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Effect of Unilateral Enucleation on Protein and Ribonucleic Acid Metabolism of Avian Brain

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The metabolic activity of brain regions at various times after unilateral eye removal has been studied in the chick where the visual pathways are totally crossed over. Protein and ribonucleic acid from cerebral hemispheres and optic lobes were assayed for radioactivity following subcutaneous injection of tritiated leucine or uridine. Cerebral hemispheres and optic lobes contralateral to the extirpated eye showed a reduction of protein and RNA synthetic rate relative to the paired ipsilateral regions. Some of these differential effects were observed in animals as early as 1 hr after enucleation and persisted during the ensuing 17 days. Results were of similar magnitude both in the optic lobes which are directly innervated by the optic nerve and in the cerebral hemispheres which receive little or no direct innervation from the optic tract. The decay rates of protein or ribonucleic acid were unaltered in the paired regions from unilaterally enucleated birds. The results suggest that rapid metabolic alterations subsequent to eye removal are not confined to directly denervated regions. A significant fall in the rate of RNA synthesis is the earliest effect noted; subsequent effects may thus be a reflection of alterations of RNA transcription at the genetic level.

Introduction

In previous reports (4, 13) we described an experimental system facilitating the study of the effects of sensory deprivation on the brain. This system utilized the fact that avian optic tracts are completely crossed over (7). Furthermore, since a structure corresponding to the mammalian corpus callosum is absent, there is a low degree of intrahemispheric communication, especially in young birds (6). Thus, unilateral manipulation of visual input resulted in differential effects in the two halves of the brain.

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Effects observed were directly due to experimental variation of sensory input and not to secondary systemic or hormonal changes which affected both experimental and control halves of the brain equally.

After unilateral enucleation of the chick, the contralateral optic lobe failed to increase in weight, protein, or ribonucleic acid. Additionally, development of a specific cerebral enzyme (acetylcholinesterase) was retarded. The contralateral cerebral hemisphere in unilaterally enucleated, light-maintained chicks also exhibited a similar but lesser impairment of development.

The studies reported here were directed to determining the underlying metabolic causes of this impaired development by examination of the rates of protein and ribonucleic acid synthesis and decay. Subcutaneous injection of labeled compounds permitted rapid symmetrical access of protein and ribonucleic acid precursors to both halves of the chick brain. Rapid and pronounced changes in patterns of macromolecule synthesis follow unilateral enucleation. These precede and may eventually result in the gross differences of composition reported previously.

Methods

Fertile eggs from white Leghorn, strain K137 (Kimber Farms, Pomona, California), were incubated in forced-draft incubator at 37.5C until hatch. The newly hatched chicks were maintained in a brooder cabinet. Within 24 hours of hatch, either the left or right eye was removed from each chick under halothane anesthesia. Mortality was less than 5%. Animals recovered from anesthesia within 10 min and were returned to the brooder with free access to food and water.

At various intervals after enucleation, labeled precursors were injected subcutaneously. Half the chicks received 50–100 μ c of 4, 5-(3 H)-leucine (19.7 c/mmole) and the other half 250–500 μ c of 5-(3 H)-uridine (21.7 c/mmole). One hour after injection of leucine or 1½ hours after injection of uridine, chicks were decapitated, and brains dissected into individual optic lobes and cerebral hemispheres (12), weighed, and frozen. In the case of long-term decay studies, precursor was injected 1 hour after enucleation and chicks killed 17 days later.

The concentration and specific activity of protein and RNA were assayed in the brain regions. In addition, the amount of unincorporated radioactivity in each region was evaluated as a measure of the precursor pool.

Protein Assay. Brain regions from the 3 H-leucine-injected chicks were homogenized in 5 ml cold 15% trichloroacetic acid and centrifuged at 0C at 600g for 10 min. The clear supernatant constituted the acid-soluble precursor pool and was retained. The precipitate was resuspended in 5 ml 15% trichloroacetic acid, heated to 90C for 10 min to hydrolyze nucleic

acids and any amino-acyl transfer RNA present, then cooled, and re-centrifuged. This precipitate was washed twice more with 5 ml cold 15% trichloroacetic acid, and the final precipitates from optic lobes or cerebral hemispheres were dissolved in 2.5 or 10 ml, respectively, of 1 N NaOH at 40C. Samples of this solution were taken for determination of protein (11) and of protein-bound radioactivity after neutralization with Beckman Bio-Solv solubilizer No. 2. Radioactivity in 0.5 ml of the acid-soluble precursor pool was assayed by adding 10 ml standard scintillation solution. Protein-bound radioactivity in 2 ml of the neutralized protein solution was counted by adding 15 ml of standard scintillation solution. Standard scintillation solution consisted of a 2:10 (v/v) solution of Beckman Bio-Solv solubilizer No. 3 and toluene, containing 0.4% 2-phenyl-5-(biphenyl-2-yl)-1, 3, 4 oxadiazole and 0.008% 1-4 bis(5-phenylxazole-2-yl) benzene. Radioactivity was measured in a Packard Tri-Carb scintillation spectrometer at an efficiency of 28%. The counting rate of all protein and nucleic acid samples was above 500 count/min.; instrument background was 18 count/min.

