

tion by ICSH because testes were maximally stimulated by saturating levels of ICSH.

Taken together these results demonstrate that FSH augments testosterone secretion in perfused rabbit testes stimulated with ICSH and thus provides direct evidence that FSH acts synergistically with ICSH to control testosterone secretion. The fact that FSH stimulates secretion of testosterone in the presence of saturating concentrations of ICSH suggests that the gonadotrophic hormones act at different sites or by different mechanisms. This suggests a degree of control of testosterone secretion in rabbit testes separate from ICSH.

BRYAN H. JOHNSON\*  
LARRY L. EWING

Department of Physiological  
Sciences, Oklahoma State  
University, Stillwater 74074

#### References and Notes

1. C. Desjardins, L. L. Ewing, B. H. Johnson, *Endocrinology*, in press; K. B. Eik-Nes, *J. Reprod. Fert.* 14 (Suppl. 2), 125 (1967); C.

- Desjardins and L. L. Ewing, *Fed. Proc.* 27, 548 (1968); E. Steinberger and M. Ficher, *Biol. Reprod. Suppl.* 1, 119 (1969); K. B. Eik-Nes, *Am. J. Physiol.* 217, 1764 (1969).
  2. D. H. Lockwood, R. W. Turkington, Y. J. Topper, *Biochem. Biophys. Acta* 130, 493 (1966).
  3. G. S. Greenwald, *Endocrinology* 80, 118 (1967).
  4. N. L. VanDemark and L. L. Ewing, *J. Reprod. Fert.* 6, 1 (1968).
  5. L. L. Ewing and K. B. Eik-Nes, *Can. J. Biochem.* 44, 1327 (1966).
  6. B. H. Johnson and L. L. Ewing, *Proc. Ann. Meet. Soc. Study Reprod.* 3rd (1970), p. 28.
  7. A. J. Lostroh, *Endocrinology* 76, 438 (1969); G. M. Connell and K. B. Eik-Nes, *Steroids* 12, 507 (1968).
  8. J. H. Exton and C. R. Park, *J. Biol. Chem.* 242, 2622 (1967).
  9. A. C. Brownie, H. J. van der Molen, E. E. Nishizawa, K. B. Eik-Nes, *J. Clin. Endocrinol.* 24, 1091 (1964); M. A. Kirschner and G. D. Coffman, *ibid.* 28, 1347 (1968).
  10. R. Ryhage, *Ark. Kemi* 26, 305 (1967); G. R. Waller, *Proc. Okla. Acad. Sci.* 47, 271 (1967).
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- \* Present address: Department of Animal Science, North Carolina State University, Raleigh 27607.

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## Transplanted Precursors of Nerve Cells: Their Fate in the Cerebellums of Young Rats

**Abstract.** *The multiplying cells of the external granular layer in 7-day-old rats were labeled with [<sup>3</sup>H]thymidine. Slabs of the cerebellum were transplanted into the same region of uninjected hosts of the same age. The transplanted, undifferentiated cells of the donors migrated actively in the cerebellar cortex of the hosts and apparently differentiated there into basket and granule cells.*

A few investigators have attempted to transplant neural tissue from one adult mammal into the brain of another, but they found that the transplants degenerated (1). Others who worked with young mammals observed that the transplants grew and differentiated to some extent (2). Recently, Wenzel *et al.* (3) reported that outgrowing processes of transplanted nerve cells of adult mice penetrated into the cerebral cortex of the host. However, to our knowledge there are no reports available to indicate that transplanted nerve cells can be structurally incorporated into the brains of recipients.

Instead of working with mature neural tissue of adult mammals, we used undifferentiated portions of the cerebellar cortex of young mammals (rats and rabbits) and transplanted this tissue into immature but developing brain regions (cerebellar cortex, hippocampus, and olfactory bulb) of

recipients. For easy identification, the donor cells were tagged radioactively. Here we report briefly the results of transplantation of slabs of the cerebellar cortex of infant rats into the cerebellar cortex of rats of the same age.

Eight laboratory-bred Long-Evans hooded rats from one litter were injected intraperitoneally with [<sup>3</sup>H]thymidine when they were 7 days old (4). A large proportion of the proliferating cells of the germinal zone of the cerebellar cortex in the external granular layer, and a smaller proportion of cells in the internal granular layer became labeled, and this material served as the transplant. One hour after injection slabs of the cerebellar cortex of the donors were surgically removed, washed twice in Ringer solution, and transplanted into the cerebellums of rats of the same age. Two or more animals were killed 3 hours

or 1, 2, 4, 10, or 16 days after surgery by transcatheter perfusion with 10 percent neutral formalin. The cerebellums of these animals were processed for histology and autoradiography in the usual manner (5).

