The Journal of Neuroscience, October 1987, 7(10): 3317–3324

Cell-Specific Regulation of Neuronal Production in the Larval Frog Retina

T. A. Reh

Department of Medical Physiology and Neuroscience Research Group, Health Sciences Center, The University of Calgary, Calgary, Alberta T2N 4N1, Canada

We have previously postulated the existence of a feedback mechanism from differentiated neurons that regulates the production of new neurons. Evidence for such regulatory feedback comes from experiments in which dopamine-containing amacrine cells, ablated in the developing retina by 6-hydroxydopamine (6-OHDA), were up-regulated in their production. To determine whether this is a general phenomenon of the developing retina, the neurotoxin kainic acid (KA) was injected intraocularly in midlarval-stage *Rana pipiens* tadpoles to produce selective lesions of certain retinal cell types. After periods of 1–21 d, the animals received intraperitoneal injections of ^{3}H-thymidine. Animals were then allowed to survive for periods of up to 3 weeks and were then fixed, the eyes embedded in plastic, sectioned at 3 μm, and processed for autoradiography by standard methods. At the dosage used, the KA produced a 52% decline in the cell density of the inner nuclear layer (INL), a 37% decline in the retinal ganglion cell layer (RGC), and no significant change in the density of cells in the outer nuclear layer (ONL).

The ^{3}H-thymidine allowed us to detect any changes in the number of new cells added to the retina after the KA lesion. Within the first week after the KA injection, there was a decrease in the number of ^{3}H-thymidine (^{3}H-Thy)-labeled cells in the lesioned eye as compared to in the control retina; however, KA treatment of slice cultures demonstrated that the toxin does not affect proliferating neuroblasts directly. By contrast, in animals that received ^{3}H-Thy injections more than one week after the KA lesion, the number of new cells produced by the germinal neuroepithelium was increased in each retinal layer in proportion to the degree to which differentiated neurons were destroyed by KA in that layer. These results suggest that feedback regulation of neuron production is a general phenomenon of retinal histogenesis.

During the development of the vertebrate CNS, an enormous variety of different neuronal types is generated. Moreover, these various kinds of neurons are produced in precise ratios with respect to one another, resulting in the appropriate numbers of the different cell types necessary for the functioning of the adult nervous system. Although little is known concerning the mechanisms that regulate the production of the appropriate numbers of neurons, in other tissues there is evidence that tissue-specific or endogenous mitotic inhibitors provide feedback from the differentiated cells to the stem cell population (Bullough, 1975; Marks, 1976; Holley et al., 1980; Bryant and Simpson, 1984). To determine whether the ratios of neurons normally produced by the germinative zone of the CNS are under the regulation of a similar kind of feedback from differentiated cells, the effects of ablating particular classes of neurons on the developing retina were studied in *Rana pipiens* larvae.

The retina of the larval frog is ideally suited for the study of factors that regulate neuroblast proliferation and differentiation. Several studies have established that new neurons are added via a zone of germinal neuroepithelial cells at the extreme periphery of the retina throughout the larval period, and even in postmetamorphic frogs (Hollyfield, 1968; Beach and Jacobson, 1979; Reh and Constantine-Paton, 1983). Therefore, it is possible to experimentally manipulate the composition of the differentiated retina using selective neurotoxins, and to examine the effects on the production of new neurons in the proliferative zone.

Previous reports, in which 6-hydroxydopamine (6-OHDA) was used to selectively destroy all retinal dopamine-containing neurons (Negishi et al., 1982; Reh and Tully, 1986) provided evidence for a specific up-regulation in the production of new neurons of this type. In the present report, cell-specific lesions of the retina were made with intraocular kainic acid (KA) injections to determine whether the specific up-regulation observed for dopamine-containing neurons could be induced in other cell types. The results support the hypothesis that neuronal cell ratios are regulated in part via feedback from already differentiated neurons onto undifferentiated neuroblasts. It is therefore possible that tissue-specific mitotic inhibitors, similar to those found in other tissues, may also be important regulators of cell proliferation in the CNS as well.

Materials and Methods

Twenty-six lab-reared *Rana pipiens* tadpoles received unilateral intraocular injections of 1 μl of a 10 mM KA solution (approximately 100 μM intraocular concentration), followed by injections of ^{3}H-Thy, i.p. (1 μCi/gm body weight; sp act, 20 Ci/mmol) (New England Nuclear) from 3 to 16 d later. After survival periods of up to 3 weeks, sufficient for the labeled cells to differentiate into cells of 1 of the 3 retinal layers, the animals were killed and the retinas were fixed, embedded in either paraffin or JB-4 and sectioned at either 10 or 4 μm, respectively, and processed for autoradiography by standard techniques, using Kodak NTB3 emulsion (Reh and Constantine-Paton, 1983). To reduce variability due to autoradiographic processing, both the experimental and control eyes were embedded, sectioned, and processed in a single block. In a given retina, nuclei with more than 5 silver grains were counted in each of the retinal layers in the 10 sections adjacent to, or containing,

Received Dec. 9, 1986; revised Apr. 23, 1987; accepted Apr. 27, 1987.