The extent of conversion of injected ^3H -leucine to other compounds during the 1-hour labeling period was determined in the acid-soluble pool with a Beckman Spinco Model 120 amino acid analyser (14).

Ribonucleic Acid Assay. Brain regions from chicks injected with ^3H -uridine were homogenized in 5 ml cold 5% trichloroacetic acid and centrifuged at 0C at 600g for 10 min. The supernatant constituted the acid-soluble nucleotide precursor pool and 1-ml portions were counted in 10 ml standard scintillation solution. The precipitates were washed at 0C successively in 5% trichloroacetic acid (twice), ethanol (twice), and once with ether. The final precipitates were suspended in 1.25 ml 0.3N KOH for 2 hours at 37C. This solution was then neutralized with 70% perchloric acid and the resulting protein and KClO centrifuged down at 0C. Aliquots of the supernatants were taken for colorimetric assay of RNA (5), and 0.5 ml portions were added to 10 ml of standard scintillation solution for radioactive assay as described in the protein section.

Statistics. At least five chicks were used for each experimental point reported, each chick constituting a set of data. Probability of these internally paired sets of data was calculated by Students' one-tailed *t* test with $P < 0.05$ taken to be significant.

Results

Birds were injected with radioactive precursors at 1 hour, 1 day, and 17 days after unilateral enucleation. Optic lobes and cerebral hemispheres contralateral to the excised eye are referred to as the blind regions, while those areas contralateral to the unoperated eye are referred to as the visual regions. Radioactivity data have been presented as the ratio Visual/Blind

(V/B) and are the averages of values calculated for each individual bird and not from the ratio of the final pooled averages.

Protein Metabolism. To evaluate differential rates of protein synthesis in blind and visual regions of enucleated chicks, V/B for the specific activity of protein and for the concentration of unincorporated precursor was examined in these regions 1 hour after injection of ^3H -leucine (Table 1). Relative concentrations of the free precursor pool were not significantly different in visual and blind regions of chicks enucleated at the time intervals studied.

Specific activity of free leucine in brain regions of enucleated chicks was examined in an amino acid analyzer equipped with a radioactive flow detector. One hour after administration of precursor, 39–55% of the labeled leucine had been converted to glutamic acid and glutamine. The specific activity of glutamine was always higher than that of glutamate indicating compartmentation was occurring early in the developing chick brain. Blind and visual regions exhibited similar degrees of conversion and specific activities of free leucine. In contrast, the specific activity of protein was significantly higher in visual optic lobes and cerebral hemispheres of chicks injected 1 and 17 days after enucleation. The concentration of protein per milligram of tissue was identical in visual and blind regions 1 hour and 1 day after enucleation (Table 2); therefore, the elevated specific activity of protein in visual regions 1 day after eye extirpation indicated a higher rate of protein synthesis. Similarly, at 17 days the higher specific activity of protein in visual cerebral hemispheres relative to the corresponding blind hemispheres was due to a higher rate of protein synthesis.

TABLE 1

INCORPORATION OF ^3H -LEUCINE INTO PROTEIN OF BRAIN REGIONS AT VARIOUS TIMES AFTER UNILATERAL ENUCLEATION OF NEWLY HATCHED CHICKS

	cpm/mg Protein		
	1 Hour	1 Day	17 Days
Optic lobes	1.04 $\pm$ 0.02	1.07 $\pm$ 0.03 ^b	1.07 $\pm$ 0.01 ^b
Cerebral hemi.	1.00 $\pm$ 0.02	1.04 $\pm$ 0.02 ^b	1.09 $\pm$ 0.06 ^b
	cpm Pool/mg wet wt tissue		
Optic lobes	1.02 $\pm$ 0.02	0.99 $\pm$ 0.02	0.99 $\pm$ 0.01
Cerebral hemi.	1.03 $\pm$ 0.02	1.00 $\pm$ 0.01	0.99 $\pm$ 0.02

^a The size of the radioactive precursor pool and specific activities of protein were determined 1 hour after precursor injection; results are expressed as the mean ratio of values in paired visual and blind regions, V/B, from five or more birds.

^b $P < 0.05$.

