the physiological significance of any central effects of this new peptide remains to be determined. The sheer abundance of NPY, in concentrations even higher than those reported for vasoactive intestinal polypeptide (*23*) and cholecystokinin octapeptide (*24*), should encourage detailed pharmacological and neurophysiological investigations.

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16. Antisera to NPY were raised in New Zealand White rabbits against natural porcine NPY conjugated to bovine serum albumin with bis-diazobenzidine. Third and subsequent monthly boosters were made with keyhole-limpet hemocyanin as carrier.
17. Total quenching of immunostaining was observed after preabsorption with 0.1 nmole of synthetic NPY per milliliter of diluted antiserum. Natural procine PYY quenched at 10 nmole per milliliter of diluted antiserum, whereas 20 nmole of natural APP per milliliter of diluted antiserum only partially reduced the intensity of staining. Preabsorption with BPP and with the unrelated peptides vasoactive intestinal polypeptide, cholecystokinin octapeptide, substance P, somatostatin, and Met-enkephalin at 20 nmole per milliliter of diluted antiserum had no effect, however. At radioimmunoassay working dilutions, no cross-reaction was seen with natural porcine PP, PYY, or APP in concentrations up to 100 pmole per tube.
18. Immunostaining was performed on serial 20-μm cryostat sections of rat brain fixed by perfusion with 4 percent paraformaldehyde in phosphate-buffered saline (*p*H 7.2) in accordance with the peroxidase-antiperoxidase method [L. A. Sternberger, *Immunocytochemistry* (Wiley, New York, ed. 2, 1979)].
19. NPY concentrations were measured by specific radioimmunoassay of tissue samples extracted immediately in 0.5*M* acetic acid. The assay could detect changes of 2 fmole between adjacent assay tubes with 95 percent confidence.
20. Immunostaining was eluted in a glycine and hydrochloric acid buffer (*p*H 3.0) adapted from the method of P. K. Nakane [*J. Histochem. Cytochem.* **16**, 557 (1968)].
21. Fractionation of NPY-like immunoreactivity was carried out on a reverse-phase HPLC column by using a linear gradient elution system and a flow rate of 2 ml/min. One-milliliter fractions were collected for subsequent radioimmunoassay. Recoveries were all between 88 and 94 percent.
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Circumventing the Blood-Brain Barrier with Autonomic Ganglion Transplants

Abstract. *Superior cervical ganglia, whose vessels are fenestrated and permeable to protein tracers such as horseradish peroxidase, were transplanted to undamaged surfaces in the fourth ventricle of rat pup brains. Horseradish peroxidase, infused systemically into the host, was exuded from the graft's vessels into the graft's extracellular stroma within 1 minute. At later times the glycoprotein reached the extracellular clefts of adjacent brain tissue, the vessels of which appeared to retain their impermeability. The blood-brain barrier to horseradish peroxide was thus bypassed where the extracellular compartments of graft and brain became confluent. The graft of autonomic ganglia can serve as a portal through which peptides, hormones, and immunoglobulins may likewise enter the brain.*

Neural transplantation is an important tool for studying aspects of brain development and neuronal interactions. Transplant attempts have largely been concerned with the survival and development of grafts and their direct functional connections with the host brain (*1*). While it appears that the brain can be a receptive and effective host for certain axonal connections, almost no attention has been paid to important vascular relations at the interface between transplant and brain that could affect the availability of, for example, circulating antibodies and hormones to the brain. On the basis of our previous studies, we postulated that the introduction of a foreign neural graft would be likely to alter one of the basic morphological and physiological characters of the mammalian brain: the blood-brain barrier. To our knowledge, there is very little information on the effects of neural tissue transplants on the blood-brain barrier (*2*). This report focuses on structural and functional interrelations in neural transplantation, namely, the changes induced in the blood-brain barrier by grafts of autonomic ganglia. We found that a systemically administered protein, horseradish peroxidase (HRP), freely permeates these grafts on the internal or external brain surfaces of rat pups. Significantly, the blood-borne glycoprotein can subsequently infiltrate adjacent brain parenchyma, where normally it would be excluded.

