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Receptor position, not nerve branch, determines electroreceptor somatotopy in the gymnotiform fish (*Apteronotus leptorhynchus*)

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The supraorbital (SO) nerve branch of some weakly electric teleosts innervates electroreceptors on the entire rostral snout and therefore excludes the infraorbital (IO) branch. A ventral twig of SO innervates the ventral snout (normally IO territory) and projects into the electroreceptive lateral line lobe in an IO pattern. This suggests that afferents to adjacent snout receptors can take widely divergent pathways (different nerve branches) to the electrosensory lateral line lobe (ELL) yet retain somatotopy centrally. We conclude: (1) that there is intrabranch somatotopy within these nerves, and (2) that receptor position, not nerve branch, determines ELL somatotopy.

Because of its anatomical and functional specificity, we have recently begun to develop the projection of primary electrosensory afferents onto the electrosensory lateral line lobe (ELL) of weakly electric teleosts into a preparation for studying the formation of ordered neuronal connections [11]. Electroreceptors of gymnotiform teleosts are distributed over the entire body surface; they are innervated by cells in the anterior lateral line nerve (ALLN) ganglion, which in turn project centrally to the ELL. Peripherally, the ALLN consists of 3 cranial nerve branches, the supraorbital (SO), infraorbital (IO), and mandibular (MN), and one trunk branch, the posterior ramus. Both electrophysiological [10] and morphological [2, 11, 12] studies have demonstrated that electroreceptors are topographically mapped within the ELL. In the ELL, electroreceptors terminate in 4 separate maps arranged in mirror image fashion. The medial segment (MS) receives ampullary electroreceptor input, the centro-medial (CMS), centrolateral (CLS), and lateral (LS) segments receive tuberous organ input.

The anatomical studies examining this morphology [2, 11] used individual nerve

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fills to map this projection and found that each peripheral nerve branch forms highly ordered fascicles within the deep fiber layer of the ELL. It is therefore possible that either the position of the receptor on the skin, or the identity of the nerve carrying the afferent fiber provides the essential information required to specify this topographical projection (interactive combinations of receptor position and nerve branch may also be used). Because the formation of ordered connections is a general issue in developmental neurobiology (e.g. ref. 5) we decided to directly address this question by combining intracellular recordings to precisely specify the peripheral receptive fields of each nerve branch with single and double labelling of small nerve fascicles. We chose to work mainly on the SO and IO nerve branches, as preliminary work suggested they have the greatest overlap.

