte Wirkung haben.

REFERENCES

1. *Allen G. S., Burns R. S., Tulipan N. B.*: Adrenal Medullary transplantation to the caudate nucleus in Parkinson's disease. Arch Neurol. (1989); 46: 487-491.
2. *Backlund E. D., Granberg P. O., Hamberger B.*: Transplantation of adrenal medullary tissue to striatum in parkinsonism. J. Neurosurg (1985); 62: 169-173.

3. *Bankiewicz K. S., Plunkett R. J., Kopin I. J.*: Transient behavioral recovery in hemiparkinsonian primates after adrenal medullary allografts. Progress in Brain Research (1988); 78: 543-549.

4. *Bjorklund A., Dunnett S. B., Stenevi U.*: Reinnervation of the denervated striatum by substantia nigra transplants: Functional consequences as revealed by pharmacological and sensorimotor testing. Brain Res. (1980); 199: 307-333.

5. *Bohn M. C., Cupit L., Marciano F.*: Adrenal medulla grafts enhance recovery of striatal dopamineric fibers. Science (1987); 237: 913-916.

6. *Carmichael S. W., Wilson R. J., Brimjoin W. S.*: Decreased Catecholamines in the adrenal medulla of patients with parkinsonism. New England J. Med. (1988); 318: 254 (letter).

7. *Fiandaca M. S., Kordower J. H., Hansen J. T.*: Adrenal medullary autografts into the basal ganglia of cebus monkeys: Injury induced regeneration. Experimental Neur (1988); 102: 76-91.

8. *Freed W. J., Morihisa J. M., Spoor E.*: Transplanted adrenal chromaffin cells in rat brain reduce lesion induced rotational behavior. Nature (1981); 2292: 350-351.

9. *Goetz C. G., Olanow C. W., Koller W. C.*: Multicenter study of autologous adrenal medullary transplantaion to the corpus striatum in patients with advanced Parkinson's disease. New Engl. J. Med. (1989); 320: 337-341.

10. *Goldstein M., Lieberman A., Helmer E.*: Biochemical analysis of caudate nucleus bioppsy samples from parkinsonian patients. Ann Neurol (1988); 24: 685-688.

11. *Hansen J. T., Kordower J. H., Fiandaca M. S.*: Adrenal medullary autografts into the basal ganglia of cebus monkeys graft viability and fine structure. Experimental Neur (1988); 102: 65-75.

12. *Hurting H., Joyce J., Sladek J. R.*: Postmortem analysis of adrenal medulla to caudate autograft in a patient with Parkinson's disease. Ann Neur (1989); 25: 607-614.

13. *Jankovic J., Grossman R., Goodman C.*: Clinical, biochemical and neuropathologic findings following transplantation of adrenal medulla to the caudate nucleus for treatment of Parkinson's disease. Neur (1989); 39: 1227-1234.

14. *Jiao S., Zhang W., Cao J.*: Study of adrenal medullary tissue transplantation to striatum in parkinsonism. Progress in Brain Research. (1988); 78: 575-580.

15. *Kelly P. J., Ahlskog J. E., Van Heerden J. A.*: Adrenal medullary transplantation into the striatum of patients with Parkinson's disease. Mayo Clinic Proc. (1989); 64: 282-290.

16. *Koller W. C., Morantz R., Vetere-Overfield B.*: Autologous adrenal medullary transplant in progressive supranuclear palsy. Neur (1989); 39: 1066-1061.

17. *Lindvall O., Backlund E. D., Farde L.*: Transplantation in Parkinson's disease: Two cases of adrenal medullary grafts to the putamen. Ann Neur (1987); 22: 457-468.

18. *Madrazo I., Drucker-Colin R., Diaz V.*: Open microsurgical autograft of adrenal medulla to the right caudate nucleus in patients with intractable Parkinson's disease. N Engl J Med. (1987); 316: 831-834.

19. *Madrazo I., Drucker-Colin R.*: Neurosurgical treatment of Parkinson's disease with adrenal medullary autografts to the caudate nucleus. (1990) In Press.

20. *Olson L., Blacklund E. O., Gerhardt G.*: Nigral and adrenal grafts in parkinsonism. Recent basis and clinical studies. Adv Neur (1986); 454: 85-94.

21. *Peterson D. I., Price L., Small C. S.*: Autopsy findings in a patient who had an adrenal-to-brain transplant for Parkinson's disease. Neur (1989); 39: 235-230.

Primary Report on Auto - and Fetal Adrenal Medullary Tissue Implantation by Open-Microsurgery for Parkinson's Disease (PD)

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Key words Parkinson's disease; microcraniactomy; fetus; adrenal medullary tissue; caudal nucleus; transplantation.

Backlund (1985) reported first on implantation of cerebral tissue to treat Parkinson's disease (PD). In 1987, Madrazo (1987) reported to change implantation technique to craniactomy and to achieve short term effect after operation. In 1987, Beijing Neurosurgical Institute improved Madrazo's method to implant auto-or fetal adrenal medullary tissues into the caudal nucleus of 7 parkinsonian patients. At present those patients have been followed for 13 to 20 months.

Clinical data

I. General materals. See Table.

II. The principle to choose patients:

1. All patients were confirmed as primary PD by neurologists; 2. They aged under 60 years without dementia and psychical disorders. 3. They have correspondence to L-dopa or madopa but the treatment result was turning to bad or there were serious side effects appeared. The patients had lost work ability and hardly to support themselves. 4. The patients and relatives strongly demand and understand the transplantation.

III. The examination and evaluation of pre-and post-operation.

1. To evaluate the patients clinically with Webster's rating scale on PD (1986). The preoperation evaluations were performed during and after stopping of the drug treatment. Every 2 weeks after operation, one evaluation was carried out. One month after the operation, the patients were appraised once every 3 months. 2. Selfevaluation was performed by patient after the operation. 3. The CSF specimen were obtained pre-and postoperatively to examine DA and its metabolic materials' concentrations with HPLC technique for comprasion.

