References and Notes

1. For fluorescent markers, see S. B. Kater and C. Nicholson, Eds. [*Intracellular Staining in Neurobiology* (Springer-Verlag, New York, 1973)]. For HRP staining, see J. Jankowska, J. Rastad, and J. Westman [*Brain Res.* **105**, 557 (1976)] and S. T. Kitai, J. D. Kocsis, R. J. Preston, and M. Sugimori [*ibid.* **109**, 601 (1976)].
2. R. W. Guillery, *J. Comp. Neurol.* **128**, 21 (1966).
3. Cells with class 4 morphology (*2*) are limited to the most ventral C laminae. They have an intermediate soma size and fairly appendage-free dendritic tree, usually oriented in a plane parallel to the laminae. They may represent the physiological class of W cells, which are also limited to these laminae [P. D. Wilson and J. Stone, *Brain Res.* **92**, 472 (1975)]. Since we have neither investigated W cells physiologically nor seen class 4 cells morphologically, they will not be considered further in this report. Also, E. Famiglietti [*Brain Res.* **20**, 181 (1970)] and B. Updyke [*Neurosci. Abstr.* **1**, 649 (1978)] have suggested a fifth class of cells, which have beaded dendrites.
4. B. G. Cleland, M. W. Dubin, W. R. Levick, *J. Physiol. (London)* **217**, 473 (1971); S. Hochstein and R. M. Shapley, *ibid.* **262**, 237 (1976); *ibid.*, p. 265; M. H. Rowe and J. Stone, *Brain Behav. Evol.* **14**, 185 (1976); P. D. Wilson, M. H. Rowe, J. Stone, *J. Neurophysiol.* **39**, 1193 (1976).
5. K.-P. Hoffmann, J. Stone, S. M. Sherman, *J. Neurophysiol.* **35**, 518 (1972).
6. C.-S. Lin, K. E. Kratz, S. M. Sherman, *Brain Res.* **131**, 167 (1977).
7. M. W. Dubin and B. G. Cleland, *J. Neurophysiol.* **40**, 410 (1977).
8. Guillery (*2*) stated that 40 percent of the Golgi-impregnated cells had morphology intermediate among the major classes described and thus could not be placed into one of these classes.
9. C. Enroth-Cugell and J. G. Robson, *J. Physiol. (London)* **187**, 517 (1966); K. E. Kratz, S. V. Webb, S. M. Sherman, *Vision Res.* **18**, 1261 (1978).
10. J. T. McIlwain and O. D. Creutzfeldt, *J. Neurophysiol.* **30**, 1 (1967).
11. Stimulating electrodes were placed in cortical areas 17 and 18 for most preparations in order to activate geniculate cells (*5-7*). After removal of cortex overlying the lateral geniculate nucleus, however, we were unable to achieve as routine electrical activation of these neurons as we could when overlying cortical extirpation was unnecessary. Perhaps this ablation, although far from the optic radiation, nonetheless damaged geniculocortical fibers sufficiently to prevent conduction of action potentials. We are concerned that these cortical lesions might affect geniculate neuronal receptive fields. In agreement with K. J. Sanderson, P.O. Bishop, and I. Darian-Smith [*Exp. Brain Res.* **13**, 159 (1971)], however, we detected no difference in such properties between preparations requiring cortical extirpation and those that did not.
12. The following criteria were used to distinguish between X and Y cells (*4, 5, 9*): conduction latency to electrical stimulation of optic chiasm, linearity of spatial summation, responses to targets moved rapidly (> 200°/sec) through the receptive field, and the tonic or phasic nature of the response to appropriate standing contrast.
13. J. H. LaVail and M. M. LaVail, *Science* **176**, 1416 (1972); *J. Comp. Neurol.* **157**, 303 (1974).
14. K. J. Sanderson, *J. Comp. Neurol.* **143**, 101 (1971).
15. T. Ogawa, T. Takimori, Y. Takahasi, *Brain Res.* **139**, 35 (1978).
16. These last two had large somata and thick primary dendrites. One had grapelike clusters at dendritic branch points, which is typical of class 2 cells, but it also had many dendrites with a beaded appearance, which is characteristic of class 5 cells (*3*). The other Y cell had numerous dendrites, which arborized mostly in the dorsoventral axis; these dendrites were covered with short processes that gave them a hairy appearance.
17. E. Famiglietti and A. Peters, *J. Comp. Neurol.* **144**, 285 (1972).
18. S. LeVay and D. Ferster, *ibid.* **172**, 563 (1977); D. Ferster and S. LeVay, *ibid.* **182**, 923 (1978).
19. J. Stone and B. Dreher, *J. Neurophysiol.* **36**, 551 (1973).

