over, with the new method, the pattern of the grid on the retina is sharper, since diffraction blurs the image of the lines along their length rather than their width.

7. For this purpose we construct two sets of polynomials that are orthogonal over the two (four- and five-line) integral sampling grids. We find a least-squares fit for the two derivative surfaces given by the $x$ and $y$ coordinate locations of the grid intersections of the subject's drawings and correct the derived coefficients for displacements of the axes of the grid relative to the center of the pupil.
8. Our subjects included approximately equal numbers of males and females with an average age of 25 ± 8 (standard deviation) years and no obvious visual pathology.
9. Circular, orthogonal Zerninke polynomials were computed from our measured Taylor coefficients [M. Born and E. Wolf, *Principles of Optics* (Pergamon, Oxford, ed. 5, 1975)]. The root-mean-square deviation of the wave aberration surface from a perfect spherocylinder (computed from the Zerninke coefficients) is useful in the image evaluation of small aberrations. Here it is convenient for ordering the nine-dimensional high-order aberration wave surfaces according to severity.
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## Transplanted Neural Tissue Develops Connections with Host Rat Brain

Abstract. *Superior collicular fragments transplanted from fetal to newborn rat brains develop complex internal organization and receive visual afferents from the host providing they lie sufficiently close to the host visual pathways. This system allows investigation in vivo of special affinities between cells of the mammalian central nervous system.*

The vertebrate nervous system exhibits predictable patterns of interconnections that persist when cut axons regenerate in vivo (*1*) and, in some cases, even when neuronal tissues are cultivated in vitro (*2*). To explain the origin of this predictability, Sperry (*3*) proposed that neurons acquire unique cytochemical labels whose selective matching leads to the formation of ordered connections between different brain regions. This general hypothesis is supported by cell aggregation experiments that demonstrate region-specific affinities between neuronal cells (*4*), but these studies are still several steps removed from explaining the development of ordered synaptic connections. Furthermore, the interpretation of any in vitro experiment is clouded by the observation that neurons are capable of substantial modification and reorganization of connections. For example, in fish, amphibia, and developing mammals, removal of afferents to certain regions often causes major reorganization of other inputs such that they invade and form functional synapses in the deprived region; conversely, removal of the target area of a growing axon population may cause these axons to innervate adjacent regions (*5*, *6*). At the level of individual neurons, such results suggest that identifying cytochemical labels are not irreversibly determined, or that factors other than special affinities between axons and their postsynaptic cells play a role in the development of ordered neuronal connections.

The present study demonstrates a way in which these problems may be systematically investigated in the developing mammalian nervous system and addresses the specific question of whether neuronal populations are automatically innervated by a group of axons if placed in the terminal field of those axons. The approach entails transplantation of tissue fragments from the superior colliculus of fetal rats into the superior colliculus region of newborn rats and the subsequent demonstration of synaptic connections between host and transplanted tissues. Two features make this feasible: (i) afferent pathways to the superior colliculus of rats are still capable of growth through lesions or of modified growth in response to specific surgical manipulation at birth (*5*); and (ii) newborn rats have not developed immunological competence and so will not reject the transplants (*7*). A similar approach involving transplantation of regions of fetal rat brain into the cerebellum of rats after birth was reported by Das (*8*) but, while demonstrating transplant survival, he did not investigate the formation of connections between transplant and host in that study.

Pregnant rats were injected with [$^3$H]thymidine on fetal day 13 or 14, the days when terminal cell division in the colliculus is most frequent. On day 15 or 16, fetuses were removed and the tectum separated from the rest of the brain in

