noreactivity in cell bodies and fibers primarily in the hypothalamus (*5*), recent studies reveal scattered CRF-positive cells in rat and sheep hippocampus (*5, 6*). Radioimmunoassay also reveals CRF in the hippocampus (*13*). Furthermore, the subependymal location of CRF-positive fibers near the third ventricle (*5*) might release sufficient CRF into the cerebrospinal fluid to activate hippocampal neurons. Indeed, central injection of as little as 10 ng of CRF activates the hippocampal electroencephalogram (*14*). If endogenous CRF is involved in activation of brain neurons for response to stress (*2*), the novel pattern of neuronal excitation seen in this study could provide the cellular basis.

Note added in proof: A study in our laboratory indicates that CRF also has excitatory actions on locus ceruleus neurons in the rat in vivo (*15*).

J. B. ALDENHOFF*
D. L. GRUOL, J. RIVIER
W. VALE, G. R. SIGGINS†
A. V. Davis Center for Behavioral Neurobiology and *Peptide Biology Laboratory, Salk Institute, San Diego, California 92138*

References and Notes

1. A. Witter and D. deWied, in *Handbook of the Hypothalamus*, P. Morgane and J. Panksepp, Eds. (Dekker, New York, 1980), p. 307; W. Vale, C. Rivier, M. Brown, in *ibid.*, p. 165.
2. W. Vale, J. Spiess, C. Rivier, J. Rivier, *Science* **213**, 1394 (1981); J. Spiess, J. Rivier, C. Rivier, W. Vale, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6517 (1981).
3. M. Brown, L. A. Fishers, J. Rivier, J. Spiess, C. Rivier, W. Vale, *Life Sci.* **30**, 207 (1982); M. Brown *et al.*, *Regul. Peptides*, in press.
4. R. E. Sutton, G. F. Koob, M. Le Moal, J. Rivier, W. Vale, *Nature (London)* **297**, 331 (1982).
5. F. E. Bloom, E. L. F. Battenberg, J. Rivier, W. Vale, *Regul. Peptides* **4**, 43 (1982); G. Bugnon, D. Fellman, A. Guoget, J. Cardot, *C. R. Acad. Sci.* **294**, 599 (1982); F. E. Bloom, E. Battenberg, J. Morrison, in preparation.
6. L. Swanson, P. E. Sawchenko, J. Rivier, W. Vale, *Neuroendocrinology* **36**, 165 (1983). In this study, larger amounts of colchicine were used than in earlier studies.
7. These data have been published in preliminary form [J. B. Aldenhoff, D. L. Gruol, G. R. Siggins, *Soc. Neurosci. Abstr.* **8**, 983 (1982)].
8. G. R. Siggins and P. Schubert, *Neurosci. Lett.* **23**, 55 (1981); Q. Pittman and G. R. Siggins, *Brain Res.* **221**, 402 (1981). After being cut on a McIlwain chopper, slices were incubated for 1 hour, with the top surface exposed to warm, moist atmosphere consisting of 95 percent O_2 and 5 percent CO_2; the slices were then submerged and superfused at 2 to 4 ml/min with ACSF gassed with 95 percent O_2 and 5 percent CO_2 (*p*H 7.4) and maintained at constant temperature (32° to 35°C). The composition of the ACSF, in millimoles per liter, was NaCl, 124; KCl, 5; $MgSO_4$, 2; KH_2PO_4, 1.3; $NaHCO_3$, 26; $CaCl_2$, 2; and glucose, 10. The superfusion rate allowed the CRF in the chamber to reach 90 percent of the reservoir concentration within 2.5 minutes (Q. Pittman and G. Siggins, unpublished data). Cells accepted for analysis had stable membrane potentials of 55 mV or greater; recording durations were 1 to 5 hours.
9. J. R. Hotson and D. A. Prince, *J. Neurophysiol.* **43**, 409 (1980); P. A. Schwartzkroin and C. E. Stafstrom, *Science* **210**, 1125 (1980); R. K. S. Wong and D. A. Prince, *J. Neurophysiol.* **45**, 86 (1981). Our experiments also support the Ca^{2+}-dependent K^+ mechanism for the afterhyperpolarizations: (i) afterhyperpolarizations and Ca^{2+} spikes were blocked by 10 m*M* Mg^{2+}; (ii) the afterhyperpolarizations were associated with decreased input resistance; (iii) the afterhyperpolarization was decreased by membrane hyperpolarization; and (iv) the afterhyperpolarization was not sensitive to chloride injection.
10. With superfusion of ACSF with 20 m*M* $MgCl_2$ to block calcium channels and synaptic transmission, a slight CRF-induced increase of discharge frequency was still observed, although the increase was much smaller than in normal ACSF. ACSF with 11.5 m*M* Mg^{2+} also somewhat reduced the depolarizations evoked by large CRF concentrations but did not block them. The posttrain afterhyperpolarizations and the "calcium spikes" seen after tetrodotoxin were antagonized by both Mg^{2+} concentrations. In CA1 neurons, CRF in normal ACSF often increased the frequency of subthreshold activity (Fig. 1), composed either of attenuated dendritic spikes or synaptic potentials. In tetrodotoxin, synaptic activation and spontaneous discharge were both blocked; under these conditions high CRF concentrations only occasionally depolarized pyramidal neurons.
11. G. Hofmeier and H. D. Lux, *Pfluegers Arch.* **391**, 242 (1981).
12. R. K. S. Wong, D. A. Prince, A. I. Basbaum, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 986 (1979).
13. A. J. Fischman and R. L. Moldow, *Peptides* **1**, 149 (1982).
14. C. L. Ehlers, S. J. Henriksen, F. E. Bloom, J. Rivier, W. J. Vale, *Soc. Neurosci. Abstr.* **8**, 1013 (1982).
15. R. Valentino, S. Foote, G. Aston-Jones, *Brain Res.* **270**, 363 (1983).
16. Supported by PHS grants AM 26741 and AA 03504 and by grants from the Alexander-von-Humboldt Foundation (to J.B.A. and G.R.S.) and the F. M. Kirby Foundation, Inc. (to J.B.A.). We thank F. E. Bloom and L. Koda for criticism, C. Franklin for technical help, and N. Callahan for manuscript preparation.
* Present address: BKH Kaufbeuren, Kemnaterstrasse 16, D-895 Kaufbeuren, West Germany.
† Address reprint requests to G.R.S.

