Table 1. Number (and percentage) of myenteric neurons in which firing was inhibited by DMH or DADLE or both.

Treatment	Inhibition by DHM only	Inhibition by DADLE only	Inhibition by DHM and DADLE	No inhibition
DHM (10 n*M*) plus DADLE (1 n*M*)	25 (19)	5 (4)	39 (30)	60 (47)
DHM (30 n*M*) plus DADLE (10 n*M*)	2 (10)	3 (16)	11 (58)	3 (16)

sequences of their selective occupation are the same. However, it is difficult to examine this in the central nervous system in vivo because the concentrations of the selective ligands must be precisely known. The myenteric plexus of the guinea pig ileum contains binding sites for both the μ ligand dihydromorphine (DHM) and the δ ligand D-Ala^2-D-Leu^5-enkephalin (DADLE) (*7, 8*). We examined the effects of low concentrations of these substances on action potential discharge of single myenteric neurons in vitro and attempted to differentiate further between the two sites by applying naloxone.

The preparation was perfused at 37°C with a physiological saline solution and the action potentials of single myenteric neurons were recorded with glass suction electrodes (*9*). Drugs were applied by changing the superfusing solution to one which differed only in its content of the drugs. Both DHM and DADLE were applied to the same neurons. In the majority of experiments (129 neurons), 10 n*M* DHM and 1 n*M* DADLE were applied. It was considered that at these concentrations the agonists might act more or less selectively on μ and δ receptors (*8*). Firing of 47 percent of the 129 neurons was not affected by either ligand and firing of 30 percent was inhibited by both (Table 1). The degree of inhibition caused by DHM was approximately the same as that caused by DADLE (Fig. 1). Twenty-five neurons were inhibited by DHM but not by DADLE, whereas the firing of only five cells were inhibited by DADLE but not by DHM. The relatively small number of cells affected only by DADLE may have resulted from the low concentration used, because a concentration ten times higher selectively inhibited 16 percent of the cells (Table 1). Increasing the concentrations of DHM and DADLE increased the proportion of cells inhibited by both ligands (Table 1), perhaps because they no longer acted selectively on their receptors.

More naloxone is required to displace tritiated DADLE than to displace tritiated DHM from their binding sites in guinea pig ileum and cow brain (*8*); median inhibitory concentrations are 13.5 and 18.5 n*M* naloxone, respectively, for tritiated DADLE and 2.3 and 3.3 n*M* naloxone, respectively, for tritiated DHM. We therefore exposed cells inhibited by both agonists to a low concentration of naloxone (1 n*M*). This prevented the action of DHM without changing the inhibition induced by DADLE (Fig. 1). Since the initial degree of inhibition caused by 10 n*M* DHM was about the same as that caused by 1 n*M* DADLE, it appears that the two receptors are distinct. This effect of naloxone was reversed after the tissue was washed for several minutes (Fig. 1). In other experiments, increasing the concentration of naloxone to 10 n*M* resulted in antagonism of the effects of DHM and DADLE.

Whereas binding experiments show distinct μ and δ sites in the guinea pig myenteric plexus (*8*), pharmacological studies on the electrically induced, nerve-mediated contractile response of the longitudinal muscle have not detected a δ receptor in this tissue (*3, 8, 10*). Our results are direct evidence for the existence of a δ receptor and show that its occupation by an agonist has an effect—inhibition of cell firing—that is the same as the effect of a μ receptor agonist acting on a μ receptor on the same neuron. Important differences in the physiological consequences of μ and δ receptor agonists may exist which are beyond the resolution of our extracellular recording technique, but one common result of both is a depression of neuronal excitability.

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11. This work was supported by PHS grants DA01730, NS06672, and RCDA DA00068.

