algesic effect of morphine in the periaqueductal gray matter (later confirmed in an additional group of ten rats) was accompanied by violent uncontrolled jumping characterized by stereotyped circular leaps; these were set off by any slight stimulus, either auditory or visual (*9*). Some animals suffered fatal self-inflicted concussions by jumping and striking their heads repeatedly against the top of the cages. Morphine blocks the release of acetylcholine in peripheral (*10*) and central (*11*) tissues. Thus, its hyperalgesic action here may be the opposite of electrical stimulation; that is, morphine may block cholinergic neural transmission in these pain-inhibitory pathways. The stereotyped circular leaps may be caused by morphine acting in addition on a dopaminergic system.

The neurochemical events underlying these diverse effects of morphine are unknown. Other investigators have implicated the cholinergic (*10*), serotonergic (*12*), and catecholaminergic (*13*) systems. A "narcotic receptor" that specifically binds naloxone was found to be concentrated in the striatum (*14*); however, Wei *et al.* (*15*) found that when naloxone was injected into brains of morphine-dependent rats, the greatest incidence of withdrawal symptoms followed injections in the medial thalamus, with few symptoms being elicited by injections in the basal ganglia. However, in both reports acetylcholine was suggested as the most likely neurotransmitter involved in the action of morphine. Further work is necessary before any firm conclusions can be made.

In conclusion, our results show that intracerebral injections of morphine have effects significantly different from those of systemic injections; this is similar to results with other centrally acting drugs (*16*). Depending on site and dose, intracerebral morphine injections result in either analgesia or hyperalgesia, the latter accompanied in some cases by violent motor activity.

In addition, these results indicate that microinjection, when used with care, is a fruitful method of investigating morphine action. Among the steps we took to eliminate the various sources of error inherent in this system were (i) use of fine-gauge cannulas—30-gauge (outer diameter 0.30 mm) for the guide cannula and 35-gauge (outer diameter 0.13 mm) for the injection cannula—to minimize damage to dorsal sites as well as to the intended site; (ii) injecting 1 mm beyond the cannula tip to minimize backwash and diffusion to other sites (via the outer walls of the cannula into ventricular spaces); (iii) slow injection of a small volume (0.5 μl) to allow fluid to be absorbed by the tissue and to avoid tissue damage that may result from greater volume; (iv) thorough habituation of the animals to the injection procedure and experimental chamber before testing; and (v) a reliable but sensitive analgesia test that also minimized stress to the subject. We believe that conflicting results obtained with the microinjection method are probably due to lack of care in any of these aspects.

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5. deGroot A-P, L, and V coordinates for these sites were as follows: posterior hypothalamus, 4.6, 0.5, and 2.5 above intraaural (IA) 0; medial septum, 8.2, 0, and 5.0 above IA 0; caudate, 7.8, 3.0, and 6.0 above IA 0; and periaqueductal gray matter, 0.6, 0.5, and 4.25 above IA 0.
6. Two rats were excluded from the final results because of questionable cannula placements.
7. This may be related to the findings of Pert and Snyder (*14*) who found significant opiate receptor binding in the corpus striatum.
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Electrophysiological Identification of a Visual Area in Shark Telencephalon

Abstract. *Optic nerve stimulation in the shark evokes short-latency telencephalic field potentials localized to the ipsilateral, posterior central nucleus. Such a well-defined visual area in elasmobranch telencephalon further challenges classical formulations of forebrain evolution. Moreover, its ipsilateral representation confirms recent evidence for a crossed thalamotelencephalic visual projection.*

The classical view of forebrain evolution (*1*) suggests that in more primitive nonmammalian vertebrates, such as cartilaginous fish, the telencephalon is largely dominated by olfactory input with less prominent representation of other sensory modalities. In the course of vertebrate phylogeny these other modalities presumably gain increasing telencephalic representation, and, with specialized exceptions, olfactory domination decreases. However, the resurgence of comparative neuroanatomy in the past decade has generated considerable data inconsistent with this view. As the sampling of nonmammalian vertebrate species increases, substantial evidence is accumulating that nonolfactory modalities have well-defined telencephalic representations. This has been most comprehensively investigated with respect to visual pathways ascending to the telencephalon, and specific thalamotelencephalic visual pathways have now been identified in amphibians, reptiles, and birds (*2*, *3*). Thus, there is little doubt that specialized visual receiving areas exist in the telencephala of nonmammalian terrestrial vertebrates, and an analogous story is developing with respect to other nonolfactory modalities (*4*).