I am indebted to Lori Bullock, Thomas Nagy, and Kori Radke for expert technical assistance during the course of this work. I also acknowledge discussions with Dr. Martha Constantine-Paton during the initial phases of this study. This work was supported by the Medical Research Council (Canada), Grant MA-9333. The author is a scholar of the Albert Heritage Foundation for Medical Research.

Correspondence should be addressed to Dr. Thomas A. Reh, Department of Medical Physiology, Health Sciences Center, The University of Calgary, 3330 Hospital Drive N.W., Calgary, Alberta T2N 4N1, Canada.

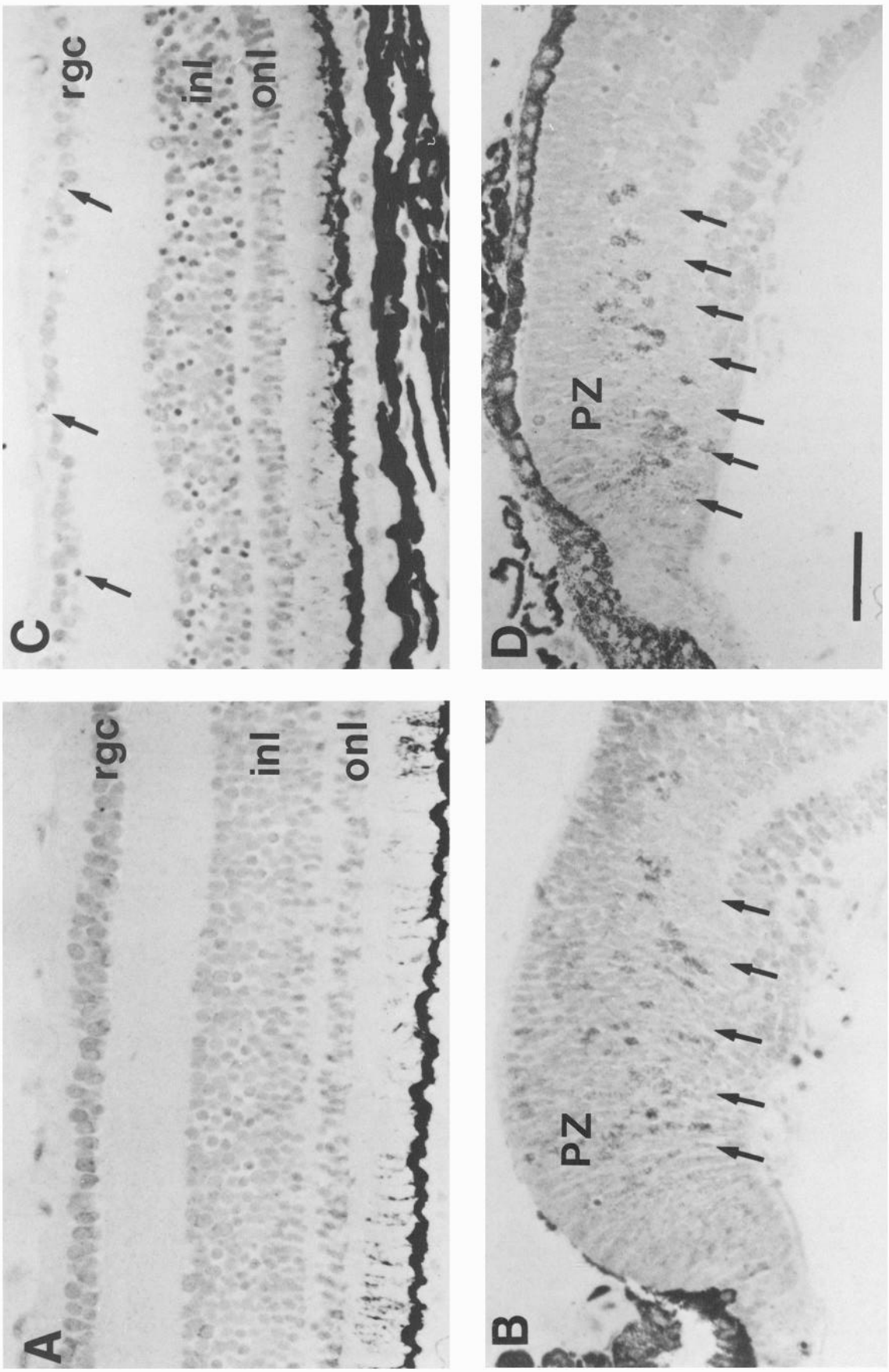

Figure 1. *A*, *B*, Central retina and proliferative zone (*PZ*), respectively, of a retinal slice culture after 24 hr *in vitro* labeling with ^{3}H-Thy. *C*, *D*, Central retina and proliferative zone, respectively, of a retinal slice culture following a 2 hr exposure to 100 μm KA and a 24 hr period of ^{3}H-Thy labeling *in vitro*. *Arrows* in *C*, degenerating cells in the RGC layer. *Arrows* in *B* and *D* denote the ^{3}H-Thy-labeled cells. Scale bar, 50 μm.