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Efferents to the Retina Have Multiple Sources in Teleost Fish

Abstract. *Multiple efferent systems project to the retina in three species of teleost fish investigated with the horseradish peroxidase technique. These animals are the first vertebrates shown to have more than one central nervous system structure projecting to the retina. The connections discovered may reflect a primitive organization of retina-brain interconnections.*

One of the questions of neurobiology is the significance of interspecific variability in brain organization. Certain cell aggregations present in some species are absent in others. An example of such variability is the absence or presence of efferent systems projecting to the retina. The most celebrated system of this kind is found in birds, in which a large, conspicuous cell group in the caudal mesencephalon projects to the contralateral eye (*1*). A comparable cell group has been observed in some reptiles (*2–4*), but has not been identified in other vertebrate classes (*5, 6*). Nevertheless, it has been argued that the cell group may exist in other vertebrates but that it lacks a projection to the retina (*7*). Optic nerve efferents have been described in mammals (*8*), although the sources of such fibers remain unknown (*6*). In reptiles a cell group in the ventral thalamus was recently identified with the horseradish peroxidase (HRP) technique. In the snake it is called the nucleus of the ventral supraoptic commissure (*4*) and in the lizard it is called the centrifugal optic thalamic nucleus (*9*). It is located near

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the ventral geniculate complex and may be part of this structure. Amphibians examined so far lack central nervous system efferents projecting to the retina (*5*). However, on the basis of physiological experiments (*10*), teleosts are thought to have such a projection from the optic tectum. [Attempts to identify neurons with efferent fibers projecting to the retina in cyprinid fish (*11*) did not allow any conclusions because of problems with the collateral circulation of the eye.]

The overall pattern among vertebrates makes little sense unless one believes that, for example, the isthmo-optic nucleus is unique to birds and some reptiles and that the projection mentioned sprouted to the eye in those vertebrate classes only. The present report describes the discovery of a spectrum of arrangements in three teleost species. These findings may explain the diversity of organizations observed in other vertebrate groups.

We used eight specimens of the upside-down catfish (*Synodontis nigriventris*), three specimens of a cichlid (*Julidochromis regani*), and eight specimens of a freshwater puffer (*Tetraodon fluviatilis*). The fish were anesthetized with tricaine methanesulfonate (Finquel) and the right eye of each was injected with HRP (Miles) in 0.2*M* phosphate buffer (*p*H 7.2), 1 percent lysolecithin, and 1 percent dimethyl sulfoxide. The HRP is taken up by retinal ganglion cells projecting to the brain and by nerve terminals in the retina and is then transported to the brain. The animals were killed by transcardial perfusion 3 to 7 days after the injections. The brains were sectioned and alternate sections were processed according to modifications (*12*) of the De Olmos and Heimer technique (*13*) or by the method of LaVail and LaVail (*14*). The results with the two techniques were similar, although the morphology of HRP-filled brain neurons is more visible in diaminobenzidine-treated material.

Cells containing HRP were found in the optic tectum (Fig. 1) in all three species. The catfish and the cichlid had a somewhat smaller number of stained cells than the puffer. The cells were mostly located in the stratum griseum et fibrosum superficiale, but some were also found in the stratum griseum centrale.

In the puffer and the catfish some of the neurons in the pretectal complex and dorsomedial optic nucleus were also filled with HRP. The puffer also has efferents to the retina from the corpus geniculatum laterale ipsum of Meader. In the cichlid (Figs. 2 and 3) all these cell groups (pretectal and tectal aggregates,

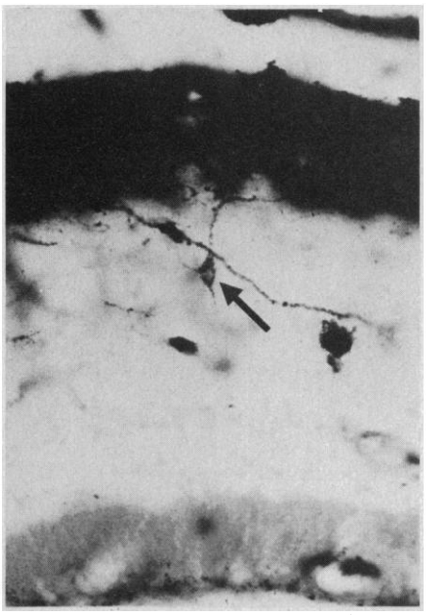

Fig. 1. Horseradish peroxidase–containing neuron (arrow) in the stratum griseum centrale of the optic tectum in the catfish *Synodontis nigriventris* after injection of HRP into the contralateral eye.

corpus geniculatum laterale ipsum, and dorsomedial optic nucleus) project to the eye, as does a hitherto unnamed nucleus in the telencephalon. This nucleus, here named the telencephalic optic nucleus (Fig. 3), is located in the ventromedial corner of the telencephalon, immediately adjacent to the medial olfactory tract (*15*). It may be homologous to an area, recently found by Münz (*16*), which provides efferents to the retina in another teleost. All the described aggregates project to the contralateral eye.

