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29. Owing to inhomogeneity of variance we used areas (instead of analysis of variance) as an index of the effect of 2DG over time. We determined least-error estimates of areas geometrically in a manner analogous to integration by the trapezoidal rule [M. C. Gemignani, *Calculus and Statistics* (Addison-Wesley, Reading, Mass., 1970), p. 169].

Area (mg/100 ml) (minutes)	
Decerebrates	Controls
1,200	1,620
4,905	3,405
4,545	6,165
6,975	8,460
7,965	10,440
9,045	10,920
9,465	12,585
11,220	12,600
14,925	16,095
*	20,220
	23,525

*120-minute sample broken in centrifugation

30. S. Siegel, *Nonparametric Statistics* (McGraw-Hill, New York, 1956), p. 116.
31. On the basis of our observations alone, we cannot determine whether glucoprivation is detected by receptors in the viscera, caudal brainstem, or both. Recent findings appear to rule out an essential contribution of peripheral receptors to the sympathoadrenal reflex [E. M. Stricker, N. Rowland, C. F. Saller, M. I. Friedman, *Science* **196**, 79 (1977)]. Adrenal catecholamine output during insulin-induced hypoglycemia was diminished by the intravenous injections of mannose or β-hydroxybutyrate, which can serve as alternate metabolic substrates for the brain. Injections of fructose, however, which does not cross the blood-brain barrier, were not effective. It appears, therefore, that if the necessary receptors for the stimulation of this reflex are contained within the central nervous system, they must reside in the caudal brainstem between the high mesencephalic transections in our subjects and the cervical cord transections used by others.
32. Supported by PHS awards Obesity Center AM-17624 to T. B. Van Itallie, AM-21397 to H.J.G., NS10150 to R. Norgren, and BMS 75018067 to Rockefeller University. A portion of this work was done at Rockefeller University where H.J.G. was a postdoctoral fellow. We thank F. X. Pi-Sunyer, for his helpful advice and the use of his glucose analyzer, and T. B. Van Itallie, R. Norgren, A. N. Epstein, S. Hashim, F. X. Pi-Sunyer, R. Bernstein, E. Coons, S. Gale, and M. L. Grundleger for their critical readings of the manuscript. We also thank R. Feller and E. Farabelli for typing the manuscript.

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Structure of Physiologically Identified X and Y Cells in the Cat's Lateral Geniculate Nucleus

Abstract. *Horseradish peroxidase injected into 18 single, physiologically identified geniculate X and Y cells permitted a detailed morphological correlate to be determined for the physiological properties of each neuron. Class 1 morphological characteristics were associated with Y cells, class 3 with X cells, and class 2 structural traits were seen in both physiological types.*

One of the major goals of neuroscience is to identify the structural basis of function at the single cell level. Recently, this has been approached in many neural loci by intracellularly injecting dyes, such as fluorescent markers or horseradish peroxidase (HRP), into physiologically identified cells (*1*). The HRP seems to diffuse throughout the cell into the finest processes, and this permits a detailed morphological view of the cell comparable to that possible with Golgi impregnation (Fig. 1).

We have begun to use this technique to relate physiological and morphological classes in the laminated portion of the cat's lateral geniculate nucleus. Previous morphological studies of this nucleus, based mostly on Golgi impregnation of cells, have identified three main classes of cells found throughout the laminae (*2*): class 1 cells are characterized by the largest somata and thick, fairly straight dendrites with occasional spinelike appendages; class 2 cells, by intermediate soma sizes, fine and somewhat curved dendrites, and frequent clusters of specializations appended at or near dendritic branch points; and class 3 cells, by small somata, very fine, wavy, and tortuous dendrites, and frequent clusters of complex stalked appendages along these dendrites (*3*). Physiological studies have identified the following main cell types distributed in all laminae (*3-5*): X cells are relay cells with slowly conducting retinal afferents and slowly conducting axons to cortex, fairly linear spatial summation, small receptive fields, and often tonic responses to stimuli of appropriate standing contrast; Y cells are relay cells with fast-conducting retinal afferents and fast-conducting axons to cortex, nonlinear spatial summation, relatively large receptive fields, and usually phasic responses; the rare interneurons (*6*) have not been extensively studied but many have receptive field properties similar to the X and Y cells that project axons to the visual cortex (*7*). Our preliminary data suggest that most Y cells are of class 1 and that the morphology of most X cells is intermediate between classes 2 and 3 (*8*).