TABLE 2
 PROTEIN AND RNA CONTENT OF CHICK BRAIN REGIONS
 FOLLOWING UNILATERAL ENUCLEATION

Time after enucleation	Optic lobes			Cerebral hemi.		
	Visual	Blind	V/B	Visual	Blind	V/B
Protein ($\mu\text{g}/\text{mg}$ tissue)						
2 hr	62.2	60.3	1.03 $\pm$ 0.04	71.2	71.8	0.99 $\pm$ 0.02
1 day	68	67	1.02 $\pm$ 0.02	65	69	0.95 $\pm$ 0.03
17 days	79	84	0.94 ^a $\pm$ 0.01	75	76	0.99 $\pm$ 0.02
RNA ($\mu\text{g}/\text{mg}$ tissue)						
2.5 hr	2.72	2.72	1.00 $\pm$ 0.01	3.27	3.27	1.00 $\pm$ 0.02
1 day	1.96	1.90	1.03 ^a $\pm$ 0.01	1.81	1.84	0.98 $\pm$ 0.02
17 days	1.46	1.58	0.92 ^a $\pm$ 0.03	1.11	1.14	0.97 ^a $\pm$ 0.004

^a $P < 0.05$.

The specific activities of protein and RNA in brain regions of chicks 17 days after unilateral enucleation and precursor injection are shown in Table 3. Isotopes were administered 1 hour after enucleation, when the rates of protein synthesis in blind and visual regions were not significantly different (Table 1). After 17 days no difference was noted in protein specific activity between blind and visual regions. However, the blind optic lobes were considerably smaller and contained less total protein than the corresponding visual lobes (4). Thus, although enucleation results in a reduced rate of protein synthesis in the blind (contralateral) brain regions, it would appear that the rate of protein decay is similar in both halves of the brain.

Ribonucleic Acid Metabolism. RNA metabolism of blind and visual regions of unilaterally enucleated chicks was studied in experiments paralleling those concerned with protein metabolism; V/B for specific activity of RNA and the size of the precursor pools in cerebral regions of enucleated chicks 90 min after injection of ^3H -uridine were determined (Table 4). No significant difference existed in the radioactive precursor pool of visual and blind cerebral hemispheres at any time, or in optic lobes 1 hour after enucleation. However, visual optic lobes had a more highly labeled pool in chicks 1 and 17 days after enucleation.

The specific activity of RNA was significantly higher in all visual regions than in corresponding blind regions. This was especially prominent in the optic lobes 1 and 17 days after enucleation, where the RNA from visual lobes had specific activities over 20% greater than those from the blind lobes. These differences are partially due to increased radioactivity in the precursor pool of visual lobes. Furthermore, in the case of the 17-day-old

TABLE 3

RADIOACTIVITY REMAINING IN PROTEIN AND RNA OF BRAIN REGIONS FROM ENUCLEATED CHICKS, 17 DAYS AFTER A SINGLE INJECTION OF LABELED PRECURSORS^a

	Optic lobes			Cerebral hemi.		
	Visual	Blind	V/B	Visual	Blind	V/B
Protein (cpm/mg)	887	874	1.02 ± .02	779	762	1.03 ± .04
RNA (cpm/μg)	390	349	1.12 ^b ± .03	481	429	1.10 ± .07

^a Chicks were enucleated within 24 hours after hatch and 1 hour later received a subcutaneous injection of 0.05 mc ³H-leucine or 0.5 mc ³H-uridine for protein and RNA determinations, respectively. Chicks were subsequently killed at 17 days.

^b $P < 0.05$.

chicks, the significant reduction of RNA concentration in the visual regions (Table 2) could have accounted for part of the higher specific activity of RNA from the visual lobes over that of blind lobes. However, neither of these two factors were of sufficient magnitude or extent to account for the consistently higher specific activity of RNA from all visual regions. Therefore, these results indicated a higher rate of synthesis in the visual regions of the enucleated chicks as was the case with protein metabolism. The rapid fall in the rate of RNA synthesis in the blind optic lobes may account for the significant difference in total RNA concentration found between the blind and visual lobes as early as 1 day after unilateral enucleation. The relatively reduced concentration of RNA and protein in the visual areas

TABLE 4

INCORPORATION OF ³H-URIDINE INTO RNA OF CHICK BRAIN REGIONS AT VARIOUS TIMES AFTER UNILATERAL ENUCLEATION^a

	1 Hour	cpm/mg RNA	
		1 Day	17 Days
Optic lobes	1.05 ± 0.01 ^b	1.22 ± 0.02 ^b	1.25 ± 0.03 ^b
Cerebral hemi	1.04 ± 0.02 ^b	1.08 ± 0.02 ^b	1.06 ± 0.01 ^b
		cpm Pool/mg wet wt. tissue	
Optic lobes	1.01 ± 0.01	1.09 ± 0.01 ^b	1.07 ± 0.02 ^b
Cerebral hemi	1.02 ± 0.01	1.01 ± 0.01	1.02 ± 0.01

^a The size of the radioactive precursor pool and specific activities of RNA were determined 1 and 1.5 hours after injection of precursor; results are expressed as the mean ratio of values in paired visual and blind regions, V/B, from five or more birds.