15 March 1983

Neuropeptide Y Distribution in the Rat Brain

Abstract. *A massive neuronal system was detected by immunocytochemistry and radioimmunoassay with antibodies to neuropeptide Y, the recently isolated peptide of the pancreatic polypeptide family. Immunoreactive cell bodies and fibers were most prevalent in cortical, limbic, and hypothalamic regions. Neuropeptide Y was extracted in concentrations higher than those of any other peptide hitherto discovered in the mammalian brain. Column chromatography of brain extracts and double immunostaining experiments indicate that neuropeptide Y is the endogenous brain peptide responsible for immunostaining of pancreatic polypeptide–like immunoreactivity in the mammalian brain.*

It has been thought for some time that a peptide chemically related to the pancreatic hormone pancreatic polypeptide (PP) is present in the mammalian central nervous system (CNS). Immunocytochemical studies with antisera to avian PP (APP) (*1–6*) and bovine PP (BPP) (*7*) have suggested a new and widespread PP-like immunoreactive neuronal system. However, no significant APP- or BPP-like immunoreactivity has been found on radioimmunoassay, so the true nature of the PP-like immunoreactivity has remained unknown. Recently, a peptide consisting of 36 amino acid residues, neuropeptide Y (NPY), was isolated from extracts of porcine brain (*8*) by a novel method for detecting peptides having a COOH-terminal α-amide (*9*), a characteristic feature of many biologically active peptides (*10*). It shares major sequence homologies with the putative gut hormone peptide YY (PYY) (*11–14*) and with PP (*14, 15*). NPY is, therefore, considered to be the latest member of the emerging PP family (*14*).

Table 1. Distribution of NPY-like immunoreactivity in the rat brain. Values (means ± standard errors; $N = 6$) are based on porcine NPY cross-reactivity.