20. Supported by PHS grant EY 01565, NSF grant BNS 77-06785, and a grant from the A. P. Sloan Foundation. Further support included PHS grant RCDA EY00020 to S.M.S., postdoctoral fellowship EY 05197 to M.J.F., and postdoctoral fellowship NS 05664 to C.S.L. We thank S. Gibson for her expert and tireless technical assistance.

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Intracephalic Implants: A Technique for Studying Neuronal Interactions

Abstract. *Implants of embryonic neural tissue from all regions of the neuraxis survive grafting to the brains of adult rats. After implantation, neurogenesis and differentiation continue, and connections are formed with the mature host brain. Thus, the intracephalic implants provide excellent model systems for studying cellular interactions that regulate synaptogenesis and determine the cytoarchitectonic organization of developing neural tissues.*

The ability of developing mammalian central nervous system (CNS) tissue to differentiate and survive in isolation has been well established in explant culture in vitro (*1*). Although culturing procedures are excellent for investigating certain types of neuronal and glial interactions, only relatively small pieces of mammalian CNS tissue can be maintained in isolated culture because nutrients from the culturing medium must be able to diffuse into the explant tissue. Moreover, since it is difficult to produce an artificial culturing medium that is functionally similar to the normal extracellular fluid of the CNS, small variations in the composition of the culturing media may also influence development and survival of the explant.

One procedure that attempts to overcome the limitations of an artificial culturing medium is the intraocular transplantation technique (*2*). The procedure demonstrates that the aqueous humor of the anterior eye chamber can provide a favorable culturing environment in which small pieces of embryonic CNS tissue can be vascularized and maintained in relative isolation from extrinsic neuronal elements. This technique also adds a further dimension to studying cellular interactions, since the transplanted CNS tissue is capable of interacting with the tissues in the anterior eye chamber of the adult host animal (*3*).

The transplantation of fetal and neonatal CNS tissue into the brain of neonatal rats has also been reported (*4*). Small pieces of neural tissue are injected into the host animals, where they are incorporated into the normal CNS environment of the host. This procedure allows the transplanted tissue to be reincorporated into a CNS environment where it can continue to develop, mature, and interact with the developing host brain. However, the technique appears to suffer the disadvantages that it is difficult to localize the transplanted tissue to a specific region of the host brain, and that, in the quoted studies, the transplanted tissue was small and sometimes difficult to identify.

Despite their drawbacks all the above techniques provide methods for studying specific types of neuronal interactions. However, none permits the study of interactions between neural elements in embryonic and mature CNS tissue, nor do they provide an environment in which larger pieces of embryonic tissue can survive and differentiate within a CNS environment. We now report on an intracephalic implantation technique in mammals that allows cellular interactions to be investigated both within the embryonic implant and between the implanted tissue and the mature neural tissue of the host animal.

We took pieces of brain or spinal cord from rat embryos measuring 9 to 30 mm from crown to rump (corresponding to embryonic days 13 to 20) and transplanted them into cavities prepared by removing either the parietal cortex (rostral transplantation site) or occipital cortex (caudal site) of adult female Sprague-Dawley rats (Fig. 1), as described by Stenevi *et al.* (*5*). The implants comprised entorhinal cortex plus subiculum, the hippocampal formation (including den-

