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Behavioral Assessment of the Ability of Intracerebral Embryonic Neural Tissue Grafts to Ameliorate the Effects of Brain Damage in Marmosets

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Abstract

The transplantation of neuronal tissue into the brains of patients with Parkinson's disease is already being assessed as an experimental treatment for the symptoms of this disease, and the possibility of using similar graft tissue to ameliorate the symptoms of other neurodegenerative diseases is being considered. In this context, a small number of transplant experiments have been carried out in monkeys with lesions of the central dopamine and cholinergic systems. These experiments make it possible to determine the optimum methods of transplantation in an animal whose brain is structurally more closely related to the human than that of the rat and to assess the behavioral consequences of transplantation on symptoms that either resemble very closely the symptoms seen in patients, or are of a complex cognitive nature and are therefore more difficult to measure in the rat. It is intended that these experiments will contribute to the development of better treatments for the neurodegenerative diseases, either by the use of transplantation as a clinical treatment, or by contributing to a better understanding of the mechanisms that normally maintain neuronal function and that fail in these diseases.

Index Entries: Cholinergic projections; dopaminergic projections; fornix transection; embryonic neural tissue transplants; Parkinson's disease; amnesia; dementia.

Introduction

Although occasional attempts to transplant neural tissue into adult brain were made during the first half of this century *(1,2)*, the modern era of neuronal transplantation began in the 1970s with the work of a group of Swedish researchers who developed the techniques necessary for graft survival (*see* refs. *3,4* for early reports). It has subsequently become clear, in many experiments, that grafted embryonic tissue can form anatomically specific and appropriate synapses with the surrounding tissue (e.g., refs. *5–7*) and that the physiological state of the host can influence the neurochemical activity of the graft (e.g., refs. *8,9*). Of considerable interest has been the extent to which, and by which mechanisms, the graft can influence both the brain activity and the behavior of the host. A wide range of effects of grafting different tissues into rat brain has been demonstrated, including hormone production *(10)*,

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sensory processing *(11)*, movement control (*see* refs. *12,13* for reviews of early work), and effects on learning and/or memory (*see*, e.g., refs. *14–16*, for early work in this area).

Subsequent work has endeavored to elucidate how grafts influence host behavior, the main approach being to define the type, condition, and placement of graft tissue that is effective in comparison to that which is not. Different mechanisms may be at work in different circumstances. It was found, for example, in studies involving the transplantation of solid tissue into surgically prepared cavities in the brain, that sprouting of host dopamine (DA) fibers, and some behavioral recovery, occurred in monkeys with lesions within the dopamine system irrespective of whether the grafted tissue contained DA cells *(17,18)*. This observation, which was seen only in animals with surgical cavitation, may depend on the tissue-stimulating effect of this extensive neurosurgery and/or the MPTP-lesion technique used (*see* Lesions of the DA System), which, in this case, left DA depletion in the adjacent nucleus accumbens incomplete. In contrast, transplantation of DA-rich cell suspensions into the unilateral 6-hydroxydopamine (6-OHDA) lesioned rat, where ipsilateral DA depletion is more extensive, resulted in substantial improvements in behavior, whereas grafts of other types of tissue produced no improvement *(19)*. It is also clear that grafts of either DA-rich or acetylcholine (ACh)-rich tissue must be placed in the target area of the lesioned projection to produce behavioral recovery *(20–22)*. This implies that the graft interacts closely with the adjacent host tissue to bring about the behavioral effects. Further evidence that the graft itself, rather than a change in the host, is responsible for improvement in behavior comes from the observation that if the DA-rich graft is destroyed with 6-OHDA, improvement is then lost *(19)*.

The early work was done primarily using rodents, which remain the species of choice for most experiments. There are, however, persuasive reasons for using primates in certain circumstances. These include the need to examine higher levels of cognitive function (e.g., certain aspects of learning and memory) or skilled movement (e.g., skilled hand as opposed to paw use), the need to investigate physiological or immunological aspects of transplantation in a system that is more comparable to the human, and the desire to study behavior that bears as close a resemblance to the clinical symptoms of human disease as possible. For these reasons, a limited number of experiments have been undertaken using primates. Several groups of investigators have used small numbers of Old World monkeys to look at the effect of DA-rich adrenal medulla chromaffin cell grafts or ventral mesencephalic tissue grafts on movement disorders in monkeys that have been rendered parkinsonian with the neurotoxin MPTP. These studies have been reviewed in detail in Dunnett and Annett *(23)*. In this article, we will describe the work that has been done using the New World monkey, the common marmoset.

Transplantation studies in the marmoset have concentrated on two systems, the DA system and the ACh system, both of which comprise diffuse projections to wide areas of the telencephalon arising from a relatively limited number of discretely placed neurons. Both systems seem to exert an enabling or activating effect on behavior, the DA system projecting mainly to the caudate-putamen and influencing the initiation and execution of movements and the ACh system projecting to the cortex, amygdala, and hippocampus (cf Old World monkeys, ref. *24*) and exerting a comparable effect on the acquisition and maintenance of memories in primates *(25)*. In humans, both systems are important clinically, degeneration of the DA system occurring in Parkinson's disease and degeneration of the ACh system being a major component of the pathology in most dementing diseases, including Alzheimer's disease *(26)*, Parkinson's disease with dementia *(27)*, and alcoholic dementia *(28)*. Transplantation of human DA-rich adrenal medulla chromaffin cells or human DA-rich embryonic ventral mesencephalic tissue has been used as an experimental treatment for the symptoms of Parkinson's disease in a number of different countries (*see* ref. *29* for review of earlier studies and *30–32*). Early reports of great success with adrenal autografts *(33)* have not been sustained in subsequent studies (see ref. *34* for review). Results of transplantation studies using embryonic tissue suggest that if the technique is carefully applied (in terms of the age of the embryonic tissue, the amount of tissue used, its placement in brain, and so forth), then it can be remarkably successful in improving symptoms and in reducing the need for medication *(32)*. The technique was, however, not always successful in earlier studies (e.g., ref. *35*), indicating that the improvement is not a general consequence of surgery and emphasizing the need to carry out further research to define the best techniques. If it is accepted that transplantation of DA-rich tissue can

be therapeutic, then the production of suitable tissue by genetic manipulation and tissue culture techniques will almost certainly obviate the need to use embryonic tissue (cf refs. *36,37* for immortalized hippocampal stem cells). There will, however, always be problems associated with the use of such an invasive treatment. Although transplantation has been useful in the treatment of a few young patients with MPTP-induced parkinsonism *(38)*, idiopathic Parkinson's disease usually occurs in elderly patients, and their physical condition may make some of them unsuitable for major surgery. It is also unlikely that transplantation will be able to arrest the disease process. However, the value of a few "good years" should never be underestimated.

Transplantation of ACh-rich tissue into demented patients has not been attempted to our knowledge, and it is unlikely that it would ever be a feasible proposition. The loss of cholinergic terminals in the target areas in dementia is so extensive that transplants over a very wide area of cortex would have to be made. Furthermore, although the stage of dementia is related to the degree of cholinergic loss statistically *(39)*, the additional neurochemical and structural damage in dementia almost certainly contributes to the cognitive impairment. There are, however, a few exceptional cases where ACh transplantation may be warranted. Iatrogenic and traumatic transection of the fornix can result in profound amnesia *(40)*, and results of experiments described below suggest that the major effect of fornix transection on memory functions occurs as a result of the loss of the cholinergic projections to the hippocampus and can be overcome by ACh-rich grafts into the hippocampus. It is also possible that, although the most common forms of dementia (Alzheimer's disease and multi-infarct dementia) are of cortical origin, some subcortical dementias may be more closely related to cholinergic dysfunction, e.g., the milder memory dysfunction associated with aging and the dementia associated with Lewy-body invasion and cell loss in the basal forebrain cholinergic nuclei in Parkinson's disease. In some milder cases of senile dementia the neurochemical defect may be confined almost completely to a loss of cholinergic activity in the temporal lobes *(41)*. It is possible that ACh-rich transplants, confined to the temporal lobe, may be able to relieve the symptoms and provide a better quality of life, at least for some time, in some of these cases. Even if transplantation of exogenous tissue does not ultimately prove to be a useful or economic treatment for neurodegenerative conditions, it is to be hoped that experiments using this technique will be of value in understanding brain function in general and, in particular, will enable a greater understanding of the processes of degeneration and regeneration to be achieved and lead to the development of methods of preventing and controlling these very common conditions.