Region	Immunoreactivity (picomoles per gram of wet tissue)
Olfactory bulb	82.2 ± 4.2
Frontal cortex	178.5 ± 23.7
Parietal cortex	183.6 ± 30.2
Occipital cortex	188.7 ± 31.5
Preoptic hypothalamus	729.8 ± 164.0
Periventricular hypothalamus	980.0 ± 162.0
Caudate	312.5 ± 68.6
Putamen	516.0 ± 104.3
Globus pallidus	311.2 ± 47.6
Nucleus accumbens	891.4 ± 80.9
Septum	762.2 ± 142.7
Amygdala	714.7 ± 56.2
Thalamus	103.4 ± 20.9
Hippocampus	205.2 ± 31.6
Periaqueductal gray region	894.4 ± 169.9
Cerebellum	27.0 ± 5.2
Pons	45.8 ± 16.0

By raising antibodies to NPY (*16*) that did not show significant cross-reactivity with PYY or APP (*17*), we have detected by immunocytochemistry (*18*) and radioimmunoassay (*19*) a widespread NPY-immunoreactive system in the rat brain that strongly resembles the reported distribution of PP-like immunoreactivity. Indeed, double immunostaining by an elution technique (*20*) confirmed that NPY and an APP-like immunoreactive material are located in the same cells. Analysis of brain extracts by reverse-phase high-performance liquid chromatography (HPLC) (*21*) showed NPY-like immunoreactivity to elute in a position similar to that of pure NPY. NPY may, therefore, be the true peptide of the PP family in the rat brain.

A salient feature of the distribution of

NPY-like immunoreactivity was the high concentration in forebrain regions, which became gradually lower toward the brainstem (Table 1). NPY-immunoreactive cell bodies were most numerous in the cortex, particularly in deeper layers V and VI, and in the caudate nucleus and putamen (Fig. 1, a and b). Cell bodies were also observed in the hypothalamus, particularly the arcuate nucleus, and in the molecular layers of the hippocampus and dentate gyrus. Scattered cell bodies were found throughout the anterior olfactory nucleus, septum, nucleus accumbens, and amygdaloid complex. In the brainstem, cell bodies and fibers were observed in the periaqueductal gray region and less densely in the locus coeruleus, nucleus of the tractus solitarius, and superficial laminae of the trigeminal nucleus. Dense plexuses of fibers and terminals alone were most apparent in the bed nucleus of the stria terminalis, the peri- and paraventricular regions of the thalamus and hypothalamus, and discrete hypothalamic nuclei (Fig. 1c). Long varicose fibers in the corpus callosum, fornix, stria terminalis (Fig. 2d), and diagonal band of Broca suggest that NPY projects in long pathways throughout the brain.

Lorén *et al.* (*1*), who studied the location of PP-like immunoreactivity in the brain by immunocytochemistry, recognized neuronal PP to be distinct from the PP that occurs in pancreatic endocrine cells. Antisera to APP identified neuronal structures in the mammalian CNS, whereas BPP and human PP stained only gut and pancreas. It is pertinent that complete amino acid sequencing (*14*) has revealed NPY to share 20 of 36 amino acid residues with APP and only 17 with BPP. Jacobowitz and Olschowka (*7*) did localize by immunocytochemistry a BPP-like immunoreactive substance in the rat brain. However, neither APP nor BPP has been extracted from mammalian brain or detected in significant quantity by assay. Column elution position after HPLC fractionation of NPY immunoreactivity from rat brain extracts showed a high recovery at the standard peak for pure porcine NPY (Fig. 2), whereas APP could not be detected. Furthermore, double immunostaining revealed an APP-like immunoreactive substance in NPY-immunoreactive cell bodies (Fig. 1e), indicating that both antisera recognize the same structures. We interpret these findings, therefore, as evidence that NPY is the endogenous PP in the rat brain. We further postulate that previous studies in which PP-like immunoreactivity was reported to have been detected with antisera to APP and BPP were identifying instead the NPY-immunoreactive system because of the major sequence homologies that APP and BPP share with the naturally occurring brain peptide.