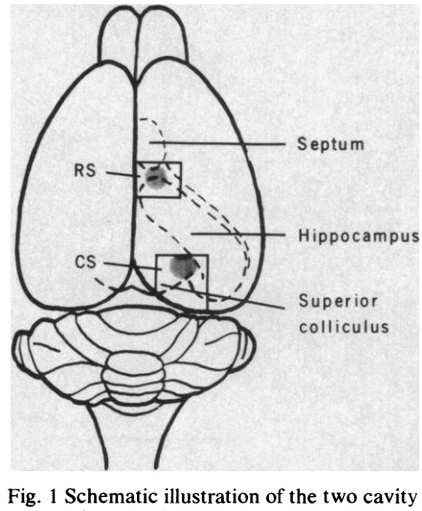

Fig. 1 Schematic illustration of the two cavity preparations used. In the rostral site (*RS*) the fimbria and septal end of the hippocampus were removed by suction and the implanted tissue (shaded area) is placed on the vessel-rich covering of the anterior thalamus. For the caudal site (*CS*) the neocortex and hippocampal tissue overlying the dentate gyrus were removed and the implant (shaded area) was placed on the dorsal surface of the caudal thalamus or superior colliculus adjacent to the dentate gyrus and choroidal fissure.

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tate gyrus), the septum-diagonal-band area, cerebellum, the locus coeruleus and substantia nigra regions of the brainstem, and the cervical spinal cord. After survival times of 1 to 14 months, the brains were fixed in formalin and stained with cresyl violet, the Kluver-Barrera stain, or the Holmes' silver stain (*6*). Some brains carrying brainstem implants were processed for monoamine histofluorescence according to the Falck-Hillarp formaldehyde method (*7*) and some septal-diagonal band and spinal cord implants were stained for acetylcholinesterase (*8*).

In the intracephalic cavities (approximately 3 by 3 mm), the implants were placed on the vessel-rich pia covering the caudal thalamus and superior colliculus (caudal site) or ependyma covering the anterior thalamus (rostral site). After 1 month all cavities were resealed with new dura and pia-arachnoid membranes and, being in direct communication with the lateral ventricle, became filled with cerebrospinal fluid. The implant itself acquired a pial or ependymal covering and received a rich vascular supply from the thalamic and collicular surface, and sometimes also from the adjacent choroid plexus. Although all types of implants survived (the overall survival rate being approximately 80 percent), each region of the CNS had a specific developmental time period during which survival was optimal.

