We conclude that the respective superiority of each lateral stereognostic sensory field derives from its anatomical relationship to the specialized contralateral cerebral hemisphere by means of the decussating afferent input to that hemisphere. The logic of this conclusion follows that used either explicitly or implicitly by others [for example (*4, 6, 7*)].

Since the accuracy of the minor system (the nonspecialized sensory field and minor hemisphere) for the processing of linguistic materials is above that of chance, the specialization of the dominant hemisphere system for stereognosis in children may be a relative one, with the difference between the functioning of the two being one of degree. Some accurate processing of linguistic material by the minor hemisphere (system) has been shown in adult commissurotomized patients (*15*). Our results appear to generalize the principle of relative specialization of a hemisphere to a function for which the minor system is the more specialized, namely for stereognosis of spatial stimuli. It is also generalized to children. However, since the corpus callosums of our subjects were presumably functioning normally, our experiment does not preclude the possibility that the greater-than-chance stereognostic processing of the different stimuli by the nonspecialized system was effected in the specialized hemisphere by means of this commissure.

While our results do not confirm the sexual dimorphism for the nonsense forms found by Witelson (*3*), we note that the differential processing of the bigrams by the girls and boys of our study is entirely consistent with her conclusion that the "brains of girls and boys may be differentially organized for the cognitive processes involved in reading" (*3*, p. 426). If accepted, however, such a conclusion must be qualified because first, both the girls and boys in this study processed the two-letter words in almost identical fashion, and second because a minority of girls processed the bigrams as most boys did and vice versa.

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9. Our subjects were identical to Witelson's (*2*) in certain characteristics. For example, all showed 80 percent right-hand preference for the ten unimanual tasks in the *Harris Test of Lateral Dominance* (Psychological Corp., New York, ed. 3). The school reported that they had no grade failures, no medical or behavioral problems, and were not in need of reading remediation. Parents gave informed voluntary consent for their child's participation. Each child gave similar consent and was at liberty to withdraw from the study at any time.
10. Witelson's (*2*) nonsense shapes comprise ten irregular forms each measuring approximately 37 by 37 by 15 mm. Ours were cut from styrofoam 15 mm thick. We paired the shapes as she did. Our words and bigrams consisted of upper case letters used for bulletin boards (Kwik Sign Polystyrene Letters, Western Speciality Manufacturing Corp., Cheyenne). They measure 20 by 18 by 3 mm per letter with a stroke width of 4 mm. The stroke has distinctly palpable edges.
11. Other significant interactions were age × stimuli ($F = 3.04$, d.f. = 4, 216, $P < .05$), and stimuli × hand ($F = 137.97$, d.f. = 2, 216, $P < .01$).
12. To protect against a type I error, we adopted an alpha level of .001 for all planned comparisons.
13. Subsidiary experiments using smaller groups of children showed that the results with the word and bigram tests of this study could not be attributed to the different instructions given the children, namely, to "show me what you read" for the words and "what you felt" for the bigrams.
14. A comparable effect, that is, more accurate identification of faces presented in the left sensory field (tachistoscopically), has been found for both boys and girls by A. Young and H. Ellis, *Neuropsychologia* **14**, 495 (1976).
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Cholinergic Neuronotrophic Factors: Intraocular Distribution of Trophic Activity for Ciliary Neurons

Abstract. *Chick ciliary ganglionic neurons require an interaction with their peripheral targets for survival during a critical period of their embryonic development in vivo. It has recently been shown that survival of these neurons in dissociated cell cultures is supported by extract from whole chick embryo. In this study, an assay system based on microwell cultures of ciliary ganglionic neurons was used to demonstrate that a very rich source of trophic factor for them is the intraocular target tissues they innervate. Out of 8000 trophic units present in a 12-day embryo, 2500 were contained in the eye. A subdissection of the eye showed its activity to be localized in a fraction containing the ciliary body and choroid coat, with a specific activity almost 20-fold higher than that of the whole embryo. This selective intraocular distribution at a time when survival or death of ciliary ganglionic neurons is decided in vivo suggests that this soluble factor may be involved in the normal development of the ciliary ganglion.*