The transplantation system we described (*3*) has been used successfully to follow the growth and development of grafted peripheral autonomic neurons (*4*), central neurons (*5*), and cardiac muscle (*6*) and is minimally traumatic to the host. In previous studies we proposed that allografted superior cervical ganglion (SCG) in this system, in which the brain surfaces are undisturbed, produces both neurotropic and gliotropic effects on the developing brain (*7*). Since a transplant must be vascularized in order to survive, we have begun to investigate vascular or angiotropic responses of the host brain. The allografted SCG is useful for analyzing vascular interactions because it normally lies outside any blood-neuronal barrier. In situ the SCG is freely permeated by blood-borne HRP (*8*). Might the permeability properties of autonomic ganglia be retained after transplantation to the brain surface, and can cerebral vessels that supply the transplant be altered?

In order to determine whether vessels of the graft provide an entry for blood-borne HRP (molecular weight 40,000) through established vascular connections with the host brain, the following experimental protocol was used. Rat pups, 6 to 10 days old, received an SCG allograft adjacent to the cerebellum and

medulla in the unaltered fourth ventricle (*3*). After 1 month to 1 year the rats were anesthetized and HRP (Sigma type VI; 1 mg per 5 g of body weight, dissolved in 0.7 ml of balanced salt solution) was injected into the femoral vein and allowed to circulate for periods ranging from 50 seconds to 1 hour. In control groups a stab wound was made with a 25-gauge needle or an SCG graft was plunged 1 to 2 mm into the cerebellum (only a few of these grafts survived). These animals were allowed to live 1 to 4 months and HRP was delivered in an identical manner.

Immediately after the interval of HRP circulation the animals were perfused, first with balanced salt solution to clear the vasculature and then with a 3 percent glutaraldehyde fixative in 0.1*M* sodium cacodylate buffer. The SCG transplants and surrounding brain tissue were removed, rinsed in the same buffer, and sectioned serially (thickness, 40 or 60 μm) on an Oxford Vibratome. The sections were incubated for HRP reaction product with 3,3′-diaminobenzidine (*9*); some sections were further processed for electron microscopy. Areas displaying reaction product were trimmed and postfixed in 1 percent buffered osmium tetroxide and processed for electron microscopy.

After intravascular HRP administration the protein was exuded from blood vessels in the SCG allograft in all specimens examined. With an HRP circulation time of 50 seconds the perivascular region and adjacent stroma around several vessels in the transplant were laden with HRP reaction product (Fig. 1A). In some specimens a slight amount of HRP appeared in the brain subjacent to the graft after 2 minutes. In most specimens, however, the underlying brain was free of HRP. With 10 to 15 minutes of HRP circulation nearly all the graft was filled by the glycoprotein, which had also spread to the adjacent brain tissue (Fig. 1B). Regenerating ganglion neurons and neurite bundles in the graft were delineated by HRP trapped in the extracellular stroma (Fig. 1C). The distribution of HRP was comparable to that in the SCG in situ (*8*). Where there was close apposition or melding of the graft with adjacent cerebellum or medulla, these areas were infiltrated with HRP for distances > 1 mm from the pial surface and at least equal to the breadth of the graft (Fig. 1B). The extracellular spaces, including synaptic clefts, were filled with HRP (Fig. 1D). Often the endothelial cell linings of capillaries in the flooded cerebellum or medulla contained far more pits and vesicles than normal (Fig. 1D) (*10*). As to the origin of vessels supplying the grafts, some may have branched from pial vessels, but others evidently arose from deep in the cerebellum, passed the glial border, and entered the cerebrospinal fluid to reach the graft (Fig. 1E). These HRP-laden vessels persisted in transplants that were at least 1 year old. In control animals no HRP was detected at the wound site after postoperative intervals sufficient to allow the injured blood-brain barrier to recover (*11*).