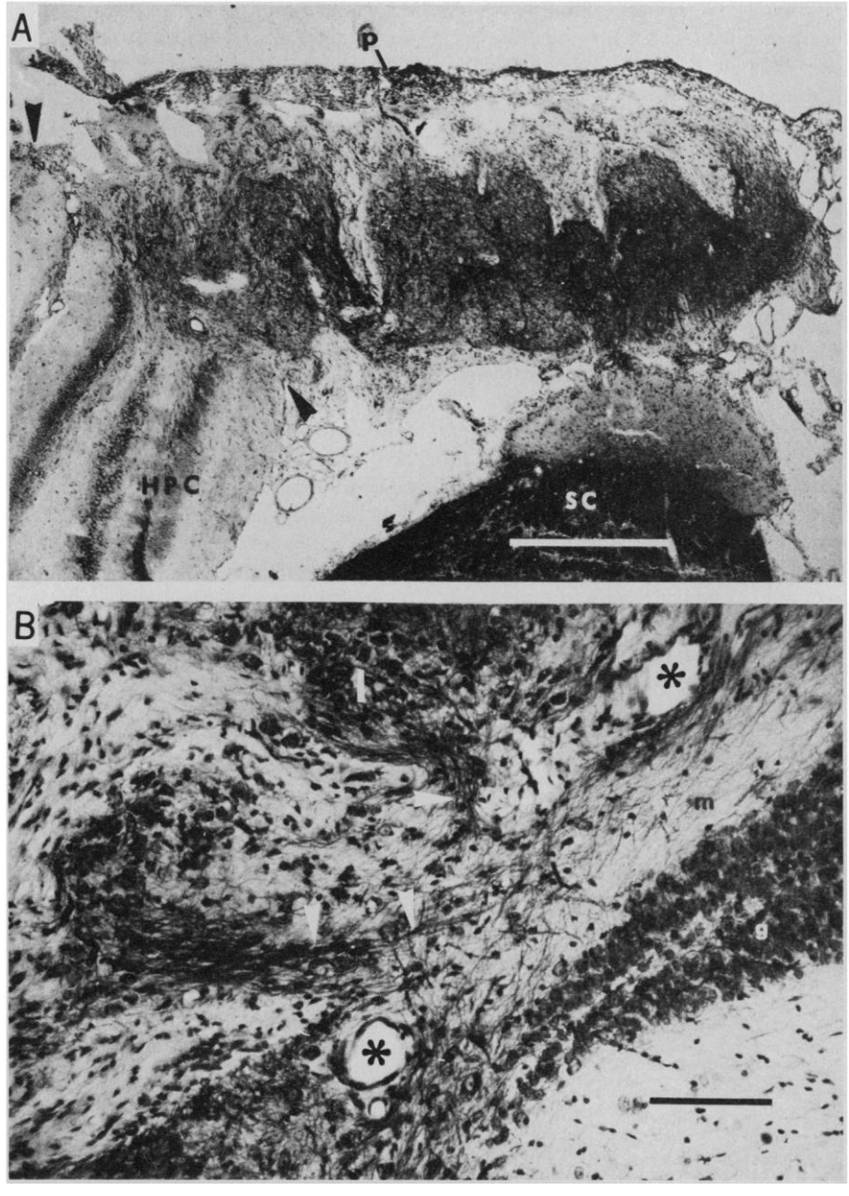

Fig. 2 (A) Coronal section through an implant of the locus coeruleus region of the brainstem, taken from an embryo 16 mm long (crown to rump). Survival time was 14 months. The implant, which is covered with pial membrane (*P*), has fused with the cut surface of the hippocampus (*HPC*) of the host brain laterally (arrows), and contacts the pia overlying the superior colliculus (*SC*), ventrally. The implant contained an abundance of darkly stained myelinated fibers that interconnected the various sublobules [Klüver-Barrera stain; (bar equals 0.5 mm)]. (B) Detail from the same locus coeruleus implant as in (A), showing the extension of myelinated fibers (arrows) between the implant (*I*) and the dentate molecular layer (*m*) of the host brain. The two blood vessels (asterisks) are located at the border between the two regions. Abbreviation: *g*, granule cell layer (Klüver-Barrera stain, bar equals 100 μm).

The implants varied considerably in size and shape. In general, the embryonic implants retained or increased their size while maturing in situ so that the final volume of tissue ranged between 0.2 and 35 mm^3. The shape of the implants depended somewhat on the age of the donor, the region dissected, and the disruption and folding of the embryonic tissue during implantation. Most implanted tissues, however, had a lobular organization partly related to the arrangement of the vascular supply. The lobules fused with each other and also with the cut surfaces of the host brain.

Numerous myelinated and unmyelinated fibers were present in all implanted tissues, where they both interconnected the implant lobules and formed connections with the recipient brain. The latter connections occurred where the implant had fused to regions of the host brain lacking an ependymal or pial covering. The presence of myelinated fibers within a locus coeruleus-brainstem implant is illustrated in Fig. 2A. After 14 months of survival in situ, heavily myelinated fibers still interconnected all lobules within the implant and also formed extrinsic connections with the host hippocampus. Figure 2B illustrates a region in which numerous myelinated fibers from the implant entered the molecular layer of the dentate gyrus. This ingrowth of myelinated fibers indicates that neurons within the brainstem implant can interact with the adjacent CNS tissue of the adult recipient even though the implanted neurons would not necessarily form connections with this region during normal development.

A number of different neuron types can be identified in the implants, and sometimes the characteristic cytoarchitectural features of the transplanted region are recognizable. Noradrenergic, serotoninergic, and dopaminergic neurons, identified with the fluorescence histochemical technique, were present in the brainstem implants, in which they were often located in clusters reminiscent of their normal nuclear configurations. These clusters were located

among numerous nonmonoaminergic neurons. Acetylcholinesterase-positive neurons occurred in both the septal-diagonal band and the spinal cord implants. Although those implants contained recognizable neuron types, their general intrinsic architecture was not so well developed as that in the normal adult brain. In contrast, the cerebellar and hippocampal implants demonstrated striking cellular differentiation and internal organization.