We collected data from normal adult cats. The physiological preparation, anesthesia, and recording techniques were identical to those described extensively in previous reports (*5, 9*), with minor exceptions noted here. We used fine micropipettes, filled with 2 to 3 percent HRP (Sigma type VI) in 0.2*M* KCl buffered with 0.05*M* tris at *p*H 8.6. The tips were beveled to a size < 0.5 μm and an impedance at 200 Hz of 100 to 200 megohms. In most experiments, it was necessary to remove a portion of the overlying cortex 5 mm in diameter and 4 to 8 mm deep to reach the lateral geniculate nucleus with these electrode tips intact (*10*), but occasionally tips reached the nucleus successfully without cortical extirpation (*11*). The electrode was inserted into the brain through a hydraulically sealed chamber.

Once a geniculate cell was isolated extracellularly, its physiological properties, including latency to optic chiasm stimulation and receptive field characteristics, were studied; the neuron was duly identified as an X or Y cell (*12*). If the cell was clearly identified as one of these types, we advanced the electrode in 1-μm steps until it impaled the neuron. Figure 2A illustrates some of the criteria for intracellular recording, which include

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(i) a rapid, 30- to 65-mV drop in the d-c potential, (ii) an increase in the action potential amplitude to 25 to 70 mV, (iii) often a change in the action potential waveform to monophasic positive with a slight afterhyperpolarization, (iv) the appearance of slow potentials, and (v) a return to the original baseline d-c level upon withdrawal of the electrode. We reinvestigated neuronal properties intracellularly to verify that we impaled the same neuron from which extracellular data were obtained. The cell was then iontophoretically filled with HRP by administering 200 msec, square-wave depolarizing pulses of 5 to 10 nA at 4 Hz for 5 to 10 minutes. In many cases, fairly normal neural activity was still evident upon cessation of iontophoresis (Fig. 2A). We then withdrew the electrode and repositioned it for a new track. The cats were maintained for 2 to 24 hours after the intracellular HRP injection. They were then deeply anesthetized and perfused intracardially with buffered 1 percent gluteraldehyde and 1 percent paraformaldehyde. The brains were stereotaxically removed, sectioned coronally at 120 μm, and reacted with diaminobenzidine (*13*). Some sections were counterstained with cresylecht violet. Each HRP-filled cell was located in the tissue and related to its functional properties by means of Sanderson's retinotopic maps of the lateral geniculate nucleus (*14*) and a determination of laminar location based on ocular dominance. In any one lamina, no more than two cells were injected, and they were spaced $>$ 1 mm apart.

Figure 1 indicates the type of morphological detail seen with these methods and represents an X cell with class 3 morphology. Figure 2D is a line drawing of the same cell, and Fig. 2, B and C, represents a Y cell with class 1 morphology. The records in Fig. 2A are taken from this Y cell. Its axon could be followed into the optic radiation, and en route it issued a collateral branch into the perigeniculate nucleus just dorsal to lamina A (Fig. 2B). Such branching was seen in six of ten Y cells, including one which had class 2 morphology. This may be the anatomical basis of physiological evidence for collateral branching of Y cell terminals into the perigeniculate nucleus (*7*).

To date, eight X cells and ten Y cells have been studied both electrophysiologically and morphologically. The X cells were either completely class 3 in morphology (one cell) or had structure intermediate between classes 2 and 3 (seven cells). These latter cells included the entire morphological spectrum of nearly total class 3 characteristics through nearly pure class 2 structure (*8*). Of the ten Y cells, six had unambiguous class 1 morphology, two were of class 2 (*15*), and the final two had different morphology (*16*). Each of the Y cells had an axon that could be traced for at least several hundred micrometers. Although X and Y cells shared class 2 characteristics, especially in the appearance of grapelike clusters at dendritic branch points, the Y cells had larger somata and