Methodological Issues

Animals

The first primate studies of transplantation in the CNS were carried out using Old World monkeys *(42)*, and these species are still the most commonly used even though they present certain difficulties in availability, housing, and handling because of their large size and demanding temperament. The animals used in the experiments that we have undertaken were all common marmosets *(Callithrix jacchus)*. The marmoset is a New World primate, and is particularly suitable for transplantation experiments because of its small size (350–450 g adult weight), relatively cooperative demeanor, and remarkable reproductive rate compared to other primates. New World monkeys form stable monogamous pairs, and engage in frequent copulation throughout the estrus cycle and pregnancy *(43)*. Female marmosets are sexually mature at 18 mo, and many animals will have 2 pregnancies/yr for >8 yr. Each pregnancy produces two to three offspring, although it is exceptional for more than two to be reared. These offspring are not monozygotic, but are usually monoplacental and are therefore tolerant to each other's tissue. The timing of reproduction is also particularly reliable. Conception usually occurs on d 12 *postpartum* and can be detected by transabdominal palpation 4 wk later *(44)*. In the absence of conception, estrus occurs every 12 d *(45)*, and at the beginning of a pregnancy, this time difference is sufficiently large for the date of most conceptions to be calculated accurately following palpation. Gestation is 147 d in the marmoset *(44)*. In comparison, macaques and other Old World monkeys do not reach sexual maturity until 2–3 yr of age, they rarely produce more than 1 offspring/yr, and pregnancies usually have to be timed by restricting mating opportunities between eligible females and a dominant male *(46)*. One possible disadvantage of the marmoset is that the small size of the brain (~3 cm length) may make it

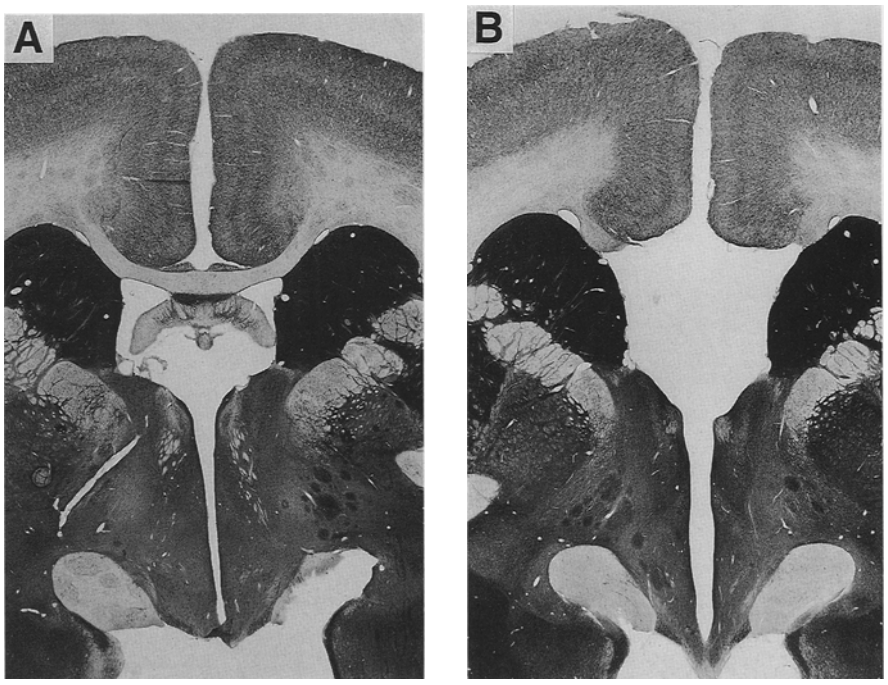


Fig. 1. **(A)** Part of a coronal section of the normal marmoset brain, stained for AChE, showing the fornix suspended below the corpus callosum in the third ventricle. **(B)** Equivalent section in marmoset following fornix transection showing absence of fornix and overlying corpus callosum, but little extraneous damage. Following craniotomy, the corpus callosum is approached from above between the two cerebral hemispheres, the corpus callosum is punctured, and the fornix is transected using a small hook.

easier for only one or two small graft deposits to reinervate most of a brain structure, e.g., the caudate nucleus. This may give an overoptimistic impression of achievable recovery in comparison to that which might be found in the much larger human brain.

Lesion Techniques

Lesions of the Cholinergic System

Fornix Transection

In primates, the cholinergic neurons of the vertical limb of the diagonal band (VDB), which are situated in the ventromedial septal area, send long axonal processes through the fornix to the greater part of the hippocampus and entorhinal cortex *(24)*, although the cholinergic projection to the most anterior part of the hippocampus arises in the basal nucleus of Meynert (NBM) and travels via a ventral pathway *(47)*. A substantial cholinergic lesion of the hippocampus can therefore be produced without additional structural damage to the temporal lobe by surgical transection of the fornix (*see* Figs. 1 and 2). Fornix transection also interrupts other hippocampal afferents, which are largely GABAergic, and efferents that are mainly glutamatergic *(48,49)*. It would appear, however, that this additional damage does not contribute to the cognitive impairment that we have studied because neurotoxic lesions of the VDB, which produces less damage to noncholinergic fibers in the fornix, produce the same degree and type of cognitive impairment as fornix transection, and the impairment produced by either of these lesions can be very substantially ameliorated by treatment with cholinergic agonist drugs *(50,51)*.

Neurotoxic Lesions

The primate cholinergic system can also be lesioned by the stereotaxic injection of neurotoxins. These neurotoxins are not neurochemically specific to cholinergic neurons; they lesion the cholinergic system only because they are injected stereotaxically into the area of the brain in which the cholinergic cell bodies are situated. The first neurotoxin to be used in primates was ibotenic acid *(52)*. Some doubt has been cast on the specificity of the behavioral impairment that this lesion has produced in