In the cerebellums of the hosts that were killed 3 hours after surgery the transplants with their labeled cells were easily identifiable (Fig. 1). A few

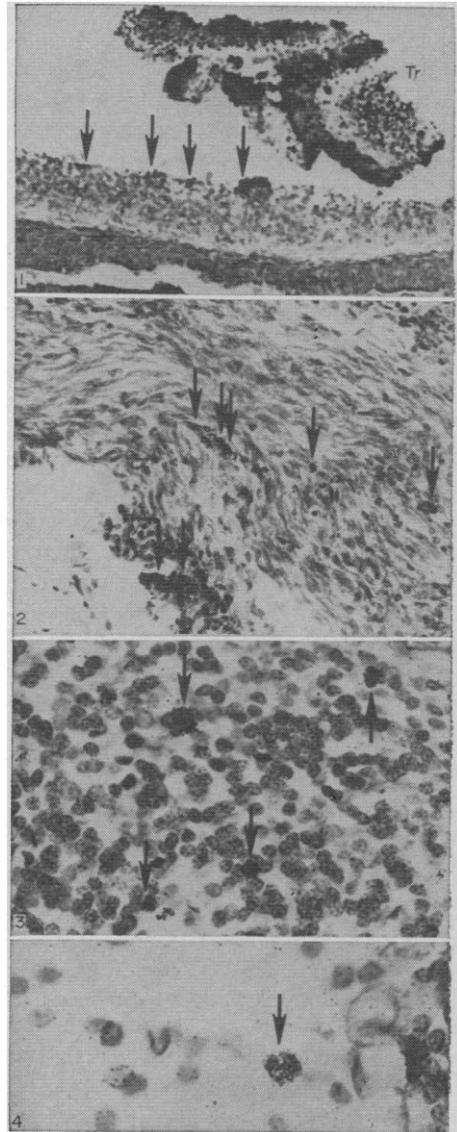


Fig. 1. Transplant (Tr) in the host cerebellum, 3 hours after surgery. Note extensive labeling of cells in the external granular layer and sparse labeling in the internal granular layer. Arrows indicate a few labeled cells attached to the exposed surface of the host cerebellum ( $\times 300$ ). Fig. 2. Stream of migrating cells in the transplant 4 days after surgery. Arrows indicate labeled cells ( $\times 325$ ). Fig. 3. Labeled cells (arrows) in the internal granular layer of the host cerebellum 16 days after the transplantation ( $\times 500$ ). Fig. 4. Labeled cell, presumably a basket cell (arrow), in the molecular layer of the host cerebellum, 16 days after the transplantation ( $\times 800$ ).

labeled cells were also seen closely attached to the exposed surfaces of the cerebellums of the hosts. In the interior of the cerebellar cortex, however, labeled cells were not seen at this time, which indicated that free [<sup>3</sup>H]thymidine was not available to the host tissue. In the rats that survived for 2 to 4 days after surgery the transplanted tissue was still identifiable. The following observations were made in this tissue. Extensive regions in the transplants were full of degenerated nerve cells and were characterized by intense phagocytic activity. In these regions very few intensely labeled cells were seen. Smaller isolated regions that appeared normal were seen and, in these, tagged cells with intense to moderate accumulation of label were present. Furthermore, in some regions of the transplants large numbers of streaming, spindle-shaped migratory cells were seen (Fig. 2). In these streams of migrating cells a few were intensely labeled, some lightly labeled, and many were unlabeled. Judging from the varying patterns of labeling over these cells and their intimate relationship with the surviving external granular layer of the transplants, we assumed that they represented an extension of the external granular layer of the transplants and that many of these had multiplied a number of times. Such streams of migrating cells, which followed tortuous courses, appeared to penetrate the host tissues either through the medullary layer or through the external granular layer of the cerebellum of the hosts.

In the brains of the animals that survived for 10 to 16 days after surgery, there remained no trace of the transplanted tissue. However, close to the site of transplantation in many regions of the host cerebellum a varied number of well-labeled cells was seen in the internal granular layer and in the molecular layer (Figs. 3 and 4). In addition to these intensely and moderately labeled cells, a large number of lightly labeled cells were found in these regions. These observations suggested that some intensely labeled cells had proliferated after having migrated into the host tissues. Although the identity of the donor cells could not be determined when they were intensely labeled, examination of the lightly labeled cells suggested that the donor cells or their progeny became differentiated in the cerebellar cortex of the hosts into granule and basket cells.