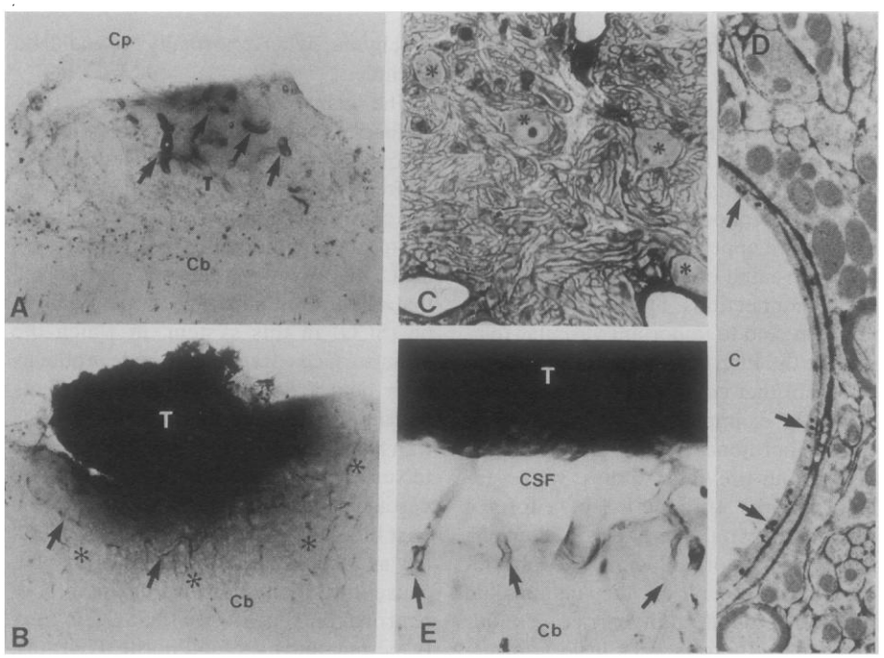

Fig. 1. (A) Perivascular region and adjacent stroma around several vessels (arrows) in an SCG transplant (*T*), shown laden with HRP reaction product after 50 seconds of HRP circulation. The adjacent cerebellum (*Cb*) is free of reaction product; *Cp*, choroid plexus. Vibratome section, 5 months after surgery (×140). (B) SCG transplant totally filled by HRP after 15 minutes of HRP circulation. Asterisks show the extent of HRP penetration of the subjacent cerebellum, which contains several HRP-laden vessels (arrows). Vibratome section, 3 months after surgery (×120). (C) HRP reaction product permeating the transplant after 15 minutes' circulation time. The product surrounds regenerating neurons (*) and axon bundles. Plastic section (1 μm) counterstained with toluidine blue, 3 months after surgery (×480). (D) Numerous HRP-containing vesicular organelles (arrows) filling an endothelial cell that lines a capillary (*C*) in the medulla subjacent to an SCG graft. After 20 minutes of circulation, HRP has infiltrated all the extracellular spaces around the capillary and surrounding neuropil. Electron micrograph, 10 weeks after surgery (×13,500). (E) HRP-laden vessels (arrows) emerging from deep in the molecular and granular layers of the cerebellum, traversing the cerebrospinal fluid (*CSF*), and entering the transplant (30 minutes' HRP circulation). Vibratome section, 1 year after surgery (×350).

These results shed light on an important aspect of neural transplantation: the access of blood-borne substances to the extracellular compartment of the brain through the extracellular spaces of the graft. A major question is how the systemically administered glycoprotein entered the adjacent brain parenchyma, where normally it would be excluded. Our experiments suggest several possibilities. First, fenestrated vessels may arise from the graft and infiltrate the brain parenchyma. However, vessels that supply cerebral regions outside the blood-brain barrier have yet to be found in the brain adjacent to the graft. Second, the proximity of the graft may cause cerebral vessels to become highly stimulated; not only do they emerge from the brain to enter the graft but their endothelial cell linings contain large numbers of HRP-laden organelles reminiscent of those found where the blood-brain barrier has been rendered "leaky" by hyperosmolarity (*12*), hypertension (*13*), experimental brain trauma (*14*), or induced brain tumors (*15*). Finally, if the graft is connected to the brain and if there is a confluence of their extracellular compartments, HRP may filter through extracellular or perivascular spaces and may also enter the cerebrospinal fluid. The last possibility may be the most likely because, after a brief circulation time, the first blush of HRP appeared in the graft whereas the brain was usually free of HRP. Only later, as a rule, was the brain infiltrated. The appearance of HRP in the brain is, therefore, time-dependent.

