Transplants as Guides to Brain Development

Brain transplant experiments are helping investigators assess the relative importance of the various factors that control brain development

For many years, neurobiologists have put transplantation techniques to good use in studying brain development in amphibians, fish, and birds. Only recently, however, have they made a sustained effort to perform similar studies on mammalian brains, although there have been sporadic reports of such experiments since early in this century.

The ability to successfully graft tissue into the brain makes it possible to selectively manipulate the many variables thought to influence development and sort out their relative contributions. "That is why the implants are so exciting," says Carl Cotman of the University of California at Irvine. "You can alter things like position or timing and see which are important."

Brain transplants, which might seem to be the stuff of science fiction, or perhaps of Gothic novels, are proving instead to be very practical tools for tackling the unsolved problems of mammalian behavior and brain development. Recent experiments show that pieces of fetal brain tissue not only survive when transplanted into the brain or other sites of recipient animals but thrive there. The transplants often form correct connections with their normal target neurons, in some cases reversing neurological deficits caused by surgical or genetic lesions.

The success of the animal work has already prompted an attempt to treat human patients with Parkinson's disease, a severe neurological disorder, by injecting into their brains tissue that has the potential of remedying the underlying chemical deficiency.

The use of tissue transplants to study fundamental questions about the nature of brain development are discussed in the first of two Research News articles in this issue. The second deals with the potential clinical applications of brain tissue transplantation.

Exactly how developing neurons form the myriad of specific connections needed to form the mammalian brain is still unclear, but some clues are emerging. Neurons may be intrinsically programmed to develop in a certain way. For example, the type of neurotransmitter they secrete may influence the kind of connections they make. Competition between fibers may be important, as some types may be able to exclude others from making connections with the same target. The initial position of the developing tissue and guidance of growing nerve fibers by degenerating tracts seem less important. Finally, the transplant methods are leading to the discovery of new trophic factors, agents that stimulate nerve growth and may direct the fibers to the right locations.

All this had to await the demonstration that mammalian brain tissue would not only survive transplantation to a new location but would develop normally. One of the hindrances to the work was the belief that mammalian brains, unlike those of amphibians and fish, would have little recuperative power if they were damaged, as they would be during transplant surgery. That turned out not to be a problem in practice. Beginning about 10 years ago, investigators, including Gopal Das of Purdue University and Anders Björklund and Ulf Stenevi of the University of Lund, showed that, when fetal brain tissue was transplanted into new sites, it would send out nerve fibers to other brain areas, and just as important, also receive incoming fibers. Often the transplanted tissue made the appropriate connections with the right targets. "Most of the time the reinnervation is normal when you look in the microscope," says Stenevi of transplants of tissue that form connections with an area of the brain called the hippocampus.

The age of the transplants is important, however. Investigators generally agree that early fetal tissue works best. "Later fetal transplants, taken around the time of birth, don't survive very well," points out Raymond Lund of the Medical University of South Carolina. This is probably related to the ability of the nerve cells to divide, which is lost with age. "A pathway that is in a growth phase seems to be more effective at forming connections." Usually the fetal brain transplants are made in newborn rats when the brains are still developing. Transplants into adult brains also work, but not as well as those in young brains.

How a particular fetal region develops appears to be intrinsically programmed to a large degree, although not completely, by the time it is taken for transplantation. Das, for example, found that tissue from any of several different parts of the fetal brain, when transplanted into the cerebellum, developed as they would have if they had been left in their original sites. And Lund showed that embryonic retinas, when transplanted into the brain, would develop their normal structures.

Moreover, Barry Hoffer and his colleagues at the University of Colorado Health Science Center in Denver, in a series of experiments performed in collaboration with Lars Olson's group at the Karolinska Institutet in Stockholm, Sweden, have been studying the development of isolated regions of brain tissue that they have transplanted to the anterior chamber of the eye (between the cornea and lens). Here the transplants can neither make nor receive the usual connections with other brain neurons, nor can they be subject to other brain influences. Nevertheless, each of the different types of fetal tissue attains the normal cellular architecture of the mature brain part and individual cells respond to stimulation in the usual way for that type of cell.