IV. Operation method

The keynotes were as follows: 1. After the right frontal small flap cranictomy, to puncture at middle front gyrus into lateral ventricle to decide the position of ventricle frontal horn and make cortical fistula (15-20 mm in diameter) at which microscope were focused so as to determine the head of

caudal nucleus. Then to make cavities of 5 mm deep and 5 mm in diameter. 2. In view of autograft of adrenal medul-lary tissue cases, to resect the right 2/3 of or total of the adrenals; as for fetal adrenal medullary transplantation, to resect the bilateral adrenal medullary tissue immediatelly after the stopping of the fetus (4.5-5 months of fetal age) heart. Under microscope, to transect and lacert the corties from medullary tissue and then to cut the latter into 1-1.5 mm^3 fragments. Then put the fragments into Ringer's aseptic culture dish (1986). 3. To cut hemotic gelatin sponge into disks of 1-1.5 mm thick and 8-10 mm in diameter and puncture them into cribriform to cover graft tissues. 4. With direct vision under microscope, to put the adrenal medullary tissue fragments into the cavities in the caudal neucleus, then cover the cavities with the cribriform gelatin sponge and press the sponge under ependyma. In this way, the grafted tissue was fixed into the cavities and could get nutrition from CVF. In each operation we organized 2-3 operative groups, the time from getting adrenal to completing implantation was 15-30 minutes.

V. Typical cases:

Case 1 Male, 32 years of age, admission number: 54087. Admission date: January 29, 1988. He had troubled with PD for 5.5 years before coming to medical attention. The initial symptom was the left hand tremor. The four extrimeties were involved within 1.5 years. On admission, the left extrimeties had identical muscular rigidity and difficulty in walk. Madopa had been administered but achieved little result. Examination: clear speech, mask face, high tension of four extremities, esp. the left extremities, four extremity tremor (5 times/second) and small gait. After admission, he was treated with antiparkinsonian drugs: Arntane, 2 mg, three times aday; amantadine, 0.1, three times a day; and madopa, 250 mg, two times a day. The Webster scores were: 11 (during drug treatment) and 13 (after stopping of drugs).

He was performed autograft of adrenal medullary tissue into caudal neucleus in April 18, 1988. After operation, only amantadine (2 mg, three times a day). One week after operation, he felt the left extremities relaxed, expression of the face became natural, the left hand tremor disappeared, upper extremities' tension reduced and the lower extremities' tension remained the same as before. The Webster score was 10. One month after operation, he felt normal muscle tension, natural walk, the left extremities' muscular tension reduction and less extremity tremor. He won 6 of Webster score. Up to now, he was followed for 13 months. He was found still using amantadine (2 mg, three times a day) and 10 of Webster score.

Case 2 Male, 18 years of age, admission number: 53257. Admission date: December 8, 1987. Before then, he had suffered PD with a history of 4 years. Limited movement and rigidity were appeared initially in the right upper extremity and one year later invaded ispilateral lower extremity with tremor. An half year before admission, his symptoms progressed to the extent of the difficulty in the right hand writing and slow speech. He had been treated with madopa but which made less and less effect. Examination: Slow speech, mask face, mandillary and tonge little tremor, increased extremities' tension. On admission, he was administered with madopa (250 mg, three times a day) and amantadine, (0.1, three times a day). His Webster scores were 11 and 14 respectively during drug administration and after drug stopping.

He was implanted with fetal adrenal medullary tissue in January 26, 1988. After surgery, he was still used drugs at doses administered before operation. One week after that, he felt identical reduction of upper extremities' tremor and four extremities's tension. When being supine, he could strecth the whole body. His Webster score was 9. The drugs were reduced one month after operation. Madopa was applied (250 mg, one time aday). Webster score was 8. Up to now, he was followed for 17 months and his conditions remained the same.

DISCUSSION

The basic pathological and histochemistric change of PD is the mesencephalic nigra dopaminergic neuronal degeneration

and absence leading to the reduction of DA in striatum. The vast studies on cerebral tissue transplantion indicate that to implant dopamine neurons of fetal and auto-adrenal medullary tissue into animal models of parkinsonism can successfully reverse the abnormal behavior of the animal models (1979, 1981). On this basis, Backlund (1985) first reported in 1985 on the graft of autograft of adrenal medullary tissue into the right caudal nucleus with CT-guided sterotaxic technique. His two cases underwent transplantation had limited and short-term lasted improvement. The dopaminergic metabolic materials in the CSF of the cases were only slightly and short increased after operation. Studies (1979, 1976) had showed that the implantation into the artificial cavities and ventricles can let the transplanted tissue get in touch with the CSF, while the latter can supply the former with nutrition in favour of living. On the other hand, to make cerebral cavities for transplantation can let the grafted tissue to set up direct functional synaptic connection with the surrounding areas; and ventricle grafting offers the opportunity for bioactive materials secreted by implanted cells transfering to other parts of central nervous system through CSF. In this way, the implanted tissue acts as biopump. Combinating the above advantages, Madrazo (1987) performed in 1986 two young patients with PD with direct vision under microscope by craniactomy to autograft adrenal medullary tissue into the artificial cavities of the head of the right caudal nucleus communicated with the lateral ventricle, which improved the patient's clinical conditions in a short time. In 1987, Allen (1987) applied Madrazo's method on 6 patients with Parkinson's disease and achieved certain effect in short term. However, no DA or NE change was found in CVF determination. Penn (1988) reported in 1988 on his improved Madrazo's method to 5 cases of PD. The patients received antiparkinsonian drug administration (preoperative dose) 3 weeks after operation. In the observation of 20 weeks after operation, the patients' symptoms had the tendency of improvement. This suggests that the results of implantation were hardly acceptable. While Wa-cheng Zhang (1988) presented his good results of 4 cases of transplantation, which had their symptoms identically improved as high as 8.5 of Webster score. The CSF and DA contents were also clearly increased. The 7 cases in present series, with a mean follow-up period of 18 months, has certified this method as quite safe so as to avoid making further neurological damage to the patients. The 4 cases with autograft of adrenal medullary tissue implanted into caudal nucleus had their age range similar to that of Allen and Penn but elder than that of Madrazo. In the followup, no improvement was observed as identical as that of Madrazo. Besides the reduction of antiparkinsonian drug in 3 cases (case 1 and case 4 were signfiicant), clinical symptoms and signs had subjective and objective improvement of different degree. However, the Webster scores were not identically changed comparised with preoperation. Pre-and postoperative CSF's DA and its metabolic material examination showed no statistic significant change.