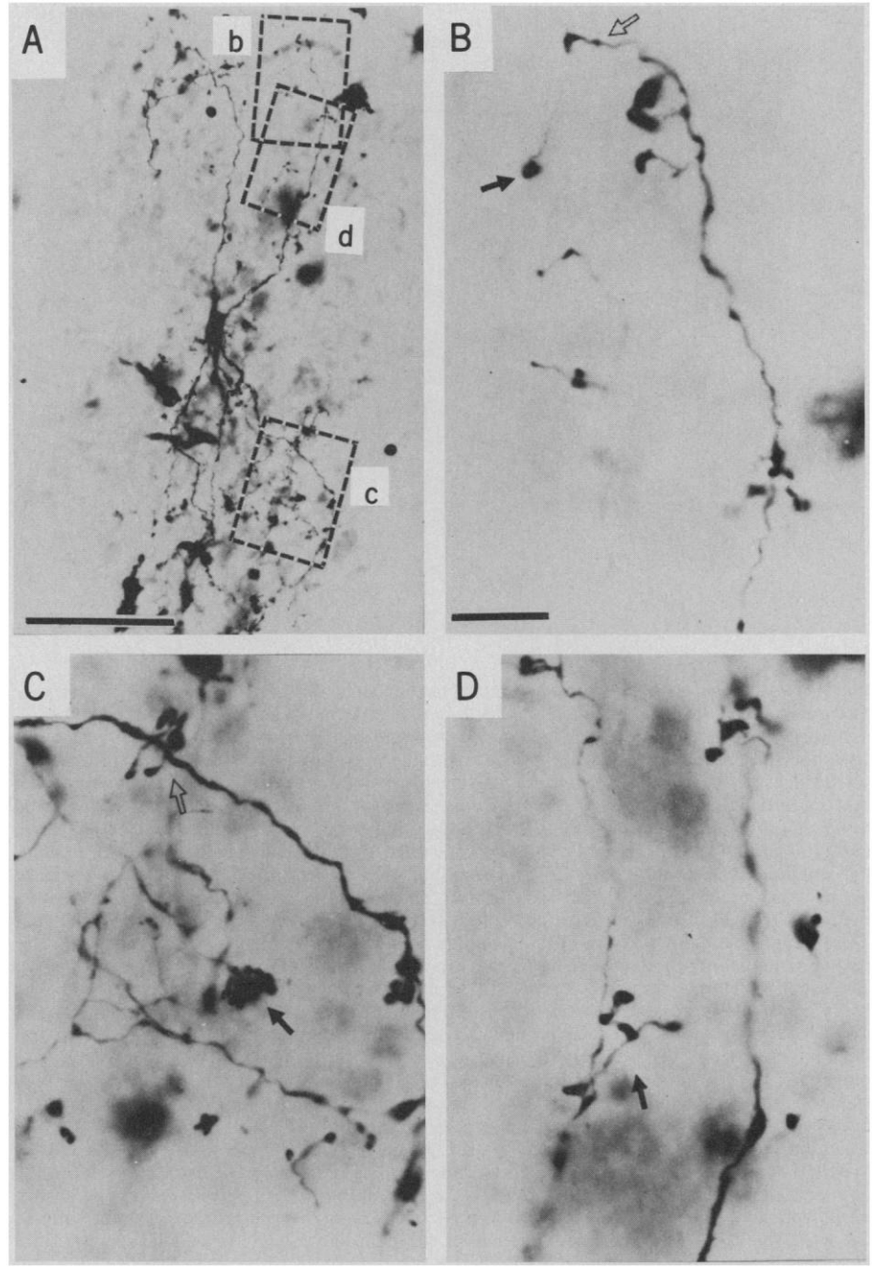

Fig. 1. Photomicrographs of HRP-filled X cell from lamina A_1; sections were counterstained with cresylecht violet and photographed through a filter (Kodak Wratten 48A) to enhance contrast. This cell, also illustrated in Fig. 2D, has class 3 morphology. (A) Low-power view of cell; scale, 100 μm. Many of the processes are out of the plane of focus and are thus not visible. The rectangles *b*, *c*, and *d* refer to the higher-power views in (B) to (D), respectively. (B) Higher-power view of dendrites and some appendages; scale, 10 μm and applies as well to (C) and (D). The filled arrow points to a specialization appended to the dendrite by a fine shaft (partly out of focus), which connects with the dendrite at the slight thickening indicated by the open arrow. This specialization is designated by the upper, filled arrow in the line drawing of Fig. 2D. (C) View of dendrites and complex appendages. The filled arrow points to a dense cluster of spheroid specializations (also indicated by the lower, filled arrow in Fig. 2D), and the open arrow, to less dense, ovoid specializations. The fine network of processes, which weave in and out of the plane of focus, can all be traced in continuity with the soma. (D) View of dendritic processes and appendages. The arrow indicates ovoid specializations appended to the dendrite by fine stalks. This same cluster is designated in Fig. 2D by the middle, open arrow.