The only vertebrates in which the classical formulation of forebrain evo-

lution has not been challenged are the cartilaginous and bony fish. Were nonolfactory sensory representation demonstrated in such species, then the view of an olfactory-dominated telencephalon as the predecessor of more evolved forms would have to be abandoned. This issue motivated recent anatomical studies of shark (elasmobranch) brain (*5*) which have clearly indicated a visual thalamotelencephalic projection and thus suggest telencephalic representation of a nonolfactory sensory modality. Paradoxically, neurophysiological studies (*3*) involving photic and optic tract stimulation failed to show the existence of any visual area in lamprey (cyclostome) and skate (elasmobranch) telencephala, although well-defined evoked responses were recorded from the optic tectum. This inconsistency between the anatomical and physiological results for cartilaginous fish constituted the impetus for our electrophysiological survey of the nurse shark telencephalon during optic nerve stimulation.

Experiments were performed on four nurse sharks (*Ginglymostoma cirratum*) ranging in length from 61 to 89 cm. Anesthesia was induced with tricaine (MS-222), and the animals were maintained in a shallow, continuously aerated seawater bath which covered the mouth and ventral half of the body. Skin and cartilaginous roof overlying the telencephalon and optic nerves were removed, and one optic nerve was dissected free of surrounding tissue and placed on a stainless steel bipolar stimulating electrode. This electrode had an intertip distance of 0.75 mm and contacted the nerve approximately 5 mm proximal to the globe. After electrode placement the entire region was bathed in mineral oil.

Recordings were made in both ipsilateral and contralateral hemispheres in vertical penetrations with tungsten microelectrodes of 1-μm tip diameter. The stimulating and recording equipment were conventional, and the amplifier time constant was set at 0.2 second. The stimuli were single monophasic square waves 0.1 msec in duration and were delivered every 1.0 second. At selected sites a 10-μa anodal current was passed for 5 to 10 seconds to permit tip localization. The animals were perfused through the heart with 10 percent formalin; the brains were removed and, after further fixation, were cut while frozen into 50-μm sections. Each section was stained with cresylechtviolett.

Electrode penetrations were made in a systematic grid as indicated in Fig. 1A. In the rostrocaudal direction penetrations were made every 2.0 mm from 1.0 to 9.0 mm anterior to the caudal hemispheric pole. At each rostrocaudal level mediolateral penetrations were made in 1.0-mm increments from the midline to the lateral hemispheric border; often these penetrations were repeated in the lateromedial direction. For each penetration recordings were taken every 0.5 mm from the dorsal surface to approximately the ventral surface, and this sequence was frequently repeated on electrode withdrawal.

In the ipsilateral hemisphere evoked field potentials were localized to an oblong region within the posterior central nucleus (Fig. 1A). The long axis of this region ran rostrocaudally, angulating toward the midline in the rostral direction (Fig. 1A). The caudal extent of the active region was located approximately 3.0 mm rostral to the posterior hemispheric margin (Fig. 1, A and E; Fig. 2, B and C), and at this level the field potentials were consistently negative (Fig. 1I). This activity could generally be recorded from 3.0 to 6.0 mm lateral to the midline and 4.5 to 6.5 mm ventral to the hemispheric surface, although the precise extent depended on brain size. The site of maximum negativity was generally 4.0 mm lateral to the midline and 5.0 to 6.0 mm ventral to the surface. In this region field potentials had amplitudes of 150 to 200 μv and latencies of 20 to 23 msec at stimulating currents twice threshold (2*T*) (Fig. 1I). (All latencies