The other 3 cases in the present study underwent fetal adrenal medullary tissue implantation with the same method discriped above. We considered that fetal tissue has more active ability to survive than matured tissue. Furthermore, the patient experiences only one operation on head so that he avoids surgical suffering and the complications followed endocrinal function depression of adrenal resection. Because the success of survival of implanted fetal tissue has been achieved in some animal models (esp. primate) of PD (1986) and the old of human was subject mainly to PD, the implantation of fetal adrenal tissue may be the suitable choice to treat PD. West (1953) reported that the adrenal medullary tissue of 15 weeks of fetal age could produce catachemine, so we selected donor fetuses of 4.5-5 months of fetal age. In addition, we administered cyclosporine A postoperatively to the patients. 3 cases had their symptoms improved identically. Until now, the Webster score improved 3 under the condition of the drugs' reducing to 1/3 doses. The other 2 cases showed unidentical effect with that of autograft of adrenal medullary tissue at followup of 18 months.

The pre-and postoperative DA examination of CSF did not indicate statistical changes in 3 cases. The concept that brain is immunologically prividged site has been accepted by most neuroscientists. A study tend in recent years, however, is hat the brain is a parted immunologically privileged site (1981). The 3 cases recived postoperatively continuous treatment of cyclosporine A. Apart 1 case showed identical functional improvement, the other 2 had no clear effect difference with those underwent autograft. It is uncertain that the survival difference of implanted tissue exists in the 3 cases caused by immunological rejection. But we can not exclude the active role of immunological inhibitor in grafted tissue's surviving.

In the present series we used HPLC method to check the concentration change of pre-and postoperative CSF's DA and its metabolic materials. The results were primarily identical with that of Backlund and Allen: no clear change between pre-and postoperation. So it is doubted whether the improvement of symptoms were the results of chromaffin cells' surviving and DA increasing. Positron emission tomography (PET) (1987) can be applied on living patients to check if the implanted tissue survives and its metabolic activity. Besides PET, it is difficult to perform other objective examination to decide whether the chromaffin cells survive and carry out normal endocrinal function. If PET is absent, clinical evaluation is the only way to appraise and compare operative results.

This series is the first group in China to perform microcraniatomy to implant self-or fetal adrenal medullary tissue under direct vision into the cavity in the caudal nucleus communicated with lateral ventricle to treat PD. The primary conclusion we make is: 1) Although some cases reduced postoperatively the doses of antiparkinsonian drugs, the actual effect is not up to expectation to improve identically the symptoms. So this technique of operation can not replace at present sterotaxic lesion technique to treat PD. 2) We used separately auto-and fetal adrenal medullary tissue as grafted materials. Apart one case who has been daily administered postoperatively with cyclosporine A achieved more distinctive improvement than that of autograft of adrenal medullary tissue, no clear difference exists between the two kind of implantations. So it may be the effort direction to treat PD by implantation of fetal adrenal medullary or mesencephalic nigra tissues.

TABLE. THE GENERAL INFORMATION OF THE PATIENTS IN THE PRESENT GROUP

Case *	Admission Nº	Sex	Age	Disease course (yr)	Webster rating scale								
					Preoperation		Postoperation (month)						
					Drug treat.	Drug stop	0.5	1	3	6	12	18	20
1	50211	M	44	5	15	17	14	13	13	11	13	13	14
2	50512	M	55	5	11	13	12	10	10	10	11	10	10
3	50723	M	46	26	27	29	26	24	25	25	26	25	26
4	54087	M	32	5.5	11	13	10	6	11	11	10		
5	51034	M	48	25	18	20	18	17	17	17	17	17	
6	51835	M	52	7	17	21	18	17	17	17	17	17	
7	53257	M	48	4	11	14	9	9	8	8	8		

Objective self-evaluation of the patients' symptoms		Postoperative dose change of antiparkinsonian drugs	Comparison of DA & HVA in CSF between pre- & postoperation
Result	Lasting time (mon)		
Four extremity muscle tension & lower extremity tremor reduced.	20	Reduction of 1/2	No change
Four extremity muscle tension reduced & the whole body relaxation.	20	Reduction of 1/2	No change
Facial & four muscle tension & tremor reduced.	4	No change	No change
Four extremity tremor amplitude decreased both upper extremity muscle tension reduced; whole body muscle relax feeling.	13	Reduction of 2/3	No change
Four extremity activity became slightly nimble than preoperation; but no significant improvement in muscle regidity & tremor.	18	No change	No change
Cervical movement became more nimble than preoperation; four extremity regidity decrease.	18	Reduction of 2/5	No change
Both upper extremity tremor decreased; four extremity muscle tension reduced; the whole body relax feeling.	17	Reduction of 2/3	No change

* Cases 5-7 accepted the implantation of fetal adrenal medullary tissue into the caudal neucleus.

SUMMARY

This is the first report of China on 7 cases of implantation of auto-(4 cases) and fetal adrenal medullary tissue (3 cases) into the cavities in the caudal neucleus communicated with the lateral ventricle with open-microsurgery. All grafted tissues were in the form of fragments. The donated fetuses were 4.5-5 month of fetal age. After operation, the recipients were administered orally cyclosporine A every day. In the present series, Webster rating scale, recipient's self-appraisal, and check of concentration of metabolic materials were adopted to examine the operative result. At present, the 7 cases have been followed for 13 to 20 months. The antiparkinsonian drugs have been reduced, but the clinical symptoms and signs have improved. There is no clear dispancy between the autograft and fetal adrenal medullary transplantation. Meanwhile, The metabolic material concentrations of DA in CSF of the 7 cases were not identically changed. The authors would like to point out as far as the treatment of Parkinson's disease is concerned, this kind of operation can not replace sterotaxic lesion in PD; it might be the direction to explore to use fetal adrenal medullary or mesencephalus nigra tissues as implantation materials.

RESUMEN

Este es el primer informe de china sobre 7 casos de implantes de auto (4 casos) y fetal (3 casos) de tejido de médula suprarenal en las cavidades del núcleo caudal comunicando con el ventrículo lateral. Esto con microcirugía abierta.

Lo fetal incorporado era de 4.5-5 meses de edad fetal.

Después de la operación a los que recibieron se las administró por vía oral ciclosporina por 13 a 20 meses. Fueron reducidas los medicamentos antiparkinsonianos

pero los pacientes mostraron mejoría clínicamente en síntomas y signos. No hay diferencias entre el transplante autográfico y el fetal de la médula suprarenal. Sin embargo la concentración del material metabólico de Dopamina en el líquido cefalo raquideo en los 7 casos no tuvieron el mismo cambio.

