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SUPPRESSION OF VENTILATORY REAFFERENCE IN THE ELASMOBRANCH ELECTROSENSORY SYSTEM: MEDULLARY NEURON RECEPTIVE FIELDS SUPPORT A COMMON MODE REJECTION MECHANISM

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Summary

Elasmobranch fishes have an electroreceptive system which they use for prey detection and orientation. Sensory inputs in this system are corrupted by a form of reafference generated by the animal's own ventilation. However, we show here that in the carpet shark, *Cephaloscylium isabella*, as in two previously studied batoid species, this ventilatory 'noise' is reduced by sensory processing within the medullary nucleus of the electrosensory system. It has been proposed that the noise cancellation is achieved by a common mode rejection mechanism. One prediction of this hypothesis is that secondary neurons within the medullary nucleus should have both excitatory and inhibitory components to their receptive fields. This prediction is experimentally verified here. Projection neurons of the medullary nucleus in the carpet shark typically have a focal excitatory, and a diffuse inhibitory, receptive field organization including a component of contralateral inhibition. This result provides strong support for the hypothesis that ventilatory suppression in the elasmobranch electrosensory system is achieved by a common mode mechanism.

Introduction

Elasmobranch fishes have ampullary electroreceptive organs specialized for the detection of the weak electric fields that occur naturally in aquatic environments (Kalmijn, 1971). Such fields can be either animate or inanimate in origin and the electrosensory system can be used in prey localization and in orientation. The sensory biology of electroreception has been reviewed by Kalmijn (1978, 1988) and Montgomery (1988). The elasmobranch electroreceptor organs are innervated by

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lateral line nerve fibers that project to the first-order electrosensory nucleus, termed the dorsal nucleus, in the medulla oblongata of the brain. For a review of central anatomy and physiology of elasmobranch electroreception see Bodznick and Boord (1986).

One important function of the dorsal nucleus is the suppression of self-generated noise (electrosensory reafference) resulting from the animal's own ventilatory activity. The primary electrosensory afferents are driven over a substantial portion of their dynamic range by the animal's own ventilation, and it seems likely that self-generated noise of this sort could interfere with the normal sensing of important extrinsic electric stimuli. However, many neurons of the dorsal nucleus show a greatly reduced ventilatory modulation despite maintaining a very high sensitivity to extrinsic stimuli (Montgomery, 1984*a*,*b*; New and Bodznick, 1990). These same studies provide evidence that the suppression of responses to ventilation could be the result of a common mode rejection mechanism operating within the medulla. A common mode mechanism is possible because all electroreceptors, regardless of their orientation or location on the body surface, are stimulated in the same phase and at the same amplitude by ventilation whereas they are affected differentially by extrinsic fields. Moreover, it has recently been shown that a common mode signal (i.e. one of the same amplitude and phase for nearly all the receptors) that is unrelated to ventilation is also suppressed in the medulla (Bodznick *et al.* 1992). One of the predictions of the 'common mode rejection' hypothesis is that secondary neurons within the dorsal nucleus that have small ipsilateral excitatory receptive fields should also receive inhibitory inputs, probably *via* interneurons, from other ampullary organs. Inhibitory input from contralateral receptors *via* hindbrain commissural pathways has been shown to contribute to ventilatory suppression (New and Bodznick, 1990) but the most direct evidence for the common mode rejection hypothesis would be a direct demonstration of inhibitory areas in the second-order neuron receptive fields.

We report here that in the carpet shark, as in the previously studied batoids, ventilatory electrosensory reafference is suppressed in projection neurons of the dorsal nucleus and that the receptive fields of medullary electrosensory neurons have both excitatory and inhibitory components.

Materials and methods

Carpet sharks, *Cephaloscylium isabella* Bonnaterre, were captured in crayfish traps and on longlines by commercial fishermen on the east coast of the North Island of New Zealand. They were held in seawater tanks at 17–19 °C at the Leigh Laboratory of the University of Auckland. Animals were anesthetized for surgery by immersion in a 0.007 % solution of tricaine methane sulphonate (MS222) in sea water. The cranium was opened, the animal was decerebrated by diencephalic section, and the spinal cord was pithed. In four animals a Ag/AgCl electrode was also introduced beneath the skin in the region of the buccal electroreceptor

capsule to measure the internal potentials created by the animal's own ventilation. The animal was then placed in a holder in a tank of chilled (10°C) sea water with the water level adjusted to just below the level of the cranial opening. In experiments in which ventilatory reafference was measured the animals were permitted to ventilate normally. However, in experiments in which medullary neuron receptive fields were studied the sharks were first paralyzed by intravenous injection of tubocurarine chloride (approximately 2–5 mg kg^{-1}) and a flow of aerated, chilled sea water was maintained across the gills.