Since the anterograde transport of HRP was truly massive, we cannot rule out the possibility of transneuronal transport. However, our tentative conclusions are based on the assumption that the labeled cells were filled by a process of retrograde transport. This conclusion is supported by the finding that cells and nearby fibers were stained equally early after eye injections.

To our knowledge, this is the first description of more than one site of origin for retinopetal connections in any vertebrate. An explanation for the interspecific variation is not readily apparent. Our data cast considerable doubt on the possibility that, earlier in evolution, the cells in question migrated to their current positions from a common ancestral site. Rather, the multiple origins of the retinal afferent system in teleosts suggest the opposite. The extensive retinopetal systems seen in the fish may reflect a primitive organization, and the absence or reduction of such connections described for other, more advanced vertebrates may be the consequence of an evolutionary loss of pathways (perhaps collaterals in this case) (*17*). This interpretation is

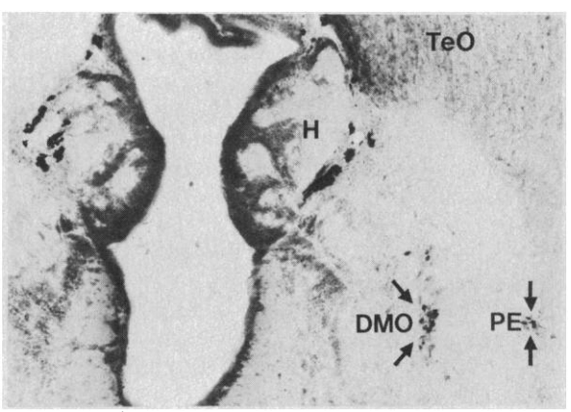

Fig. 2. Horseradish peroxidase–containing neurons in the dorsomedial optic nucleus (*DMO*) and an efferent subnucleus of the pretectal complex (*PE*) in the cichlid *Julidochromis regani* after injection of HRP into the contralateral eye (*H*, habenular nucleus; *TeO*, tectum opticum).

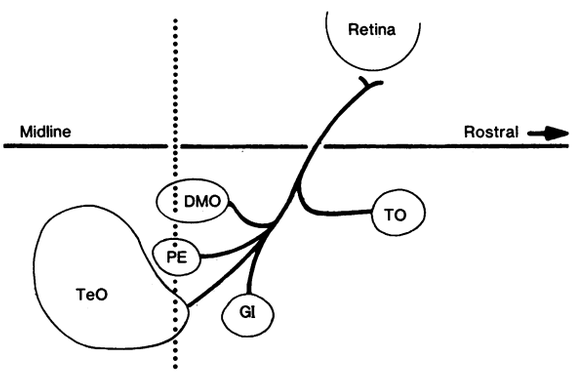

Fig. 3. Schematic drawing of the efferent connections to the retina in *Julidochromis regani*. All structures indicated in the lower part of the figure send fibers to the contralateral retina. The dotted line represents the level at which the brain shown in Fig. 2 was sectioned. (*GI*, corpus geniculatum laterale ipsum of Meader; *TO*, telencephalic optic nucleus).

consistent with the view that the retina and brain had more extensive reciprocal connections earlier in evolution and agrees with the parcellation theory (*17*), according to which neuronal systems evolve by a process that involves the loss of connections rather than the creation of new connections with hitherto unrelated targets.