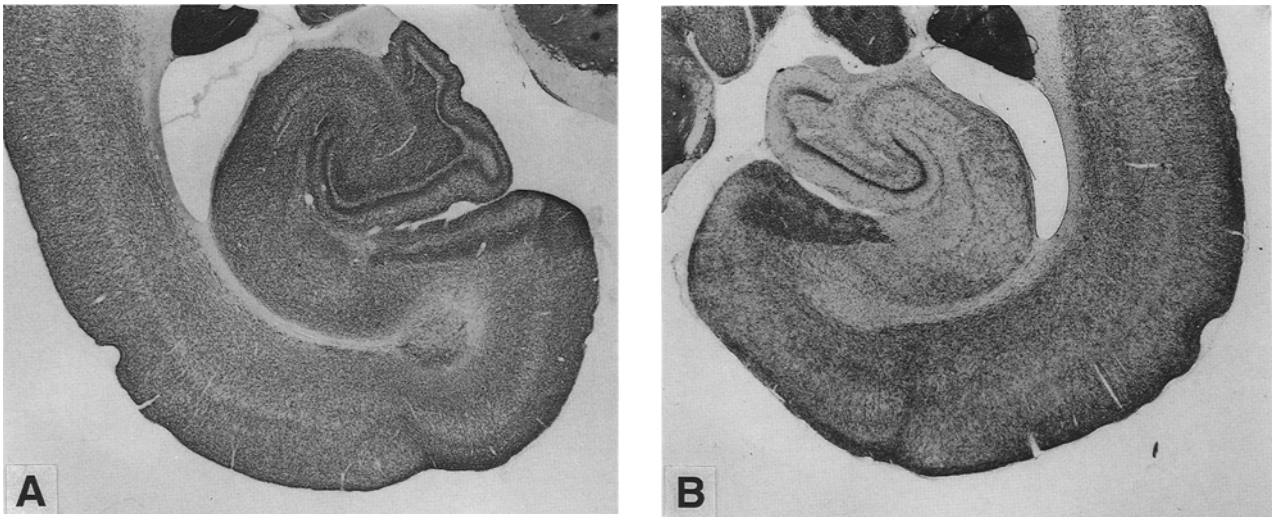


Fig. 2. **(A)** Part of a coronal section of the normal marmoset brain, stained for AChE and counterstained with cresyl violet, showing the pattern of distribution of AChE-positive nerve terminals in the hippocampus. This enzyme is a marker for cholinergic nerve terminals. **(B)** Equivalent section in a marmoset following fornix transection. Note marked diminution of AChE staining within the hippocampus and dentate gyrus, but normal staining in cortex. The cresyl violet-stained granule cells of the dentate are now readily seen.

rodents, mainly because other toxins, e.g., *n*-methyl-*d*-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and quisqualate, which produce larger depletions of the cholinergic system, did not produce such a broad behavioral deficit as ibotenic acid *(53)*. The similarity, however, between the cognitive effects of lesions made with ibotenic acid and NMDA in the marmoset *(54,55* and Ridley et al., unpublished), the more compact distribution of the cholinergic neurons of the VDB and NBM in the marmoset *(56)*, which makes it easier to produce a more specific cholinergic lesion in the marmoset than in the rodent, and the ameliorative effect of cholinergic agonists in marmosets with neurotoxic lesions of the NBM *(57)* and VDB *(50)* all suggest that ibotenic lesions in the marmoset exert their influence on learning and memory by virtue of their effect on the cholinergic system. The cognitive effects of ACh-rich grafts placed in the cortex of marmosets with NMDA-induced lesions of the NBM (*see* Figs. 3 and 4) are currently being investigated.

Lesions of the DA System

Primate models of Parkinson's disease are produced by destroying the ascending dopamine pathways from the substantia nigra to the caudate-putamen, either bilaterally or unilaterally, using the neurotoxins 6-hydroxydopamine (6-OHDA) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (*see* ref. *23* for review). The peripheral administration of MPTP, which produces bilateral symptoms, has been the method most frequently used with Old World monkeys (e.g., refs. *58,59)*. It has also been used in marmosets *(60)* and, accidentally, in humans. In all primate species, it produces a very profound syndrome that is very similar both behaviorally and neurochemically to severe idiopathic Parkinson's disease. When beneficial effects of DA-rich grafts are observed in this model *(60–65)*, they are clinically particularly relevant. The disadvantage of this model, however, is that the behavioral symptoms can be so severe that not all the animals are capable of self-care.

Unilateral models have the advantage that near total lesions can be achieved in half the brain without incapacitating or killing the animal. More complete lesions may be less prone to spontaneous recovery than partial lesions if recovery involves sprouting of remaining nerve terminals. MPTP can be administered unilaterally either by injection into one of the ascending carotid arteries (e.g., refs. *66,67)* or by unilateral stereotaxic injection directly into the brain (e.g., refs. *68,69)*. The motor symptoms resulting from this are confined largely to the side of the body contralateral to the lesion, while

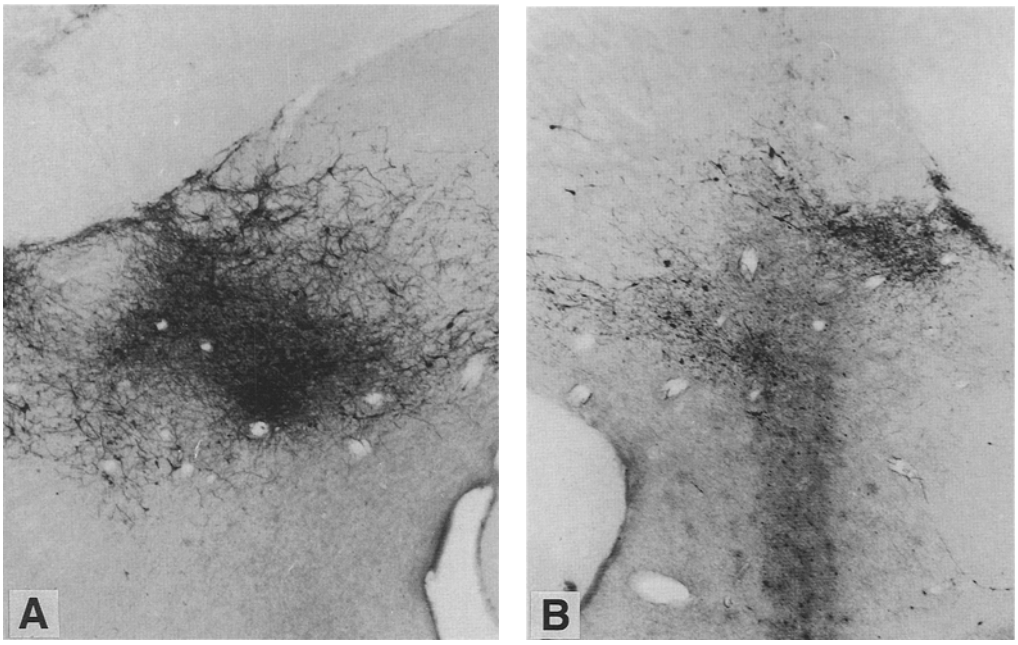


Fig. 3. **(A)** Cholinergic neurons in the NBM of a normal marmoset, stained with antibody to human nerve growth factor receptor. **(B)** Equivalent section in a marmoset following NMDA-neurotoxic injection in the NBM. Note loss of neurons.

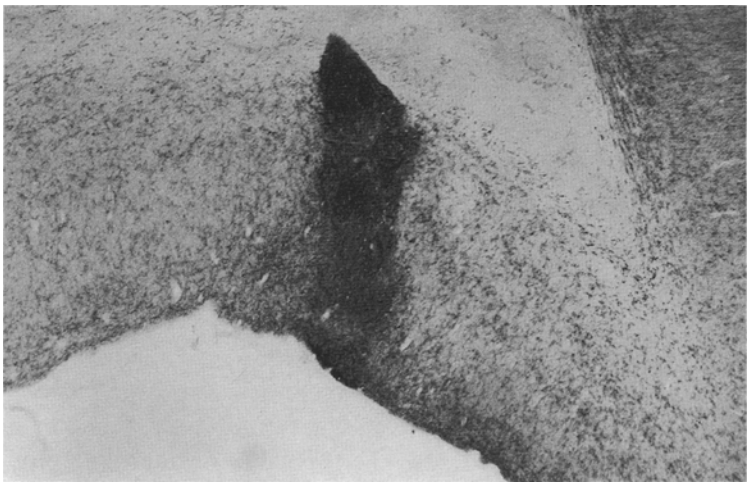


Fig. 4. Part of coronal section of the frontal lobe of a marmoset with NMDA lesion of the NBM and a subsequent transplant of ACh-rich tissue in the frontal lobe, stained for AChE.

appetite, interest, and self-care are maintained almost intact. Unilateral intracerebral injection of 6-OHDA has similar effects and has been the model most often used in marmosets to study the efficacy of DA-rich grafts. Unilateral injection of 6-OHDA into the nigrostiatal pathway in the marmoset produces unilateral depletions of dopamine in the basal ganglia of 98–99% and motor symptoms that show little spontaneous recovery *(70)*. One disadvantage of the unilateral model is that since the behavioral effects (e.g., rotation) do not bear such a close resemblance to the clinical symptoms of

Parkinson's disease as do the effects of the bilateral lesion, improvements in these behavioral measures by putative therapeutic treatments may require further validation.

