These integrated light optical, histochemical, microspectrographical, and ultrastructural studies have revealed the widespread occurrence in the rabbit lung of intramucosal, innervated, densecored cellular corpuscles. Although these corpuscles probably have a variety of functions, they appear to be the source of an intrapulmonary production of serotonin. While the argyrophilia, argentaffinity, and constant yellow fluorescence suggest the occurrence of serotonin within the DCV's, the positive ultrastructural reaction after an FGD fixation (*15*) and the microspectrographic emission and excitation spectra indicate the high concentration of 5-hydroxytryptamine in these specialized epithelial cells. Moreover, most of the intrapulmonary bronchial capillaries are fenestrated in the vicinity of the NEB's and are drained off via the bronchopulmonary veins to the pulmonary veins (*20*). Thus the serotonin produced by the NEB's—which may be stimulated, for example, by the partial pressure of the oxygen or carbon dioxide of the intralumenal gas—may reach the pulmonary veins directly. This anatomical pathway offers an explanation for the previously obscure morphological mechanism of hypoxic pulmonary venoconstriction (*21*).

We realize that the granulated cells probably produce substances other than serotonin, whose metabolism may be combined with a variety of cellular activities in the amine and peptide spectrum. Indeed, they share many characteristics of the bronchial and bronchiolar AFG (argyrophil, fluorescent, and granulated) cells which we have identified in the human infant (*5*, *22*). Although the corpuscular appearance and the obvious innervation of the NEB's are characteristics establishing a separate morphological entity, they may also exhibit various local secretory functions (*5*), modulating not only vasomotion but also other bronchial and bronchiolar functions such as mucosal secretion, smooth muscle tone, or the general integration of the activities of the pulmonary unit lobules (*6*).

The distinct innervation of the NEB's indicates their direct connection with the central nervous system, probably in an afferent as well as efferent manner. The typical synaptic end formations with a practically homogeneous population of small and agranular vesicles suggest an efferent innervation with probably a centrofugal modulating activity which may be compared to similar properties of the carotid body. The immediate contact of these NEB's with the airway lumina, their occurrence throughout the most peripheral airway branchings, their high incidence, and finally their corpuscular appearance are all indicative of a neuroreceptor function. The possibilities include a response to various stimuli and a chemo-, stretch-, baro-, or tactile-receptor function. It may be that they react to the $P_{O_2}$, the $P_{CO_2}$ and/or other constituents in the airway gas, or are stimulated by inhaled pollutants or particles in the inspired air, which have passed the larger bronchi.

Additional biochemical, physiologic, and pharmacologic investigations are needed to elucidate the precise function of the identified (*23*) NEB's (*24*).

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18. The emission and excitation spectra have been measured microspectrographically on the NEB's of six rabbit fetuses, eight term rabbits, and three adult rabbits, and on the enterchromaffin cells of six adult rabbits and three human adults.
19. A variance analysis was performed on the dimensions of the DCV's. It revealed that the longitudinal axis and the elongation index of the two types of DCV's are significantly different ($P < .001$). The vertical axis does not exhibit a significant difference ($P > .05$). Hence and though both types of DCV's are morphologically, morphometrically, and histochemically different, it may not be excluded that in individual cases "rounded vesicles with dense core" represent a lateral cut of a type 1 vesicle.
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## Visual Discrimination in Sharks without Optic Tectum

Abstract. *After complete removal of the optic tectum, nurse sharks can learn to discriminate black versus white and horizontal versus vertical stripes. This finding is contrary to the traditional belief of exclusive tectal control over visuomotor behavior in lower vertebrates and suggests a role for the telencephalon in the vision of these primitive animals.*

The optic tectum has long been supposed to be the sole repository for the mechanisms involved in the visuomotor behavior of nonmammalian vertebrates. Indeed, Herrick referred to the tectum of lower vertebrates as the "supreme center of regulation of motor responses to the exteroceptive system of sense organs" (*1*). Based partly on this belief and related neuroanatomical data, the theory of encephalization asserts that there is a gradual shift of most visual function from midbrain optic tectum to visual cortex as one ascends the phylogenetic scale (*2*). Furthermore, the shift in locus of function is generally thought to reflect the evolutionary development of the human visual system.

Several investigators have recently questioned whether this theory of encephalization can be legitimately applied to vision within the class Mammalia (*3*). However, until now no one has seriously challenged the more widespread belief that the functional organization of the visual system of other vertebrate classes reflects a progressive "encephalization." By implicating nontectal central neural structures in shark vision, the present findings force us to reconsider this traditional concept of comparative neurology. The traditional concept was based, of course, on the expansion of diencephalic and telencephalic centers, and if this expansion cannot be explained by the transfer of functions from the brainstem, then the facts of comparative anatomy become an even greater challenge to investigators than they were at the turn of the century.