Table 1. Percentages of cells in retinal layers following kainic acid treatment

	RGC (%)	INL (%)	ONL (%)
Degenerating cells in layers, 1 d after KA (%) ($n = 4$)	10.3 ± 4.5	23.3 ± 8.7	<1
Change in cell density (%), 2–6 weeks after KA ($n = 16$)	−37.6 ± 3.8	−52.3 ± 4.2	−5.8 ± 2.9
Change in ^{3}H-Thy-labeled cells/layer (%): KA/control (<1 week) ($n = 5$)	−2.1 ± 19	−9 ± 12	−10 ± 20
Change in ^{3}H-Thy-labeled cells/layer (%): KA/control (>1 week) ($n = 5$)	+15.2 ± 2.7*	+22.2 ± 2.4**	+1.6 ± 3

Asterisks signify level of statistical significance using the nonparametric randomization test: * $p < 0.01$; ** $p < 0.005$.

the optic nerve head. Mueller cells were identified by their elongate, darkly staining nuclei (Ingham and Morgan, 1983), and were not included in the cell counts. In most cases mitotic figures were also counted at the sclerad surface of the germinal zone.

For the *in vitro* experiments, larval *Rana pipiens* retinas were dissected free from surrounding ocular tissue under sterile conditions, embedded in agarose (4%), quickly chilled on ice (<2 min), and sectioned at 100 μm on a Vibratome (Oxford Instruments, Oxford). The slices were then incubated for 2 hr either in Ringer's alone or in the same solution with 100 μM KA added. Next, the slices were rinsed in Ringer's and placed in 50% L-15 media with 10% fetal calf serum and 2 μCi/ml of ^{3}H-Thy (6.7 Ci/mmol) added, incubated for 24 hr, fixed in 2% paraformaldehyde, embedded in JB-4 plastic, and sectioned at 4 μm. The sections were then processed for autoradiography as described above. Previous work has shown that the cells of the germinal neuroepithelial zone of the tadpole retina continue to proliferate for up to 2 weeks at approximately normal rates under these culture conditions (Reh, 1985).

Results

Effects of kainic acid on the cells of the differentiated retina

Kainic acid had profound effects on the cells of the inner nuclear layer (INL) both *in vivo* and *in vitro*. Many pycnotic nuclei were observed in the INL 1–3 d following the neurotoxin administration. The pycnotic nuclei were particularly apparent in the *in vitro* slice cultures 1 d after KA incubation (Fig. 1*C*), and the percentage of degenerating cells in each layer is listed in Table 1. While the cells of the outer nuclear layer (ONL) were virtually unaffected at the dosages used, some degenerating cells were observed in the ganglion cell layer (RGC; Fig. 1*C*, arrows; Table 1). This decline in cell number in the RGC has been attributed to the loss of displaced amacrine cells in other species (Erlich and Morgan, 1980), and a similar explanation may be applicable with the frog (Beazley et al., 1986; Stelzner and Strauss, 1986). The laminar specificity of the neurotoxic effects of KA has been observed previously in several other species and is thought to represent the extreme sensitivity of some neuronal types, particularly those that depolarize in response to transmitter, to intense and prolonged activation of glutamate receptors on these cells (Erlich and Morgan, 1980; Hampton et al., 1981; Ingham and Morgan, 1983). Ganglion cells, photoreceptors, and ON bipolar cells appear to be relatively insensitive to the toxin, while OFF bipolar cells, horizontal cells, and most classes of amacrine cells are very sensitive to KA activation.

By one week after the KA injection, most of the degenerating cells have been cleared from the retina, and the density and total number of INL cells in the retinas are markedly reduced (Fig. 2, *A, B*; Table 1). In addition, there is also a decrease in cell number and cell density in the RGC, which might be attributable to loss of displaced amacrine cells; however, there is no change in either of these parameters for the photoreceptor layer (Fig. 2, *A, B*; Table 1).

Short-term effects of kainic acid on new retinal neuron production

As in the normal tadpole, new cells continue to be added to the retinal ciliary margin following the KA lesions. However, in the first week after KA injections, there is a decline in the number of mitotic figures in the KA-treated retina (Fig. 3). A decrease in mitotic figures could indicate either that the number of proliferating neuroblasts has decreased or that the mitotic phase of the cell cycle has been shortened (Jacobson, 1978). In an attempt to determine which of these alternatives was correct, after ^{3}H-Thy injections the number of labeled cells was counted in the KA-treated and control eyes. As Figure 3 shows, there is also an initial decline in the number of ^{3}H-Thy-labeled cells following KA treatment. While a greater number of mitotic figures in a histological section might represent increased numbers of dividing cells, or a lengthening of the M phase of the cell cycle, counts of ^{3}H-Thy-labeled cells directly reflect the rate of cell proliferation, since they measure the number of cells produced during the period ^{3}H-Thy was available for incorporation. While considerable cell death might confound this measurement, the fact that the 2 independent measures of cell proliferation respond in parallel, coupled with the fact that little, if any, neuronal death is observed in larval *Rana* retina (Reh and Constantine-Paton, 1983), suggests that the figures given in this study accurately reflect the changes in rates of neuronal proliferation following KA treatment.