These results indicated that transplantation of the precursors of neurons is possible in the maturing nervous system of postnatal mammals. At least two factors facilitated this process. First, the precursors of nerve cells of the donor tissue, because of their active migratory behavior, escaped from the local degenerative processes as they migrated into the host tissue. This observation suggested that the transplantation of harvested undifferentiated cells of the external granular layer devoid of other cerebellar tissue should further facilitate transplantation. Second, the ongoing neurogenetic processes in the host cerebellum, whereby the primitive cells that migrate in large numbers become incorporated into the evolving architecture of the cerebellar cortex, facilitates the acceptance of exogenous cells of similar properties. This consideration suggested that depletion of the local supply of competing migratory cells by x-irradiation (6) may further facilitate acceptance of the donor cells.

The uses of this technique in studying some of the principles of neurogenesis, particularly in mammals, must be obvious. For instance, it has been debated for some time whether the undifferentiated cells of the external granular layer are pluripotent and can differentiate into neurons as well as glia, and whether some inherent properties of the multiplying cells or the time of their origin and the milieu in

which they differentiate determine whether they will become basket, stellate, or granule cells. Indeed, can a cell destined to become a granule cell in the cerebellar cortex become differentiated into a granule cell in the hippocampus with totally different chemical and morphological properties?

GOPAL D. DAS

JOSEPH ALTMAN

Department of Biological Sciences,  
Purdue University,  
Lafayette, Indiana 47907

#### References and Notes

1. S. Saltykow, *Arch. Psychiat.* **40**, 329 (1905); S. W. Ranson, *J. Comp. Neurol.* **24**, 547 (1914); R. M. May, *Arch. Anat. Microscop. Morphol. Exp.* **26**, 433 (1930); C. W. Tidd, *J. Comp. Neurol.* **55**, 531 (1932); P. Glees, *Regeneration in the Central Nervous System*, W. F. Windle, Ed. (Thomas, Springfield, Ill., 1955), p. 94.
2. R. Altobelli, *Gazz. Intern. Med. Chir.* **17**, 25 (1914); E. H. Dunn, *J. Comp. Neurol.* **27**, 565 (1917); W. E. Le Gros Clark, *J. Neurol. Neurosurg. Psychiat.* **3**, 263 (1940); *J. Anat.* **77**, 20 (1942).
3. J. Wenzel, E. Bärlechner, M. Wenzel, D. Ilius, *Z. Mikrosk.-Anat. Forsch.* **81**, 1, 32 (1969).
4. [<sup>3</sup>H]Thymidine (New England Nuclear Corp., Boston, Mass.) had a specific activity of 6.7 curie/mole (1 mc dissolved in 1 ml of sterile physiological solution). The donors were injected with [<sup>3</sup>H]thymidine at a dose of 10  $\mu$ c per gram of body weight.
5. The blocks of brains were embedded in Paraplast and cut serially on the coronal plane at thicknesses of 5 to 7  $\mu$ m. The paraffin was removed and sections were processed for histology and autoradiography. The slides were dipped into Kodak NTB-3 nuclear emulsion by the dipping technique. The autoradiograms, which were sealed in the light-proof boxes containing Drierite as the desiccant, were kept at 5°C for 10 weeks for exposure. At the end of the exposure period the slides were developed and stained with cresyl violet.
6. J. Altman and W. J. Anderson, *Exp. Neurol.*, in press.

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## Shift in Binocular Disparity Causes Compensatory Change in the Cortical Structure of Kittens

**Abstract.** *Kittens were raised with prisms in front of their eyes which introduced a vertical disparity. At 4 months of age the disparity necessary to maximally stimulate a sample of binocular cortical cells was determined. The distribution of optimal disparities was abnormal, and shifted in a direction which would tend to compensate for the prism-induced disparity.*

Ample evidence has been amassed that the environment exerts a degree of control over the development of the nervous system (1-5), making it necessary to suppose that activity in a nerve fiber can effect the functional connections made by that fiber. It has been proposed that an ingrowing axon tends to make contact with a cell when the activities in the axon and the target cell are correlated (6). Synapses already present are strengthened by the existence of such a correlation, while syn-

apses that exhibit weak or no correlation between pre- and postsynaptic activity either decay or are displaced by axon terminals with higher correlations. Axon terminals which either alone or in concert with others exert a large degree of control over a postsynaptic neuron will tend to increase in their control to an even greater extent. As a result, axons with highly correlated activities among each other (such as those receiving inputs from nearly the same part of the visual field) will tend to