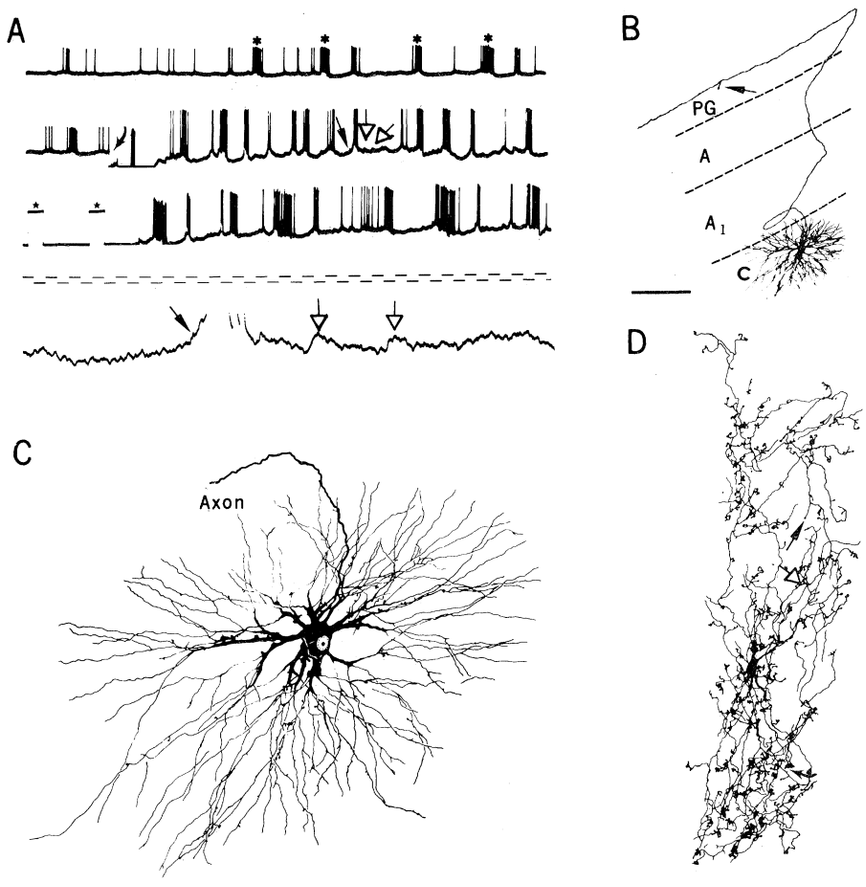

Fig. 2. Typical electrophysiological and morphological data. The line drawings in (B) to (D) are tracings at 1000× made with the aid of a drawing tube attached to a microscope with a 100× oil-immersion objective. For these drawings, we used a Kodak Wratten 48A or 49B (blue) filter to enable us to see the finest processes possible with our optical system. These filters were chosen on the basis of their complementary spectral density with respect to the HRP-diaminobenzidine reaction product. All drawings are from coronal sections. (A) Electrophysiological records from the Y cell illustrated in (B) and (C). The top trace illustrates recording as the electrode came close to and perhaps touched the cell membrane (quasi-intracellular record). The action potential amplitude was about 18 mV in this condition, whereas just prior to this, during standard extracellular recording, it was about $\leq$ 2 mV. There was a slow 10 mV drop in the resting potential as the electrode was advanced. The asterisks indicate responses to a flashing stimulus. The second trace shows actual intracellular recording as the electrode impaled the cell. The resting potential appeared as a rapid 55 mV drop in the d-c level (indicated by curved arrow). The oscilliscope trace dropped off scale at this point and had to be manually repositioned. The spike amplitude increased to > 50 mV. Both spontaneous subthreshold slow potentials and those leading to a burst of action potentials were seen in the intracellular records (open and filled straight arrows, respectively, in this trace). In the third trace, the last two iontophoretic pulses for HRP injection (stars) are shown. These pulses swamped the amplifier. The subsequent recording on line 3 records the cell's activity after 5 minutes of HRP injection. A slight decrease ($\simeq$ 5 mV) in resting potential was seen after HRP iontophoresis. The fourth line is a calibration pulse (5 mV and 100 msec for lines 1 to 3, and 1.5 mV and 5 msec for line 5). Line 5 is a higher-gain, faster-sweep recording taken from line 2. The filled and open arrows refer to the corresponding points in line 2. The noise in the recording is due largely to our use of an f-m adapter on an a-m tape recorder to store our d-c records; thus these examples do not indicate the actual quality of the recording. Nonetheless, the main elements of intracellular recording are clear. (B) Lower-power tracing of HRP-filled Y cell with class 1 morphology; scale, 200 μm. Geniculate laminae A, A_1, and C are indicated, as is the perigeniculate nucleus (*PG*). The soma lies in lamina C as shown, but dendrites extend into the ventral portion of lamina A_1. After a curious loop in lamina A_1, the axon was followed vertically to the perigeniculate, at which point it turns abruptly to the left (laterally). At least one fine collateral was seen in the perigeniculate (arrow). (C) Higher-power tracing of soma and dendrites of same cell as shown in (B); scale is 50 μm and applies as well to (D). Class 1 morphology is indicated by the large, spherical soma, cruciate appearance of the dendritic tree which crosses laminar borders, and spinelike dendritic appendages (*2*). (D) Tracing of HRP-filled X cell with class 3 morphology. Antidromic activation of this cell was not possible (*11*). Since it was the only unambiguous class 3 cell in our sample, we cannot exclude the possibility that it is an interneuron with monosynaptic input from the optic tract and with X cell receptive field properties (*7*). The soma was found near the center of lamina A_1, and the dorsal and ventral limits of the dendritic tree occur at the borders of this lamina. The arrows indicate structures illustrated by photomicrographs in Fig. 1. Class 3 morphology is indicated by a small soma, fine, sinuous dendrites with complex appendages, and a dendritic tree that remains within a lamina (*2*).