Neuronal cell death is a widespread phenomenon in the normal development of the nervous system (*1*). In the chick ciliary ganglion, half of the neurons present at embryonic day 8 die before embryonic day 14 (*2*). This cell death occurs at the time when ciliary ganglionic (CG) neurons are connecting with their target tissues, the ciliary body and choroid coat of the eye. Prior removal of an eye results in the complete loss of CG neurons in the ipsilateral ganglion, at the very time when cell death occurs during normal development (*3*). Conversely, neuronal death can be reduced by implanting an additional eye primordium, which increases the amount of target tissue available to the CG neurons (*4*). Neurons have been hypothesized to compete for a limited trophic supply from the tissues they innervate (*1*). Excess neurons fail to receive sufficient trophic support and do not survive.

In monolayer cultures, CG neurons seem to require special trophic support, which can be provided by coculture with skeletal muscle cells (*5*) or by medium conditioned over heart cells or supplemented with extract of chick embryos (*6, 7*). The presence, in extract of embryonic tissues, of a soluble material capable of supporting CG neurons, all of which are cholinergic, suggests that such extracts can be profitable sources for the isolation and identification of possible cholinergic neuronotrophic factors. We report here that (i) the highest concentration of trophic activity for CG neurons was found in the tissues that are their physiological targets and (ii) the active agent in these tissues exists in amounts sufficiently high to make its purification feasible.

Tissues to be assayed for trophic activity were homogenized in 6 ml of distilled water per gram of wet weight of the sample, with 20 strokes of a homogenizer (Potter-Elvehjem). The crude homogenates were spun at 108,000*g* at 0° to 4°C for 2 hours. Supernatant fractions from these spins were collected and assayed for protein concentration (*8*). To determine the amount of trophic activity in an extract, a dilution series was tested with a microwell bioassay, which we developed by modifying and scaling down

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to 16-mm microwell plates the culture procedures previously described for 35-mm dishes (*7*). Figure 1 gives the details of the bioassay and shows a dose-response curve obtained with an extract of whole 12-day embryos. No neurons survived for 24 hours without the addition of extract, either on the substratum or in the medium (by microscopic inspection), and abundant cell debris was present in the cultures. With increasing amounts of extract, the number of neurons increased to a plateau. At the cell densities used in the assay (2500 cells per square centimeter), the plateau corresponds to approximately 30 percent of the neurons seeded. Higher recoveries (approximately 80 to 100 percent) can be achieved through the use of higher seeding densities (*7*), but the lower densities were considered more convenient for an assay to be routinely applied to large numbers of samples. We have defined one trophic unit as the amount of material present in 1 ml of medium that allows the survival of 50 percent of the neurons. Since the extract assayed in Fig. 1 was diluted 400-fold to give a response of 1 unit per milliliter, the titer of the extract was 400 units per milliliter.

To study the intraembryonic distribution of trophic activity, we dissected 12-day embryos into four different portions: the heart and other viscera, eyes, brain, and carcass. Extracts were prepared from whole embryos, from each of the portions, and from the portions recombined before homogenization (Table 1). The relatively good agreement among the values for the whole embryo, recombined embryo, and the sum of the individual portions suggests that there was no differential extraction from the fractions. Whole embryos were found to contain approximately 8000 units with a specific activity of 125 units per milligram of protein. Of these 8000 units, roughly 1/3 and 2/3 were in the eyes and the carcass, respectively. The other fractions showed little, but detectable, activity. The eyes showed the highest specific activity, with 875 units per milligram of protein. This was sevenfold higher than the specific activity found in either the whole embryo or the carcass portion.

If the tissues normally innervated by CG neurons were indeed the richest source of trophic activity, one would expect to find a high specific activity in the portions of the eye containing the ciliary body and the choroid. To examine this possibility, we subdivided eyes from 12-day embryos into neural retina, cornea, vitreous and lens, iris, and a fraction containing the choroid, ciliary body, pigmented retinal epithelium, and sclera (henceforth referred to as the "choroid" fraction). Extracts from each of these fractions were prepared and assayed according to the procedures described above. Titration of the trophic activity (Table 2) showed that the choroid fraction has 80 percent of the activity present in extract of the whole eye and has a specific activity of 2400 units per milligram of protein, which is three times that of the eye and almost 20 times that of the whole embryo. The retina, vitreous and lens, and cornea all have specific activities comparable to those of the whole embryo or the carcass portion. The iris, a structure closely associated with the ciliary body, has a specific activity intermediate between those and the choroid fraction.

