Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 6958–6960, October 1987
Neurobiology

Retinal transplants can drive a pupillary reflex in host rat brains

(olivary pretectal nucleus/neural specificity)

HENRY KLASSEN AND RAYMOND D. LUND*

Department of Neurobiology, Anatomy and Cell Science, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261

Communicated by James M. Sprague, June 22, 1987

ABSTRACT **Retinae taken from embryonic rats were transplanted over the midbrain of neonatal rats, from whom one eye had been removed. After 5 months, the optic nerve of the remaining eye was cut, and the transplant was exposed. Illumination of the transplant caused pupilloconstriction of the host eye, a response abolished by damaging the transplant. Thus neural transplants are capable of driving specific reflexes in response to natural stimuli.**

Various studies have shown behavioral recovery following transplantation of embryonic tissue to the brains of mammals with neurological deficits resulting from specific lesions or genetic disorders (1). In all these cases, the essential function of the transplant seems to be to produce an appropriate chemical in approximately the correct location. The donor cells need not be the normal ones innervating the region: adrenal medulla, for example, may serve as an alternative source to substantia nigra of dopamine-producing cells (2). The connections made between transplanted cells and host brain also need not be normal for functional recovery to occur (14).

A second role for neural transplants that has so far received much less attention is to mediate functions that require precise patterns of connections as their substrate. One such system is the pupillary reflex. In intact animals, this is mediated through a subdivision of the pretectum, the olivary pretectal nucleus (4, 5). This receives optic input and projects directly or indirectly to the Edinger–Westphal nucleus of the oculomotor complex, which in turn innervates the eye through the ciliary ganglion (refs. 6–10; Fig. 1*A*). The reflex is abolished by destruction of the pretectal nuclear complex (11), and specific stimulation of tonic "on" cell clusters in the olivary pretectal nucleus leads to pupilloconstriction (4). In this study we have examined whether a reflex response can be elicited in the eye of a host rat by illuminating a retinal transplant placed over the brain stem.

METHODS

Retinae were dissected from rat embryos of 13 days of gestation and transplanted over the superior colliculus of neonatal rats, as described (12). Effort was made to place the transplants either just caudal to the pretectum or more caudally such that they would ultimately come to lie over the cerebellum and be readily accessible for experimentation. The right eye was removed at the time of transplantation to ensure a heavier innervation of the subcortical visual centers by the transplant (12). After 5 months, the optic nerve of the remaining eye was sectioned intracranially to prevent relay of visual information to the brain from that eye but to maintain intact the parasympathetic outflow to the eye carried in the oculomotor nerve. Two days later, the transplant was exposed either by drilling off the bone over the cerebellum (posterior location) or by removing the bone, occipital cortex, and hippocampus on both sides to expose the midbrain (anterior location). The experimental preparation is shown schematically in Fig. 1 *B* and *C*. A set of normal animals served as controls. Their right eye was removed, their left optic nerve was sectioned, and then their midbrain was exposed as for the anteriorly located transplants. The efficacy of optic nerve section was confirmed by ensuring that the host eye failed to pupilloconstrict on illumination and by direct examination both during surgery and at the time of fixation. Horseradish peroxidase (HRP) was injected into the orbit of controls to ensure that the oculomotor nerve had been spared. The pupil was examined with a surgical microscope (Wild M650) under normal illumination conditions. At the end of each experiment, animals were fixed by perfusion with 4% (wt/vol) paraformaldehyde or 1% gluteraldehyde/1% paraformaldehyde (HRP experiments). The brains were stained with Nissl and a silver stain for normal axons or with HRP histochemistry using tetramethylbenzidine as a chromagen. Animals were anesthetized with ether for neonatal surgery and subsequently with Nembutal or tribromoethanol. No significant difference was noted in pupillary response with the two anesthetics.

