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Effects of Prevention of Afferentation of the Development of the Chick Optic Lobe

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BONDY, S. C., M. E. HARRINGTON AND C. L. ANDERSON. *Effects of prevention of afferentation on the development of the chick optic lobe.* BRAIN RES. BULL. 3(5) 411–413, 1978.—The effects of unilateral extirpation of the right optic cup of the three-day incubated chick embryo upon the rate of synthesis and the stability of DNA in the non-innervated optic lobe, have been studied. This surgical procedure prevents innervation of the optic lobe contralateral to the removed eye, while the other optic lobe is normally innervated by retinal ganglion cells of the remaining eye. At the 20th day of incubation, the DNA content of the non-innervated lobe was below that of the paired lobe receiving normal innervation. This deficiency of cell number was caused by two events; death of an excess number of neurons formed early in embryogenesis and a reduced rate of glial proliferation in the later stages of incubation.

Non-innervation Enucleation Chick embryo DNA synthesis Cell death Brain development

EARLY removal of the chick optic vesicle results in an arrest of development of the contralateral optic lobe which would normally receive fibers from the retinal ganglion cells of the removed eye [12]. After this operation, a few retinal ganglion cells from the remaining eye project to the ipsilateral optic tectum. However, by the time of hatch, these aberrant fibers have disappeared [11]. Otherwise, the development of the ipsilateral optic lobe appears to be normal [12]. Abnormal morphogenesis in the contralateral lobe is first apparent shortly after the time when outgrowing fibers from the retinal ganglion cells innervate the outer layers of the tectum. Hypoplasia then becomes increasingly obvious so that at the time of hatch, the non-innervated lobe is much smaller than the partner lobe which appears to develop normally [14]. The experimental lobe has a reduced weight, DNA, RNA, and protein content [1,2]. However, a considerable amount of biochemical differentiation can occur in the concentrations of choline acetyltransferase, tyrosine hydroxylase and nerve specific protein (NSP) in the developing non-innervated optic lobe. In addition, the blood-brain barrier of the non-innervated optic lobe appears to develop normally [2]. A partial maturation of high affinity neurotransmitter uptake mechanisms in the experimental lobe also occurs [1,2].

The reduced DNA content of experimental lobes could be due to failure of tectal cell survival or to a reduced rate of cell proliferation in the affected lobe. The purpose of this study was to determine the relative contributions of these two possible causes, in bringing about the reduced cell number in the non-innervated optic lobe. Since polyploidy is uncommon in nerve tissue, the DNA content has been taken to reflect cell number.

METHOD

Surgical Removal of the Optic Cup

Fertile chick eggs of a White Leghorn strain were maintained at 38° for 48 hr in a rotating incubator, after which they were incubated in an upright position (with the blunt pole upward) for several hours preceding surgery. An opening of approximately 1 cm dia. was then cut over the air space and the embryo was exposed by peeling back the allantoic membrane. A Bausch and Lomb dissecting microscope was used to visualize the embryo. The right eye, which almost invariably faced upward, was electrocoagulated using a Malis Bipolar Coagulator (Codman Inc., Randolph, MA) at a setting of 3, attached to a sharpened bipolar forceps insulated except at the tip. The egg was then resealed with a circle of paraffin wax (m.p. 56°) upon which a flame-heated coverslip was placed. The remainder of the incubation was carried out with the egg in a vertical position. The overall mortality up to the 20th day of incubation was 63% of all operated eggs.

Injection of ^{3}H-Thymidine

Ten μl of (6–^{3}H) thymidine (15 Ci/mmole, New England Nuclear Corp.) was injected into the air space over operated embryos at various developmental stages. Ten μCi were injected in long-term experiments while 100 μCi were used for determination of synthetic rates during 1 hr. After different intervals, embryos were removed and examined to ensure that the right eye had completely failed to develop and that the left eye appeared grossly normal. Embryos with partially surviving right eyes, formed as a result of incomplete extir-

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pation of the optic vesicle, were rejected. Left and right optic lobes were dissected out, weighed and stored at −12°C.