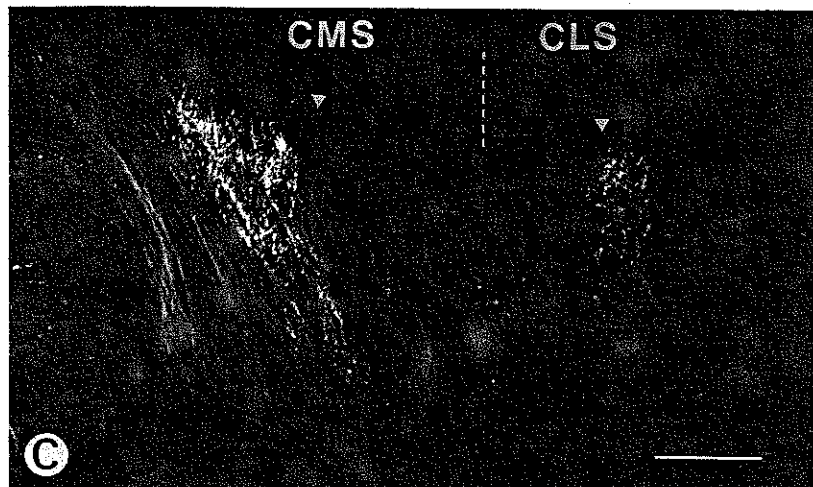
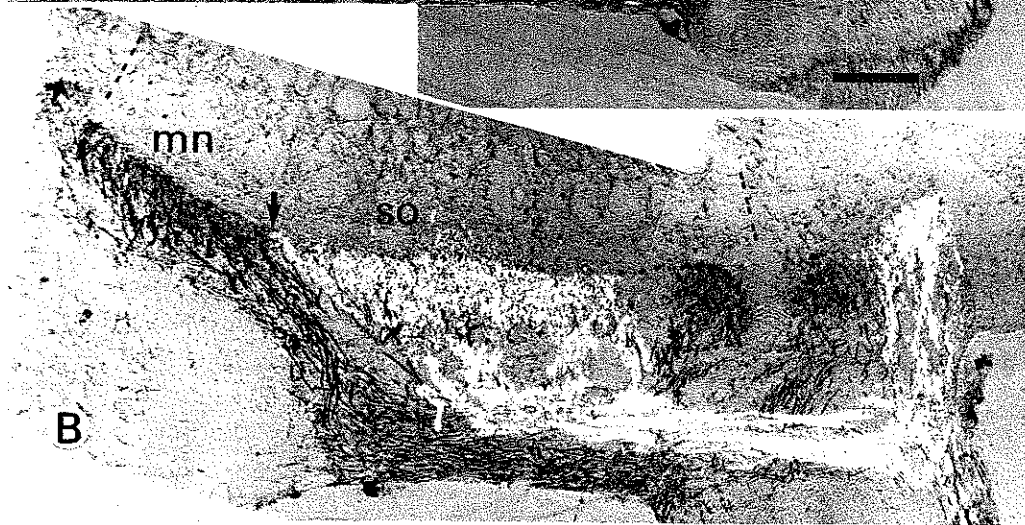
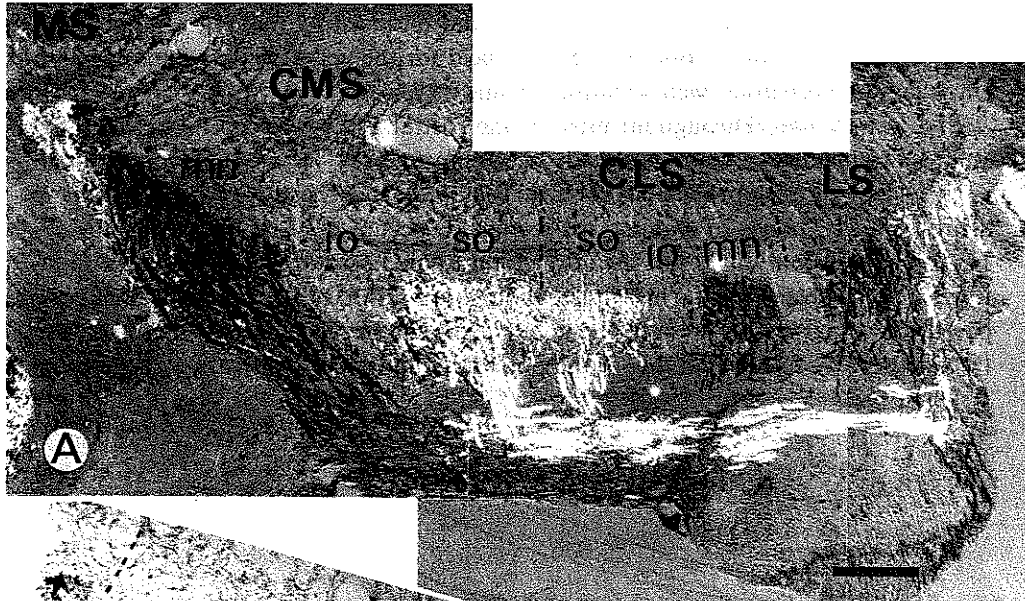
Animals. Our primary study animals were brown ghost knifefish (*Apteronotus leptorhynchus*) between 12 and 16 cm total length. These fish are hardy and inexpensive but males especially, have elongated snouts that may have affected our results. To control for snout morphology we also examined black ghosts (*A. albifrons*) of the same size, which have stubby snouts more typical of gymnotiform teleosts. We found no interspecific differences in primary electrosensory afferent projections to the ELL. Likewise, there were no sexual differences in ELL morphology.

HRP-cobalt chloride double labelling. All combinations of SO, IO and MN nerve branches were double-labelled with horseradish peroxidase (HRP) and cobalt chloride (as detailed in ref. 7). Because cobalt must remain in contact with the proximal portion of the cut nerve and because the jaw adductor muscles create a pocket around the MN nerve, with experience we found that cobalt best labelled MN. Fish were sacrificed after two days survival by deeply anesthetizing them with MS 222 (Sigma, St. Louis), and perfused intracardially with 0.25% ammonium sulfate, phosphate buffer (pH 7.4) and 3% glutaraldehyde. Brains were removed, blocked and placed in 20% sucrose overnight. ELLs were cut at 30 μm in a plane transverse to the ELL long axis [2]. Sections were reacted for cobalt chloride and HRP (as detailed in ref. 7) and were not counterstained.