To determine whether the KA was having a direct effect on the mitotically active germinal neuroepithelial cells at the retinal ciliary margin, the following experiment was carried out. Slices of larval retina that contained proliferating neuroblasts were incubated in 100 μM kainic acid, as described above. Next, the sections were rinsed and cultured in media for 24 hr with ^{3}H-Thy to label the mitotically active cells. Following fixation, sectioning, and autoradiography, the counts (mean of 5 sections) of ^{3}H-Thy-labeled cells in KA-treated slices were compared with those of control slices. While there was considerable variation between the total numbers of labeled cells per slice, the means for the experimental ($n = 4$) and control ($n = 4$) conditions were virtually identical (KA, 2131 ± 965 cells/slice; control, 1901 ± 1008 cells/slice). In addition, the density of labeling and histological appearance of the proliferative zone was also normal (Fig. 1, *B, D*). Therefore, it is unlikely that the KA is having any direct effect on the mitotically active retinal precursor population.

The laminar specificity of the decrease in labeled cells immediately following the KA treatment was also examined *in vivo*. The percentages of ^{3}H-Thy-labeled cells in each retinal

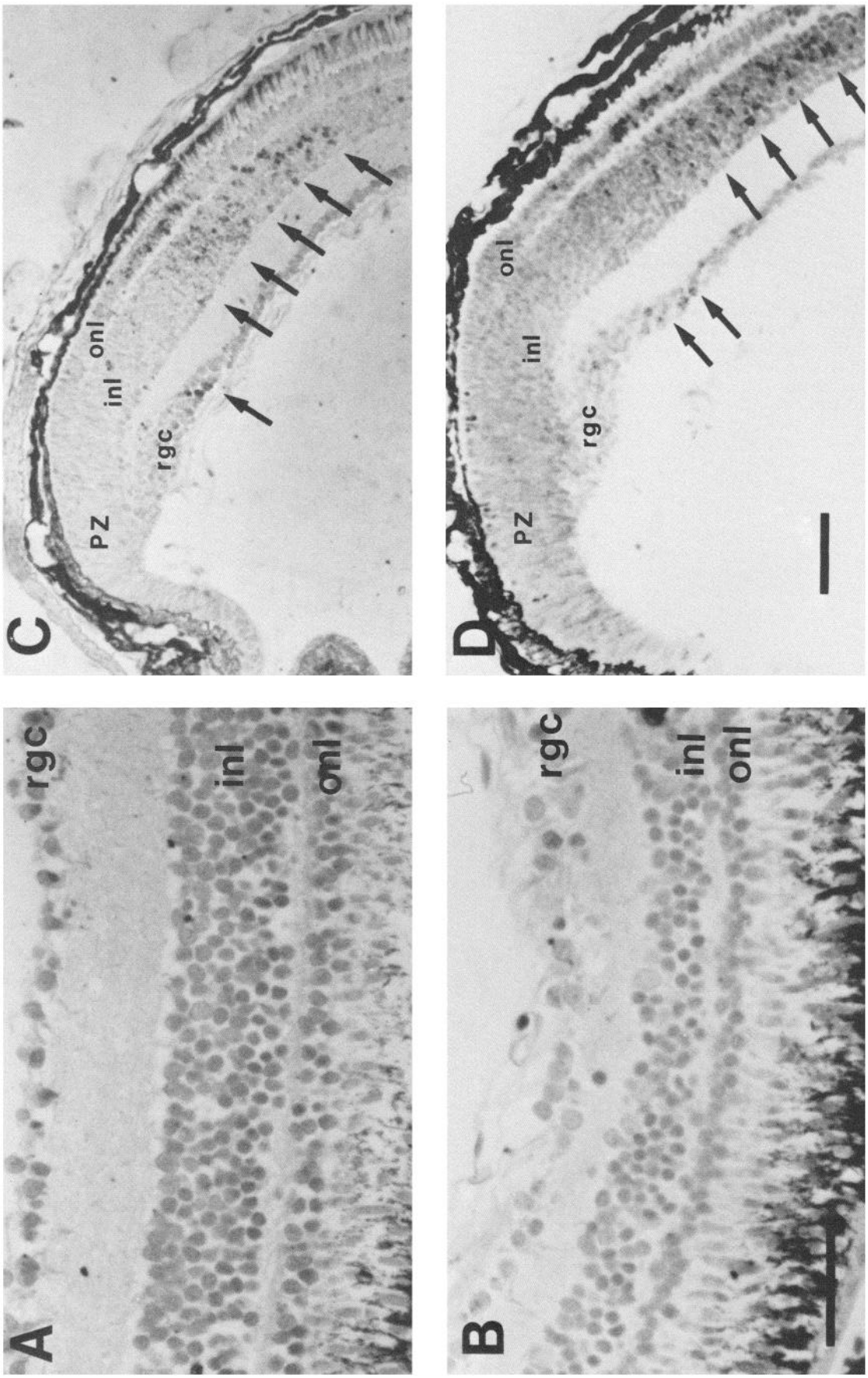

Figure 2. Photomicrographs of 4 μm plastic sections of larval frog retina. *Arrows* denote ^{3}H-Thy-labeled cells. *A, C,* Central retina and proliferative zone (*PZ*), respectively, of an untreated control eye 2 weeks after ^{3}H-Thy injection. *B, D,* Central retina and proliferative zone, respectively, of an experimentally treated eye 4 weeks after KA injection and 2 weeks after ^{3}H-Thy labeling. Note the large increase in the size of the proliferative zone in *D*.