More recently, the Hoffer and Olson groups have progressed to transplanting two types of fetal brain tissue into the same eye. Their questions were, Hoffer explains, "What would happen if we put in multiple areas, especially those that communicate with one another? Do they form normal connections?" The answer turns out to be yes. For example, in one experiment, the locus coeruleus and hippocampus were transplanted together.

As Hoffer describes the results, "In the eye, fibers grow out of the locus

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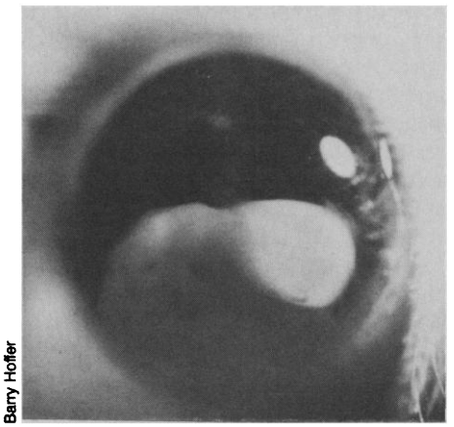
coeruleus into the hippocampus and exert an effect, decreasing the activity of the hippocampal cells." Similarly in the brain, nerve fibers from locus coeruleus inhibit firing of the hippocampal cells. Because of the simplicity of the eye transplant system and the ease with which the activities of the transplants can be monitored, the system is ideal for exploring the way specific connections form between neurons, Hoffer says.

The type of neurotransmitter secreted by a neuron may be one of the intrinsic properties that influences its developmental fate. Björklund and Stenevi have examined the innervation produced in the adult hippocampus by implants of four nerve tracts from fetal brain, each of which produces a different neurotransmitter. No two transplants produced the same pattern.

Three of the transplanted tracts normally make connections with the hippocampus. All three, which secrete either acetylcholine, noradrenalin, or serotonin, form their typical patterns, provided that the endogenous pathways of the same type had been completely severed before the implants were made. Stenevi says, "The simple rule is that as the noradrenergic fibers grow in, they will mimic the normal noradrenalin innervation. It is the same for cholinergic and serotoninergic fibers." The fourth type of fiber tract, which produces dopamine and does not normally innervate the hippocampus, formed a pattern that resembled the serotoninergic one.

Cotman and Ellen Lewis, who was a graduate student at Irvine, produced further evidence for the importance of neurotransmitter type in influencing the fate of developing neurons. After cutting the endogenous cholinergic pathways to the hippocampus, they transplanted into the brains either septal or striatal tissue. Both tracts contain cholinergic fibers but only the striatal fibers ordinarily innervate the hippocampus. Nevertheless the two types of implants formed similar—and normal—patterns of cholinergic innervation there. Victoria Holets, who also works with Cotman, showed that transplants of serotoninergic neurons from the Raphé area produced a different result, however. Cotman says, "It certainly doesn't form the same pattern as a cholinergic system does. The pattern formed seems to be associated with transmitter type."

The long-held idea that competition between neurons for targets might be one of the factors guiding brain development has also received support from the transplant experiments. For example, Lund has found that fibers that grow out from retinas that have been transplanted into brain occupy a greater area in their target, the superior colliculus, if the eye on the side opposite (contralateral) to the transplant is removed. This has the effect of removing the fibers that the contralateral eye would send to the superior colliculus and, presumably, provides more space for the fibers from the transplant.



Barry Hoffer

Intraocular transplants

Two types of brain tissue have been transplanted into the anterior chamber of a rat eye. The larger, grey mass, which is located just below the pupil, is a piece of cerebral cortex. The white graft to its right is locus coeruleus.

Moreover, in Björklund's and Stenevi's experiments, the innervation patterns formed in the hippocampus by the various transplants were very abnormal when the endogenous tracts were left intact. The fibers from the transplants apparently could make few connections with their usual targets when these were already occupied by endogenous fibers.