Los autores desean puntualizar en lo que atañe al tratamiento de la enfermedad de Parkinson, que esta operación no puede reemplazar la intervención estereotáxica. Debe orientarse en la exploración del uso de médula adrenal fetal o tejido mesencéfalo nigra, como materiales de implante.

RÉSUMÉ

Ceci est le premier raport chinois, sur 7 cas de gréfes (auto-gréfes 4 cas) grèfes foetales (3 cas) de tissus de moéle, suprarenales, à l'intérieur de la cavité du moyau caudé, qui comunique avec le ventricule latéral. Technique de chirurgie "ouverte".

Le grèfe foetal avait 4.5.5 mois (âge-foetal). De la ciclosporine a été administré pandant 13' a 20 mois après l'opération. Les anti-Parkinsoniens furent diminués, malgrés cela il y eut une ameilloration clinique des simptones et des signes. Il n'y a aucune différence entre l'autogrèfe et la grèfe embryonaire. La concentration des métabolites de la Dopamine dans le liquide rachidien des 7 cas a diferé dans chaque des cas.

Les anteurs veulent faire remarquer que la traitement de la maladie de Parkinson ne peut pas remplacer l'intervention stéréotaxique. Les traitement doit être orienté vers l'exploratation de l'usage de la moele adrenal foetale ou du tissus de "substancia nigra" comme materiau de grèfe.

ZUSAMMENFASSUNG

Dies ist die erste Mitteilung aus China ueber sieben Faelle von Suprarenalmerk-Transplantationen in die Hoehle des Nucleus Caudatus, wovon 4 Faelle von tautologem Gewebe waren und drei Faelle von foetalem Gewebe. Der Nucleus Caudatus war in Verbindung mit del Ventriculus lateralis. Cie Operation wurde mit oofener Mikrochirurgie durchgefuehrt.

Das einverleibte foetale Gewebe war 4,5 und 5 Monate alt.

Nachder Operation bekamen die Empfaenger Cyclosporina per os waehrend 13 bis 20 Monate. Sie warenauf die antiparkinsonischen Medikamente beschraenkt Doch zeigten die Patienten klinisch die Symsome und Zeichen der Krankheit.

Man bemerkte keinen Unterschied zwischeen den Trans plantaten autologen und foetalen Ursprungs des Geweben. Immerhin, die Konz entration der Metaboliten von Dopamina im der Encephalo-medullaeren Fluessigke it in den sieben Faellen, hatten nicht die gleichen Veraenderungen.

Die autoren betonen bezueglich der Behandlung der Perkinsonachen Krankheit, dass diese Operation nicht die Stereotaxische Intervention ersetzen kann. Man muss untersuchen den Nutzen der Benutzung von foetalem Supraraenalmark o Mesenecephales Gewebe der Substantia Nigra fuer die Transplantationen.

REFERENCES

1. *Allen G. S., et al.*: Vanderbilt Medical News. June 30, 1987.
2. *Apuzzo M. L., Mitchell M. S.*: Immunological aspects of intrinsic glial tumors. J. Neurosurg. 1981; 55: 1.
3. *Backlund E. O., et al.*: Transplantation of adrenal medulla tissue to striatum in parkinsonism: First clinical trials. J. Neurosurg. 1985; 62: 162.
4. *Freed W. J., et al.*: Transplanted adrenal chromaffin cells in rat brain reduce lesion-induced rotational behaviour. Nature. 1981; 292: 351.
5. *Madrazo I., et al.*: Open microsurgical autograft of adrenal medulla to the right caudate nucleus in two patients with intractable Parkinson's disease. N Engl J. Med. 1987; 316: 831.
6. *Penn R. D., et al.*: The adrenal medullary transplant operation for Parkinson's disease: Clinical observations in five patients. Neurosurgery. 1988; 22: 999.
7. *Perlow M. J., et al.*: Brain grafts reduce motor abnormalities produced by destruction of nigrostriatal dopamine system. Science. 1979; 204: 643.

8. *Redmond D. E., et al.*: Fetal neuronal grafts in monkey given methylphenylterrahydropyridine. Lancet. 1986; 1: 1125.

9. *Stenevi U., et al.*: Transplantation of central and peripheral monoamine neurons to the adult rat brain: Techniques and conditions for survival. Brain Res. 1976; 114: 1.

10. *Wa-cheng Zhang, et al.*: Implantation of adrenal medulla into brain to treat tremor and spasity. IV clinical application study. Chinese J. Surg. 1988; 26: 1.

11. *Webster D. D.*: Critical analysis of the disability in Parkinson's disease. Mod. Treat. 1986; 5: 257.

12. *West C. B., et al.*: The function of the organs of Luckerkandl. Clin. Science. 1953; 12 317.

GABAergic Modulation of Cholinergic Septal Neurons Transplanted into the Hippocampal Formation

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INTRODUCTION

Intrahippocampal transplants of fetal cholinergic neurons have been shown to innervate the hippocampal formation of adult rats with fimbria-fornix lesions, and to improve impaired learning and memory function (Low et al., 1982, 1985; Dunnett et al., 1982). Histological investigations using acetylcholinesterase (AChE) staining and choline acetyltransferase (ChAT) immunocytochemistry have demonstrated that cholinergic fibers grow from the graft into the host hippocampus (Bjorklund and Stenevi, 1977; Dunnett et al., 1982; Low et al., 1982, 1983, 1985;) and form synaptic connections (Clarke et al., 1987). Various studies have reported that septal transplants are functional in terms of the electrophysiology of neural connectivity (Low et al., 1982; Buzsaki et al., 1987). In addition, neurochemical studies have shown that ChAT activity (Bjorklund and Steveni, 1977; Bjorklund et al., 1983) in the host hippocampus is restored, and ^{14}C-acetylcholine (^{14}C-ACh) synthesis from ^{14}C-glucose is also reinstated (Bjorklund et al., 1983b) following the transplantation.

Simon et al. (1976) have shown that the rate of high affinity choline uptake (HACU) is related to the activity of cholinergic neurons where activated neurons exhibit higher rates of uptake. This property of HACU can be used to determine whether afferent systems can modulate the activity of transplanted cholinergic neurons. The purposes of this study were therefore to determine whether septal transplants can restore the HACU system in the hippocam-

Table 1. Effects of septal transplants on HACU in synaptosomal fractions from the hippocampal formation following fimbria-fornix lesions.