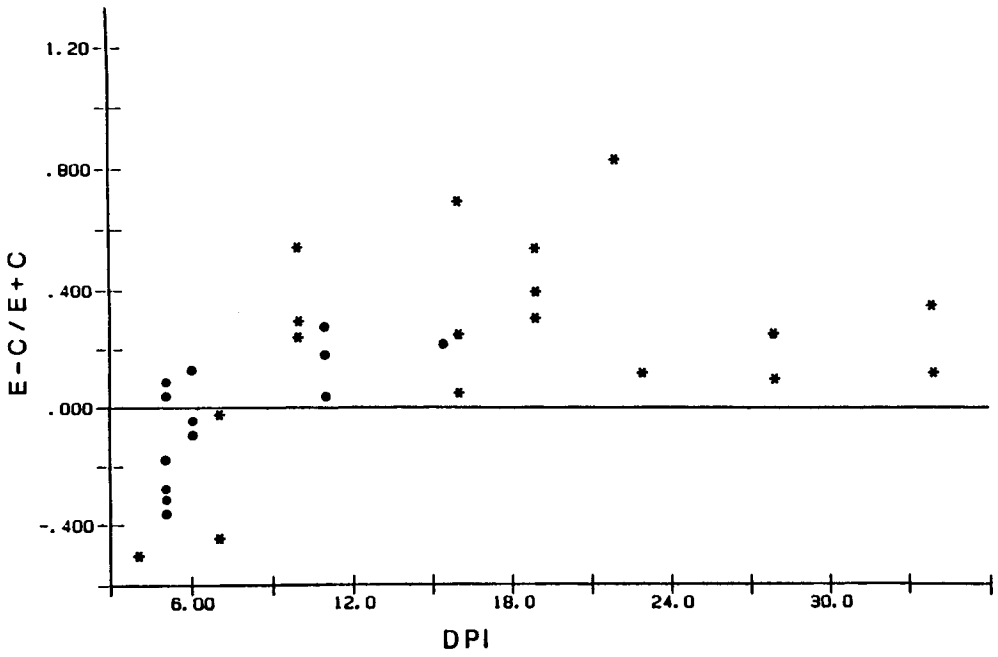

Figure 3. Ratios of mitotic figures (*asterisks*) and ^{3}H-Thy-labeled cells (*black dots*) (for day of ^{3}H-Thy injection) for the KA-treated and untreated control eyes at various days post-KA injection (*DPI*). Note that the initial decrease in mitotic figures and labeled cells is followed by a large increase in both parameters by 1 week after the KA treatment. $(E - C)/(E + C)$ = experimental minus control, divided by experimental plus control.

layer were quantified in those animals that received the isotope less than one week after the KA treatment. These data, listed in Table 1, suggest that the decline in ^{3}H-Thy-labeled cells that occurs immediately following the KA treatment is not restricted to the cells of the INL, but appears to be a general effect on the production of new cells of all types. Since the *in vitro* experiment rules out a direct effect of the KA on the germinal neuroepithelial cells in the ciliary margin growth zone, the KA may be affecting some other aspect of the physiology of the eye in the first week after the injection.

Long-term effects of kainic acid on new neuron production

As can be seen in Figure 2, *C, D,* as well as in Figure 3, after the initial decline in mitotic figures and ^{3}H-Thy-labeled cells in the KA-treated retina, there is a large increase in both parameters. By 20 d after the KA injection, the number of labeled cells in the experimental eye is approximately 40% greater than that of the control retina. This increase in new cell production persists for several weeks, and levels are still above normal at 36 d after the KA administration (Fig. 3).

To determine whether the production of any particular retinal cell type was selectively increased after the KA treatment, the laminar distribution of the newly produced cells was quantified in those cases in which ^{3}H-Thy was received more than 1 week after the kainic acid. As Figure 4 and Table 1 demonstrate, the cells that show the greatest increase in their numbers are those of the INL, which were most affected by the KA. Figure 4 shows the data for the 5 cases that received ^{3}H-Thy injections more than 7 d after the KA lesion; there is a consistent, selective increase in the number of labeled cells in the INL of the KA-treated retina, compared to the normal retina. By contrast, there is a smaller, but also consistent, increase in the number of labeled cells in the RGC, while no such increase was observed in the ONL cells, with some cases showing a decrease or no difference in ONL cell labeling. From these data, the percentage of cells in each retinal layer of the experimental and control retinas was calculated and is given in Table 1 and graphed in the lower-right-hand plot in Figure 4.

Discussion

The primary objective of this study was to determine whether the cell-specific up-regulation of neuronal production observed in dopamine-containing amacrine cells following 6-OH-DA lesions to the developing retina is a general phenomenon of CNS neuroblasts. To this end, certain classes of retinal cells were selectively destroyed in the larval frog retina, and ^{3}H-thymidine autoradiography was used to determine whether these cell types were selectively replaced. This appears to be the case, since the number of new cells produced by the germinal neuroepithelium was increased in each retinal layer in proportion to the degree to which differentiated neurons in that layer were destroyed by the neurotoxin.