From a review of the evidence for the theory of visual encephalization, it is surprising how little behavioral research has been done on central visual functions in nonmammalian species. Investigations with fish have been almost entirely restricted to teleosts (*4*). Sharks have received even less attention, although they could easily be considered of special interest since they appeared almost 200 million years before the first teleosts and apparently have changed relatively little since the Jurassic period (*5*). Thus, these cartilaginous fish can possibly provide us with important clues about the evolution of visual functions (*6*).

While it appears that complete tectal ablation produces permanent blindness in teleosts (*7*), the same may not be true for sharks. A few early investigators did report blindness following bilateral tectal ablation in sharks (*8*); however, these findings are difficult to assess, since they consisted of general observations made within the first 1 or 2 days after surgery and, in some cases, included evidence of severe oculomotor, locomotor, and pupillary deficits.

To investigate the effects of tectal lesions, six juvenile nurse sharks (*Ginglymostoma cirratum*), about 90 cm long, were trained on a black-white discrimination task and then on a horizontal versus vertical stripes problem. The details of the apparatus and procedure have been described elsewhere, so a brief description here will suffice (*9*). The discriminanda were two 30.4-cm-square painted targets located at the end of a Y-shaped alley and separated by a 15.2-cm baffle. The position of the correct target was varied quasi-randomly. Subjects were given six trials per day, 45 seconds apart, according to a correction procedure (*10*). The correct target-door opened when the shark crossed the choice line drawn 15.2 cm in front of the positive discriminandum. The subject was then rewarded with a 5-g piece of thawed Spanish mackerel impaled on a stiff wire. Afterward, the shark swam through a corridor back to the start pen. The fish rewards given during training constituted the shark's total daily food intake. To assess any possible motivational deficits, response latency was also measured.

Table 1. Number of trials (T) and errors (E) to criterion on the black-white (BW) and horizontal-vertical (HV) discrimination tasks. Parentheses indicate that the subject did not reach criterion.

| Shark | BW |  | HV |  |
|---|---|---|---|---|
|  | T | E | T | E |
|  | *Controls* |  |  |  |
| 144 | 96 | 25 | 48 | 9 |
| 146 | 183 | 49 | 219 | 78 |
| 147 | 108 | 38 | 186 | 63 |
|  | *Operated* |  |  |  |
| 143 | 203 | 72 | 213 | 75 |
| 184 | 378* | 109 | (114) | (51) |
| 187 | 318 | 106 | 264 | 112 |

* Subject performed significantly above chance ($P < .005$) after 107 trials (41 errors).

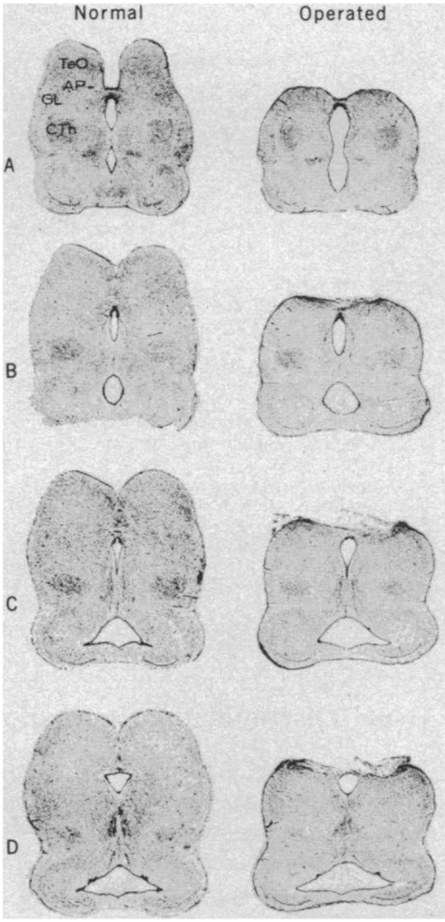

Fig. 1. Transverse Nissl-stained sections through comparable levels of the mesencephalon of a normal nurse shark (left) and of subject 143 with a total tectal ablation (right). Sections are ordered rostrocaudally (A to D), 600 $\mu$m apart. Abbreviations: *AP*, pretectal area; *CTh*, central nucleus of thalamus; *GL*, lateral geniculate nucleus; *TeO*, optic tectum.

Three of the sharks were operated before beginning discrimination training. The optic tectum was removed bilaterally by aspiration with the subject anesthetized in a bath of tricaine methanesulfonate solution (*11*). The postoperative recovery period before training lasted for 9 to 27 days. At the conclusion of the experiment, these sharks were killed by intracardiac perfusion with 10 percent formalin. After removal, the brains were frozen and sectioned transversely every 50 $\mu$m (*12*). Every 12th section was subsequently stained for cell bodies with cresyl-violet.