Group	Dorsal Hippocampus	Ventral Hippocampus
Sham	20.1 ± 1.7 (n = 6)	23.3 ± 0.9^{+} (n = 6)
Lesion	5.6 ± 0.9* (n = 6)	8.8 ± 0.9$^{+,*}$ (n = 6)
Transplant	17.8 ± 3.1 (n = 5)	17.3 ± 1.8** (n = 4)

* $p < 0.01$ vs. sham and transplant

** $p < 0.01$ vs. sham

$^{+}$ $p < 0.01$ vs. dorsal hippocampus of the same experimental group

HACU assays were performed using 0.5 uM 3H-choline as described in text. Mean ± SEM values (pmole/4min/mg protein) are presented.

pal formation in rats with fimbria-fornix lesion, and whether HACU can be activated by a gamma amino butyric acid (GABA) antagonist, picrotoxin, and thus provide evidence that transplanted cholinergic neurons receive functional inputs from GABAergic afferents.

METHODS

Pregnant Sprague-Dawley rats were anesthetized with Nembutal (50 mg/kg, i.p.). Eight to 14 embryos were taken from each pregnant rat at a gestational age of 15 to 17 days (crown-rump length 13-19 mm). The septal-diagonal band area was dissected and pooled in chilled CEM-2000 tissue culture medium (Scott Labs) and dissociated into a cell suspension. Recipient rats (male Sprague-Dawley 240-340 g) were anesthetized with Nembutal (50 mg/kg, i.p.), and placed in a stereotaxic apparatus. The transplanted (TP) group received injections of cell suspension (2 ul) bilaterally. The lesion (LES) group and sham operated (SHAM) group received bilateral injections of 2 ul of CEM-2000 alone. Following the injections, the fimbria-fornix pathway was aspirated in the TP and LES animals, whereas SHAM animals received aspirations of cortex overlying the fimbria-fornix fiber projection.

High affinity choline uptake was determined 9-10 weeks following transplantation or surgery as previously described (Simon, 1982). For each assay, one rat from each group was decapitated, and the brain was rapidly removed. Both hippocampi were dissected on ice. The brain tissue was weighed and homogenized in 30 volumes of 0.32 M sucrose. A crude nuclear pellet (P1) was obtained by centrifugation at 1,000 g for 10 min, and the supernatant (S1) was centrifuged at 17,000 g for 15 min to obtain a crude mitochondrial pellet (P2). The P2 pellet was resuspended in

Table 2. Effects of picrotoxin on HACU in synaptosomal fractions from the hippocampal formation following fimbria-fornix lesions and septal transplants.

Group	Saline		Picrotoxin		% of saline
SHAM	23.3 ± 1.6	(n=6)	29.8 ± 2.0**	(n=6)	128%
LES	8.1 ± 0.5	(n=6)	7.6 ± 0.3	(n=6)	94%
TP	13.5 ± 0.9	(n=4)	18.3 ± 1.4*	(n=4)	136%

* $p < 0.05$ vs. saline

** $p < 0.01$ vs. saline

Rats injected with picrotoxin (12 mg/kg, i.p.) were decapitated during the convulsion. HACU was determined at 0.5 uM 3H-choline. Mean ± SEM values (pmole/4 min/mg protein) are presented.

25 volumes of 0.32 M sucrose and used for uptake.

HACU assays were performed in triplicate. Fifty microliters of the resuspended P2 pellet was incubated in Krebs-Ringer phosphate buffer PH 7.4 (KRP: 126 mM NaCl, 4.75 mM KCl, 1.27 mM $CaCl_2$, 15.8 mM Na_2HPO_4, 1.42 mM $MgCl_2$, 10 mM dextrose) containing a final concentration of 0.5 uM [^{3}H]choline. The incubation was performed at 30ºC for 4 min, and blank values were determined from samples maintained at 0ºC for 4 min. After the incubation, the tubes were centrifuged at 6,000 g for 15 min. The supernatant was removed by aspiration, and the pellets were rinsed with 2 ml of cold saline and solubilized with 0.5 ml TS1 (Research Products International). Following 20 min of digestion, 5 ml of 3a20 (Research Products International) was added to each tube and the contents transferred to plastic vials. Radioactivity was counted in a Beckman LS 2800 scintillation spectometer.

HACU was calculated by subtracting blank values from the total uptake values. Protein was measured (Lowry et al., 1951), and HACU was expressed as pmole/4 min/mg of protein.

The ability of GABAergic afferents to modulate the activity of transplanted cholinergic neurons was determined by the use of picrotoxin, a GABA antagonist. Picrotoxin was dissolved in 0.9 % NaCl to obtain a concentration of 3 mg/ml and was injected, i.p., at a dose of 12 mg/kg. Control rats were injected with 0.9 % NaCl (5 ml/kg, i.p.). The rats injected with picrotoxin were decapitated during the tonic phase of convulsion, which generally occurred within 7 min after injection. The hippocampus was dissected and HACU assays were performed.