Behavioral Assessment

Learning and Memory

The standard method for assessing learning, memory, and other cognitive processes in primates uses the Wisconsin General Test Apparatus (WGTA). This apparatus has been in use for >50 yr *(71)*, and has never been surpassed in its simplicity and versatility. We prefer to use a manual apparatus, since this permits us to use real objects (e.g., small toys, bottle tops, and so on) as stimuli between which the marmosets can distinguish on the first presentation *(72)*. This makes it possible to design experiments where the primary difficulty for the animal is learning of, and memory for, stimulus-reward and stimulus-response associations rather than discriminating between the stimulus objects *(25)*. Automated versions of the WGTA can also be used *(73)*, in which stimuli are presented on a VDU permitting modification of the stimuli along different dimensions. This can be useful for studying perception and attention as well as learning. The WGTA has been used extensively to test Old World monkeys (usually macaques), but to our knowledge, no other studies of the cognitive effects of ACh-rich transplants have been undertaken using Old World primates.

Tasks presented in the WGTA can be designed to tax specific aspects of cognition. Tasks that require discrimination between patterns or between very similar objects place heavy demands on perceptual analysis, and monkeys with ablations of the posterior temporal cortex find these tasks difficult *(74)*. Tasks that require concurrent discrimination between many different pairs of objects tax memory more specifically. Monkeys with ablations of the anterior temporal cortex are impaired on acquisition of these tasks *(75)*. Performance of reversal tasks, where the stimulus-reward contingency is abruptly reversed, is disrupted by lesions of the frontal lobes *(76,77)*. Marmosets with neurotoxic lesions of the NBM (the origin of cholinergic projections to those areas of cortex just mentioned) are impaired on the acquisition and reversal of these types of visual discrimination learning (*52,54,55,57; see* Fig. 5A). Transection of the fornix does not produce impairment on these tasks, but it does produce impairment on the acquisition of tasks that require stimulus-response association, where the response can be spatial (e.g., if Stimulus A, go left; if B, go right; *see* Fig. 5B), conditional (if A, choose X; if B, choose Y; *see* Fig. 5C), or any other rule of responding (*see* ref. *25* for an explanation of the neuropsychological theory). These tasks are also impaired by neurotoxic lesion of the VDB *(50,78)*, which is the origin of the cholinergic projection through the fornix to the hippocampus.

Movement Disorders

The measurement of movement disorders provides a particular challenge to the ingenuity of the experimenter. Overall levels of activity can be measured in any of several commercially available animal-activity monitors, but such devices can usually only indicate how much rather than what kind of movement is occurring. In some cases, an assessment of the quality of movement can be made by the use of small items of apparatus that the animal must interact with or manipulate to obtain reward (e.g., refs. *63,64,79*).

Monkeys whose dopamine systems have been damaged unilaterally with either MPTP or 6-OHDA are much less incapacitated than monkeys with bilateral lesions. They also tend to rotate (i.e., to run or walk around in circles) toward the lesion side, both spontaneously and in response to amphetamine treatment, and to rotate away from the lesion side when treated with direct dopamine receptor agonists, such as apomorphine *(67,70)*. This occurs because DA release on the intact side stimulates activity on that side and drives the animal around toward the lesion side (i.e., ipsilaterally). Peripheral amphetamine treatment increases the release of available dopamine on the intact side and increases the amount of ipsilateral rotation. However, the denervated DA-receptors on the lesion side become supersensitive so that, if the animal is treated with a direct DA-receptor agonist, such as apomorphine, it will rotate away from the lesion side (i.e., contralaterally). Rotation in response to different types of dopaminergic drugs can therefore be used as a sensitive and quantifiable measure of levels of dopamine activity. Low levels of rotation can be measured by videotaping the animal's behavior over any period of time and then measuring the degree of rotation quickly by speeding up the replay of the video. This measure of the asymmetry of behavior allows animals with different levels of overall activity, or the same animals on different days to be compared. It also has the advantage of being sensitive to excess dopamine activity, which

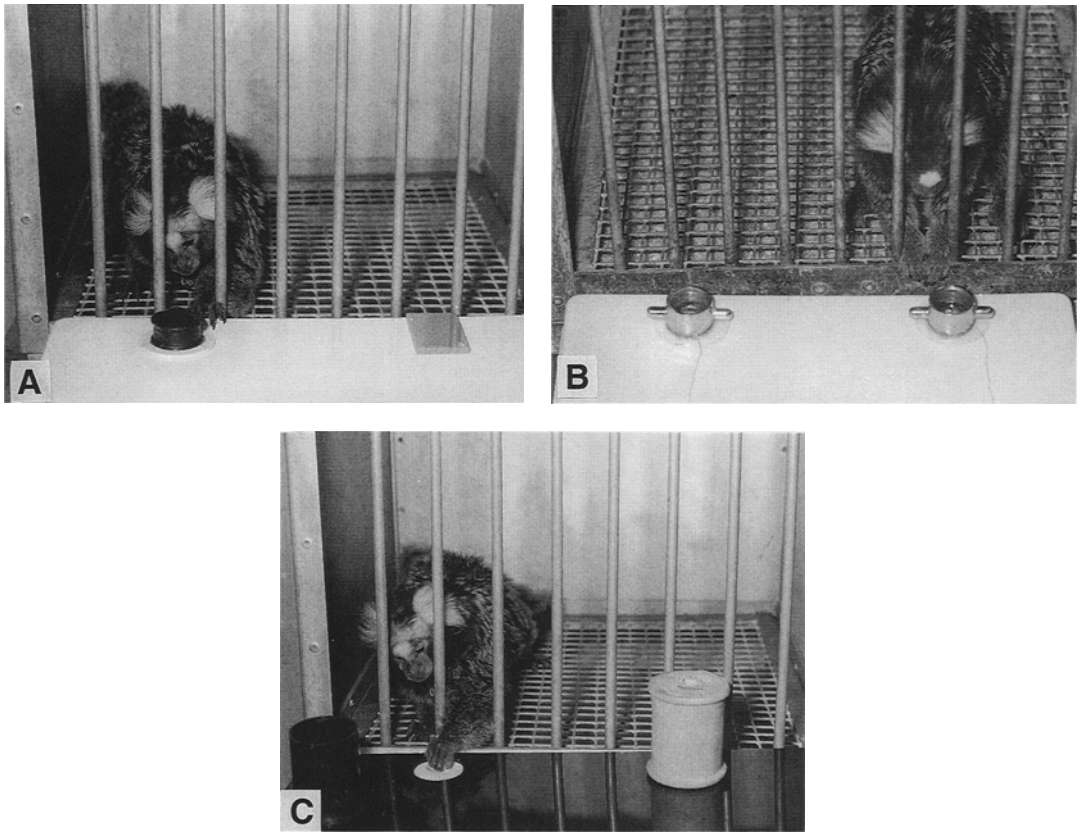


Fig. 5. **(A)** Marmoset performing a simple visual discrimination task in the WGTA. On each of a series of trials, an opaque shutter is raised to reveal the two stimuli that cover the two food wells, one of which contains a small piece of food reward. The marmoset moves one of the stimuli in search of the food. The left/right position of the stimuli varies according to a pseudorandom schedule, but the reward is always to be found beneath the same stimulus object. **(B)** Marmoset performing a visuospatial task. Trials on which this pair of identical objects is presented are interspersed pseudorandomly with trials on which a different pair of identical objects is presented. Food reward is to be found under the left stimulus object when one of the pairs of objects is presented, and reward is to be found under the right stimulus object when the other pair of objects is presented. **(C)** Marmoset performing a visual conditional task. When the two stimulus objects are presented on this black test-board, the reward is to be found under the bottle top, but when the same stimulus objects are presented on a white test-board, the reward is to be found under the cotton reel. The black and white boards and the left/right position of the two stimulus objects are presented according to two different, superimposed pseudorandom schedules.