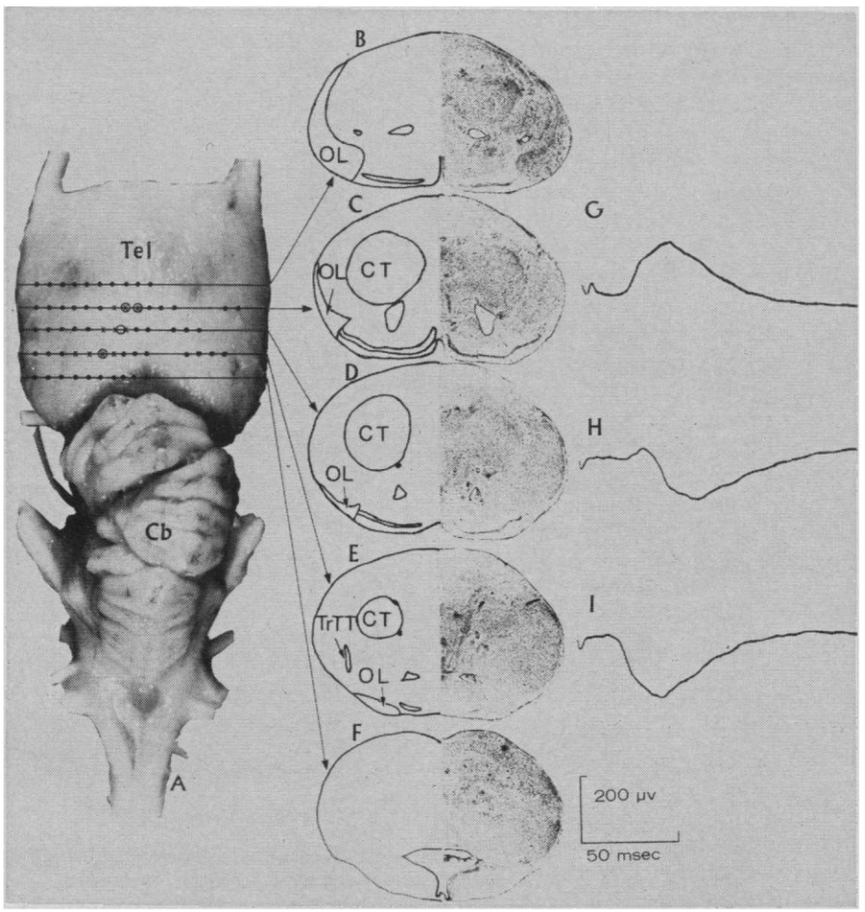

Fig. 1. (A) Dorsal view of the nurse shark brain with a schematic illustration of the sites of electrode penetrations in the telencephalon (*Tel*). The horizontal lines are separated by 2 mm, and each symbol indicates a penetration site. Mediolateral symbol separations are equivalent to 1 mm. The crosses on the ipsilateral (left) telencephalon indicate penetrations where field potentials were evoked. The large open circles indicate penetrations where maximum activity was obtained. The cerebellum is labeled *Cb*. (B to F) Transverse sections through telencephalic levels indicated by the leaders from (A). The right half of each section is stained with cresylechtviolett to illustrate the prominent central telencephalic nucleus (*CT*). The other abbreviations are: *OL*, lateral olfactory area; *TrTT*, thalamotelencephalic tract. (G to I) Representative waves elicited in penetrations at levels corresponding to (C), (D), and (E), respectively. Downward deflections indicate negativity.

indicate the time to the leading edge of the evoked wave.)

The rostral pole of the active ipsilateral region was approximately 7.0 mm anterior to the caudal pole of the hemisphere (Fig. 1, A and C; Fig. 2, A and C). However, at this rostrocaudal level the field potentials were consistently positive (Fig. 1C), and the active area extended from 1.0 to 3.0 mm lateral to the midline and 4.5 to 7.0 mm ventral to the surface. Maximum positivity occurred in an area 1.0 to 2.0 mm lateral to the midline (Fig. 1A) and 5.0 to 6.5 mm below the dorsal surface. In this region field potentials had amplitudes of 125 to 175 μv and latencies of 25 msec at 2T (Fig. 1G).

Midway between these caudal and rostral boundaries of the active zone—that is, 5.0 mm rostral to the caudal hemispheric pole (Fig. 1A)—the field potentials were biphasic, positive-negative waves (Fig. 1H) which were maximum in amplitude 2.5 mm lateral to the midline and 5.0 to 6.0 mm ventral to the surface. With stimuli of 2T the peak negativity was approximately 100 μv with a latency of 20 to 25 msec (Fig. 1H).