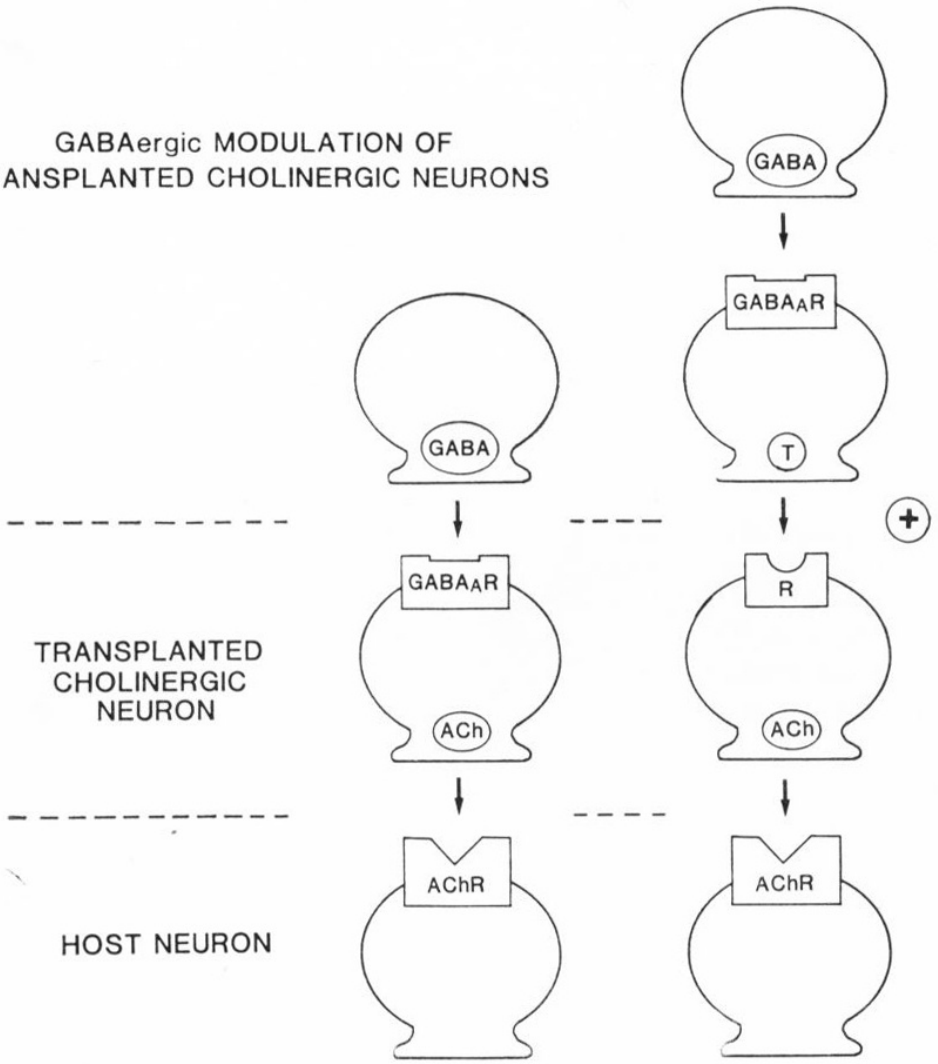

Fig. 1. — *Schematic illustration of possible GABAergic modulation of transplanted cholinergic neurons. Left panel indicates direct innervation of transplanted cholinergic cell by GABA synthesizing neuron. Right panel indicates indirect modulation of transplanted cholinergic cell by a GABAergic neuron via an excitatory interneuron.*

RESULTS

Lesions of the fimbria-fornix produced significant decreases in HACU for both dorsal and ventral hippocampus (Table 1). HACU in the dorsal hippocampus in animals with fimbria-fornix lesions decreased to 28 % of that in SHAM animals. Similarly, HACU in the ventral hippocampus in animals with lesions fell to 38 % of that in SHAM animals. Rats with septal transplants, however, exhibited HACU near normal values. Septal transplants restored HACU back to 89 % and 74 % of SHAM values in the dorsal and ventral hippocampus, respectively. No significant differences in HACU were found between the SHAM and TP groups in the dorsal hippocampus. Significant differences were found, however, between these two groups in the ventral hippocampal formation. HACU in the TP group was significantly lower than that of the SHAM group only in the ventral hippocampus ($p < 0.05$).

Picrotoxin administration increased hippocampal HACU by 36 % and 28 % in the TP and SHAM groups respectively (Table 2). There was no effect of picrotoxin on HACU in the LES group although convulsions were seen in all rats. In both saline and picrotoxin-treated rats, HACU was significantly higher in the TP group than in the LES group.

PROPOSED GABAergic MODULATION OF SEPTAL CHOLINERGIC NEURONS

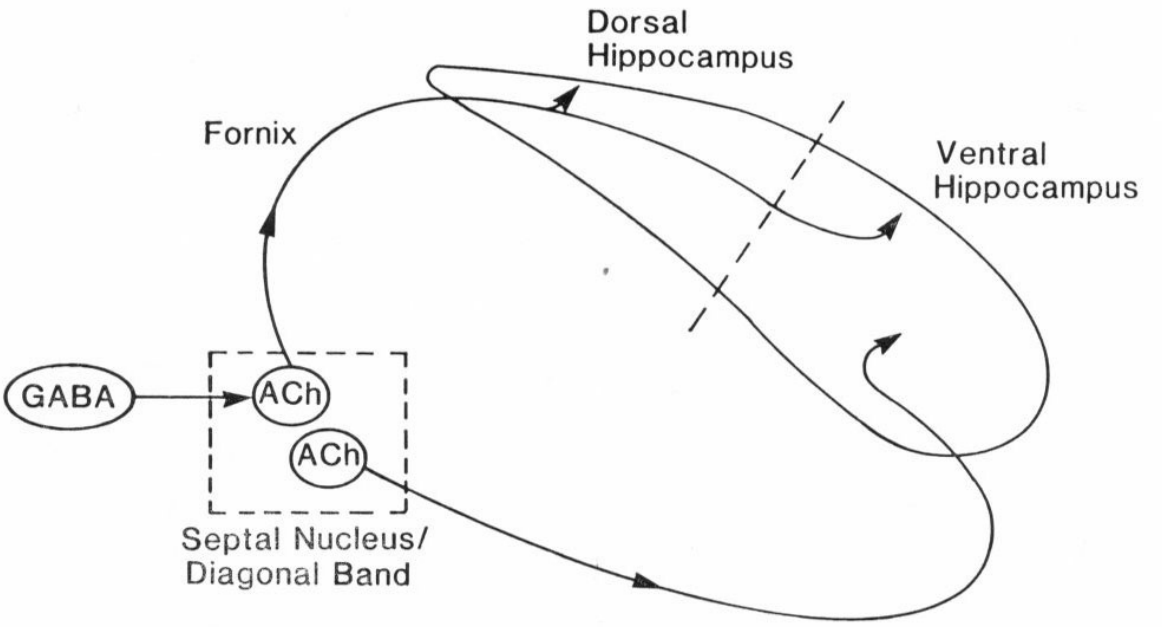

Fig. 2. — *Schematic illustration of proposed GABAergic modulation of cholinergic septal/diagonal band neurons. Note GABAergic innervation of only those cholinergic neurons that project to the hippocampal formation by way of the fornix pathway.*

DISCUSSION

Our results demonstrate that HACU can be restored by fetal septal cells transplanted into the host hippocampal formation in animals with fimbria-fornix lesions. We have also found that HACU in hippocampi with implanted cholinergic neurons can be activated by picrotoxin, a GABA antagonist which is known to increase hippocampal HACU when injected intraperitonealy (Richter et al., 1982). The existence of GABA receptor complexes on cholinergic cells in the medial septal nucleus is suggested by a decrease in hippocampal HACU following the intraperitoneal injection of the GABA agonist muscimol (Millner and Richter, 1986) and the local injection of muscimol into the septum (Richter and Gormley, 1986). Picrotoxin, however, increases hippocampal HACU when injected locally in the lateral septum but not in the medial septum (Richter and Gormley, 1986). From these findings, Richter and Gormley (1986) have suggested that there may be a putative lateral to medial septal excitatory pathway which is tonically inhibited by GABAergic terminals in the lateral septum. The source of GABAergic innervation in the septum may be derived from GAD positive cells in both the medial and lateral septum (Panula et al., 1984).