RESULTS

Six rats that had received transplants at birth and optic nerve cuts at maturity form the basis of this study, with four controls that had received no transplants. Optic nerve section prior to testing generally resulted in a pupil of ≈3.5 mm in diameter that was totally unresponsive to light. The diameter was unaffected by the surgery necessary to expose the transplant.

In all six animals with transplants, the pupil in the remaining host eye constricted when the transplant was exposed to light (Figs. 1*C* and 2*B*) and dilated when it was covered (Figs. 1*B* and 2*A*). In most animals, constriction began after 5 sec: in one it followed a small dilation that occurred predictably after 1 sec of light exposure. The greatest change in pupil diameter was from 1.5 to 4 mm and the least from 0.5 to 0.9 mm. The smallest diameter was achieved between 4 and 13 sec after initial light exposure. Dilation after covering the transplant (Figs. 1*B* and 2*A*) was somewhat slower, beginning after 1–5 sec and taking 10–60 sec to reach completion. While there was some variability in response times and magnitude of response among animals, these were constant for each individual when tested over a period of time. Indeed the behavior was extremely robust and was still brisk after 5 hr of testing. The constriction effect was not diminished by placing a heat filter between the light source and transplant, but the degree of constriction could be reduced by placing barrier filters in between. In cases in which the transplant could be visually localized, it was selectively masked leaving the rest of the brain exposed to light: this resulted in

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: HRP, horseradish peroxidase.

*To whom correspondence should be addressed.

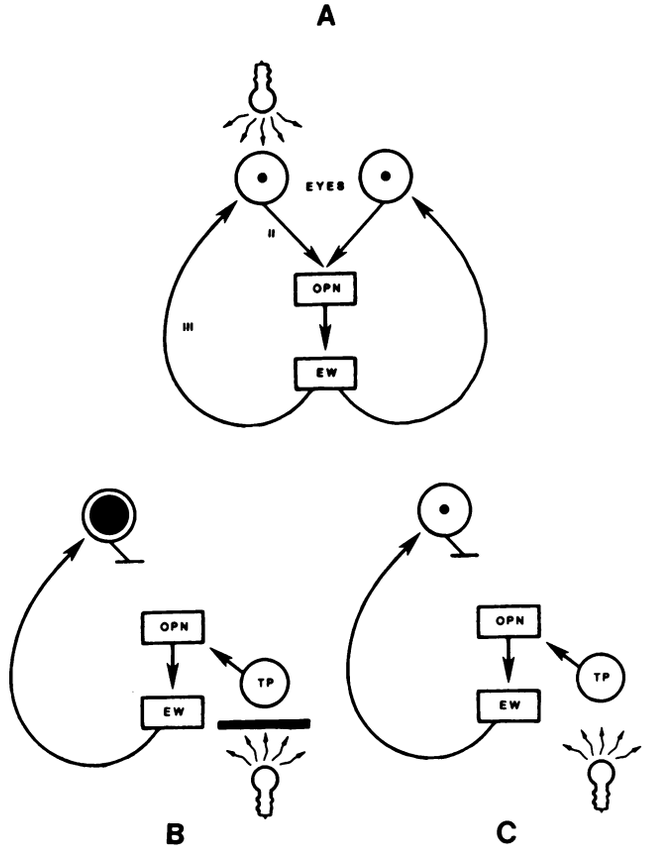

FIG. 1. Schematic of pathway for pupilloconstriction. In normal rats (*A*), light activates neurons that pass from the eye by way of the optic nerve (II) to the olivary pretectal nuclei (OPN) on each side. From there, axons project to the Edinger–Westphal (EW) nuclei. These project by way of the oculomotor (III) nerve through the ciliary ganglion to the pupilloconstrictor muscles. (*B*) Experimental preparation used here with the transplant (TP) screened from light and with the pupil dilated. (*C*) Effect of illuminating the transplant.

pupillodilation. Thus, it would appear that the illumination of the transplant is the essential stimulus for pupilloconstriction of the host eye and that neither heat effects nor incidental stimulation of host brain structures are confounding factors. In two animals, the transplant was lying over the cerebellum, and during testing it was removed without involvement of host brain structures. In both cases, the host pupillary reflex to visual stimulation of the transplant immediately disappeared and did not return. The control animals without transplants showed no indication of pupillary change in response to light.

