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Effect of Unilateral Visual Deprivation by Eyelid Suturing on Protein and Ribonucleic Acid Metabolism of Avian Brain

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Rates of protein and ribonucleic acid (RNA) synthesis in brain regions have been examined following suturing of the eyelids of one eye immediately after hatching. Although capable of receiving diffuse light, this eye could not be used effectively. Tritiated precursors were injected subcutaneously, and the extent of incorporation of radioactivity into macromolecules derived from symmetrically paired cerebral hemispheres and optic lobes was determined. One day after suture both the lobe and the hemisphere contralateral to the occluded eye manifested a reduced rate of RNA synthesis relative to the corresponding ipsilateral region. This effect persisted for over 17 days. Parallel long-term differences in protein synthetic rates or concentrations were not observed. This suggests that the major effect of varying RNA production was to modify the type of proteins synthesized rather than their amounts. The use of paired internal controls removed the possibility that asymmetric effects were due to nonexperimentally controlled variables such as differences in systemic hormone levels. The results indicate that modification of the information content of visual input to the brain can result in widespread cerebral metabolic differences.

Introduction

The preceding paper reported reduced rates of protein and ribonucleic acid synthesis in cerebral regions of the chick, following extirpation of a single eye (3). We extended these finding by examining the effect of monocular suture upon the metabolic differential in cerebral regions in an otherwise identical experimental system. Monocular suture involves less trauma than enucleation and has been shown to result in long-term

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asymmetrical changes in avian brain composition. These changes differed both quantitatively and qualitatively from those resulting from eye extirpation (4).

After subcutaneous injection of radioactive protein and ribonucleic acid precursors, we have found that suture of the eyelids of chicks rapidly resulted in a reduced rate of ribonucleic acid synthesis in the cerebral regions contralateral to the sutured eye. This effect was widespread and persisted throughout the period studied.

Methods

Eggs from white Leghorn, strain K137 (Kimber Farms, Pomona, California), were incubated in a forced-draft incubator at 37.5C and newly hatched chicks maintained in a brooder. Within 24 hours of hatch, chicks were anesthetized with an intramuscular injection of 1 mg Nembutal (Abbott). Eyelids of either the left or right eye were sutured with Nylon monofilament and then painted with a collodion film to insure that the eyelids remained sealed. Animals were then returned to the brooder with free access to food and water and maintained in a lighted room.

At 1 or 17 days after suture, half the chicks received 50–100μc of 4,5-(^{3}H)-leucine (19.7 c/mmole) and the remainder 500μc of 5-(^{3}H)-uridine 21.7 c/mmole) by subcutaneous injection. Chicks were killed 1 or 1.5 hours after injection of leucine or uridine, respectively, and optic lobes and cerebral hemispheres dissected out. The content and specific activities of protein and RNA and also the amounts of unincorporated precursors in each region were determined as previously described (3).

Optic lobes and cerebral hemispheres contralateral to the sutured eye are referred to as occluded regions while the ipsilateral areas are referred to as normal. Data are presented as the ratio Normal/Occluded (N/O). Averages are calculated for each individual bird and not derived from ratios of final pooled averages. Five chicks were used for each experimental point reported. Probability of internally paired sets of data was calculated by Students' one-tailed t test with $P<0.05$ taken as significant.

Results

One hour after administration of ^{3}H-leucine to birds 1 or 17 days after eyelid suture the specific activity of protein from paired normal and occluded optic lobes was not significantly different (Table 1). However, the specific activity of protein from the normal cerebral hemisphere was significantly higher than that in the corresponding occluded hemisphere of chicks 1 day after monocular suture. This difference could not be attributed to variations in size of the radioactive precursor pool which

was similar in occluded and normal brain regions at 1 or 17 days after suture. A reduced rate of protein synthesis in the occluded cerebral hemispheres was implied. Unlike the effect of enucleation on cerebral protein synthesis (2), this effect was transient, disappearing 17 days after suture and was not observed in the optic lobes. Ninety minutes after administration of ^{3}H-uridine, the specific activity of RNA of the occluded lobes and hemispheres was markedly depressed relative to the corresponding normal areas both 1 and 17 days after suture (Table 2). Only the occluded optic lobes 1 day after suture showed any significant relative depression of precursor pool size. This was not of sufficient magnitude to account for the lower specific activity of RNA. Thus, the rate of RNA synthesis of occluded lobes and hemispheres was reduced within 1 day after suture in comparison with the respective normal areas. This effect was of similar size in both areas and persisted for at least 17 days.

The relative concentrations of protein in cerebral regions of either 1- or 17-day-old sutured birds were unchanged. However, RNA concentrations in the occluded optic lobes of 17-day sutured birds were significantly less than that of the normal lobes (Table 3). This was in marked contrast to the elevated RNA concentration in the contralateral optic lobes 17 days after unilateral eye extirpation (3). Both procedures result in a relative reduction of the rate of RNA synthesis in the optic lobe contralateral to the treated eye. Eye removal severely retards the growth of cells of the contralateral optic lobe, while eyelid suture has no gross effect (4). This may account for the different results from the two procedures.