DNA Determination

Optic lobes were homogenized in 5 ml of 0.5N $HC1O_4$ and centrifuged (20,000 g 10′) and the supernatants decanted. In the case of 1 hr labeling times, 1 ml aliquots of the supernatants were retained for assay of soluble, unincorporated radioactivity. Precipitates were then resuspended in 5 ml cold ethanol to remove lipids, and recentrifuged. The ethanol extraction step was repeated. Washed precipitates were taken up in 5 ml 0.3N KOH and incubated (2 hr, 37°C) in order to hydrolyze RNA. DNA and protein were reprecipitated by neutralization of this solution at 0°C with 12.5N $HC1O_4$ and centrifuged. Precipitates were heated in 1.3 ml 0.5N $HC1O_4$ (90° 15′) in order to hydrolyze DNA. Aliquots of the supernatant derived from centrifugation of this mixture, were counted in order to determine radioactivity within DNA. Measurements of the optical density of these solutions at 268 nm (which was invariably an absorbance maximum of supernatants) allowed the DNA content to be calculated. The absorbance of 1 mg/ml hydrolyzed calf thymus DNA at 268 nm was 32.3, when its water content was taken into consideration [2].

RESULTS

DNA Content/Lobe

At 20 days age, immediately prior to hatch, weights of experimental (non-innervated) lobes were 32% below those of control lobes while DNA content was only 14% below control values (Table 1). Thus, the cell number in experimental lobes was decreased while the cell density was increased. Innervated lobes were slightly lighter than those from unoperated embryos of the same age. This may be due to a minor developmental delay caused by surgery. At 14 days age there was a very small difference in the DNA content of wet weight of left and right optic lobes [14]. Thus, the major part of the cellular deficiency of the experimental lobe occurred after the complete innervation of the control optic tectum by the contralateral intact eye.

TABLE 1

DNA CONTENT AND WEIGHT OF OPTIC LOBES FROM CHICK EMPRYOS

	Wet Weight (mg)	DNA (μg)
20 d Embryos		
Experimental Lobe	49.3 ± 1.1*	39.9 ± 1.5*
Control Lobe	67.2 ± 1.3	46.3 ± 1.6
Lobes of Unoperated Chicks	71.2 ± 1.3	47.8 ± 1.2
14 d Embryos		
Experimental Lobe	43.0 ± 2.2	38.9 ± 2.1
Control Lobe	47.2 ± 1.7	41.3 ± 1.8
Lobes of Unoperated Chicks	47.9 ± 1.0	42.0 ± 2.2

Experimental embryos had their right optic cup removed on the third day of incubation.

Values are average ± SEM of data from between six and 10 embryos.

* $p<0.05$ that experimental values are below control values.

Rate of DNA Synthesis

A short duration of labeling (1 hr) was used to estimate rates of DNA synthesis in 14 and 20 day old embryos that had an optic vesicle removed on the third day of incubation. When overall incorporation rates were compared, they were depressed in both 14 and 20 day old embryos (Table 2). This deficit was more marked in 20 day old embryos (28%) than in 14 day embryos (15%). Similar values were apparent when data was expressed as incorporated radioactivity per mg DNA. The wet weight and soluble radioactive pool was reduced to a similar extent in experimental lobes of the 20 day old embryo. Thus, the rates of DNA synthesis expressed on the basis of tissue content or radioactive pool, were similar in experimental and control lobes.

Stability of DNA

^{3}H-thymidine was injected into operated eggs. By measuring the amount of label remaining within DNA after 5–15 days, the survival of those cells synthesized shortly after isotope administration, could be estimated.

Radioactivity within DNA was measured in 14 day embryos unilaterally enucleated at 3 days, and injected with 10 μCi ^{3}H-thymidine at 5 days incubation. No significant asymmetry between left and right optic lobes was found (Table 2). However, if embryos labeled at 5 days of age were allowed to live 20 days, the optic lobes contralateral to the removed eye had less label in DNA than the control lobes, innervated by and opposite to the intact eye (Table 2). This implied that many of the cells generated in the non-innervated lobe around Days 5 to 6 of incubation had died between 14 and 20 days.