The developing neural tube has been shown to undergo gross tissue "regulation" in the same way that other areas of the vertebrate embryo compensate for surgical ablation of small regions of tissue (Hughes, 1968; Bryant and Simpson, 1984). This process of "regulation" is thought to be analogous to wound healing, in the sense that removal of a piece of tissue results in a discontinuity in positional information, which must then be filled in by new cells. The effect on neuroblast proliferation observed following kainic acid lesions may involve similar mechanisms. However, it is clear from the selective KA lesions that the mitotic stimulation is not a general effect, but rather that the new cells produced specifically replace those that were ablated by the neurotoxin.

Previous experiments in which classes of monoamine-containing amacrine cells were destroyed in developing retina using selective neurotoxins showed an up-regulation in the production of new cells of the ablated type (Negishi et al., 1982; Reh and Tully, 1986). We proposed that the new cells were produced as a result of the removal of an inhibitory signal that normally limits either mitosis of an amacrine precursor or differentiation of unspecified neuroblasts to a monoaminergic pathway (Reh and Tully, 1986). The evidence presented in this study supports the existence of an inhibitory feedback affecting the production of new cells of particular types, and extends previous findings

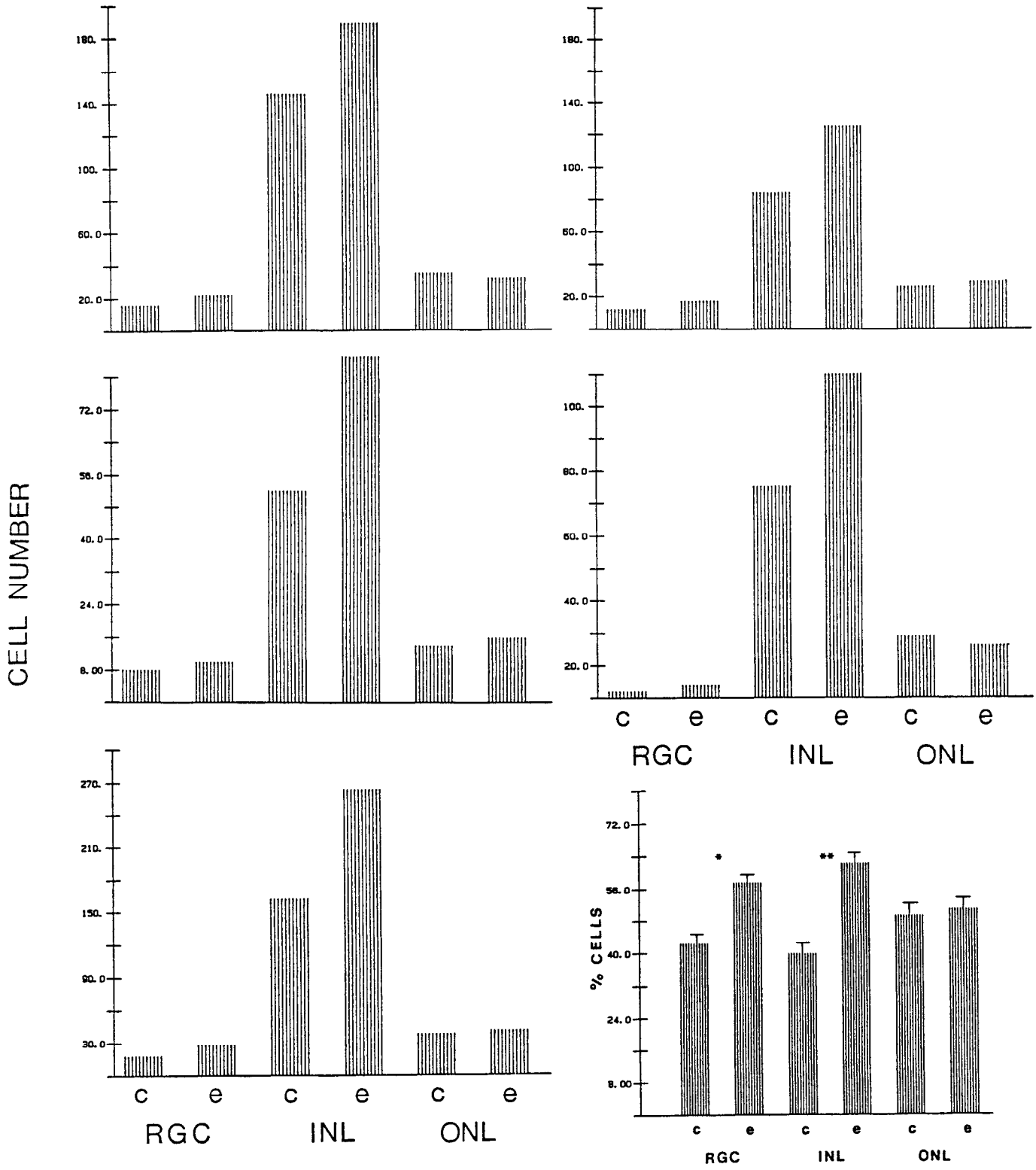

Figure 4. Histograms showing the numbers of ^{3}H-Thy-labeled cells in each retinal layer in those cases labeled with ^{3}H-Thy more than 1 week after the KA treatment for control (*c*) and experimental (*e*) eyes. The *lower-right-hand histogram* shows the normalized data from these 5 cases and the percentage change in each layer. *Asterisks* signify levels of statistical significance using the nonparametric randomization test: $*p < 0.01$; $**p < 0.005$.

in suggesting that this feedback operates to regulate neuroblast proliferation as well as differentiation.