The hemisphere contralateral to the stimulated optic nerve was explored much less systematically (Fig. 1A), but no evoked responses were ever observed. Particular attention was given to contralateral sites homotopic to the most active ipsilateral sites, and still no evoked activity could be recorded.

Finally, to ensure that the observed ipsilateral activity was, in fact, due to optic nerve activation and not to spread of current to surrounding tissue, the nerve was sectioned following location of a telencephalic site giving maximum negative responses. This completely abolished all evoked activity.

The findings reported above clearly indicate the existence of a well-defined visual area in the nurse shark telencephalon, an area confined to the posterior portion of the ipsilateral, central telencephalic nucleus. While an ipsilateral representation is unusual, it is what one would anticipate in view of the findings that the retinothalamic projection is totally crossed (*6*) and that the subsequent thalamic projections to the central telencephalic nucleus are very largely crossed (*5*). Thus, after a total decussation of the retinal fibers there appears to be a substantial recrossing in the thalamotelencephalic projections. Further, we obtained evidence of a dipole across this nucleus in the rostrocaudal direction with evoked negativity approximately 3 mm from the caudal hemispheric pole, positivity 4 mm rostral to this, and biphasic positive-negative activity in between. This may imply that the soma-dendritic complex receiving the visual thalamic projection is restricted to the more caudal aspect of this 4-mm active region and that the axons of these neurons collect in the more rostral portion. This would provide a current source for a soma-dendritic sink in the more caudal part of the nucleus. However, this is speculative and requires anatomical evaluation. Another point concerns the absence of evoked activity in the contralateral hemisphere. While suggestive of a restricted homolateral representation, this negative finding must be viewed with caution, since we believe that more extensive exploration of the contralateral hemisphere is dictated. Finally, although we are confident about the existence of a well-defined visual receiving area in the central nucleus, the specificity of this area for visual input remains moot, pending studies which demonstrate the absence of inputs to this region from other sensory modalities.

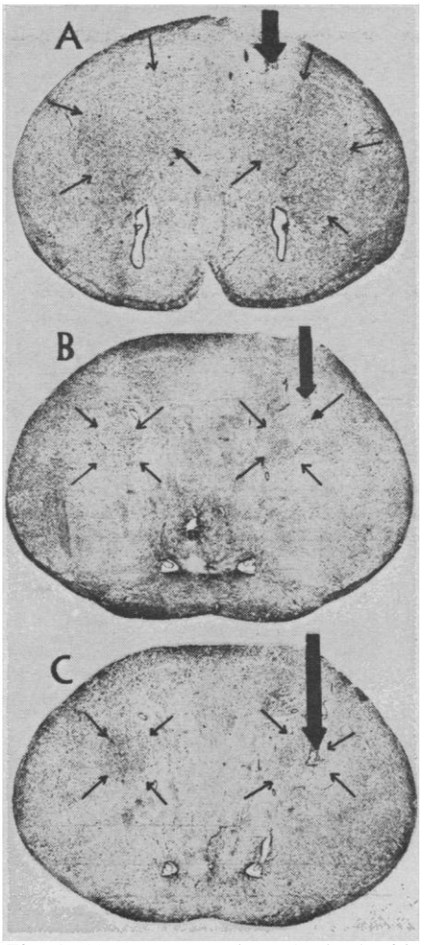

Fig. 2. Transverse sections (stained with cresylechtviolett) through the central telencephalic nucleus, which is indicated by small arrows. (A) shows the most rostral level of evoked field potential activity (positive wave) and corresponds approximately to Fig. 1C. The dorsal boundary of the active area was marked with a small lesion (large arrow). (B) and (C) show the most caudal level of evoked activity (negative wave) and correspond approximately to Fig. 1E. The dorsal and ventral boundaries are marked by lesions indicated by the large arrows in (B) and (C), respectively.

In conclusion, our findings demonstrate that the cartilaginous fish fit the emerging story of substantial, specific representation of nonolfactory sensory modalities in the nonmammalian telencephalon. This presents serious difficulty for the classical concept that in primitive nonmammalian vertebrates the telencephalon is primarily olfactory, with specific and distinct nonolfactory sensory fields being a more recent phylogenetic development.

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