The distribution of NPY immunoreactivity in the rat brain and its presence in long projecting pathways suggest that NPY is one of a growing list of neuropeptides that probably act as neurotransmitters or neuromodulators in nervous system functioning. In the peripheral nervous system NPY has been shown to be a potent vasoconstrictor and to inhibit pancreatic secretion (*8*, *22*). However,

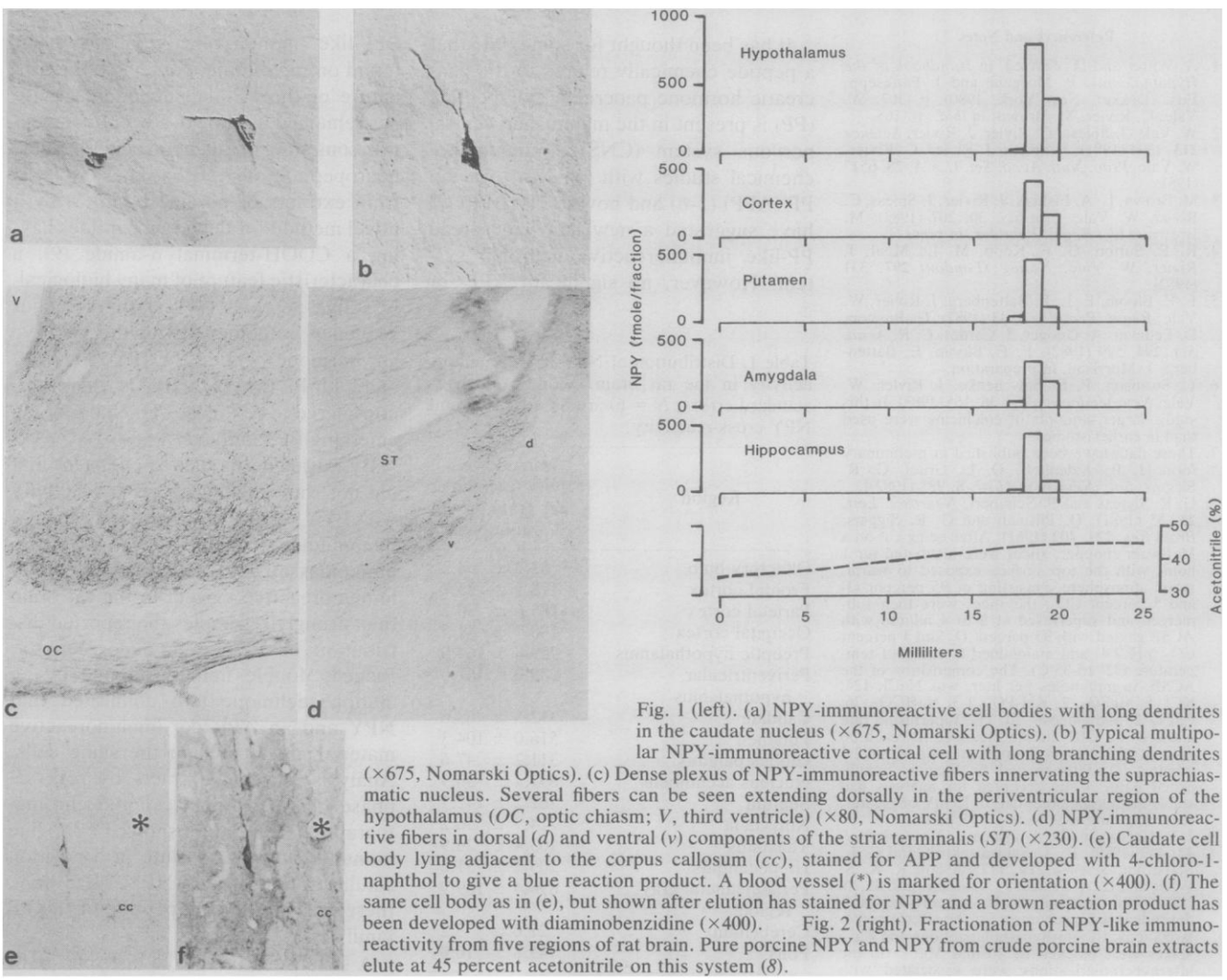

Fig. 1 (left). (a) NPY-immunoreactive cell bodies with long dendrites in the caudate nucleus (×675, Nomarski Optics). (b) Typical multipolar NPY-immunoreactive cortical cell with long branching dendrites (×675, Nomarski Optics). (c) Dense plexus of NPY-immunoreactive fibers innervating the suprachiasmatic nucleus. Several fibers can be seen extending dorsally in the periventricular region of the hypothalamus (*OC*, optic chiasm; *V*, third ventricle) (×80, Nomarski Optics). (d) NPY-immunoreactive fibers in dorsal (*d*) and ventral (*v*) components of the stria terminalis (*ST*) (×230). (e) Caudate cell body lying adjacent to the corpus callosum (*cc*), stained for APP and developed with 4-chloro-1-naphthol to give a blue reaction product. A blood vessel (*) is marked for orientation (×400). (f) The same cell body as in (e), but shown after elution has stained for NPY and a brown reaction product has been developed with diaminobenzidine (×400). Fig. 2 (right). Fractionation of NPY-like immunoreactivity from five regions of rat brain. Pure porcine NPY and NPY from crude porcine brain extracts elute at 45 percent acetonitrile on this system (*8*).

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.

Y. S. ALLEN
Department of Histochemistry, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 OHS, United Kingdom
T. E. ADRIAN
J. M. ALLEN
Department of Medicine, Royal Postgraduate Medical School
K. TATEMOTO
Department of Biochemistry II, Karolinska Institute, S-104 01 Stockholm, Sweden
T. J. CROW
Division of Psychiatry, Clinical Research Centre, Harrow, Middlesex HA1 3UJ, United Kingdom
S. R. BLOOM