A small opening was made in the posterior choroid plexus to permit microelectrode access to the dorsal nucleus and a dense, inert oil with high oxygen solubility (FC-77, 3M Co.) was introduced into the IVth ventricle to prevent blood seeping from the choroid into the ventricle. A concentric bipolar stimulating electrode was placed in the lateral mesencephalic nucleus (LMN) on one side and its depth was adjusted to maximize the evoked antidromic field potential in the contralateral dorsal nucleus. The activity of primary electrosensory afferents was recorded extracellularly from the intracranial portion of the anterior lateral line nerve with glass microelectrodes (20–30 MΩ) filled with 4 mol l^{-1} sodium chloride. Extracellular recordings of neurons within the dorsal nucleus were made with platinum-black-tipped indium electrodes, and neurons were identified as ascending efferent neurons (AENs) by antidromic stimulation from the contralateral LMN. Neurons within the dorsal nucleus that did not respond antidromically to LMN stimulation were called dorsal nucleus neurons (DNs).

The ventilatory electrosensory reafference was measured in primary afferents and neurons of the dorsal nucleus as previously described (Bodznick *et al.* 1992). The ventilatory modulation, or *noise* level, was the change in discharge rate occurring during ventilation measured from histograms of 30 ventilatory cycles. As a basis for comparison, a standardized response of the afferents and medullary neurons to uniform field stimulation was taken as the peak-to-peak change in discharge rate measured in response to 2 μV cm^{-1}, 2 Hz longitudinal and transverse fields from peristimulus time histograms of 60 cycles. The uniform field stimuli were presented without a fixed phase relationship to the shark's normal ventilation, which normally occurred at a rate of 0.3–0.5 s^{-1}. The response of the neuron, or *signal* level, was taken as the square root of the sum-of-squares of the individual responses to the longitudinal and transverse fields. In effect, this provides a characterization of the response to uniform field stimulation which should be independent of the orientation of the receptor canal. The signal-to-noise ratio for each neuron was then defined as the ratio of these signal and noise levels.

Dipole electrodes used in receptive field determinations were made from seawater/agar-filled polyethylene tubing with a separation of 0.5 cm between the poles. The electrodes were positioned with the dipole axis normal to the skin surface and with the closest electrode 1 cm distant from the skin. Specified dipole field intensities were those measured in open water along the dipole axis at a distance of 1 cm from the closest pole relative to a distant reference electrode. Thus, the specified intensities were the approximate intensities at the skin surface

when the dipole was in place. For experiments on the receptive field of the central neurons, up to five dipoles were placed around the head of the fish. Dipole 1 was located in the excitatory receptive field and the neuron was activated by a 5 μV, 1 Hz stimulus. Dipoles located over other areas of the head were then activated singly, or in concert, by a 2 μV, 100 ms square pulse with the cathode, which is excitatory for the electroreceptors, located towards the skin of the fish. These pulses were timed to coincide with the excitatory response evoked by the first dipole. Control experiments were carried out during recordings from electrosensory afferents to observe what stimulus strengths were required outside the excitatory receptive field to inhibit directly the afferent firing by cathodal stimulation of the capsular region through the skin.

Intensity–response functions were determined for a small sample of primary afferents and central neurons to uniform field and dipole field stimulation.

Results

Suppression of ventilatory reafference

A slow electrical potential modulation 1–3 s in duration and coincident with ventilatory movements was recorded between a Ag/AgCl electrode placed beneath the skin of the head and a similar electrode in the sea water. These so-called ventilatory potentials measured in four animals were normally 10–20 μV peak-to-peak but ranged as high as 150 μV in one animal in the hour immediately following surgery. As in skates (Bodznick *et al.* 1992), the ventilatory potentials of carpet sharks were variable in waveform as well as in amplitude among fish and at different times in the same fish. Primary electroreceptor afferents of the anterior lateral line nerve were strongly modulated by the ventilatory activity. The average peak-to-peak modulation of all electroreceptors recorded was 46.5±17.9 impulses s^{-1} (S.D.; N=75). In accordance with the ventilatory potential itself, the extent of modulation and the times of excitation and inhibition during the ventilatory cycle varied among animals and within a single animal at different times.