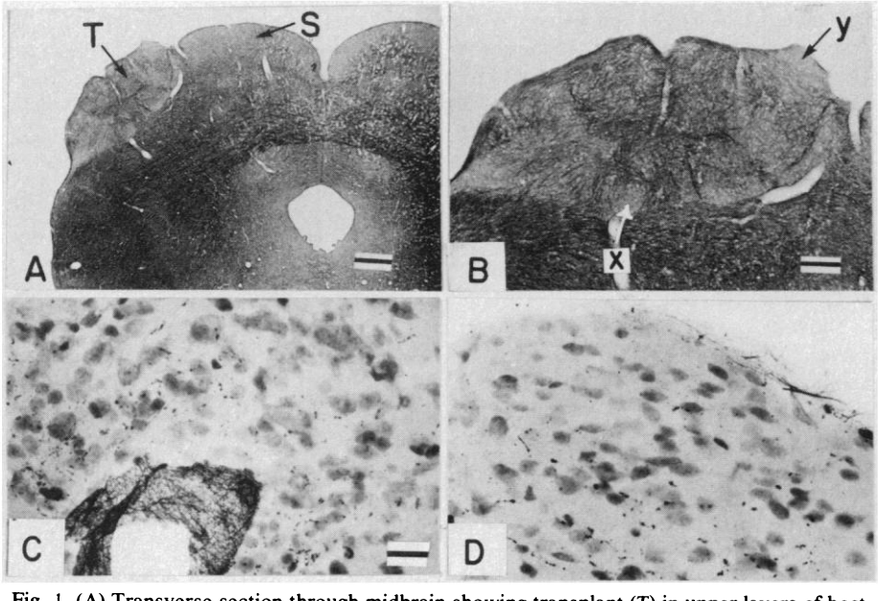


Fig. 1. (A) Transverse section through midbrain showing transplant (*T*) in upper layers of host superior colliculus (*S*); neurofibrillar stain; scale bar, 450 μm. (B) Detail of transplant in (A). Adjacent Fink-stained sections show an optic projection to the two areas ($x$ and $y$); scale bar, 200 μm. (C and D) Details from section adjacent to that in (A) and (B), stained by Fink-Heimer method and showing degenerating optic terminals in regions $x$ and $y$, respectively. No other part of this transplant showed terminal degeneration; scale bar, 20 μm.

Ham's F10 medium. The overlying mesodermal elements, including the pia mater, were dissected off and transverse strips of tectum approximately 0.2 mm wide and 1 mm long were cut. At day 15 the tectal plate is 0.2 mm thick and is not differentiable into the various layers characterizing the mature superior colliculus. Since visual afferents from both retina and visual cortex end mainly in the upper two layers at the adult colliculus (stratum zonale and stratum griseum superficiale) and inputs from a variety of nonvisual regions end in deeper laminae (*9*), the tissue taken for transplantation contains cells that will ultimately achieve a spectrum of different connections. Optic axons do not arrive at the superior colliculus until day 16 to 17 (*10*). The strips of tissue were sucked into a capillary tube with a tip diameter of about 0.2 mm by use of a 50-$\mu$l syringe. They were placed near the tip of the tube and together with about 3 $\mu$l of fluid were injected into the left superior colliculus through a slit made in the skin and skull of ether-anesthetized newborn rats. More than 90 percent of the animals survived for 4 to 6 weeks, when the right eye or the left visual cortex was removed under ether anesthesia. Four days after this surgery, animals were killed and brains were fixed in paraformaldehyde and cut as frozen sections. Every third section was processed by the Fink-Heimer method for degenerating axons resulting from the lesion, with a neurofibrillar stain for demonstrating fiber patterns, and by autoradiography to provide definite identification of the [$^3$H]thymidine-labeled transplant (*11*).

A few animals were killed 5 to 10 days after transplantation. At these survival times the transplants appear as aggregates of tightly packed cells. Cell death has occurred within transplants, as judged by the presence of autoradiographic grains not associated with cell nuclei, but the surviving cells look healthy and vascularization of the transplant by the host is well advanced even by day 5. By 1 month, the transplants show a complicated internal organization with localized differences in cell body size and packing density. In addition, they show an elaborate and intricate fiber organization (Fig. 1, A and B) consisting of fiber-dense zones surrounding fiber-sparse regions. While the formal lamination characteristic of the adult superior colliculus is not present, it is possible that various zones may represent fragments of laminae whose development has been disrupted by the transplantation procedure. Characteristic synapses can be identified with the electron microscope.

