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EFFECT OF LIGHT ON THE LABELING OF OPTIC TECTUM GANGLIOSIDES AFTER AN INTRAOCULAR INJECTION OF N-[^{3}H]ACETYLMANNOSAMINE

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Summary. Axonally transported gangliosides from retina were more labeled in the optic tectum of chickens exposed to light compared to those maintained in the dark. No differences were observed between the labeling of retinal gangliosides from the two groups. These results indicate that light modifies either the labeling of ganglion cell gangliosides or their axonal transport.

Introduction. Exposure to light of chickens that received in the dark a subcutaneous injection of labeled glucosamine brings about changes in the labeling of their retinal and brain gangliosides (1, 2). The changes in brain and retina are in opposite directions. Two hours after the injection, the brain gangliosides of the exposed animals increased whereas the retinal gangliosides decreased their labeling compared with their counterparts that remained in the dark. The opposite behaviors of the retinal and brain gangliosides are reexamined in the present report to take into consideration recently acquired information on the subcellular site of ganglioside synthesis. In the goldfish and other animals, gangliosides synthesized in retina move by axonal transport to the contralateral tectum (3, 4). It was also shown that most of the gangliosides in rat brain and in the ganglion cell nerve endings in the chicken optic tectum are synthesized in the neuronal soma, in agreement with the finding that the pertinent glycosyl transferases are not localized in the synaptosomal fraction but presumably in smooth microsomes and Golgi membranes (5, 6, 7, 8). According to this information, in chickens,

Abbreviations: D-N [^{3}H] acetylmannosamine, [^{3}H] ManNAc; N-[^{3}H] acetylneuraminic acid, [^{3}H] NeuAc; trichloroacetic acid, TCA; phosphotungstic acid, PTA.

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in which the optic nerve fibers cross at the optic chiasma (9), the difference in labeling between the gangliosides of the contralateral and ipsilateral optic tecta after inoculation of labeled precursor into a single eye, should reflect the gangliosides synthesized in retina and carried to the contralateral tectum by axonal transport.

Materials and Methods. Cobb Hardig Concord or Cobb Hardig sex linked chickens (white and black varieties respectively) were used in different experiments. From hatching to the eighth day of age they were exposed to cycles of illumination and darkness of 12 h each and then put in the dark for 48 h. Ten microliters of [^{3}H]ManNAc (specific activity 300 µCi/µmol; 3 µCi/µl) prepared from mannosamine and [^{3}H] acetic anhydride (10) was injected into one eye (usually the right eye) and the animals were alternatively included in a group that remained in the dark, or into another that was exposed to light (1000 lux). Animals from each group were decapitated at different times and their retina and optic tecta were dissected.

Each retina was washed with 2 ml of 0.25 M sucrose and homogenized by sonication in a Branson Model B12 sonifier in 0.01 M phosphate buffer, pH 7.4. Tissues from animals in the dark were obtained under the light of a red photographic safety lamp and maintained in tubes covered with black tape. Samples were taken to measure protein content by Lowry's method and the remaining homogenate was centrifuged at 100000xg for 30 min. Radioactivity in [^{3}H]ManNAc and [^{3}H]NeuAc was determined in the supernate. Labeling of [^{3}H]ManNAc was quantified after passing a sample of the supernate through a column (0.5 x 3 cm) of Dowex 1 - x8, formate form, in the unretained fraction. At 5 h after injection about 80% of the radioactivity in this fraction co-cromatographied with authentic N-acetylmannosamine. Labeling of [^{3}H] NeuAc was determined after treatment of another sample of the supernate with NeuAc-aldolase (Sigma Chemical Co.) for 15 h in a toluene atmosphere and passage through Dowex 1. The difference between the radioactivity found in [^{3}H]ManNAc after and before the treatment with NeuAc-aldolase gives the radioactivity of NeuAc in the supernate (11). Retinal pellets were resuspended and precipitated with TCA 5 % (w/v) - PTA 0.5 % (w/v) and the pellets were washed twice with 5 ml of TCA 5%- PTA 0.5 % and twice with 5 ml of water to eliminate any soluble contaminant (12). Gangliosides were extracted and purified from the pellet by the method of Folch (13). Contra- and ipsilateral tecta were homogenized in 2 ml of 0.01 M phosphate buffer, pH 7.4; a sample was taken for determination of protein content and TCA 5% (w/v) - PTA 0.5 % (w/v) was added (12). Acid soluble radioactivity from tecta was measured in dried samples of its TCA-PTA supernatant fraction. Contra- and ipsilateral tecta gangliosides were obtained as described for retina.

Results and Discussion. Retinas were analyzed for the radioactivity in the total acid soluble fraction, [^{3}H]ManNAc and [^{3}H]NeuAc. The total acid soluble and the [^{3}H]ManNAc labelings were presumably of extra and intracellular origins whereas the [^{3}H]NeuAc found was con - sidered an expression of intracellular activity. No difference was found between the retinas in dark and in light in the cpm per mg of protein for any of these determinations in a period in which their

Table I. Labeling of NeuAc and gangliosides from the retina of chickens injected intraocularly with [^{3}H] ManNAc