could occur, for example, if a graft grew too large. It is not clear, however, on the basis of neuroanatomical substrate, which symptoms of Parkinson's disease would be equivalent to rotation. As is shown under Results, rotation may result from unilateral dopamine depletion in the caudate rather than the putamen, but the anatomical basis of the different symptoms of Parkinson's disease is not entirely understood.

Monkeys with unilateral dopamine lesions also show impairment of the use of the contralateral limbs. This could be because of either sensory neglect of stimuli impinging on the contralateral side of the body, difficulty in initiating or fulfilling movements using the contralateral side of the body, or both. In marmosets, neglect has been assessed by measuring the time taken to contact and remove sticky-paper labels attached to the hand and feet *(70)*. Skilled hand use has been assessed by measuring the maximum distance inside a tube from which a marmoset can retrieve a small piece of reward and the success with which a marmoset can pick up a piece of food reward that is moving past the cage,

in either direction, on a conveyor belt (*70*). Side preferences, e.g., in the spontaneous choice of which hand to use to reach for objects (*17*) and in the preferred position of the head relative to the body can also be measured (*70*).

Tissue Transplantation Techniques

Two different methods of intracerebral transplantation have been attempted in primates. The first involved the placement of solid tissue into cavities in the basal ganglia by a direct surgical approach (e.g., ref. *17*), whereas the second method required the stereotaxic injection of fragments of appropriate tissue (e.g., ref. *61*) or dissociated cell suspensions (e.g., ref. *62*). The stereotaxic injection of dissociated cell suspensions is much less invasive than the open surgical method and is the only method that has been used in marmosets.

There are two principal sources of DA-rich tissue for transplantation. The first source to be used in primates was adrenal medulla tissue (*42,80*) usually taken from the same animal into which these cells were then transplanted. These cells contain dopamine as a precursor of adrenalin and noradrenalin, which this endocrine organ normally secretes. It has become clear from subsequent work, however, that graft tissue survival is not good. Improvements in behavior are not greatly better than in lesioned animals without grafts, who may show a degree of spontaneous recovery, or in animals that have received grafts of various other types of non-DA-rich control tissue (*see* refs. *33,81* for review). Recent developments improving the viability of adrenal grafts by cotransplantation with nerve growth factor secreting tissue, such as peripheral nerve, however, may rescue the utility of adrenal transplant techniques.

The second source of DA-rich tissue is the embryonic ventral mesencephalon, which contains the cells of the substantia nigra. Experiments have demonstrated that the optimum age for transplantation of rodent tissue is at 13–14 d of gestation for DA-rich ventral mesencephalic tissue and 14–15 d gestation for ACh-rich ventral forebrain (*82*). By comparing the "Carnegie" stages of embryonic development across species (*83*), it can be estimated that these ages are equivalent to gestational ages 74 and 80 d, respectively, in the marmoset. The optimal age range for donor tissue may be confined to just a few days. At an earlier age, the neurons appear not to have undergone their final mitosis and differentiation, so that there is the risk that transplanted tissue will not secrete the required neurotransmitter, whereas if the tissue is taken too late, then the neurons will have developed long axonal processes that will be damaged by the dissection process (*23*).

Marmoset embryos are taken by hysterotomy from breeding females at the appropriate stage of gestation. In order to comply with UK regulations, each female is used only once for this procedure and is then returned to the breeding colony (*see* ref. *44* for details of the surgical procedure). Most females conceive on the first ovulation after the surgery, and their long-term breeding capacity is not affected. DA-rich tissue is dissected, from the ventral mesencephalon, under a low-power microscope (*see* ref. *60* for details). ACh-rich tissue is dissected from the septal and adjacent area, which contains the cells of the VDB and NBM.

Results

Histological Examination of Grafts in Marmosets

Figure 6 shows a coronal section of marmoset brain stained for acetylcholinesterase (AChE). This enzyme is found in cholinergic nerve terminals and is therefore used as a marker for these terminals, although it also occurs in other neural tissue (*84*). Grafted tissue can be seen in the temporal lobe. Figure 7A shows ACh-rich tissue graft, and Fig. 7B shows an ACh-poor tissue graft adjacent to the dentate gyrus in fornix transected animals. By comparison with Fig. 2A (normal control) and Fig. 2B (fornix transected), it can be seen that the dentate gyrus in Fig. 7A appears normal and has therefore probably been reinnervated with the appropriate pattern of terminals by ACh-fibers growing out of the graft (*see* ref. *51* for more details). The AChE staining in the dentate gyrus of Fig. 7B is clearly abnormal and probably demonstrates an increase in the noncholinergic, AChE-positive staining seen in hilar cells of the dentate gyrus, which may have been stimulated by growth factors in the graft embryonic hippocampal tissue (*see* ref. *85* for more details).

Figure 8 shows a coronal section of the marmoset brain stained with antibody to tyrosine hydroxylase (TH). TH is found particularly (but not exclusively; *86–88*) in DA neurons in the marmoset and can therefore be used as a marker for DA neurons. Grafts of DA-rich tissue can be seen in the caudate and putamen in this section.

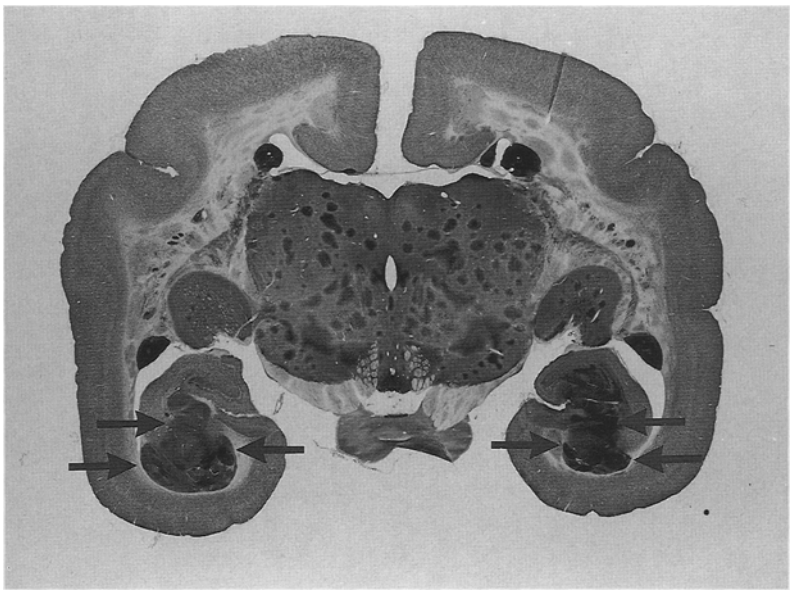


Fig. 6. Coronal section of a marmoset brain, stained for AChE, showing AChE-positive grafted tissue in the temporal lobes bilaterally.

