One explanation for the unsuccessful attempts to reactivate insulin-inactivated *N*-acetyltransferase is that insulin reacts with the enzyme to form an inactive mixed disulfide and that the disulfide bond is buried and inaccessible to DTT. The inability to reduce mixed disulfides formed by disulfide exchange reactions is well known in other systems (*10*). Failure to reactivate the insulin-inactivated *N*-acetyltransferase is consistent with the observations that it is not possible to reactivate physiologically inactivated *N*-acetyltransferase by any in vivo or in vitro treatment (*4, 11, 12*) and that stimulation of enzyme activity appears to require new protein synthesis (*4*).

Insulin is known to activate phosphoprotein phosphatase (*13*), and a phosphorylation-dephosphorylation mechanism has been speculated to regulate *N*-acetyltransferase activity (*14, 15*). Thus, it is possible that insulin or closely related peptides could regulate *N*-acetyltransferase activity indirectly via a phosphorylation-dephosphorylation mechanism. Insulin appears to undergo thiol-disulfide exchange with a number of systems including the insulin receptor and thioredoxin, a dithiol peptide (molecular weight, 12,000) (*16*).

The finding that disulfides can inactivate *N*-acetyltransferase (*5*) leads to the formulation of an attractive hypothesis to explain the inactivation of *N*-acetyltransferase by a regulatory disulfide compound. A rapid reduced-oxidized shift would cause an immediate increase in the concentration of the inactivating (S–S) form of this compound. A shift of this sort might result from the specific action of an oxidase (*17*) or from a shift in the general redox state of the cell. The hypothetical shift in the redox state of the cell could be related to the change in membrane potential required for the adrenergic control of *N*-acetyltransferase activity (*18*).

Although it is unreasonable at this time to conclude that insulin is the active S–S compound regulating *N*-acetyltransferase activity, our findings raise the possibility that an S–S peptide participates in the physiological inactivation of this enzyme. Such a peptide could have far greater reactivity toward *N*-acetyltransferase than insulin, yet share a somewhat similar primary structure.

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8. Insulin A and B are the two chains of insulin joined by disulfide bonds in the native molecule. The preparations used were the periodic acid–oxidized forms and hence should not contain any SH or S–S bond; sulfur should exist primarily in the SO_3H form. [Tyr^1]Somatostatin is a modified form of somatostatin in which the alanine residue is replaced by tyrosine. Tyr-Somatostatin has an additional tyrosine added to the NH_2-terminal alanine residue. Pressinoic acid is a disulfide-containing peptide with six amino acids, Cys-Tyr-Phe-Gln-Asn-Cys (Cys, cysteine; Phe, phenylalanine; Gln, glutamine; Asn, asparagine). [$Asu^{1,6}$]Oxytocin is a modified oxytocin containing aminosuberic acid [$NH_2(COOH)CH(CH_2)_5COOH$]. Aminosuberic acid and tyrosine are linked by a peptide bond; the molecule does not have an S–S bond (Tyr-Ile-Gln-Asn-Asu-Pro-Leu-Gly-NH_2) (Ile, isoleucine; Pro, proline; Leu, leucine; Gly, glycine). [$Asu^{1,6}$, Arg^8]Vasopressin is a similar compound (Tyr-Phe-Gln-Asn-Asu-Pro-Arg-Gly-NH_2).
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Early Removal of One Eye Reduces Normally Occurring Cell Death in the Remaining Eye

Abstract. *During normal development of the hamster eye, there is a substantial loss of cells from the retinal ganglion cell layer in the first two postnatal weeks. If one eye is lost at birth, this cell death is reduced in the remaining eye. This may account for the increased ipsilateral projection from this eye to the thalamus and midbrain observed in these animals.*

Damage to the brain early in development can result in a variety of reorganized patterns of connectivity (*1*, *2*). Relatively little is known about the qualities of the growing brain that permit this pronounced plasticity. We have investigated one possible source of plasticity, the ubiquitous overproduction and subsequent death of neurons during normal development (*3*).

When one eye is removed at birth from hamsters or rats, the remaining eye shows at maturity a markedly increased ipsilateral projection to a variety of retinal targets (*2*, *4*). Several mechanisms could produce this effect: failure to retract axons that initially projected ipsilaterally; diversion of contralaterally going fibers; increased bilateral branching of optic nerve axons at the optic chiasm; or hypertrophy of the normal ipsilateral projection. Of these mechanisms, increased bilateral branching has been demonstrated (*5*). The demonstration that early in development retinal axons distribute more broadly ipsilaterally than they do at maturity supports the hypothesis of failure of retraction but does not prove it (*6*).

In chicks and anurans, enlargement of the terminal field available to a population of growing neurons markedly reduces neuron death in that population. For example, if an additional leg is grafted onto a developing chicken embryo, cell death is substantially reduced in the anterior horn of the spinal cord projecting to that limb (*7*). Removing one eye of a growing mammal may be an equivalent experiment, since it results in an increased terminal field available to the remaining eye. Reduced ganglion cell death in the remaining eye might thus be expected. If so, this result could account for failure of axon retraction or hypertrophy of the normal ipsilateral pathway.

Degenerating cells, which can be identified by their liquefied, darkly staining and pycnotic nuclei (*8*), can be seen in the retinal ganglion cell layer of the hamster eye in the first ten postnatal days of normal development (*9*). In this period, retinal axons are establishing their central connectivity (*10*). Studies in chick

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have provided evidence that these cells have sent axons into the optic nerve prior to their death and removal, and that cell number declines by 40 to 50 percent during this period (*11*). Using a clearance time estimate of an observable degenerating cell based on calculations from mouse spinal cord, 64 percent of cells in the retinal ganglion cell layer in hamster would be lost in the course of normal development (*12*).