In light of these studies, the following model is proposed: Mitotic neuroblasts of the germinal neuroepithelium of the vertebrate neural tube are exposed to a complex microenvironment produced by dividing cells, postmitotic migrating immature neurons, and some differentiating neurons (Hinds and Hinds, 1979). The differentiating cells produce some type of signal that

inhibits the mitotic rate of a committed precursor responsible for generating cells of that particular type. Alternatively, a multipotent precursor might be channeled into a particular pathway for more divisions than normal by the absence of this signal. In either case, this inhibitory signal regulates the rate of production of particular cell types, such that their numbers and densities develop normally. When the density of a given cell type is reduced, there is a reduction in the inhibitory signal, and the mitotic neuroblasts respond by producing greater numbers of that cell type than normal.

This mechanism could thus provide a means, in addition to the death of differentiated neurons, by which the appropriate number of neurons is generated during development. Since cell death in the CNS is primarily observed between neuronal populations that connect with one another over long distances via their axonal projections (Rager and Rager, 1978; Oppenheim, 1981; Sengelaub and Finlay, 1982), this type of interaction—and consequent matching of cell numbers—can occur only after the cells have differentiated to the point of growing axons. By contrast, local circuit neurons can interact prior to differentiation, since their precursors are in close physical proximity. Moreover, it is not likely that local circuit neurons regulate their numbers primarily by cell death, since the requisite amount of neuronal loss is not observed in these cell populations (Glucksman, 1940; Rager and Rager, 1978; Sengelaub and Finlay, 1982; Spira et al., 1984). For example, when chick RGCs reach their targets in the optic tectum, a massive cell loss of approximately 500,000 ganglion cells ensues, while at the same time there is little, if any, loss of other retinal cells (Rager and Rager, 1978).

The molecular basis for the inhibitory feedback proposed to regulate neuronal production can only be speculated upon. Recently, a 13 kDa growth-regulatory polypeptide has been isolated from medium conditioned by mouse 3T3 cells; it reversibly inhibits DNA synthesis and proliferation of those cells (Hsu and Wang, 1986). Several studies of transforming growth factor-β (TGF-β) have also demonstrated inhibitory effects on cell proliferation (Roberts et al., 1985). In addition, tissue-specific proliferation inhibitors have been purified from hepatocytes (26 kDa; McMahon et al., 1982) and mammary gland cells (13 kDa; Bohmer et al., 1984). However, it is not essential that the growth inhibition observed in the present study be mediated by a soluble, diffusible substance secreted by the cells. A recent study of the growth inhibition between transformed and normal mouse 10½ cells indicated that gap-junctional communication is necessary for this inhibitory interaction (Mehta et al., 1986). Therefore, it is possible that inhibitory growth regulators can be transferred intracellularly if these cells are coupled via gap junctions. This type of mechanism may be particularly important in the germinal cells of the vertebrate CNS, in which the extensive dye and electrical coupling of the cells has been well documented (Jacobson, 1978).

A very different kind of mechanism is suggested by the evidence of Lauder and her colleagues (Lauder and Krebs, 1976; Lauder et al., 1981). They have proposed that serotonin and dopamine act as "differentiation signals" for CNS germinal neuroepithelial cells. However, attempts to demonstrate this action using monoamine agonists and antagonists have produced inconclusive results; while Lauder et al. (1981) report an increase in ^{3}H-thymidine labeling, Lewis et al. (1977) and Patel et al. (1980) report a decrease. In addition, while dopamine-containing amacrine cells are present in the retina, their destruction does not result in an overproduction of all cell types, as Lauder's hypothesis would predict (Reh and Tully, 1986). Nor is there any evidence for neurotransmitter receptors on germinal neuroepithelial cells, and the results of the present study strongly suggest that these cells do not possess kainate receptors.

Whatever the mechanisms, the regulation of the production of the various cell types that comprise the very complex tissue of the CNS is likely to involve several levels of control. The results of this study and of previous, similar experiments suggest that the microenvironment of central neuroblasts is likely to play a role in specifying their commitment to a particular phenotype.

References

Beach, D. H., and M. Jacobson (1979) Patterns of cell proliferation in the retina of the clawed frog during development. J. Comp. Neurol. *183:* 603–614.

Beazley, L. D., J. E. Darby, and V. H. Perry (1986) Cell death in the retinal ganglion cell layer during optic nerve regeneration for the frog *Rana pipiens.* Vision Res. *26:* 543–556.

Bohmer, F. D., W. Lehmann, H. E. Schmidt, P. Langon, and R. Grosse (1984) Purification of a growth inhibitor for Ehrlich ascites mammary carcinoma cells from bovine mammary gland. Exp. Cell Res. *150:* 466–476.

Bryant, P. J., and P. Simpson (1984) Intrinsic and extrinsic control of growth in developing organs. Q. Rev. Biol. *59:* 387–415.

Bullough, W. S. (1975) Mitotic control in adult mammalian tissues. Biol. Rev. *50:* 99–127.

Erlich, D., and I. G. Morgan (1980) Kainic acid lesions chick retina displaced amacrine cells. Neurosci. Lett. *17:* 43–48.