Peripheral nerve anatomy. The course of the main twigs of the peripheral nerve branches were detailed by removing the epidermis of animals preserved in 10% paraformaldehyde and staining the nerves with osmium tetroxide [6]. Nerves were drawn using the snout outline and the positions of the anterior and posterior nares and the eye as guidelines.

Receptive field location. Receptive fields for SO and IO nerves were determined by recording from their axons caudal to the eye while stimulating receptors on the rostral snout. To accomplish this, spinal cords were cut to block ongoing electric organ discharge (EOD). Electroreceptor units were encountered with a search stimulus at

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 Fig. 1. Projection patterns in the rostral electrosensory lateral line lobe after an HRP fill of the supraorbital (SO), and a cobalt fill of the mandibular (MN) branches. The infraorbital (IO) branch is unlabelled, Note 4 segments (MS, medial; CMS, centromedial; CLS, centrolateral; LS, lateral) oriented in mirror image fashion. Afferent entrance is lower right. A: normal projection pattern. B: pattern far rostral showing loss of IO projection (arrow). C: IO projection pattern after labelling ventral twig of SO branch. Scale bar = 250 μm .



the EOD frequency (see ref. 21 for details) and localized by moving a dipole with a tip separation 1 mm across the receptive field for the nerve. Sixty receptors, both ampullary and tuberous, were identified in this way.

ELL somatotopy. Throughout most of the rostral ELL, IO projects between SO and MN terminal zones, as has already been reported [2, 11] (Fig. 1A). Double labeling of ALLN branch projections revealed that in the most rostral ELL the SO nerve displaces the IO nerve, so that in cross-section SO occupied 2/3 of each map and MN 1/3 (Fig. 1B). From this result, two explanations are possible: (1) that ELL somatotopy is not as precise as was previously thought; or (2) that there is an aberrant peripheral nerve pattern in these fishes and that the SO nerve in some way innervates IO territory. We focused on the second possibility. Indeed, peripheral nerve anatomy

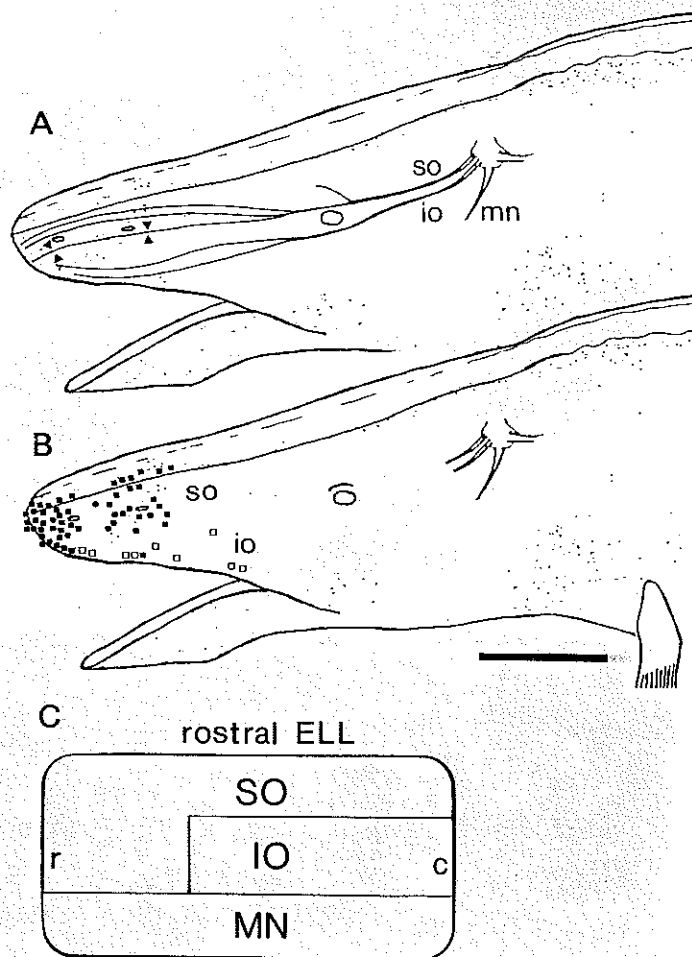


Fig. 2. A: the distribution of supraorbital (SO) and infraorbital (IO) branches and their twigs innervating the snout. Ventral twig of SO is indicated by arrowheads. B: receptive field locations for SO (filled squares) and IO (open squares) branches. C: a schematic drawing of the ELL projection in a single segment aligned to correspond to the pattern of peripheral branches in A and B. Scale bars in A and C = 1 mm.

shows that the SO nerve innervates not only the area dorsal to a line from the eye through the nares, but also rostral and ventral to the anterior nares, including the rostral oral margin of the snout (Fig. 2). A ventral branch of SO courses below the nares and may be responsible for much of this aberrant innervation (see arrows, Fig. 2). The IO nerve innervates receptors ventral and caudal to the posterior nares (Fig. 2).