Finally, according to Cotman, fibers from a region of the hippocampus, which is designated CA4, seem to be able to exclude septal cholinergic fibers from occupying the same targets. "For some reason," Cotman remarks, "a one-on-one battle of the septal and CA4 fibers is a losing proposition for the septal fibers." He points out that the septal fibers can coexist with other types and there must be an element of specificity in their apparent inability to compete with the CA4 fibers. Cotman has proposed that the CA4 fibers help to establish the laminated structure of the hippocampus by excluding from their terminal field other incoming fibers.

Factors that appeared less important than intrinsic programming and competition in guiding the fate of transplanted neurons include degenerating nerve pathways and the position of the implant. Cotman has shown that implants can make the appropriate connections with the hippocampus even when they are placed in the brain long enough after the endogenous pathway was severed so that the debris from degenerating nerves was no longer detectable.

Putting some implants in the wrong brain locations does not interfere with their ability to make normal connections with their targets. Cotman and Lewis found this to be the case for innervation of the hippocampus by cholinergic septal fibers. Septal tissue placed in a new site where the nerve fibers had to grow in the direction opposite to the one they usually follow produced a typical innervation pattern in the hippocampus. "We get a really remarkable demonstration of specificity," Cotman explains. "Cells from a younger animal in a foreign place somehow manage to grow in and form their normal connections." In addition, Lund found that retina that was transplanted into brain behind its target, the superior colliculus, still formed appropriate connections. He says, "The projections grow forward in the wrong direction to make normal connections."

Nevertheless, if the transplants are put in abnormal sites, they may receive incoming fibers from parts of the brain that would not otherwise innervate them.

The ability of fetal transplants to form appropriate connections with their targets, even when they are placed in unusual locations, implies that chemical factors may be guiding the growth of the nerve fibers. Such trophic factors have long been suspected of playing an important role in nervous system development, but with the exception of nerve growth factor, which was identified some 30 years ago by Rita Levi-Montalcini and Viktor Hamburger of Washington University, investigators have had little luck in isolating the materials. Transplant experiments may help here, too.

Cotman and Lewis noted that certain of their transplants, when placed in the brain immediately after a cavity was made to hold them, did very poorly. But if they waited for 3 to 6 days before inserting the tissue, it thrived. At first the investigators were puzzled by this observation. "It took us a long time to figure out what was happening, but when the explanation dawned, it was surprisingly simple," Cotman remarks. The injured tissue was producing a growth factor that took a few days to accumulate.

By implanting Gelfoam into the cavities, instead of tissue, Cotman and his colleague at Irvine, Manuel Nieto-Sampedro, were able to collect the material and then extract it from the Gelfoam. With Silvio Varon's group at the Univer-

sity of California School of Medicine in San Diego, they found that a factor (or factors) that stimulates the growth of peripheral nerve fibers attained high concentrations in the Gelfoam by 3 to 6 days after it was implanted. This is the same time at which they see good growth of transplanted tissue. The factor appears to be a protein, but it is not identical to nerve growth factor. The Gelfoam extracts may also contain factors that inhibit the growth of some nerve cells, but these have not yet been characterized, Cotman says.

The Hoffer group has evidence for trophic effects in their intraocular transplantation system. If cortex is transplanted after another tissue, such as locus coeruleus, tectum, or cortex itself, the development of the cortex is enhanced. The enhancement involves both an increased number of neurons and larger neuronal size. Hoffer says, "There are trophic influences; one brain area augments the growth of a second. There are a couple of interesting things about the influences. They do not depend on innervation. The increased growth occurs before the fibers grow out. And physical contact is required. I think it may be some nondiffusible chemical factor."

In addition, Björklund and Stenevi, on the basis of their own transplant and other experiments, have suggested that the hippocampus has the capacity of producing a trophic factor that stimulates the growth of both peripheral and central adrenergic neurons. Because they do not see the growth stimulation when the cholinergic nerve tract from the septum to the hippocampus is left intact, they propose that the septal neurons may ordinarily suppress formation of the factor, which would be released only when the tract was damaged.