Glucksman, A. (1940) Development and differentiation of the tadpole eye. Br. J. Ophthalmol. *24:* 153–178.

Hampton, C. K., C. Garcia, and D. A. Redburn (1981) Localization of kainic acid sensitive cells in mammalian retina. J. Neurosci. Res. *6:* 99–111.

Hinds, J. W., and P. L. Hinds (1979) Differentiation of photoreceptors and horizontal cells in the embryonic mouse retina: An electron microscopic serial action analysis. J. Comp. Neurol. *187:* 495–512.

Holley, R. W., P. Bohlen, R. Baldwin, J. H. Kleeman, and R. Armour (1980) Purification of kidney epithelial cell growth inhibitors. Proc. Natl. Acad. Sci. USA *77:* 5989–5992.

Hollyfield, J. G. (1968) Differential addition of cells to the retina in *Rana pipiens* tadpoles. Dev. Biol. *18:* 163–179.

Hsu, Y.-M., and J. L. Wang (1986) Growth control in cultured 3T3 fibroblasts. V. Purification of an M_r 13,000 polypeptide responsible for growth inhibitory activity. J. Cell Biol. *102:* 362–369.

Hughes, A. F. W. (1968) *Aspects of Neural Ontogeny,* Academic, London.

Ingham, C. A., and I. G. Morgan (1983) Dose-dependent effects of intravitreal kainic acid on specific cell types in chicken retina. Neuroscience *9:* 165–181.

Jacobson, M. (1978) *Developmental Neurobiology,* Plenum, New York.

Lauder, J. M., and H. Krebs (1976) Effects of *p*-chlorophenylalanine on time of neuronal origin during embryogenesis in the rat. Brain Res. *107:* 638–644.

Lauder, J. M., P. Y. Sze, and H. Krebs (1981) Maternal influences on tryptophan hydroxylase activity in embryonic rat brain. Dev. Neurosci. *4:* 291–295.

LeDouarin, N. M. (1980) The ontogeny of the neural crest in avian embryo chimeras. Nature *286:* 663–669.

Lewis, P. D., A. J. Patel, G. Bendek, and R. Balazs (1977) Effect of reserpine on cell proliferation in the developing rat brain: A quantitative histological study. Brain Res. *129:* 299–308.

Marks, F. 1976) The epidermal chalones. In *Chalones,* J. C. Houck, ed., North-Holland, Amsterdam.

McMahon, J B., J.-G. Farrelly, and P. T. Iype (1982) Purification and properties of a rat liver protein that specifically inhibits the proliferation of nonmalignant cells from rat liver. Proc. Natl. Acad. Sci. USA *79:* 456–460.

Mehta, P. P., J. S. Bertram, and W. R. Loewenstein (1986) Growth inhibition of transformed cells correlates with their junctional communication with normal cells. Cell *44:* 419–428.

Negishi, K., T. Teranishi, and S. Kato (1982) New dopaminergic and

indoleamine-accumulating cells in the growth zone of goldfish retinas after neurotoxic destruction. Science *216:* 747–749.

Oppenheim, R. W. (1981) Neuronal cell death and some related regressive phenomena during neurogenesis: A selective historical review and progress report. In *Studies in Developmental Neurobiology,* W. M. Cowan, ed., pp. 74–133, Oxford U. P., New York.

Patel, A. J., Z. Vertes, P. D. Lewis, and M. Lai (1980) Effect of chlorpromazine on cell proliferation in the developing rat brain. A combined biochemical and morphological study. Brain Res. *202:* 415–428.

Patterson, P. H. (1978) Environmental determination of autonomic neurotransmitter functions. Annu. Rev. Neurosci. *1:* 1–17.

Rager, G. H., and U. Rager (1978) Systems-matching by degeneration. I. A quantitative electron microscopic study of the generation and degeneration of retinal ganglion cells in chicken. Exp. Brain Res. *33:* 65–78.

Reh, T. A. (1985) Frog retinal neuroblasts continue to proliferate *in vitro.* Neurosci. Lett. (Suppl.) *22:* 347.

Reh, T. A., and M. Constantine-Paton (1983) Qualitative and quantitative measures of placticity during the normal development of the *Rana pipiens* retinotectal projection. Dev. Brain Res. *10:* 187–200.

Reh, T. A., and T. Tully (1986) Regulation of tyrosine hydroxylase-containing amacrine cell number in larval frog retina. Dev. Biol. *114:* 463–469.

Roberts, A. B., M. A. Anzano, L. M. Wakefield, N. S. Roche, D. F. Stern, and M. B. Sporn (1985) Type β transforming growth factor: A bifunctional regulator of cell growth. Proc. Natl. Acad. Sci. USA *82:* 119–123.

Sengelaub, D. R., and B. L. Finlay (1982) Cell death in the mammalian visual system during normal development. I. Retinal ganglion cells. J. Comp. Neurol. *204:* 311–317.

Spira, A. W., S. Hudy, and R. S. Hannah (1984) Ectopic photoreceptor cells and cell death in the developing rat retina. Anat. Embryol. *169:* 293–301.

Stelzner, D. J., and J. A. Strauss (1986) A quantitative analysis of frog optic nerve regeneration: Is retrograde ganglion cell death or collateral axonal loss related to selective reinnervation? J. Comp. Neurol. *245:* 83–106.