TABLE 1

INCORPORATION OF ^{3}H-LEUCINE INTO PROTEIN OF CHICK BRAIN REGIONS AFTER MONOCULAR SUTURE[a]

	cpm/mg Protein	
	1 Day	17 Days
Optic lobes	0.99 ± 0.03	1.00 ± 0.01
Cerebral hemi.	1.07 ± 0.03[b]	1.01 ± 0.02
	cpm Pool/mg wet wt tissue	
Optic lobes	0.99 ± 0.07	0.94 ± 0.05
Cerebral hemi.	0.92 ± 0.03	1.00 ± 0.02

[a] Specific activities of protein were determined 1 hour after injection of precursor; results are expressed as the mean ratio of values in paired normal and occluded regions, N/O, from five or more birds.

[b] $P < 0.05$.

TABLE 2

INCORPORATION OF ^{3}H-URIDINE INTO RNA OF CHICK BRAIN REGIONS AFTER MONOCULAR SUTURE[a]

	cpm/mg RNA	
	1 Day	17 Days
Optic lobes	1.05 ± 0.02[b]	1.10 ± 0.03[b]
Cerebral hemi.	1.06 ± 0.01[b]	1.07 ± 0.02[b]
	cpm Pool/mg wet wt tissue	
Optic lobes	1.02 ± 0.02	0.99 ± 0.02
Cerebral hemi.	1.03 ± 0.01[b]	1.03 ± 0.01[b]

[a] One and one-half hours after injection of precursor, the size of the radioactive precursor pool and specific activities of RNA were determined; results are expressed as the mean ratio of values in paired normal and occluded regions, N/O, from five or more birds.

[b] $P < 0.05$.

Discussion

Eyelid suture, unlike enucleation, does not involve nerve section or serious surgical trauma. The eyelids are translucent, and thus the eye continues to receive photic stimulation. Therefore, the possibility of any observed effects being due to early stages of transneuronal degeneration is minimized, particularly in the cerebral hemispheres which receive little or no direct innervation from the eye (5). Visual stimulation received from the sutured eye is very low in meaningful information content. This deficit may be the cause of the differential effects observed. Eyelid suture affects the size of the contralateral cerebral hemisphere and its

TABLE 3

PROTEIN AND RNA CONTENT OF CHICK BRAIN REGIONS FOLLOWING MONOCULAR SUTURE

Time after suture (days)	Optic lobe			Cerebral hemi.		
	Normal	Occluded	N/O	Normal	Occluded	N/O
Protein (μg/mg tissue)						
1	63.1	63.7	0.99 ± 0.01	70.4	72.3	0.98 ± 0.02
17	73.3	73.9	1.01 ± 0.03	79.4	78.5	1.01 ± 0.01
RNA (μg/mg tissue)						
1	2.64	2.65	1.00 ± 0.03	3.08	3.07	1.00 ± 0.01
17	2.22	2.15	1.03[a] ± 0.01	2.38	2.40	0.99 ± 0.01

[a] $P < 0.05$.

rate of RNA synthesis. These effects are of similar magnitude to those seen following enucleation. In contrast to the effects of enucleation on optic lobes, asymmetric effects of suture on growth of the occluded cerebral hemispheres have been shown to be totally light dependent (4). Furthermore, the reduced RNA synthetic rate of the occluded cerebral hemispheres is not paralleled by permanent changes in the rate of protein synthesis or by changes in the concentration of protein, as is the case with the optic lobes following unilateral enucleation (3, 4). The altered synthetic rate may be a reflection of the failure of new RNA species to appear that would otherwise be induced by the complete integrative functioning of the brain. Increased RNA synthesis has been correlated with higher cerebral function such as learning (9), and this increase has been associated with qualitative changes in RNA production (6), which may imply the ultimate production of new protein species.

Visual stimulation has been reported to have effects on occipital cortical areas, leading to an increased rate of protein synthesis (1, 7) and to the appearance of new protein species (8). More complex phenomena which are mediated by the visual system, such as imprinting, may produce effects not confined to the optic centers (2). Our monocular suture system produces two brain halves in the experimental animal, one of which receives and then utilizes patterned information for higher associative cerebral functioning to a much greater extent than the other. The widely distributed differential metabolic effects reported here cannot be due to peripheral hormonal influences, since both brain halves are subject to an identical systemic humoral environment. Therefore, these results demonstrate the need for both complex information input and integration in order to develop optimal cerebral capacity. The magnitude of effects on complex behavioral parameters may be much greater than the extent of reduced cerebral metabolism reported here.

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