Histological examination of all six transplanted animals showed healthy transplants lying over the superior colliculus or (in the case of the two lesioned animals) small fragments of retina over the cerebellum. All showed bundles of axons emanating from the transplant and running across the surface of the host midbrain. While it has not been possible to trace the projection of the transplants in detail, previous studies (12) and current experiments involving injection of HRP into similarly placed transplants showed projections to the olivary pretectal nucleus as well as to the superior colliculus, posterior pretectum, lateral geniculate nucleus, and accessory optic nuclei. Examination of the brains of the control rats showed that the exposure of the midbrain was similar to that performed in the experimental animals and that the oculomotor nucleus was labeled with HRP, indicating that failure of response was the result of neither a difficult exposure nor inadvertent damage to the oculomotor nerve.

It appears from these results that retinal transplants placed over the brain stem are capable of driving a reflex pathway in the host rat. The response is somewhat more sluggish than in normal rats. In normal rats (3), the pupil starts to constrict 400 msec or less after stimulus onset rather than the 1 sec or more found here and reaches a minimum diameter after 1 sec rather than between 4 and 13 sec observed in our animals. Dilation is a slower process in normal animals, taking 30 sec or more to reach a stable point, and this was also the case for the transplanted animals. The transient dilation before constriction seen in one animal may reflect an anomalous pattern of connections in the olivary pretectal nucleus, but the possibility of incidental stimulation of the sympathetic system, although unlikely, cannot be disregarded.

Studies have emphasized (4, 5) the role of the pretectum, and in particular a specific cell class in the olivary pretectal nucleus, in the normal animal as the center for pupilloconstriction. While we do not know whether the reflex demonstrated here is also mediated through the olivary