TABLE 2

^{3}H-THYMIDINE INCORPORATION INTO DNA OF NON-INNERVATED AND CONTROL CHICK EMBRYO OPTIC LOBES

		Experimental / Control	
Age of Embryo at Death	Prior Labeling Period	Counts in DNA/Lobe	Soluble Pool/Lobe
20 d	1 h	0.72* ± 0.05	0.66* ± 0.06
	6 d	1.04 ± 0.05	—
	15 d	0.79* ± 0.03	—
14 d	1 h	0.85* ± 0.04	0.91 ± 0.05
	9 d	0.94 ± 0.06	—

The stability of labeled DNA and rate of incorporation of ^{3}H-thymidine into DNA were determined in non-innervated, experimental (E), relative to control (C) optic lobes. Chick embryos were unilaterally enucleated at 3 days incubation. Radioactivity in DNA was measured after injection of 10-100μCi ^{3}H-thymidine into each egg. Acid soluble label was also assayed in the short term anabolic studies.

Values are averages ± SEM of data from between six and 10 embryos.

* $p<0.05$ that experimental values are below control values.

Fourteen day old operated embryos were also injected with 10 μCi ^{3}H-thymidine and label incorporated into DNA was determined on Day 20. In this case the total label in DNA of the experimental optic lobes was 21% below that of the control lobes. Allowing for the 15% reduced rate of DNA

synthesis in the experimental lobes (see above), no significant excess death of cells synthesized around 14 days incubation was found in experimental lobes.

DISCUSSION

Excess cell death and a reduced mitotic rate probably both occur in the avian optic tectum if it is not allowed to receive its normal innervation from the retinal ganglion cells. Such mortality in the non-afferented lobe seems to consist largely of cells generated during the early part (first third) of incubation, rather than of elements appearing later in embryogenesis. Since neurogenesis is active during early ontogeny while gliogenesis occurs later, most of the cells that did not survive were probably neurons. This supports the finding that neurons failing to complete normal circuitry in the chick visual system die during the last week of incubation [11]. However, death of many tectal neuroblasts occurs at 10–11 days incubation [9], a time when all mitotic activity is unaffected by early enucleation [13]. This early neuroblast death appears unrelated to peripheral input from optic fibers or to failure of neuronal connectivity. Excess neuronal cell death has been clearly shown in the ecto-mammillary nucleus, following prevention of the development of its major innervation from the retinal ganglion cells [14].

Glial cell proliferation in the optic tectum may be less severely affected by prevention of innervation. Thus, parameters of neuronal development (e.g., increased levels of acetylcholinesterase, choline acetyltransferase and the appearance of high affinity transport mechanisms) of several putative neurotransmitters are more dramatically impaired than are indices of glial maturation (e.g., levels of nonspecific cholinesterase) [2, 7, 8, 15].

The growth of the non-innervated optic lobe can be contrasted with the effect of denervation subsequent to the establishment of functional connections between the eye and the optic lobe. In this latter case, tectal cell survival is unimpaired for a considerable time [5,10] while cell growth and the maturation of glial-related processes such as myelination are virtually arrested [3,4].

The DNA content of the 20 day old non-afferented optic lobe is 14% below that of the paried control lobe, while the number of cells synthesized in the experimental lobe around Day 6 of incubation that survive to the 20th day of incubation, is 21% below the control value. It may be that excess neuronal cell death could be sufficient to account for the difference in DNA content of experimental and control optic lobes at hatch. However, the reduced rate of DNA synthesis seen in the non-afferented optic lobe may also play a role. Although major mitotic activity within the optic tectum is over at 11 days of age [13], the DNA content of normal chick optic lobes rises a further 13% between 14 days incubation and hatch (this paper, [17]).

The reduced DNA content of the non-innervated optic lobe probably results from two processes: a heightened extent of neuronal cell death and a reduced rate of gliogenesis. As the difference in size between experimental and control lobes becomes marked, the decreasing proportion of nutrients received by the smaller lobe may exacerbate the difference in overall rates of DNA synthesis between experimental and control regions. A similar decrease in the protein synthetic capacity of the non-innervated 18 day old chick embryo optic tectum [16] may also be related to the growth failure and reduced blood supply [1].

There is evidence that neuronal cell death may result from lack of critical trophic supply from innervating neurons [9]. A continuing interdependence between neurons and targets and between neurons and glia seems to characterize cerebral maturation. Such reciprocity may also exist between brain tissue and the developing vascular bed. It may be that the depressed glial proliferation of the non-innervated optic tectum represents a secondary trophically or nutritionally mediated adjustment to the reduced number of neurons.

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