thicker primary dendrites than any of the X cells.

These data are not in complete accord with previous proposals for relationships between structure and function among cat geniculate cells. In this regard, the preliminary nature of our data, as well as the more indirect and circumstantial evidence of earlier approaches must be emphasized. Many [see, for example (*2*, *17*, *18*)] have suggested that cells with class 3 morphology are probably interneurons. Since we were unable to activate our only pure class 3 cell antidromically from the cortex, this point cannot yet be resolved. However, five of our X cells were identified as relay cells because of antidromic activation from the cortex, and these all displayed some class 3 morphological characteristics. Therefore, some cells with at least partial class 3 morphology are indeed relay cells. This conclusion does not preclude the possibility that these cells also participate as presynaptic elements within the lateral geniculate nucleus (*17*). That is, some X cells may be relay cells that also participate in local geniculate circuits. Among the ten Y cells, five were antidromically activated from cortex, and all displayed an axon that we traced well into the optic radiation.

To date, the most specific structural-functional relationships in the cat's lateral geniculate nucleus were suggested by LeVay and Ferster (*18*). They proposed that morphological class 1 cells are Y cells, class 2 cells are X cells, and class 3 cells are interneurons. They did not, however, consider intermediate or unclassified morphological types (*8*). This proposal sprang from a number of converging lines of evidence, including the observation that only class 1 cells seem to project axons to cortical area 18, whereas cells of both classes 1 and 2 project axons to cortical area 17; it had previously been shown that only Y cells project to area 18, whereas both X and Y cells project to area 17 (*19*). Our preliminary evidence suggests a slightly different structural-functional correlation. That is, class 1 cells are Y cells, and cells with at least some class 3 characteristics are X cells. The major class 2 characteristic—grapelike clusters at dendritic branch points—can be shared by X and Y cells, but the Y cells have larger somata and conspicuously thicker primary dendrites than the X cells. Other morphological types (*16*) may also occur.

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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.
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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.
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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.
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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-

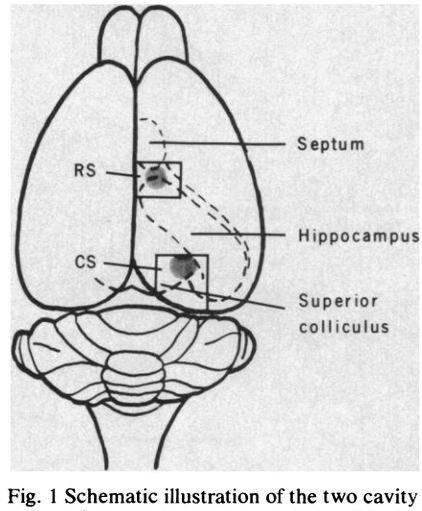

Fig. 1 Schematic illustration of the two cavity preparations used. In the rostral site (*RS*) the fimbria and septal end of the hippocampus were removed by suction and the implanted tissue (shaded area) is placed on the vessel-rich covering of the anterior thalamus. For the caudal site (*CS*) the neocortex 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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