Hippocampal HACU in the present study was activated by picrotoxin in the TP group but not in the LES group. This suggests that the transplanted cholinergic neurons receive afferent input from a GABAergic system. The transplanted cholinergic neurons might be directly inhibited by GABAergic terminals or innervated by excitatory neurons which receive tonic inhibition from GABAergic neurons (Figure 1). Although it is not clear if the neuronal input to the transplanted cholinergic cells is from the host neurons or from other transplanted neurons, our results suggest the existence of functional connections with the transplanted cholinergic cells. In the LES group, the hippocampal HACU was not changed by picrotoxin although convulsions were seen in all animals. This might

suggest that the cell bodies of the residual cholinergic fibers after fimbria-fornix lesions do not receive GABAergic input (Figure 2). The dorsal pathway which runs over the corpus callosum (Swanson and Cowan, 1979; Milner et al., 1983) and the ventral pathway (Milner and Amaral, 1984; Gage et al., 1983, 1984) originate mainly from nucleus of the diagonal band (Swanson et al., 1987). The data from our lesion studies therefore suggest that the cholinergic neurons in the nucleus of the diagonal band are not innervated by GABAergic afferents.

SUMMARY

Previous studies have shown that transplants of cholinergic cells from the fetal septal-diagonal band are capable of innervating the hippocampal formation and improve learning and memory function in rats with lesions of the cholinergic septohippocampal pathway. The present study was carried out to assess the functional integrity of graft-derived cholinergic terminals and to determine whether the activity of the transplanted cholinergic cells could be modulated by GABAergic afferents. These issues were addressed by using high-affinity choline uptake (HACU) to assess the activity of cholinergic neurons and terminals. Picrotoxin, a GABA antagonist, was administered to determine whether it could modulate graft-mediated HACU.

Transplants of cholinergic septal-diagonal band neurons into rats with lesions of the septo-hippocampal pathway were found to elevate significantly hippocampal HACU in comparison to animals with lesions alone. This increase in HACU returned to near normal levels in both the dorsal and ventral hippocampus (89 % and 74 % of control values, respectively). The administration of picrotoxin in animals with transplants resulted in a 36 % increase in HACU. This was comparable to the 28 % increase exhibited by sham operated controls. These results demonstrate that graft-derived cholinergic terminals are functionally intact and that the transplanted neurons are modulated by GABAergic afferents.

RESUMEN

Estudios previos han puesto en evidencia que transplantes de células colinérgicas provenientes de la banda septal diagonal están en condición de inervar la formación hipocámpica y mejorar la función de aprender y memorizar en ratas con lesiones de la vía colinérgica septo-hipocámpica. Nuestro estudio se propone valorar la integridad funcional de las terminales colinérgicas originadas de injertos y determinar si las células colinérgicas transplantadas puede ser modulada por aferentes gabaérgicas. Para estudiar estos problemas fue utilizado el test de la colina de alta afinidad para valorar la actividad de las neuronas y de las terminales colinérgicas. Administrando la Picrotoxina un antagonista GABA se pudo determinar si esta substancia podía modular el test de la colina HACU por intermedio de transplantes.

Los transplantes de neuronas colinérgicas que provienen de la banda septal diagonal en ratas con lesiones del pasaje septo-hipocámpico elevan en forma apreciable el test hipocámpico de la colina (HACU) en comparación con animales que solo tienen lesiones. Esta elevación del test de la colina (HACU) volvió a su nivel normal para el hipocampo dorsal, y para el hipocampo ventral (85 % y 74 % respectivamente de los valores de control). Administrando picrotoxina en animales que habían soportado un transplante se logra una elevación del 36 % del test de la colina (HACU). Estos resultados demuestran que las terminales colinérgicas derivados de los transplantes, están intactas del punto de vista funcional y que las neuronas transplantadas están moduladas por los aferentes Gabaérgicas.

RÉSUMÉ

Les études passées ont démontré que les greffes de cellules cholinergiques provenant de la bande fétale septale diagonale peuvent innerver la formation hippocampale et améliorer l'acquisition du savoir et la mémoire des rats ayant des lésions du passage cholinergique septohippocampal. Notre étude se propose d'évaluer l'intégrité fonctionnelle des terminaux cholinergiques, issus de greffes, et de déterminer si l'activité des cellules cholinergiques transplantées peut être modulée par les afférents GABAergiques (liés à l'acide biturique amino gamma). Pour étudier ces problèmes, nous avons utilisé le test de la choline à grande affinite pour évaluer l'activité des neurons et des terminaux cholinergiques. En administrant de la picrotoxine, un antagoniste GABA (de l'acide biturique amino gamma), nous avons pu déterminer si cette substance pouvait moduler le test de la choline à grande affinite (HACU) par l'intermédiaire de greffes.

Les greffes de neurons cholinergiques provenant de la bande septale diagonale sur des rats ayant des lésions du passage septo-hippocampal élèvent de façon remarquable le test hippocampal de la choline à grande affinite (HACU) retourne pratiquement au niveau normal pour l'hippocampe dorsal bien que pour l'hippocampe ventral (89 % et 74 % respectivement des valeurs de contrôle). En administrant de la picrotoxine à des animaux ayant subi une greffe, on obtient une élévation de 36 % du test de la choline à grande affinite (HACU). Ces résultats sont comparables au taux d'amélioration de 28 % que l'on observe chez les sujets de contrôle. Ces résultats démontrent que les terminaux cholinergiques, issus de greffes, sont intacts du point de vue fonctionel et que les neurons transplantés sont modulés par les afférents GABAergiques (liés à l'acide biturique amino gamma).