A discrimination task was considered learned ($P < .01$) when the subject made 17 correct responses during three successive training sessions, that is, 18 trials (*13*). Table 1 summarizes the number of trials and errors to criterion for the two groups of sharks. None of the subjects experienced any difficulty in learning to discriminate white from black. Even tectal subject 184 performed significantly above chance, $P < .005$, after only 107 trials (*14*), and continued to do so for the next ten training sessions, when training was halted for 16 days because of logistical difficulties. There was also no difference between the two groups in median daily response latency. In all cases it dropped to 5 to 10 seconds after the first few days of training.

The similar performances of the two groups on the pattern discrimination further indicate that tectal ablation does not produce blindness in nurse sharks. As shown in Table 1, the one tectal subject which did not reach criterion experienced relatively few training sessions because of lack of time. The other two tectal subjects initially performed at chance levels but eventually learned to choose either the horizontal (subject 187) or vertical stripes (subject 143).

Some additional control procedures

emphasize the sharks' dependence on visual clues for solving the second problem. For instance, to eliminate any target-specific cues, each target was painted with three black and three white stripes and served as the correct and incorrect target an equal number of times daily for each subject. Consequently, a target remained on the same side during alternate sessions, with correctness being determined by its orientation. Furthermore, when a subject was within one session of reaching criterion on either task, the experimenter randomly varied which target, positive or negative, was lowered first before each trial. Thus, any sound-related cues could not have served as a basis for learning. The possible use of such cues is also ruled out by the fact that the sharks' performances reverted to chance levels at the beginning of training on the second task. During the same precriterion session, both targets were rotated 180° from their standard training positions. A shark was therefore unable to reach criterion by using as a cue a specific stripe always located in the same position relative to the target.

Histological analysis revealed that the optic tectum was totally removed in all subjects except for a small lateral portion (approximately 5 percent) of the right tectum in subject 187. The lesions completely abolished both the upper layers of the tectum which receive the retinal input as well as the lower layers which receive the telencephalic input (*15*); however, there was only minimal damage to the underlying tegmentum. The thalamus remained undamaged except for portions of the pretectal area in subject 184. There was no evidence of any tectal regeneration (Fig. 1).

The present findings demonstrate that the optic tectum is not necessary for some types of visual discriminations. Consequently, we have begun to examine other portions of the shark brain, especially the posterior telencephalon. Bilateral lesions in this region produce severe visual dysfunction (*9, 16*). The observation of short-latency, visually evoked potentials in the same area (*17*) suggests that this deficit can be related to the recently reported similarities between sharks and mammals in some of their afferent and efferent telencephalic connections (*15*). Moreover, these facts suggest that portions of the nonlaminated posterior telencephalon of sharks are remarkably similar to the laminated visual cortex of mammals. Such a view, if correct, would necessitate a revision of our notions on the evolution of the brain, especially the view that the primitive telencephalon is dominated by olfaction (*18*).

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# DNA Base Composition of Rickettsiae

*Abstract.* *There is a small but distinct difference in DNA base composition between the typhus and spotted fever groups of rickettsiae. The molar percentages of guanine plus cytosine for* Rickettsia prowazeki, R. typhi, *and* R. canada *are approximately 30, for* R. rickettsi, R. conori, *and* R. akari *they are about 32.5. The percentage for trench fever rickettsia,* Rochalimaea quintana, *is 38.6.*

Rickettsiae, as other obligate intracellular parasites, have relatively few phenotypic characteristics that can be used for the differentiation of species and strains without undue difficulty. These microorganisms are classified primarily on the basis of arthropod vectors, antigenic differences, and a relatively small number of biological activities (*1*). A need exists, therefore, to establish an objective index of genetic relatedness among rickettsiae. We are describing here an attempt to proceed toward this goal by examining the molar percentage of guanine plus cytosine (percent G + C) in the DNA of a number of strains of the typhus and spotted fever groups, and of a strain of the trench fever rickettsia. Scrub typhus rickettsiae were not included because these organisms are fragile and are separated from host components with considerable difficulty (*2*). Q fever rickettsiae have already been examined (*3–5*).

The species and strains of the organisms studied are listed in Table 1. The rickettsiae were grown in the yolk sac of embryonated chicken and, in some cases, duck eggs. Typhus group rickettsiae, including *Rickettsia canada*, were harvested from moribund embryos, while rickettsiae of the spotted fever group were harvested from embryos that had died 1 or 2 days previously (*6*). The rickettsiae were separated from host cell constituents as described (*7*), except that 0.5 percent trypsin (Difco) was used instead of Pronase. Rickettsiae were grown also in irradiated L cell monolayers and purified as described by Weiss *et al.* (*8*). *Rochali-*