Behavioral Effects of Grafts in Marmosets

Grafts of Embryonic Ventral Forebrain Cholinergic-Rich Tissue into Marmosets with Fornix Transection

The marmosets were given a series of tasks in the WGTA before and after surgery. These tasks were divided into those on which impairment following fornix transection was predicted (experimental tasks) on the basis of neuropsychological theory *(25)* and those on which impairment was not predicted (control tasks), but that were intended to be sensitive to the deleterious effects of extraneous damage associated with either fornix transection or overgrowth of the transplant. In the first experiment *(51)* (*see* Fig. 9), it was shown:

1. That prior to any surgery, the groups of animals destined to be controls, fornix-transected, or fornix-transected plus ACh-rich grafts in the hippocampus did not differ on any behavioral measure;
2. That after fornix transection, animals were severely impaired only on learning visuo-spatial tasks; and
3. That, when tested 3–9 mo after transplantation of ACh-rich tissue into the hippocampus, animals were no longer impaired on any task, although animals with fornix transection alone remained impaired throughout this test period, indicating that the ACh-rich tissue had had a substantial therapeutic effect in these animals without any obvious adverse effects.

In a subsequent, similar experiment (*85*, *see* Fig. 10), the observation of a substantial beneficial effect of ACh-rich grafts in fornix transected animals was replicated, whereas the transplantation of ACh-poor tissue (from embryonic hippocampus) into the hippocampus of these animals had no beneficial effect at all. The two animals with the worst behavioral scores of all the animals after transplantation were animals with ACh-poor grafts, and one of these developed overt epileptic seizures some months after transplant surgery. The development of epileptiform activity after transplantation of hippocampal tissue into hippocampus has also been observed in rats *(89)*. This suggests that transplanting inappropriate tissue is not beneficial and may actually be harmful.

Grafts of Embryonic Ventral Mesencephalic DA-Rich Tissue into the Caudate-Putamen of Marmosets with Lesions of the Rising Dopamine Projections

The first attempts to graft DA-rich tissue into marmosets were made using animals treated systemically with MPTP *(60)*. Two animals with bilateral

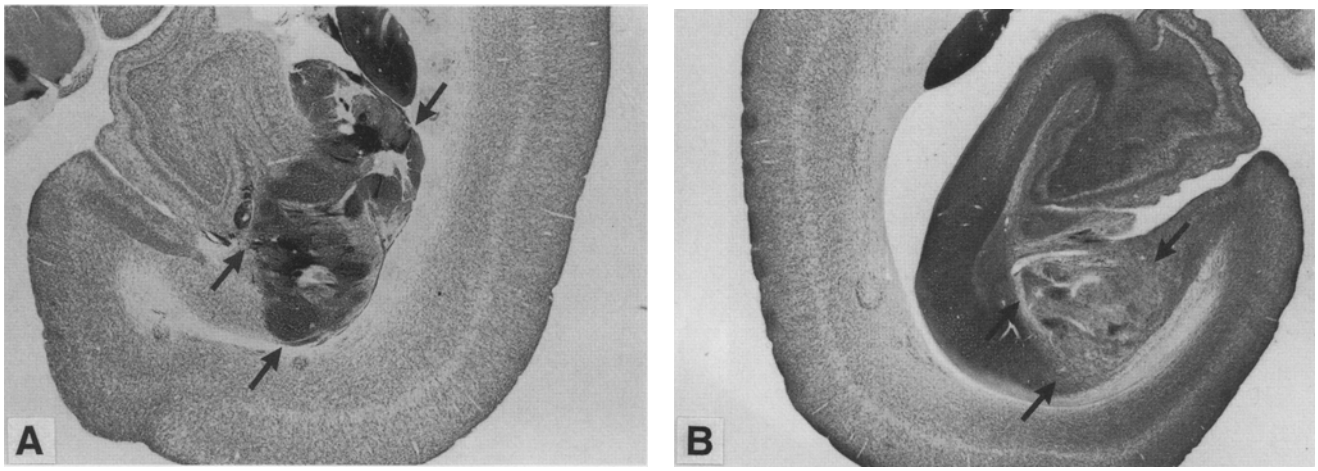
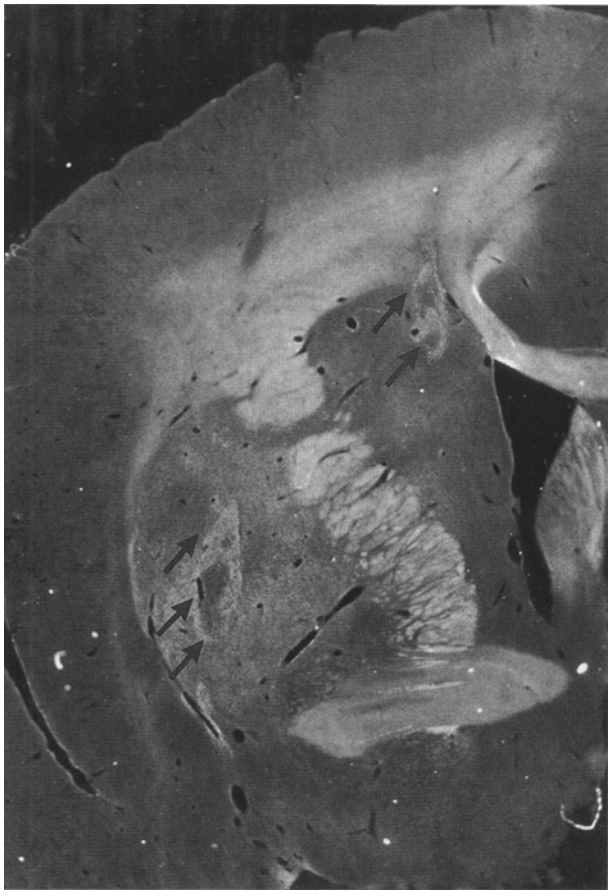


Fig. 7. **(A)** Part of a coronal section of a marmoset brain, stained for AChE, showing ACh-rich embryonic marmoset septal tissue grafted into the temporal lobe following fornix transection. Note the near-normal appearance of the AChE staining in the adjacent hippocampus (cf Fig. 2A and B). **(B)** Part of a coronal section of a marmoset brain, stained for AChE, showing ACh-poor embryonic marmoset hippocampal tissue grafted into the temporal lobe following fornix transection. Note abnormal appearance of the adjacent hippocampus.



grafts in the putamen showed increases in general activity when compared to two animals with lesion alone and two animals with lesion and grafts of DA-poor tissue into the putamen. One of four animals with bilateral MPTP treatment and unilateral grafts in the putamen developed spontaneous rotation away from the grafted side. This is consistent with greater dopamine release on the grafted side. In light of the subsequent finding of the greater effect on rotation of grafts in the caudate rather than putamen (*see* Fig. 11) it is of interest that this animal not only had the largest number of TH-positive neurons, but also showed evidence of the outgrowth of TH-positive fibers into the caudate nucleus.

Behavioral assessment of marmosets with unilateral OHDA lesions and unilateral grafts of DA-rich tissue in the caudate and/or putamen showed that these grafts improved some, but not all symptoms produced by the lesion. Specifically, grafts in the caudate and caudate plus putamen produced a

Fig. 8. Part of a coronal section of a marmoset brain, stained with antibody to TH and viewed under dark field illumination. The marmoset had received a unilateral 6-OHDA lesion of the nigrostriatal bundle followed by unilateral grafts of embryonic marmoset mesencephalic tissue in the caudate and putamen.