Opening the blood-brain barrier with ganglionic grafts is advantageous in that the opening can be confined to a selected region, a superficial region is affected

with minimum trauma, and the opening persists as long as the graft is in place (*16*). In this transplantation system, in which the brain is left as undisturbed as possible, vessels appear to emerge from the brain parenchyma to supply the graft. This vascular response suggests that some angiotropic factor produced by the graft induces changes in the host endothelium. The vasculature in the graft of autonomic neural tissue is highly permeable to blood-borne protein, just as it is in situ. We previously found that intravascular administration of the catecholamine analog 5-hydroxydopamine (molecular weight 205), which normally cannot cross the blood-brain barrier, labeled noradrenergic boutons and storage sites in the graft (*12*). Direct anastomoses were formed between the vessels of the graft and adjacent tissue; India ink, infused into the aorta, filled vessels from the choroid plexus, medulla, and cerebellum that were directly confluent with graft vessels. It is not known whether the graft vessels are replaced by those growing from the surrounding central nervous system (*17*). Electron microscopy reveals fenestrated vessels in the SCG transplant that resemble those of the SCG in situ (*12*). This finding and the fact that SCG grafts are vascularized within 18 to 24 hours (*12, 17*) would argue against the inference proposed for skin and iris implants (*2*): that the original capillary bed of the graft dies and is completely replaced by vessels from the host. Rather, some of the blood vessels that persist within the graft may be derived from original, intrinsic vessels, inasmuch as they are readily permeable to protein and amine.

Jacobs *et al.* (*18*) recently suggested that the lack of a barrier system in ganglion tissue exposes it to environmental toxins that are excluded from the central nervous system. We have determined that the difference between peripheral and central neurovasculature is apparently maintained indefinitely in this transplantation system. Transplants of autonomic neural tissue can provide a permanent biological portal whereby blood-borne substances, including biologically active peptides, hormones, and immunoglobulins, can be delivered directly to adjacent brain areas.

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Otosclerotic Lesions in the Inbred LP/J Mouse

Abstract. *Inbred LP/J mice develop abnormal bony lesions that are grossly and histologically similar to the lesions of human otosclerosis. This is the first known occurrence of spontaneous otosclerosis-like lesions in an animal. As in the human disease, these lesions impair audition by immobilizing the ossicles of the middle ear. The LP/J mouse may be an animal model for this common human disease.*

Otosclerosis, an inherited disease in which foci of abnormal bone develop in the middle and inner ear, affects 14 to 15 million people in the United States (*1*). Ten percent of affected individuals experience progressive hearing loss because of gradual immobilization of the stapes by abnormal bone. In addition, when otosclerotic bone involves the endosteum of the otic capsule, progressive damage to the inner ear may result in sensorineural hearing loss. Although highly successful surgical procedures have been devised to correct the ossicular fixation, there is no treatment for the cochlear damage. This disease has been thought to occur spontaneously only in humans, and attempts at experimental induction in animals have not been entirely successful. Therefore, knowledge of its pathophysiology is poorly understood. In this report we describe spontaneous development of otosclerosis-like foci in an animal (*2*).

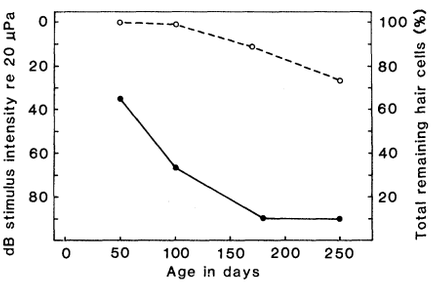

Fig. 1. Mean hair cell loss in the entire cochlea (○) and mean threshold of the auditory evoked response (●) in LP/J mice of various ages. Auditory acuity diminishes rapidly with age while the hair cell population changes little. These data were derived from cytocochleograms and electrocochleograms.

The inbred LP/J mouse has a progressive loss of auditory function (*3*); however, examination reveals that the hair cells of the cochlea remain remarkably intact when the animal has little or no auditory function (Fig. 1) (*4*). The scanning electron microscope confirms the normal appearance of the hair cells in animals with marked shifts in auditory threshold. While examining the middle ear structures of these mice, we observed abnormal bony structures on the ossicles (Fig. 2). Since these lesions are grossly similar to the lesions of human otosclerosis, we performed a histological and ultrastructural study of these ossicles.

Adult LP/J mice between 100 and 250 days of age were reared in a colony in which ambient noise was less than 40 dB over the range of their hearing. These animals were first- to third-generation descendants of parental stock obtained from the Jackson Laboratory at Bar Harbor, Maine. They were given unlimited water and Simonson mouse food. Each animal was deeply anesthetized intraperitoneally with pentobarbital and its bulla was flooded with 2 percent paraformaldehyde and 2 percent glutaraldehyde in 0.08*M* cacodylate buffer (*p*H 7.4