ZUSAMMENFASSUNG

Aus der Literatur ist bekannt, dass Ratten, deren der cholinerger Tractus septohippocampalis zerstoert worden ist, eine Verbesserung der Lern-und Merkvermoegens zeigen, wenn in die Hippocampus-Formation solcher Tiere cholinerge Zellen des fetalen septal-diagonalen Bandes transplantiert werden.

Diese Studie soll die funktionelle Bedeutung der aus dem transplantierten Gewebe entstandenen cholinergen Nervenverbindungen erlaeutern und zu erklaeren versuchen, ob die Aktivitaet der transplantierten cholingergen Zellen durch Gamma-amino-buttersaeure (GABA)-Afferenzen moduliert werden kann. Dazu wurde auf die Methode des Hoch-Affinitaets-Cholin-Uptakes (high-affinity-choline-uptake, HACU) zurueckgegriffen. Ausserdem wurde Picrotoxin als GABA Antagonist in unsere Versuche miteinbezogen.

Es zeigte sich, dass nach Transplantation cholingerger Neuronen des septal-diagonalen Bandes eine HACU des Hippocampus erreicht wurde, die signifikant hoeher war als die der Kontrollgruppe. Der Anstieg der HACU erreichte nahezu normale Werte (89 % im dorsalen Hippocampus, 74 % im ventralen Hippocampus). Durch Picrotoxin konnte die HACU gesenkt werden (36 % bei transplantierten Tieren, 28 % bei scheinoperierten Kontrolltieren). Zusammenfassend kann gesagt werden, dass fetale cholinerge Zellen durch GABA-Afferenzen moduliert werden und dazu befaehigt sind, funktionell intakte Synapsen auszubilden.

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REFERENCES

1. *Bjorklund A., Gage F. H., Schmidt R. H., Steveni U.*, and *Dunnett S. B.*: Intracerebral grafting of neuronal cell suspensions. VII.k Recovery of choline acetyltransferase activity and acetylcholine synthesis in the denervated hippocampus reinnervated by septal suspension implants. Acta Physiol. Scand (Suppl.) 522: 59-66, 1983.
2. *Bjorklund A., Stenevi U.*: Reformation of the severed septohippocampal cholinergic pathway in the adult rat by transplanted septal neurons. Cell Tiss. Res. 185, 289-302, 1977.
3. *Buzsaki G., Czopf J., Kondakor I., Bjorklund A., Gage F. H.*: Cellular activity of intracerebrally transplanted fetal hippocampus during behavior. Neurosci. 22, 871-883, 1987.
4. *Clarke D. J., Gage F. H., Dunnett S. B., Nilsson O. G.*, and *Bjorklund A.*: Synaptogenesis of grafted cholinergic neurons. Ann. N. Y. Acad. Sci. 495, 268-283, 1987.
5. *Dunnett S. B., Low W. C., Iversen S. D., Steveni U.*, and *Bjjorklund A.*: Septal transplants restore maze learning in rats with fornix-fimbria lesions. Brain Res 251, 335-348, 1982.
6. *Gage F. H., Bjorklund A.*, and *Steveni U.*: Reinnervation of the partially deafferented hippocampus by compensatory collateral sprouting from spared cholinergic and noradrenergic afferents. Brain Res. 268, 27-37, 1983.
7. *Gage F. H., Bjorklund A.*, and *Steveni U.*: Cells of origin of the ventral cholinergic septo-hippocampal pathway. Neurosci. Lett. 44, 211-216, 1984.
8. *Low W. C., Lewis P. R., Bunch S.T., Dunnett S. B., Thomas S. R., Iversen S. D., Bjorklund A.*, and *Steveni U.*: Function recovery following neural transplantation of embryonic septal nuclei in adult rats with septohippocampal lesions. Nature 300, 260-262, 1982.
9. *Low W. C., Lewis P. R.*, and *Bunch S. T.*: Embryonic neural transplants across a major histocompatibility barrier: survival and specificity of innervation. Brain Res. 262, 328-333, 1983.
10. *Low W. C., Daniloff J. K., Bodony R. P.*, and *Wells J.*: Cross-species transplants of cholinergic neurons and the recovery of function, in Neural Grafting in the Mammalian CNS (Bjorklund A. and Steveni U., eds.), pp. 575-184. Elsevier Science Publishers, B. V., 1985.
11. *Miller J. A.* and *Richter J. A.*: Effects of GABAergic drugs in vivo on high-affinity choline uptake in vitro in mouse hippocampal synaptosomes. J. Neurochem. 47, 1916-1918, 1986.
12. *Milner T. A., Loy R.*, and *Amaral D. G.*: An anatomical study of the development of the septo-hippocampal projection in the rat. Dev. Brain Res., 8, 343-371, 1983.
13. *Milner T. A.* and *Amaral D. G.*: Evidence for a ventral septal projection to the hippocampal formation of the rat. Exp. Brain Res. 55, 579-585, 1984.
14. *Panula P., Revuelta A. V., Cheney D. L., Wu J.-Y., Costa E.*: An immunohistochemical study on the location of GABAergic neurons in rat septum. J. Comp. Neurol. 222, 69-80, 1984.
15. *Richter J. A., Gormley J. M., Holtman J. R. Jr.*, and *Simon J. R.*: High-affinity choline uptake in the hippocampus: Its relationship to the physiological state produced by administration of barbiturates and other treatments. J. Neurochem. 39, 1440-1445, 1982.
16. *Richter J. A.* and *Gormley J. M.*: Inhibition of high-affinity choline uptake in the rat hippocampus by in vivo injection of phenobarbital in the medial septum. J. Pharm. Exp. Ther. 237, 563-568, 1986.
17. *Simon J. R., Atweh S.*, and *Kuhar M. J.*: Sodium-dependent high affinity choline uptake: A regulatory step in the synthesis of acetylcholine. J. Neurochem. 26, 909-922, 1976.
18. *Simon J. R.*: Cortical modulation of cholinergic neurons in the striatum. Life Sci. 31, 1501-1508, 1982.
19. *Swanson L. W.* and *Cowan W. M.*: The connections of the septal region in the rat. J. Comp. Neurol., 186, 621-665, 1979.
20. *Swanson L. W., Kohler C.*, and *Bjorklund A.*: The limbic region. I: The septohippocampal system, in Handbook of Chemical Neuroanatomy Vol. 5: Integrated systems of the CNS, Part I, Hypothalamus, Hippocampus, Amygdala, Retina (Bjorklund A., Hokfelt T., and Swanson L. W., eds), pp. 125-227, 1987.