	R E T I N A			
	NeuNAc cpm/mg protein x 10^{-3}		Gangliosides cpm/mg protein	
Time (hours)	Dark	Light	Dark	Light
	EXPERIMENT 1			
5	-	-	(11) 2306 ± 533	(11) 3271 ± 509
	EXPERIMENT 2			
1	n.d.	n.d.	(2) 691 ± 246	(2) 557 ± 87
3	(3) 38 ± 7	(3) 37 ± 11	(3) 3274 ± 802	(3) 2703 ± 815
5	(3) 62 ± 7	(3) 55 ± 9	(3) 5626 ± 558	(3) 5604 ± 494
8	(3) 102 ± 28	(3) 110 ± 28	(6) 12853 ± 1843	(6) 18323 ± 3207
	EXPERIMENT 3			
1	n.d.	n.d.	(8) 587 ± 42	(10) 543 ± 71
3	(3) 27 ± 3	(3) 28 ± 5	(7) 3716 ± 442	(8) 3865 ± 142
5	(3) 32 ± 3	(3) 28 ± 4	(7) 5860 ± 421	(8) 6704 ± 447

Figures are means ± S.E.M.; figures in parenthesis are number of animals; n.d. non detectable.

values were increasing (see Table I, exp. 2 and 3. The radioactivity values for total acid soluble fractions and [^{3}H] ManNAc are not shown), indicating that light does not modify the rate of entrance or synthesis of ganglioside precursors in retina when the precursor is given intraocularly.

No difference was found between the ganglioside labeling per mg of protein of the two groups of retinas.In previous experiments in which radioactive glucosamine was injected subcutaneously the labeling of retinal gangliosides was decreased in animals exposed to light compared to those in dark when calculated per mg of protein or the cpm of the acid soluble fraction of the retina (1). We have no explanation for the different results, which may be due to the variations of the radioactive precursor and the route of injection.

Attempts to determine in tecta the radioactivity of the ganglioside precursors that originate in [^{3}H] ManNAc were unsuccessful due

to the insufficient radioactivity of the fractions (for the method of fractionation see Materials and Methods). We measured instead the radioactivity in the dry residue of the material that remained soluble after treatment of the homogenate with TCA-PTA which precipitates proteins and glycolipids (12). Since in the chicken the ipsilateral tectum does not receive innervation from the inoculated eye any difference between the labeling of the contralateral and ipsilateral tecta could only be due to material that arrived to the contralateral tectum by axonal transport. The labeling of the dry acid soluble material showed no significant difference between the contralateral and ipsilateral tecta at 3 and 5 h (Table II). At 8 h a difference apparently appeared which because of the delay was interpreted as reflecting degradation of higher molecular weight compounds. Consequently, no evidences of axonal transport of ganglioside precursors were found in these experiments (for an alternative conclusion by others in earlier works, see 14,15). Furthermore, exposure of chickens to light did not influence the systemic transport of precursors from the eye to the tecta, as measured in the acid soluble fraction of the ipsilateral tecta from animals in dark and light.

The ganglioside fraction in the tectum contralateral to the eye that received the injection of labeled precursor was significantly more labeled in the animals exposed to light than in those maintained in the dark (Table II). Discounting the labeling of gangliosides in the ipsilateral tectum the differences in labeling between the gangliosides from chickens in light and dark amounted to 60 and 44 per cent, at 3 and 8 h post injection, respectively. The appearance of more labeled gangliosides in the tectum from illuminated animals after an intraocular injection of radioactive precursors suggests either that an effect of light on the ganglion cells of the optic system is to increase the axonal transport of their gangliosides to the tectum or that light enhances the labeling of ganglion cell gangliosides. Since the radioactive gangliosides of a retinal homogenate are in a pool that originates from a variety of cells that cannot be separated by current methods, any difference occurring in the ganglion cells only, could be masked.

It has been reported that gangliosides are involved in metabolic responses to learning tests (16, 17, 18). To our knowledge this is the first example in which a cell constituent synthesized in the neuronal perikarya localized in an organ of sensory perception appears modified by the stimulus in the first station of the via of

Table II. Labeling of acid soluble material and gangliosides from the optic tecta of chickens injected intraocularly with [^{3}H] ManNAc

	OPTIC TECTA							
	CONTRALATERAL				IPSILATERAL			
	TCA-PTA soluble material		Gangliosides		TCA-PTA soluble material		Gangliosides	
	cpm/mg protein				cpm/mg protein			
Time (hours)	Dark	Light	Dark	Light	Dark	Light	Dark	Light
			EXPERIMENT 1					
5	-	-	(9) 35±2	(9) 53±5**	-	-	-	-
			EXPERIMENT 2					
3	-	-	(3) 53±16	(3) 67±17	-	-	-	-
5	-	-	(3) 114±16	(3) 165±19	-	-	-	-
8	(3) 93±6	(3) 96±4	(6) 253±43	(6) 355±21*	(3) 63±21	(3) 60±17	(6) 25±3	(6) 26±8
			EXPERIMENT 3					
3	(3) 52±3	(3) 47±2	(8) 45±8	(8) 70±2**	(3) 40±2	(3) 48±10	(3) 4±0.6	(3) 4±0.7
5	(3) 61±6	(3) 53±13	(10) 128±10	(11) 164±8**	(3) 60±1	(3) 55±8	(9) 6±0.6	(9) 6±0.4

Figures are means ± S.E.M.; significance was calculated by Student's t test; *$p < 0.05$; **$p < 0.01$; figures in parenthesis are number of animals.

its perception. It is not known at present whether the effect is specific for gangliosides or will be shown by other cell constituents. In any of both cases, the possibility that this effect plays a role in the influence that sensory perception has on the brain, cannot be disregarded.

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