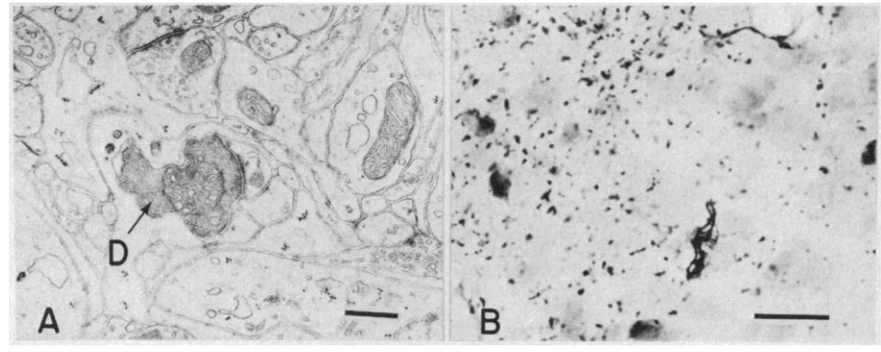


Fig. 2. Degeneration in transplant due to removal of the contralateral eye of the host. (A) Degenerating terminal (*D*) seen with electron microscope; scale bar, 0.5 $\mu$m. (B) Adjacent tissue stained with Fink-Heimer stain and showing degenerating axons and terminals; scale bar, 20 $\mu$m.

While we attempted to place the transplants within the most superficial layers at the front of the superior colliculus, they eventually came to lie in a variety of positions. Some replaced part of the superficial layers (Fig. 1A); others were found lateral to the superior colliculus, between superior and inferior colliculus, or on the inferior colliculus or cerebellum. A few were situated deep in the superior colliculus. No deep transplants received visual afferents, yet among the more superficially situated transplants, all but one of the 14 transplants lying close to or within the superior colliculus were innervated by the eye or visual cortex.

The pattern of innervation observed in the 13 innervated transplants shows a number of interesting features: (i) visual afferents never fill the whole transplant, even if this is lying in a position normally occupied by host colliculus (Fig. 1, B to D); instead, they occupy usually less than 25 percent of its volume, distributed in one or more patches; (ii) innervation is not necessarily to the zone immediately adjacent to the host optic fibers, nor is it necessarily at the surface of the transplant; (iii) the amount of innervation (judged by density of degeneration after lesions) does not appear to correlate with the closeness of the transplant to the host optic fibers; (iv) host axons do not necessarily run directly to the innervated region of the transplant, but often follow a circuitous course within the transplant to reach a zone of terminal distribution; (v) the region innervated nearly always appears pale on adjacent fiber preparations, much like the stratum griseum superficiale in intact animals; and (vi) tissue taken from the center of a transplant and processed for electron microscopy shows numerous degenerating axon terminals resulting from contralateral eye removal of the host (Fig. 2A), and light microscopy of the transplant area adjacent to that studied for electron microscopy shows typical degeneration (Fig. 2B). From these observations, it is apparent that distinct regions of the transplant receive innervation from host axons.

Considerable numbers of axons are seen in the fiber preparations running between host and transplant. Some of these are visual afferents, as shown above; some may be afferents from other sources, while others may be efferents from the transplant to the host. Evidence suggestive of the last possibility comes from animals in which the transplant directly apposes the host superior colliculus. In these animals a thick fiber bundle is often found between the transplant and the upper layers of the host colliculus. Within the stratum griseum superficiale the bundle breaks into several smaller bundles that run deep into the colliculus to be lost among the deep fiber layers or break up into a network of fine fibers. Given the way that these axon bundles cascade away from the transplant, they seem likely to be composed of efferent axons. Their pattern of termination is as yet unknown.

These studies show, as have those of Das (*8*), that fetal nervous tissue can be successfully transplanted to newborn rat brains and undergo maturation of a complex internal structure. This is perhaps not surprising since the host nervous system might be regarded as providing an ideal tissue culture medium. Das' transplants were all into the cerebellum on the premise that this tissue is still embryonic in newborn rats. The present study shows that even a relatively mature region such as the superior colliculus can still receive transplants. The significant feature of our work is that the transplants interact with the host tissue and that, at least with respect to the visual afferents, this interaction is not totally ran-

dom. While transplants are innervated by the host's visual afferents only if they lie sufficiently close to them, they are never totally innervated from the eye or visual cortex even if embedded in the tissue these afferents normally supply. As such they exhibit a form of specificity. It is tempting to suggest that those areas of the transplant that receive visual inputs correspond to the visual layers of normal animals and that specific affinities which some cells appear to demonstrate for visual afferents in intact tissue are preserved in the transplantation procedure. Conversely, those areas lacking innervation correspond to cell layers that would not normally be innervated by visual afferents.