^b $P < 0.05$.

of the chick 17 days after enucleation may be a consequence of retarded or arrested developmental increase of cell volume in blind regions.

Long-term studies on the rate of RNA decay were carried out by injection of 1-day-old chicks with ^3H -uridine 1 hour after enucleation. After 17 days, RNA specific activity was found to be higher in visual regions than in the corresponding blind regions (Table 3). However, this difference was only significant in the case of optic lobes and could reasonably be attributed to the significantly higher rate of RNA synthesis in visual areas at the time of precursor injection. Thus, as with the protein data, this indicated that the rate of RNA decay was similar in both visual and blind regions, although RNA synthesis was more rapid in the visual regions.

Discussion

Considerable metabolic differences have been demonstrated between two symmetrically equivalent regions within single animals following unilateral eye removal. These differences appeared rapidly after enucleation and persisted throughout the period of brain maturation studied. These effects were not confined solely to the optic lobes (the primary site of termination of the optic nerve) but were also found in the cerebral hemispheres which receive very few direct connections from the optic nerve (7).

Decreased rates of incorporation of precursors into protein and RNA were observed in regions contralateral to the excised eye. The major part of this decrease was due to differential rates of macromolecule synthesis. In the case of the chick optic lobe, 1 day after enucleation the radioactivity of the precursor pool in the blind lobes was depressed relative to the corresponding visual lobes. This partially accounted for the higher specific activity of RNA from the visual lobe. These concurrent differential effects on both RNA synthetic rate and precursor pool size may be analogous to effects found in tissues resulting from hormonal activation. Estrogen administration has been reported to increase the rates of uterine uptake of RNA precursor and RNA synthesis (2, 3). Thus, denervation of a cerebral region may result in the cessation of supply by axon flow, of a neurohumor essential for complete nervous development and differentiation. An equivalent difference in precursor pool size was not found in parallel experiments with ^3H -leucine. Thus, there was a relatively higher uptake of uridine by the visual lobes rather than a general difference in permeability between the visual and blind lobes. Interestingly, increased blood flow through the mammalian visual cortex has been observed after retinal illumination (15).

Our previous studies (4) have shown that failure of optic lobe development following enucleation is largely due to cessation of cell growth

rather than any marked change in cell number in the denervated lobe. Therefore, most of the higher synthetic rate of the visual lobe contributed to cell growth or to an increased concentration of cellular components rather than to division. The concentration of protein and RNA per milligram wet weight of tissue was actually lower in visual than blind lobes 17 days after enucleation. Long-term studies on decay rates of labeled macromolecules provided evidence that this higher synthetic rate in the visual regions was not related to a more rapid turnover of protein or RNA. Similar decay rates were shown for these molecules from blind and visual regions, suggesting that their half-lives were of similar magnitude in both regions.

Significant differences in rates of RNA synthesis in visual and blind regions appeared rapidly following unilateral enucleation. Similar effects on protein synthetic rates appeared later. This delay indicated that the primary effect of denervation may have been on the cerebral genome and thus on RNA output. This modified RNA output could then have resulted in differential rates of protein synthesis at the polysome level. Increased rates of protein synthesis were observed in polysomes prepared from light rather than dark-maintained rats (1). Changes in RNA output may be qualitative as well as quantitative and thus alter the types as well as amounts of protein being synthesized.

There is evidence that afferent nerves can exert direct control over the RNA and protein metabolism of muscle (8, 9). In this case also, rates of RNA synthesis are initially the most responsive to denervation. We have not observed transient metabolic increases in denervated regions as reported in studies of the concentration of RNA and the rate of protein synthesis *in vitro* in the lateral geniculate nucleus of monkeys following optic nerve section (10). The early dynamic metabolic differences in the optic lobes reported here lead initially to failure of normal growth and development and ultimately to degeneration. In the case of cerebral hemispheres, growth is only slightly reduced, and no major degenerative effects have been detected. However, a continuing significant deficit in metabolic activity may ultimately lead to an impairment of optimal cerebral functioning. Because of the widespread effects of unilateral enucleation in the avian brain, nonvisual integrative functions of the cerebral cortex may also become involved in the impairment.

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