For this experiment, three normal hamsters and four hamsters with one eye removed on the day of birth (*3*, *13*) were examined for each postnatal day 2 through days 8 and 10. Pups were given an overdose of urethane and perfused through the heart with a 4 percent Formalin–45 percent alcohol solution. The eyes were left in the snout to provide unambiguous orientation information, embedded in paraffin, and cut horizontally at 10 μm. In three sections, one each from superior, middle, and inferior retina, counts of degenerating and normal cells were made and corrected for frequency of encounter by the method of Abercrombie (*14*). Since the augmented ipsilateral projection in the hamster derives primarily from the temporal retina (*2*, *4*), we separately compared differences in cell death rate in monocularly enucleated animals from the normal rate for temporal and nasal retina (Fig. 1).

An analysis of variance in cell death rate in normal and monocularly enucleated animals in the temporal retina showed a significant diminution of cell death in the early enucleates [F (1, 47), = 4.43, $P < .04$], which is entirely accounted for by the reduction in rate on postnatal days 3 to 5. The nasal retina revealed no significant differences between groups, but the pattern of results suggests an early diminution of cell death, perhaps caused by a transient retention of the diffusely distributed ipsilateral pathway and its subsequent loss on days 6 to 10.

According to the clearance time estimate for cell debris of $1^1/_2$ hours, cell loss in the temporal retina would be reduced from 64 to 56 percent. This increase in cell number corresponds well to the apparent magnitude of the enhanced ipsilateral projection (*2*, *4*).

If cell death is reduced in the temporal retina during early development, cell density should be increased in the temporal retina relative to the nasal retina at late developmental stages and at maturity. We compared the ratio of live cell numbers in horizontal sections in the temporal retina to numbers in the nasal retina on postnatal day 10 for the three normal animals and four monocular enucleates whose data appear in Fig. 1 (*15*). The average of the ratios for normal animals was .81 ± .04; for monocular enucleates, 1.06 ± .11, which is significantly different [t (4) = 4.23, $P < .02$]. Thus, the diminution of cell death in temporal retina appears to produce an increase in cell density in the temporal retina in the late postnatal period.

These results demonstrate one possible mechanism for the augmentation of the ipsilateral pathway. Since the diminution of cell death occurs so early in postnatal development, it is likely that at least some of the diminution is accounted for by the maintenance of neurons that normally send axons ipsilaterally and later die. Since more cells are maintained in the retina, increased bilateral branching cannot be the only mechanism responsible for the increased ipsilateral pathway. However, we do not know from these results which population of cells contributes to the augmented ipsilateral pathway—excess neurons destined to go ipsilaterally or excess neurons that normally branch at the optic chiasm.

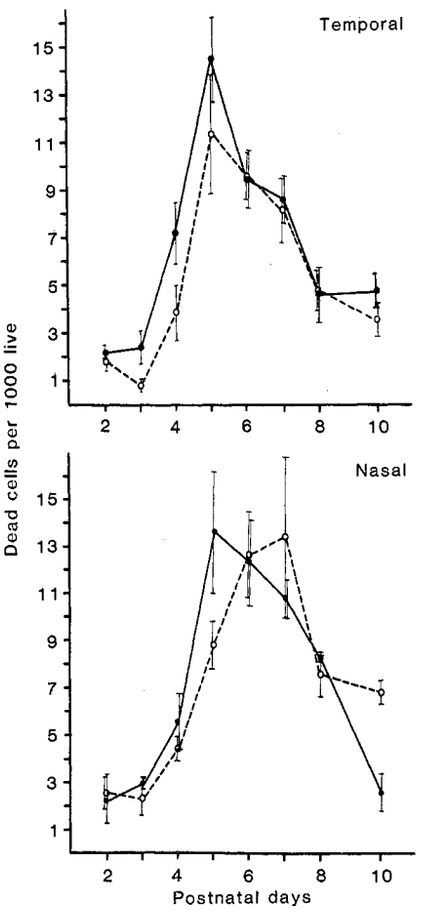

Fig. 1. Rate of cell death in the temporal and nasal retina for normal (N = 24, solid lines) and unilaterally enucleated hamsters (N = 32, dashed lines). Points represent averaged values ± standard errors of the mean. In the temporal retina, the cell death rate in enucleates was significantly lower than that of normal subjects. The nasal retina showed no such difference.

This study suggests that one mechanism by which reorganizations are possible in the damaged neonatal brain is alteration of normally occurring cell death through changing availability of terminal fields. Unlike branching or sprouting of terminal arbors of axons which occur in the adult as a result of damage (*16*), cell overproduction and death is a uniquely developmental phenomenon that may underlie much uniquely developmental plasticity.

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13. Within 8 hours of birth, the right eye was removed with the aid of a dissecting microscope. A small slit was made under the line of the prospective eyelid, and the eye was withdrawn with fine forceps with care to remove all pigmented fragments.
14. Drawings of retinal cross sections with locations and a complete count of all degenerating cells were made at ×500. The ganglion cell layer is distinguishable by postnatal day 1 and becomes progressively more defined. Because of the relatively small numbers of cells in the cross section of the ganglion cell layer, complete live cell counts of the sections were possible. Ratios of live and degenerating cells were made after correcting for cell size and section thickness [M. Abercrombie, *Anat. Rec.* **94**, 239 (1946)]. The total length of each retinal cross section was measured and bisected into nasal and temporal halves.
15. Ratios rather than absolute counts of cell numbers were used to eliminate the variability in cell numbers in horizontal retinal sections due to differential shrinkage, small deviations in section thickness, and retinal level.
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