Neurobiologists hope that identification of trophic factors and a better understanding in general of brain development will eventually lead to more effective treatments for spinal cord or brain injuries, which all too often produce permanent damage. Damaged brain neurons are not supposed to regenerate, or at least they do not form new connections that are sufficiently normal to restore lost functions.

But, researchers postulate, if they can learn how the connections are formed in the first place, they might be able to reverse the damage. One day perhaps, older brain tissue may be induced to form appropriate new connections just as the transplanted fetal tissue does. Cotman, for one, sounds an optimistic note, "The brain has some remarkable regrowing properties and we are just beginning to see its capacity to restore lost connections."—JEAN L. MARX

Grafts Correct Brain Damage

In experiments with rats and monkeys brain grafting works surprisingly well; human testing has begun

About 2 months ago, physicians at the Karolinska Hospital in Stockholm tried an unprecedented medical experiment—the first transplant of tissue into a human brain. The patient was a man so seriously affected by Parkinson's disease that, without medication, he simply could not move. He agreed to the operation, and the hospital's ethics board gave its permission. The procedure is one that works in rats but has not been extensively tested in other animals.

Neurosurgeon Olof Backlund and histologists Lars Olson and Aki Seiger first removed about two-thirds of the patient's adrenal medulla, which makes dopamine as a minor product. Then they transplanted this adrenal gland tissue directly into the caudate nucleus in the man's brain. There, they hoped, the tissue would grow and produce dopamine.

No one knew quite what to expect. Olson recalls that he and the other Swedish scientists were somewhat relieved when they saw "no immediate or dramatic symptoms when [the patient] woke up. If anything, he was somewhat better." Although, according to Olson, the outcome of the experiment cannot yet be fully evaluated, the man does seem to require less medication—80 to 85 percent of the amount of L-dopa he previously needed. Says Olson, "There was a slight improvement, but nothing sensational." The Swedish scientists are encouraged to try more of these transplants, and at least one U.S. researcher, Don Gash of the University of Rochester School of Medicine, predicts that brain transplants will be clinically feasible in this country within 5 to 10 years.

Brain transplants exploit the blood-brain barrier, which is usually considered a hindrance to medical treatments because it prevents many drugs and other substances from reaching the brain. But the blood-brain barrier also keeps cells of the immune system from reaching the brain, making the brain an immunologically privileged site. It is for this reason that brain grafting may be more feasible than grafting in other parts of the body. Brain grafts seem unlikely to be rejected.

Attempts to transplant brain tissue go back at least 50 years, when the French scientist R. May put brain tissue into the anterior chamber of rats' eyes. Only recently, however, have researchers begun to use brain grafts to correct biochemical and behavioral deficits in laboratory animals. Thus far, they have gotten grafts to make the neurotransmitters dopamine, acetylcholine, norepinephrine, and serotonin as well as the hormones vasopressin and gonadotropin-releasing hormone and have corrected brain damage resulting in movement disorders, memory loss, hyperactivity, and over-responsiveness to stimuli in animals. In addition, one group of investigators is now attempting to give blinded animals light perception by grafting retinas or whole eyes directly into the superior colliculus of the brain.

Most of the work on brain transplants involves grafts of tissues that make dopamine. At the back of the researchers' minds is the thought that their work may lead to a treatment for Parkinson's disease, a progressive neurological disorder characterized by tremor, rigidity, and difficulty in initiating movement. The disease is caused by degeneration of the substantia nigra, a major dopamine-producing region of the brain, which supplies dopamine to the caudate nucleus.

Patients with Parkinson's disease usually are treated with L-dopa, which crosses the blood-brain barrier and is converted in the brain to dopamine. But this drug treatment is far from ideal. Because L-dopa arrives at the brain only periodically, just after the drug is taken, the brain does not make dopamine in response to its needs. Investigators have

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