The cytoarchitecture of cerebellar implants mimicked that of the normal adult. These implants developed a cortex composed of molecular, Purkinje cell, and granule cell layers. This cortical region was easily identified from the adjacent regions of the implant by its characteristic foliation. The hippocampal implants often had identifiable subdivisions, such as the dentate gyrus, regio inferior and superior, and a subicular complex. The dentate gyrus developed into its normal crescent shape and contained both a dorsal and a ventral blade of granule cells surrounding a hilar region containing CA4 pyramidal cells (Fig. 3). As in the normal dentate gyrus, the granule cell layer was about five to six cells thick and bordered on a distinct molecular layer of about one-third to one-half the normal thickness. The pyramidal cells were mainly aggregated in a band that could be traced along a curvature from the dentate hilar region to the subicular part of the implant. This hippocampal region had a partial trilaminar appearance, whereas the subicular complex was characterized by a wider band of more loosely arranged cells. Specimens stained for myelin and neurofibrils demonstrated the establishment of several of the normal axonal connections in the hippocampal implants, such as the mossy fiber system and an alveus-like formation of myelinated fibers connecting the CA3 and CA4 pyramidal cells with the CA1 implant neurons and the CA1 region of the host hippocampus.

The complex organization of the hippocampal implants demonstrates the ability of an isolated immature brain region to undergo a relatively normal development in its new location in the adult rat brain. The well-developed dentate gyrus, in particular, demonstrates that neurogenesis can continue within the embryonic tissue after transplantation, since the granule cells were just beginning to be formed at the stages we studied. Also, the implanted neurons and neuronal precursors were able to interact in a complex manner not only within the implant but also with the host brain in

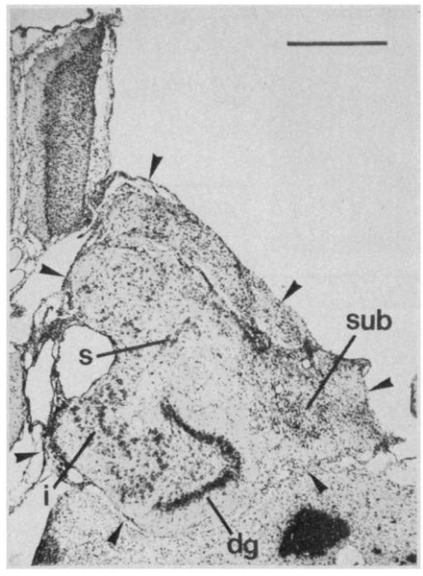

Fig. 3 Overview of a 4-month-old hippocampal implant (outlined by arrows) taken from an embryo 28 mm long. Survival time was 4 months. Although much smaller than normal, the implant contained a well-developed dentate gyrus (*dg*), hippocampal regio inferior (*i*) and superior (*s*), and a subicular region (*sub*). (Cresyl violet stain; bar equals 0.5 mm).

order to establish a normal cytoarchitecture and intrinsic and extrinsic axonal connections. These connections between the implant and adult host brain represented both an ingrowth of the embryonic implant fibers into the host and the growth of regenerating axons from the mature host CNS into the implant (*9*). Therefore, our observations indicate that the intracephalic implantation technique provides a unique system in which to study both development of embryonic neural tissue and regeneration in the adult mammalian CNS.

The intracephalic culturing technique has an advantage over both in vitro explant cultures and the intraocular transplantation technique in that the intracerebral implants are incorporated into the normal blood and cerebrospinal fluid circulation of the host brain. This creates an optimum environment for normal development and long-term survival of the implants. In addition, the preparation of a cavity within the host CNS provides a culturing chamber in which the implanted embryonic tissue can mature and grow without being restricted by the surrounding neural tissue of the host. The implants can be readily identified within the intracephalic cavity where they are available for anatomical, physiological, and biochemical studies.