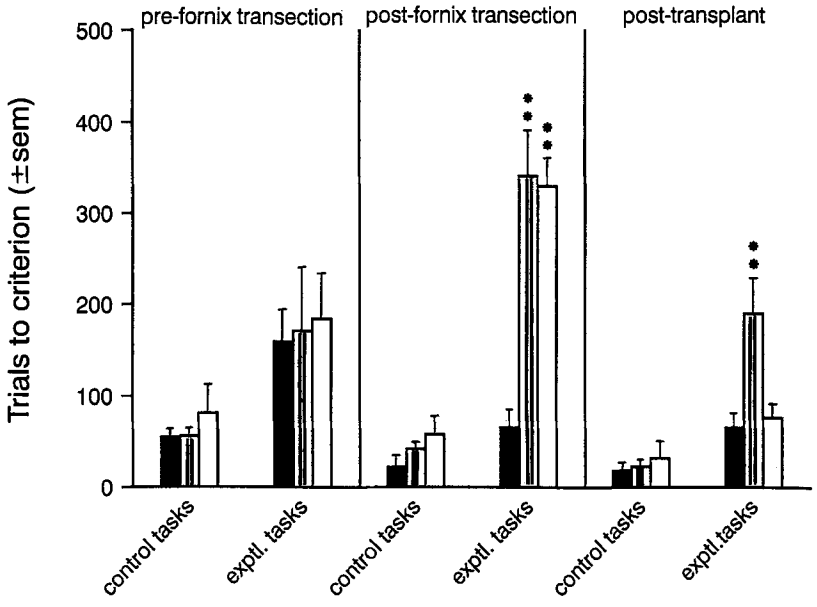


Fig. 9. Mean number of trials required for marmosets to reach a predetermined criterion of correct trials (usually 27/30 correct on each task) on control tasks (on which it was not predicted that marmosets with fornix transection would be impaired) and on experimental tasks (on which impairment was predicted), before and after fornix transection and 3 mo after transplantation of ACh-rich tissue into the temporal lobe of fornix transected marmosets. ■ = seven unoperated marmosets or marmosets with control ablations of part of the corpus callosum only; ▥ = five marmosets with fornix transection; □ = four marmosets with fornix transection plus ACh-rich grafts. $^{**}p < 0.01$ compared to control animals *(49)*.

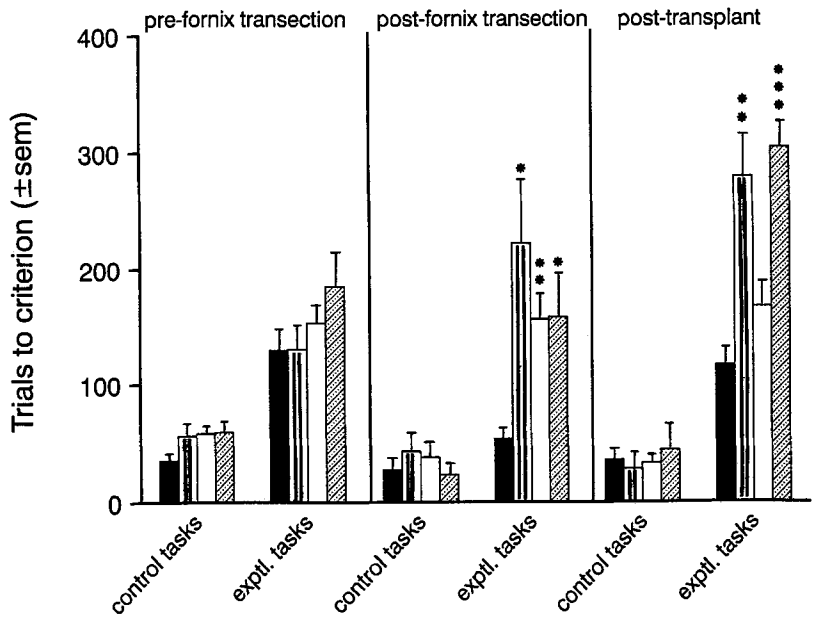


Fig. 10. (*See* Fig. 9). ■ = five marmosets with control ablation of part of the corpus callosum; ▥ = five marmosets with fornix transection; □ = four marmosets with fornix transection and ACh-rich grafts in the temporal lobes; ▨ = four marmosets with fornix transection and grafts of ACh-poor grafts in the temporal lobes. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to control animals. Different animals were used in this experiment *(83)* from those shown in Fig. 9.

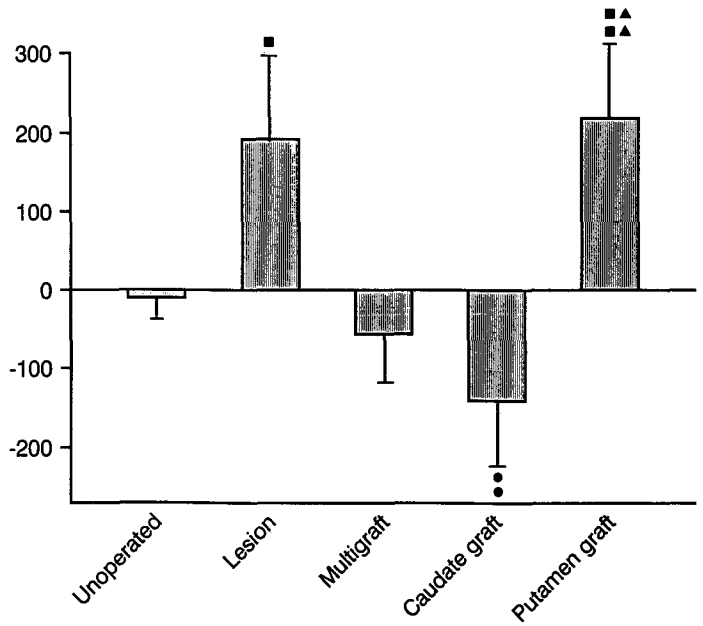


Fig. 11. Mean ipsilateral-contralateral rotation (30 min after 0.5 mg/kg amphetamine had been injected im) in marmosets 5–7 mo after unilateral 6-OHDA lesion of the nigrostriatal bundle and, in some animals, 4–6 mo after transplantation of DA-rich tissue into the ipsilateral caudate and/or putamen. Unoperated control animals, $n = 10$; lesion only, $n = 10$; multigraft (lesion plus DA-rich grafts in the caudate and putamen) $n = 6$; caudate (lesion plus DA-rich grafts in the caudate) $n = 4$; putamen (lesion plus DA-rich grafts in the putamen) $n = 5$. When compared to the unoperated group, the lesion and putamen groups rotate ipsilaterally (■ $p < 0.05$, ■■ $p < 0.01$, respectively). When compared to the lesion group, the caudate group rotates contralaterally (●● $p < 0.01$); the caudate group also differs from the putamen group (▲▲ $p < 0.01$). Data subjected to log transformation to reduce variance prior to statistical analysis.

reduction, and in some cases a reversal, of spontaneous and amphetamine and apomorphine rotation, whereas grafts in the putamen did not (*see* Fig. 11).