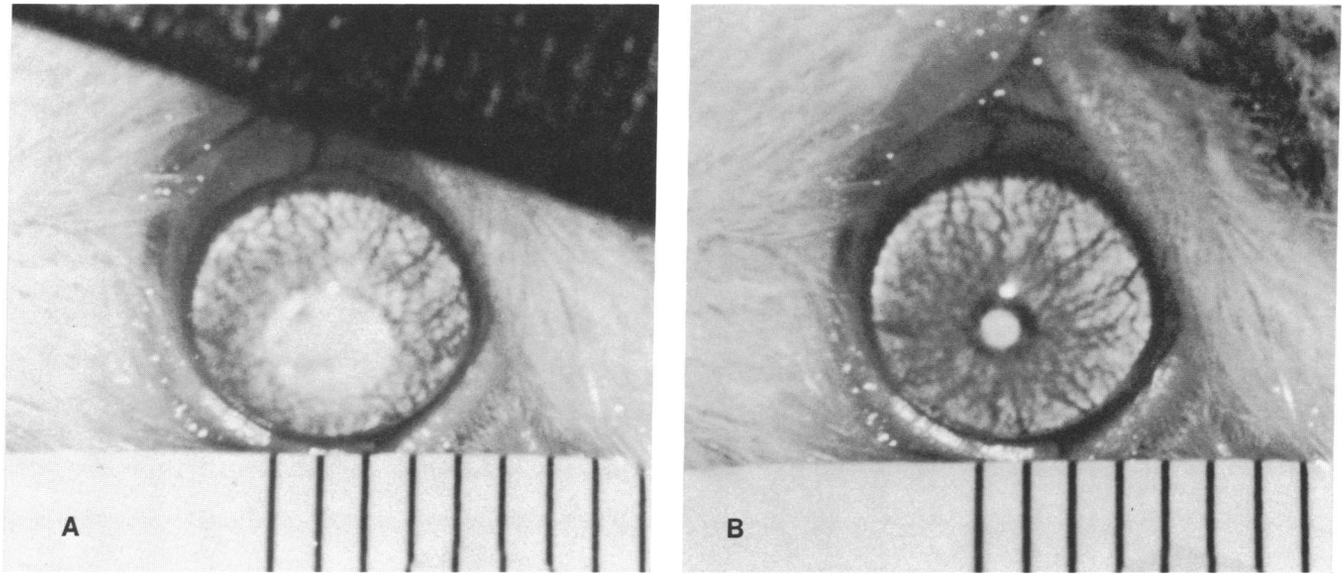

FIG. 2. Photographs of eye under conditions shown in Fig. 1*B* (*A*) and of eye under conditions shown in Fig. 1*C* (*B*) with the transplant exposed. Scale is marked in millimeters.

pretectal nucleus, there are a number of reasons to suspect that this might be the case. Retinal transplants placed on the dorsal midbrain project to the same regions in the host brain as a normal retina (12). The only region to which they project that normally subserves a pupillary reflex function is the olivary pretectal nucleus; and this is the only region in which retinal axons terminate that has a projection to the Edinger–Westphal nucleus (10), the next relay in the reflex. There is little evidence to support the possibility that by altering an input to a region the output can be substantially modified, and indeed there are studies in developing rodents showing this does not happen (13). Thus it would be unlikely that transplant input would modify the output projections of other subcortical visual centers. It appears likely, therefore, that the transplants are driving the reflex through the normal pathways.

This study shows that besides modulating complex behavior patterns, neural transplants can also drive simple reflex pathways in response to natural stimuli. This observation is important in examination of the possibility that transplantation may be an effective approach to recovering damaged neural circuitry. The present preparation is also of value in providing a simple functional assay for studying the efficiency of connectivity underlying a reflex circuit, and how this can be modified by altering various parameters of transplant innervation.

The authors thank Dr. T. Takimori for assistance in development of the preparation, Mary Houston and Fran Shagas for technical assistance, and Sharon Wesolowski for secretarial help. The research was supported by Grant EY05283 from the National Institutes of Health. H.K. is a Mellon Fellow.

1. Bjorklund, A. & Stenevi, U., eds. (1985) *Neural Grafting in the Mammalian CNS* (Elsevier, Amsterdam).
2. Freed, W. J. (1983) *Biol. Psychiatry* **18,** 1205–1267.
3. Trejo, L. J. & Cicerone, C. M. (1982) *Vision Res.* **22,** 1163–1171.
4. Trejo, L. J. & Cicerone, C. M. (1984) *Brain Res.* **300,** 49–62.
5. Clarke, R. J. & Ikeda, H. (1985) *Exp. Brain Res.* **57,** 224–232.
6. Benevento, L. A., Rezak, M. & Santos-Anderson, R. (1977) *Brain Res.* **127,** 197–218.
7. Hultborn, H., Mori, K. & Tsukahara, N. (1978) *Brain Res.* **159,** 255–267.
8. Steiger, H.-J. & Büttner-Ennever, J. (1979) *Brain Res.* **160,** 1–15.
9. Weber, J. T. & Harting, J. K. (1980) *Brain Res.* **194,** 1–28.
10. Breen, L. A., Burde, R. M. & Loewy, A. D. (1983) *Brain Res.* **261,** 303–306.
11. Legg, C. R. (1975) *Neuropsychology* **13,** 373–376.
12. McLoon, S. C. & Lund, R. D. (1980) *Exp. Brain Res.* **40,** 273–282.
13. Frost, D. O. & Metin, C. (1985) *Nature (London)* **317,** 162–164.
14. Freund, T. F., Bolan, J. P., Björklund, A., Dunnett, S. B. & Smith, A. D. (1985) in *Neural Grafting in the Mammalian CNS*, eds. Bjorklund, A. & Stenevi, U. (Elsevier, Amsterdam).