The trophic material under study may play a physiological role in the normal development of the ciliary ganglion. This soluble material is present in the eye at a high specific activity, and its intraocular distribution parallels the pattern of innervation by CG neurons at a develop-

Table 1. Distribution of trophic activity (± standard deviations) for ciliary ganglionic neurons in different portions of a 12-day chick embryo.

Embryo portion	Extracts assayed (No.)	Trophic units per embryo	Protein per embryo (mg)	Trophic units per milligram of protein
Eye	3	2515 ± 135	2.88 ± 0.46	875
Brain	4	195 ± 92	4.74 ± 1.32	40
Viscera	4	525 ± 290	7.25 ± 1.15	70
Carcass	4	4770 ± 1050	40.50 ± 7.2	120
Sum of portions	15	8005	55.4	145
Recombined tissues	3	8310 ± 3157	57.9 ± 11.8	145
Whole embryo	3	7375 ± 970	59.1 ± 1.6	125

Table 2. Intraocular distribution of trophic activity (± standard deviations) for ciliary ganglionic neurons of 12-day chick embryos. The "choroid" comprises choroid, ciliary body, pigmented retinal epithelium, and sclera.

Eye subportion	Extracts assayed (No.)	Trophic units per embryo	Protein per embryo (mg)	Trophic units per milligram of protein
Choroid	4	1965 ± 395	0.820 ± 0.24	2400
Cornea	2	8 ± 2.8	0.055 ± 0.0028	145
Vitreous and lens	4	100 ± 80.0	0.580 ± 0.099	170
Iris	4	33 ± 3.5	0.065 ± 0.007	510
Retina	4	125 ± 35.0	1.270 ± 0.140	100
Sum	18	2231	2.79	800
Recombined tissues	4	2855 ± 790	2.81 ± 0.55	1015
Whole eye	3	2515 ± 135	2.88 ± 0.46	875

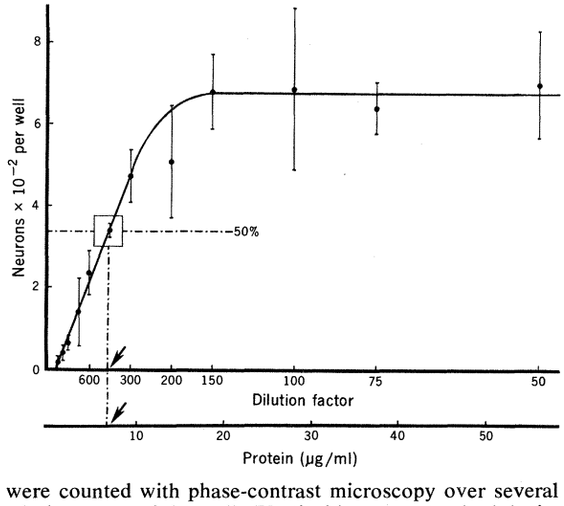

Fig. 1. Titration of trophic activity for chick embryo ciliary ganglionic neurons by use of monolayer cultures of these cells. Extract from 12-day whole chick embryo (weight/volume, 1/6) was diluted with modified Eagle's medium (*7*) and 200-μl portions were dispensed into 16-mm, collagen-coated wells. Five thousand ciliary ganglion cells, approximately 50 to 60 percent of which were neurons (*7*), were added to each microwell in 200 μl of medium supplemented with 20 percent horse serum. After 24 hours at 37°C in 5 percent CO_2, media were replaced with 2 percent glutaraldehyde in Eagle's medium, and surviving neuronal elements were counted with phase-contrast microscopy over several 0.5- to 15-mm strips passing through the center of the well. (Vertical bars are standard deviations within counts.) Half-maximum survival (50 percent horizontal line) was achieved with a 400-fold final dilution of the extract (upper arrow), assigning to the original extract an activity of 400 trophic units per milliliter. The lower arrow represents the concentration of extract protein in the 50 percent effective medium.

mental stage during which the survival of CG neurons seems to be regulated by target-derived trophic support (*2–4*). Crucial experiments necessary to verify the role of this material in vivo await the purification of the active factor or factors and the development of antibody against it. We have already found in preliminary experiments that this material is nondialyzable, thermolabile, and capable of being fractionated by chromatographic procedures (*9*). The large amounts and high specific activity of this material in the eye make eye tissue an excellent source for the purification of the active molecule or molecules involved.