Skilled use of the hands was also improved by the grafts; marmosets with grafts in the putamen and putamen plus caudate were able to reach further into a tube to obtain food using the impaired arm contralateral to the lesion compared to marmosets with the lesion, but no graft (*see* Fig. 12). Since Figs. 11 and 12 show data from the same animals, it can be seen that the lack of effect of putamen grafts on rotation (in Fig. 11) is not the result of failure of graft survival or a deleterious effect of extraneous damage, since these grafts produced the greatest improvement on skilled arm movement (Fig. 12). Neglect, measured as the ipsilateral bias to contact and remove sticky labels attached to the feet, and neglect of food pieces approaching from the contralateral side on a conveyor belt, postural biases measured as the inclination of the head at rest, and hand preference when reaching for food were not improved by any of the grafts assessed so far (*79*). These failures may have occurred for some technical reason, e.g., because the graft was not in the optimum position to affect this type of behavior, but it is also possible that, since hand preference seems to be idiosyncratic in the marmoset, a shift of hand preference brought about by the lesion would not necessarily be changed again if the neurochemical effect of the lesion were removed by a graft.

Concluding Remarks

In order to be of clinical relevance, experiments in primates should address several questions, for example: Are the grafts behaviorally therapeutic? Do the grafts cause deleterious effects? Is the behavioral recovery that is observed the result of the secretion of neurotransmitter from the graft or is it the result of some other nonspecific effect of the transplant procedure? The case for recovery of learning ability following transplantation of ACh-rich tissue into marmosets with fornix transection would seem to be clear-cut. The absence of improvement following ACh-poor grafts suggests that it is the cholinergic nature of the transplanted tissue that

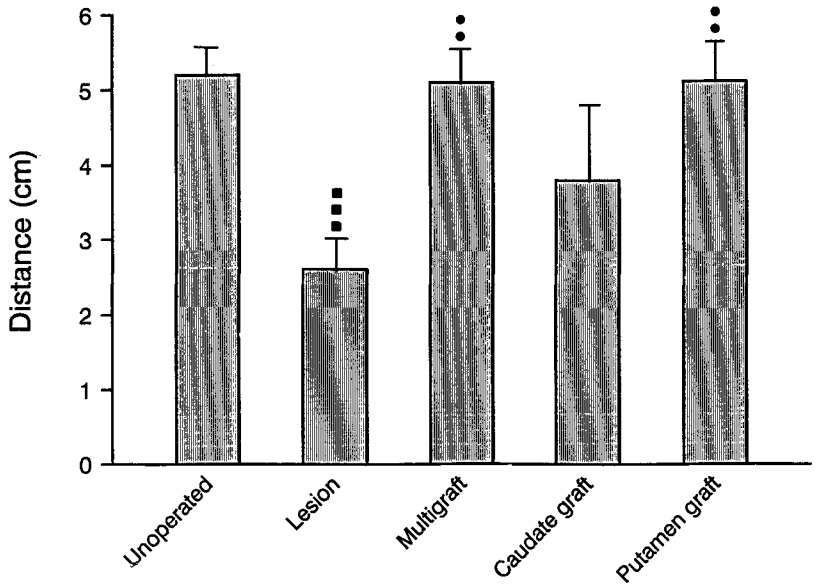


Fig. 12. Mean furthest distance up a narrow tube that the marmosets were able to reach to obtain food reward using the arm contralateral to the lesion. *See* Fig. 11 for numbers of animals. Compared to the unoperated group, the lesion group was impaired (■■■ $p < 0.001$), but none of the grafted groups was impaired. The multigraft group and the putamen group were significantly improved relative to the lesion group (●● $p < 0.01$). Comparison of Figs. 11 and 12 demonstrates the importance of putting the DA-rich graft in the appropriate place to produce improvements in different types of behavior.

is responsible for the improved learning ability. Since similar improvements are also seen in fornix transected marmosets treated systemically with cholinergic receptor agonists *(51)*, it can be supposed that in both cases, the improvement in learning is brought about by a diffuse increase in cholinergic tone in the temporal lobes. Unlike exogenous drugs, however, acetylcholine is broken down by acetylcholinesterase rapidly on release into the synaptic cleft. From this, it may be supposed that the acetylcholine released from the AChE-positive cells in the graft is interacting at a synaptic level with the host. Thus, the conclusion from much work done in the rodent, that ACh-rich grafts form functional synaptic connections with the host, may also be drawn from the primate experiments. It cannot be assumed, however, that ACh-rich grafts are always without deleterious effects. Grafts can grow very large and, possibly because they contain poorly differentiated cells, they can form tumor-like masses that may ultimately prove fatal *(90)*. Transplants in the temporal lobes may also be epileptogenic *(85)*.

The evidence that DA-rich grafts improve the motor behavior of marmosets with DA-lesions is also substantial. There is some evidence from rotation studies that DA-rich grafts may release excess dopamine. This does not appear to be greatly deleterious to the marmoset, but similar dopamine excess in a patient might have adverse psychiatric consequences. More problematic is the demonstration, in all the primate studies, that the DA-rich grafts are beneficial because they secrete dopamine. A problem with most of the studies using Old World monkeys treated bilaterally with MPTP is that the symptoms have been measured in individual animals against a clinical rating scale of severity rather than objectively, quantitatively, or statistically across groups. An important exception to this is the statistical analysis of behavioral achievement by grafted monkeys carried out by Taylor et al. *(65)*. A further problem with the bilateral MPTP model is that, although depletion of dopamine in the caudate-putamen can be nearly complete, depletion in the adjacent nucleus accumbens may be no more than 50% *(91)*, so there is considerable potential for nerve terminals in the nucleus accumbens to reinnervate the striatum, either spontaneously, or in response to the tissue-stimulating effect of either the open neurosurgical technique or the grafted tissue itself. Bankiewicz et al. *(92)* provide evidence that all these mechanisms

may contribute to a different extent to behavioral recovery, although it is unlikely that all these mechanisms always operate since they may depend on the technique that is employed. Neurosurgery within the basal ganglia is also known to have a poorly understood, but sometimes beneficial effect, in patients with Parkinson's disease *(93)*.

Work in the marmoset can go some way to determining the specificity of the effect of DA-rich transplants. The unilateral 6-OHDA method produces near total depletion of dopamine in both the dorsal and ventral striatum on one side, so there is little potential for reinnervation from host DA terminals. No evidence of DA-outgrowth from ventral striatum was seen in lesioned or lesioned plus grafted marmosets. A correlation was also observed between the number of TH-positive cells in the graft and the degree of recovery from rotation *(79)*. The stereotaxic injection of tissue-suspension grafts is much less invasive than the cavitation and solid-graft technique employed in some studies with Old World monkeys. Injection of saline instead of transplant tissue into two 6-OHDA-lesioned marmosets was without beneficial effect *(79)*, suggesting that the neurosurgical procedure itself is not responsible for the improved behavior in the grafted animals. The dissociation of the effect of DA-rich grafts in the caudate and putamen on rotation and skilled hand use (Figs. 11 and 12) strongly implies a specific interaction between graft and adjacent host tissue. Fine et al. *(60)* found that two marmosets with bilateral MPTP lesions and grafts of DA-poor striatal tissue did not improve in activity levels compared to marmosets with lesion alone, even though substantial increases in activity were seen in two marmosets with lesion plus DA-rich transplants. Overall these experiments suggest that the beneficial effects of embryonic tissue grafts in marmosets with dopamine lesions depend specifically on the release of dopamine into the appropriate part of the basal ganglia. Current experiments are examining the critical age at which embryonic tissue is most beneficial.

CNS transplantation is a drastic treatment for a very debilitating disease. The possibility of adverse outcome is quite substantial, especially if the optimum technique is not precisely defined. The symptoms of Parkinson's disease are, of their very nature, subject to fluctuation across time making behavioral assessment of the efficacy of treatments difficult. Because of the extensive surgery involved, it is not ethical to treat some patients, in a scientific experiment, with control tissue that is not expected to be effective. For all these reasons, it is important to continue experimental analysis of this technique in rodents and primates.

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