Perhaps the most significant aspect of the intracephalic implantation technique is that it provides a positive environment in which to analyze factors regulating axonal regeneration in the adult mammalian CNS. The implanted embryonic tissue thus creates an environment in which regenerating axons from the adult CNS can interact with immature neurons and glia; conversely, the CNS tissue of the host animal permits axons from the embryonic neurons to interact with cells in the mature postsynaptic environment of the host. Therefore, the implantation technique provides an experimental tool that can be used to identify factors that regulate onset and cessation of axonal growth, guidance of the growing axons to their appropriate target neurons, and synaptogenesis with specific postsynaptic sites on the target neurons.

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References and Notes

1. M. K. Wolf and K. Merrill, *J. Cell Biol.* **22**, 259 (1964); S. M. Crain, *Neurophysiologic Studies in Tissue Culture* (Raven, New York, 1976); ——— and M. B. Bornstein, *Exp. Neurol.* **10**, 425 (1964); S. M. Crain and E. R. Peterson, *Ann. N.Y. Acad. Sci.* **228**, 6 (1974); W. Hild, *Z. Zellforsch.* **69**, 155 (1966); H. M. Sobkowicz, R. W. Guillery, M. B. Bornstein, *J. Comp. Neurol.* **132**, 365 (1968); A. Privat and M. J. Drian, *ibid.* **166**, 201 (1976); R. P. Bunge, in *Neuronal Recognition*, S. H. Barondes, Ed. (Chapman & Hall, London, 1976), p. 109.
2. R. M. May, *Arch. Anat. Microsc.* **26**, 433 (1930); *ibid.* **43**, 42 (1954); L. Olson and Å. Seiger, *Z. Zellforsch. Mikrosk. Anat.* **135**, 175 (1972); B. Hoffer, Å. Seiger, T. Ljungberg, L. Olson, *Brain Res.* **79**, 165 (1974); L. Olson, R. Freedman, Å. Seiger, B. Hoffer, *ibid.* **119**, 87 (1977); D. J. Woodward, Å. Seiger, L. Olson, B. Hoffer, *Exp. Neurol.* **57**, 984 (1977).
3. Interactions occur between the transplants and the host brain resulting in specific patterns of axonal outgrowth from the transplant into the iris. [Å. Seiger and L. Olson, *Cell Tissue Res.* **179**, 285 (1977); *Exp. Brain Res.* **29**, 15 (1977)]. In addition, peripheral nerve fibers located in the iris can innervate the transplants [Å. Seiger and L. Olson, *Cell Tissue Res.* **159**, 325 (1975); B. Hoffer, L. Olson, Å. Seiger, F. Bloom, *J. Neurobiol.* **6**, 565 (1975)].
4. G. P. Das and J. Altman, *Brain Res.* **38**, 233 (1972); R. D. Lund and S. D. Hauschka, *Science* **193**, 582 (1976). These studies demonstrate that the embryonic precursor neurons are capable of undergoing differentiation after transplantation and that they can attract growing axons from the adjacent regions of the neonatal host.
5. U. Stenevi, A. Björklund, N.-Aa. Svendgaard, *Brain Res.* **114**, 1 (1976).
6. H. M. Ralis, R. A. Beesley, Z. A. Ralis, *Techniques in Neurohistology* (Butterworth, London, 1973), pp. 24 and 82.
7. A. Björklund, B. Falck, Ch. Owman, in *Methods of Investigative and Diganostic Endocrinology*, vol. 1, *The Thyroid and Biogenic Amines*, J. E. Rall and I. J. Kopin, Eds. (North-Holland, Amsterdam, 1972), p. 318.
8. B. Holmstedt, *Acta Physiol. Scand.* **40**, 331 (1957); L. L. Butcher and L. Bilezikjian, *Eur. J. Pharmacol.* **34**, 115 (1975).
9. A. Björklund, U. Stenevi, N.-Aa. Svendgaard, *Nature (London)* **262**, 787 (1976); A. Björklund and U. Stenevi, *Cell Tissue Res.* **185**, 289 (1977); A. Björklund, L. F. Kromer, U. Stenevi, *Neurosci. Lett. Suppl.* **1**, S23 (1978); L. F. Kromer, A. Björklund, U. Stenevi, *ibid.*, p. S33.
10. We thank T. Sandros for her excellent technical assistance. Supported by grants from the Swedish MRC (04X-3874) and NIH (NS 05528-02).

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