Department of Medicine, Royal Postgraduate Medical School
J. M. POLAK*
Department of Histochemistry, Royal Postgraduate Medical School

References and Notes

1. I. Lorén, R. Alumets, R. Håkanson, F. Sundler, *Cell Tissue Res.* **200**, 179 (1979).
2. J. M. Lundberg *et al.*, *Acta Physiol. Scand.* **110**, 107 (1980).
3. S. P. Hunt, P. C. Emson, R. Gilbert, M. Goldstein, J. R. Kimmel, *Neurosci. Lett.* **21**, 125 (1981).
4. T. Hökfelt *et al.*, *Peptides* **2**, 81 (1981).
5. S. R. Vincent *et al.*, *Neuroscience* **7**, 439 (1982).
6. J. P. Card and R. Y. Moore, *J. Comp. Neurol.* **206**, 390 (1982).
7. D. M. Jacobowitz and J. A. Olschowka, *Peptides* **3**, 569 (1981).
8. K. Tatemoto, M. Carlquist, V. Mutt, *Nature (London)* **296**, 659 (1982).
9. K. Tatemoto and V. Mutt, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 4115 (1978).
10. L. T. Hunt and M. O. Dayhoff, in *Atlas of Protein Sequences and Structure*, M. O. Dayhoff, Ed. (National Biochemical Research Foundation, Silver Spring, Md., 1976), vol. 5, suppl. 2, pp. 113–145.
11. K. Tatemoto and V. Mutt, *Nature (London)* **285**, 417 (1980).
12. K. Tatemoto, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2514 (1982).
13. J. M. Lundberg *et al.*, *ibid.*, p. 4471.
14. K. Tatemoto, *ibid.*, p. 5485.
15. J. R. Kimmel, L. J. Hayden, H. G. Pollock, *J. Biol. Chem.* **250**, 9369 (1975).
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.
22. J. M. Lundberg and K. Tatemoto, *Br. J. Pharmacol.* **77**, 335P (1982).
23. I. Lorén, P. C. Emson, J. Fahrenkrug, A. Bjorklund, J. Alumets, R. Håkanson, F. Sundler, *Neuroscience* **4**, 1953 (1976).
24. I. Lorén, J. Alumets, R. Håkanson, F. Sundler, *Histochemistry* **59**, 249 (1979).
25. Y.S.A. is the recipient of a Medical Research Council studentship award. J.M.A. is supported by a Wellcome Trust training fellowship. We thank J. Kimmel for the generous supply of APP antiserum.
* To whom correspondence should be addressed.

27 December 1982; revised 7 March 1983

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