A second hypothesis must also be considered. In our sample of teleost species, retinopetal systems originating in the tectum and pretectum are best developed in the puffer; they are slightly less obvious in the cichlid and much less striking in the catfish. In the weakly electric fish *Eigenmannia virescens* (*18*) they are even more poorly developed than in the catfish. It may be accidental that the magnitude as well as the frequency of spontaneous eye movements in the species studied follow the same sequence. The puffer has most extraordinary ocular movements and continuously scans its environment, whereas *E. virescens* has almost none of these movements. The catfish and cichlid are somewhere in between, with the cichlid having distinctly more pronounced spontaneous ocular movements than the catfish. Perhaps retinopetal connections are involved in preventing apparent movements of the environment during voluntary eye movements. Of course, further investigations are required to determine whether the correlation between eye movements in a given species and the development of its retinopetal systems is due to our selection of experimental subjects or whether this correlation is generally present.

Note added in proof. The recent finding of two mesencephalic nuclei projecting bilaterally to the eye in a lamprey (*19*) has just come to our attention.

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Regenerating Axons Reclaim Sensory Targets from Collateral Nerve Sprouts

Abstract. *There is a critical period for the sprouting of intact low-threshold mechanosensory cutaneous nerves in rats; functional invasion of adjacent denervated skin does not occur in animals older than about 20 days of age, and it is largely confined to denervated skin within the "domain" of the parent dermatome. These nerves can regenerate readily in the adult, however, and such regenerating nerves do not respect domain borders; moreover, they functionally displace endings of intact nerves that earlier had sprouted into denervated skin.*

One approach to the study of how nerves can establish appropriate connections, and of possible plasticity intrinsic to the connections themselves, is to permit nerves (both intact sprouting and regenerating ones) to compete for targets. In our studies of the reinnervation of denervated cutaneous targets in the rat, we have found that intact low-threshold mechanosensory nerves sprout new functional endings into adjacent denervated skin only during a brief critical period in early life and that these endings are functionally replaced by regenerating nerves that later arrive at the same region of skin.

We electrophysiologically mapped the low-threshold mechanosensory fields (*1*) of the two divisions (medial and lateral) of the segmentally arising dorsal cutaneous nerves (DCN's) (*2*). A hand-held bristle was used to deform skin and to displace hair shafts and so stimulate a variety of low-threshold mechanoreceptors (*3*). Among these were the "touch domes"—epidermal elevations that become visible after depilation. Each dome is supplied by one to three axons that branch to innervate a plate of Merkel cells at its base; displacement of the tylotrich hair associated with the dome or direct mechanical deformation of its surface evokes an irregular, slowly adapting discharge of impulses (*4*). The DCN mechanosensory subfields overlapped only slightly and were similar in size and shape from animal to animal (Fig. 1). In normal animals, the number of touch domes within the field of a given nerve remains stable from at least 15 days of age to maturity (Table 1); as the animal grows and its surface area increases, the domes become farther

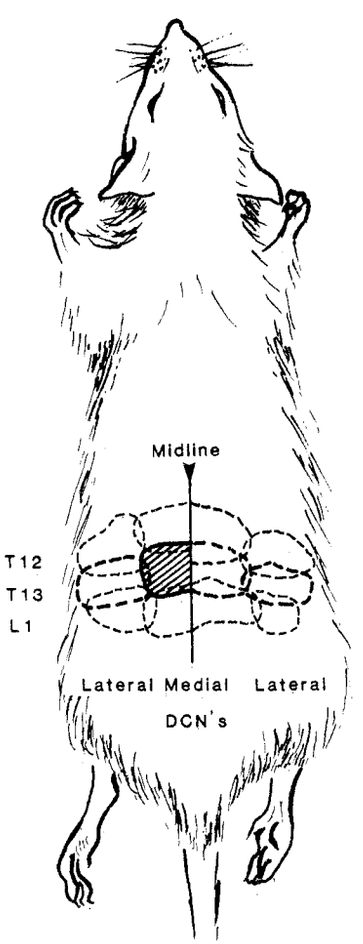

Fig. 1. Typical map of the low-threshold mechanosensory fields of the dorsal cutaneous nerves. The medial and lateral T13 subfields are shown with bold outline; the medial one (mDCN-T13) on the left is hatched.

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