The question arises whether the special affinities suggested by this work are still expressed if tissues are taken at progressively earlier ages or if they can be abolished by various manipulations in vitro before transplantation. In addition, it is not known whether tissues such as visual or somatosensory cortex also display any affinity for optic axons if placed close to their area of distribution. These questions are approachable with the present system since further studies have indicated that younger tectal tissue and tissue from various cortical areas exhibit interconnections with the host colliculus (*12*), and that such interconnections occur even if cortical or tectal tissue is maintained in vitro prior to transplantation. Ultimately, it should be possible to assess the extent to which specific connections between brain regions reflect special affinities between certain cell types (for instance, "visual" cells as opposed to "somatosensory" cells) and whether such affinities may be limited in their expression by the particular growth patterns of the fiber tracts interconnecting them.

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## Potential New Artificial Sweetener from Study of Structure-Taste Relationships

Abstract. *4-(Methoxymethyl)-1,4-cyclohexadiene-1-carboxaldehyde* syn-*oxime is a new sweetening agent developed by systematic synthesis and taste evaluation of 80 new oximes analogous to the little-used oxime sweetener, perillartine.*

Sweet tasting substances display such a diversity of chemical structure that it has been difficult to relate sweetness with structure in any unified theory, or design a new sweetener on a rational basis. Consequently, most sweetening agents were discovered by accident. All have imperfect properties as sugar substitutes, and new sweeteners are in demand. Aldoxime **44** (Fig. 1) is a new noncaloric sweetener that has 450 times (on the basis of weight) the sweetening power of sucrose, lacks the bitterness of saccharin, and has potential applicability for all sweetener uses (*1*). It was designed as an analog of perillartine **1** (*2, 3*), which has been used only as a sweetener for tobacco because of its very low water solubility, appreciable bitterness, and menthol-licorice off-tastes.

Perillartine was a useful starting point for a systematic study of taste-structure relationships (*4*). We assumed that the aldoxime moiety in **1** was the functional group responsible for sweetness. Then the rest of the molecule could be designated a "carrier" group, where changes in structure might be made. Perillartine has a high calculated potency of taste (*5, 6*), but because of its low solubility in water only a weak taste is actually attainable in solution. In designing and synthesizing new analogs (Fig. 1) our objective was to maintain high potency, increase the solubility in water, and minimize or eliminate nonsweet tastes (*7, 8*).

Both the aldoxime moiety and the $\alpha$, $\beta$-olefinic unsaturation [as in **2**, where X = H, but $R_\alpha \neq H$ (*4, 9*)] were found to be essential for sweetness. Rather drastic changes were permitted in the carrier group, such as removal of the side chain (**26**), change of ring size (**17**), or even cleavage of the ring (**4**) (*10*). Unpleasant phenolic medicinal off-tastes developed in **26**, **17**, and **4**, but appreciable sweetness and taste potency were retained. Complete loss of sweetness in **40** and **41** (ketoxime analogs of **4** and **26**) confirmed that an aldoxime is required. Need for the free oxime OH group was confirmed by the complete insolubility and tastelessness of the *O*-methyl oxime **5** of perillartine.

Fig. 1 (right). Chemical structures of oximes and their properties. Numbers under each structure are oxime solubilities (in molarity, *M*), taste potencies indicated by × (times sucrose), and ratios of the percentage of sweetness to that of bitterness estimated from the taste qualities (total 100 percent) observed for each compound. Taste screening data were single responses from four to six experienced panelists, on oxime solutions at one or two concentrations producing observed taste intensities from 0.2 to 2.0 (usually 0.5 to 1.5) relative to 0.25*M* sucrose as 1. On compounds of further interest, up to ten replications at three to four concentrations were obtained. Maximum water solubilities are given in molarity at 25°C. The potency times sucrose on a mole/mole basis = [observed intensity of oxime solution/molarity of oxime] ÷ [observed intensity of sucrose reference solution/molarity of sucrose]. The ratios listed = (percent of taste identified as sweet)/(percent identified as bitter). Other tastes = 100 percent − (sweet + bitter). There was no sourness (except for **6**) or saltiness; but menthol, anise, licorice, coconut, and mint qualities were common. Fruit-berry tastes were noted on **11** and **44**. Unpleasant off-tastes were variously described as chemical, oily, phenolic, medicinal, or peppery on **3**, **4**, **8**, **9**, **13**, **17**, **18**, **25**, **26**, **27**, **34**, **38**, **39**, **46**, **47**, **49**, and **51**. Although not predominantly sweet, **3** and **27** are listed with sweet compounds for comparison. One-time responses on sodium saccharin and calcium cyclamate gave potencies of 185 and 30, and ratios of percent sweet to the percent bitter were 93/7 and 80/0.