Electrophysiological recordings from single SO and IO axons confirmed that receptors were innervated as specified by the nerve distributions (Fig. 2B). Again, the pattern was for SO to innervate rostroventral receptors; the area rostral to the anterior nares appears to be SO territory exclusively.

Intra-nerve somatotopy. To determine whether receptors occupying a rostroventral ('IO') position on the snout, but innervated by the SO nerve branch terminated in an IO or SO pattern, HRP-soaked gel foam pellets were inserted subepidermally onto electroreceptor afferent twigs in the rostroventral snout. To prevent the IO projection from interfering with our results we cut this nerve behind the eye, insuring that only SO fibers reached the ELL. After 3 days survival, fish were perfused with 4% paraformaldehyde, brains were processed as above except HRP was visualized using the TMB method of ref. 14. Sections were counterstained with Neutral red.

HRP labelling of the nerves to rostroventral snout receptors showed that these nerves course back to the ELL through the SO nerve but project in a typical IO pattern to the central portion of each map (Fig. 1C). From this result we suggest that at the SO-IO boundary, a caudal receptor is innervated by an IO fiber, while a receptor immediately rostral to this is innervated by an SO fiber, that in turn projects to the ELL in the area immediately rostral to the IO projection (Fig. 2C).

Discussion. Because ELL rostrocaudal snout somatotopy remains intact despite fibers being carried by multiple nerve branches, we come to two conclusions concerning the formation of electrosensory somatotopy: (1) that cranial somatotopy is resolved at a level below that of nerve branches, and that (2) somatotopy is independent of the course that nerve fibers take as they project to the ELL. Both of these conclusions bear on the developmental mechanisms underlying the formation of somatotopy, including the processes of axonal guidance and target selection.

The distributions of SO and IO nerve branches in *Apteronotus* are unusual for non-electroreceptive fishes. In most fishes, SO innervates the dorsal snout and IO the ventral snout (see refs. 1, 3, 4, 9, 13, 16, 17 and 19). In these fishes, an imaginary line drawn between the center of the eye and the nares approximates the area of overlap between SO and IO branches. Another gymnotiform electric fish, *Eigenmannia virescens*, shows the *Apteronotus* pattern, suggesting that it may be common in this order. Why this pattern appears is not clear, although photomicrographs of developing *Eigenmannia* on day 12 show that the nasal placode arises on the ventral snout, near the oral margin [20]. This position may not allow enough clearance for the IO organ primordium or growth cone to pass between them; which would be necessary to establish the usual SO-IO pattern.

From developmental studies [8, 15, 18, 20] one can infer that somatotopy is established, in a general sense, along a rostrocaudal axis and conclude, as a first approxi-

mation, that during development the playing out of peripheral nerves from the otic region distally is mirrored in their central fibers to the developing ELL. Because our results show somatotopy within individual nerve branches, the story must necessarily be more complex than this, with dorsal and ventral regions within cranial nerve branches also being mapped correctly. In fact, Heiligenberg and Dye [10] showed this physiologically in the posterior ramus innervating organs on the trunk and tail, but it is more difficult to demonstrate on the head, and one could conclude that intra-branch somatotopy is not necessary on the head, as branches stack dorsally to ventrally.

Additionally, we conclude that ELL somatotopy is established independently of the course of the afferent neuron. During development, axonal guidance mechanisms and target selection cues insure correct somatotopy [11]. Because ventral snout receptors, innervated by SO, project to the rostral IO position of the ELL, these organs are represented in the somatotopically correct position in the ELL. This suggests that somatotopic divisions based on nerve branches, or on the growth of nerve branches, are not as important in establishing ELL topography as the receptor position itself. Supporting this suggestion are data showing that within nerve branches, axons do not retain a somatotopic order as they course back to the ELL, likewise ALLN ganglion cells are not somatotopically ordered [11]. The proximal factors that determine ELL somatotopy are not yet known. What is becoming increasingly clear, however, is that active axonal guidance mechanisms are required for the establishment of this orderliness, nerve branches may merely serve as facultative conduits for axons.

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