Carcass extract also contains substantial amounts of soluble trophic agents for CG neurons, although at a low specific activity. The carcass is rich in tissues, such as skeletal muscle, that are normally innervated by neurons sharing the cholinergic nature of the CG neurons. In addition, detailed studies of some of these neurons, located in the lateral motor column of the spinal cord, have shown a well-defined period of developmental cell death (*10*), which is regulated by their peripheral territory of innervation, the limb bud (*11*). In culture, skeletal muscle cells support cholinergic activities of spinal cord neurons (*12*) as well as the survival of CG neurons (*5*). These parallel features in the development of CG and spinal cord neurons, together with the distribution of trophic activity for CG neurons reported here, suggest at least three hypotheses for the nature of the trophic factor or factors:

1) A single cholinergic neuronotrophic factor is present in both eye and carcass. Limb tissues would use the same factor to regulate survival of their motoneurons that the choroid and ciliary body use to regulate survival of CG neurons. The factor may be produced by all peripheral targets for cholinergic innervation, and specificity of interactions would be conferred only by the spatial and temporal relationships between neurons and peripheral targets. The high concentration of such a common factor in the intraocular periphery of CG neurons compared with all other putative cholinergic territories, however, would require additional hypotheses, for example, temporal differences in the accumulation of trophic activity in different target tissues.

2) A ciliary neuronotrophic factor is present in both eye and carcass. The low specific activity in the carcass may represent a low-level expression of the genetic information regulating the synthesis of this factor. The substantial total activity would merely reflect the large relative mass of the carcass at this stage of embryonic development. The high specific activity found in the eye would be due to some signal turning on the transcription of the gene in those tissues to be innervated by CG neurons.

3) A ciliary neuronotrophic factor is present in the eye and a spinal neuronotrophic factor is present in the carcass. The two factors would be distinct molecules, each used for regulating survival in the respective neuronal population. There would be enough cross-activity between the two molecules for the carcass factor to show an attentuated activity in the ciliary bioassay. Conversely, one may expect the ciliary factor to display some activity on spinal cord motoneurons, although its apparent specific activity on a motoneuron bioassay would be much lower than that demonstrated in the ciliary bioassay.

The three hypotheses are amenable to experimental verification; their relative validity can be ascertained by additional research.

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Goldfish Retina: A Correlate Between Cone Activity and Morphology of the Horizontal Cell in Cone Pedicules

Abstract. *In the cone pedicules, the digitations of horizontal cell process lateral to the synaptic ribbon disappear after dark adaptation. This disappearance is correlated with the loss of color opponency and cone function shown in ganglion cell recordings in isolated retinas. Cone function and color-opponent responses are restored by reapplying background light.*

For most of the animal kingdom, the night phase of the circadian cycle constitutes a period of prolonged dark adaptation. During that period, the cones of duplex retinas are assumed to remain functional even though the rod system takes over control of the retinal output except at the fovea. It is further thought that no major synaptic modifications take place in the retina during this time (*1, 2*).

Since rod and cone inputs converge onto the same ganglion cells, it is very difficult, in whole-animal preparations, to test cone activity at the level of the optic nerve after dark adaptation because the rod receptor system comes quickly into play, masking the cone system. In the isolated retina, however, it is possible under special conditions to study cone function during dark adaptation without the intrusion of the rod system that would normally occur. After suitable bleaching, the rods will not regenerate their visual pigment in the absence of the enzymatic system present in the pigmented epithelium, which is left in the eyecup after retinal isolation (*3*). Under these conditions, cone function can be studied for an extended period under complete darkness without rod intrusion.

We report here that in the goldfish (*Carassius auratus*) the cone system becomes nonfunctional after prolonged dark adaptation, and that major synaptic modifications occur in the cone pedicules, which can be related to the change in activity observed at the ganglionic cell level and to the state of adaptation.

Comet goldfish were subjected to an artificial day-night cycle of 12 hours of light (125 lux, incandescent) and 12

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