The response to ventilation is suppressed to varying degrees in neurons of the dorsal nucleus (both AENs and DNs) (Fig. 1). While the impulse rates of most of the dorsal nucleus neurons were modulated to some extent during the animal's ventilation, many were clearly less affected than the primary afferents. Primary electrosensory afferents typically had signal to noise (S/N) ratios less than 1 (mean 0.7, S.D. 0.3, N=21). Some AENs and DNs had S/N ratios this small, but the majority were substantially higher. There was no difference between the ventilatory suppression of AENs and DNs (AEN S/N mean 8.1, S.D. 14.1, N=12; DN mean 5.2, S.D. 10.2, N=22).

AEN receptive fields include inhibitory areas

Recordings from electrosensory afferents showed that dipole stimulation of 2 μV outside the region of the receptive field produced little or no effect on

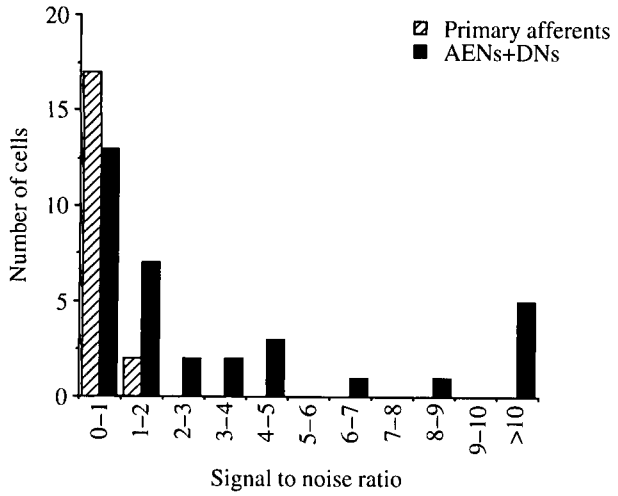

Fig. 1. A comparison of signal to noise ratios, where signal is the response to a $2\,\mu V\,cm^{-1}$ uniform electric field and noise is the modulation in discharge rate during ventilation, for primary electroreceptor afferents (AENs) and neurons of the dorsal nucleus (DNs) in the medulla.

spontaneous activity. In a few instances a very weak inhibition of spontaneous activity could be produced by a $2\,\mu V$ cathodal field located over the skin in the region of the capsule. Typically, field strengths of at least 5–10 μV were required to produce a noticeable effect, and these field strengths increased with distance from the capsular area.

In contrast, all nine AENs tested were inhibited by dipoles positioned near ampullary organs outside the neuron's excitatory receptive field. One example is shown in Fig. 2. Dipole 1 positioned over the center of the receptive field produced a distorted sine-wave response to the $5\,\mu V$ 1 Hz stimulus. Activation of dipoles 2, 3 or 4 with a $2\,\mu V$, 100 ms pulse during the excitatory portion of the response revealed a weak inhibitory input from each of these locations. Simultaneous activation of dipoles 2, 3 and 4 produced a complete suppression of firing for a period after stimulation. It is notable that dipole 4 is located on the contralateral side of the head. Primary afferents measured from the same animal, including one with a receptive field the same as this AEN (Fig. 2B), were little affected by stimulation through the dipoles outside their excitatory receptive fields. A second example of surround inhibition in an AEN is shown in Fig. 3. Here dipoles, 2, 3 and 4 individually show weak inhibitory effects, with the combination of dipoles 2, 3 and 4 producing a more pronounced inhibition. These examples were the typical pattern observed, with a discrete excitatory receptive field and a diffuse inhibitory receptive field including contralateral inhibition. One AEN (20) appeared to have a localized inhibitory input from the oppositely oriented ampullae from the same receptor group (Fig. 4). S/N ratios were only obtained for three of the nine neurons for which receptive field information was

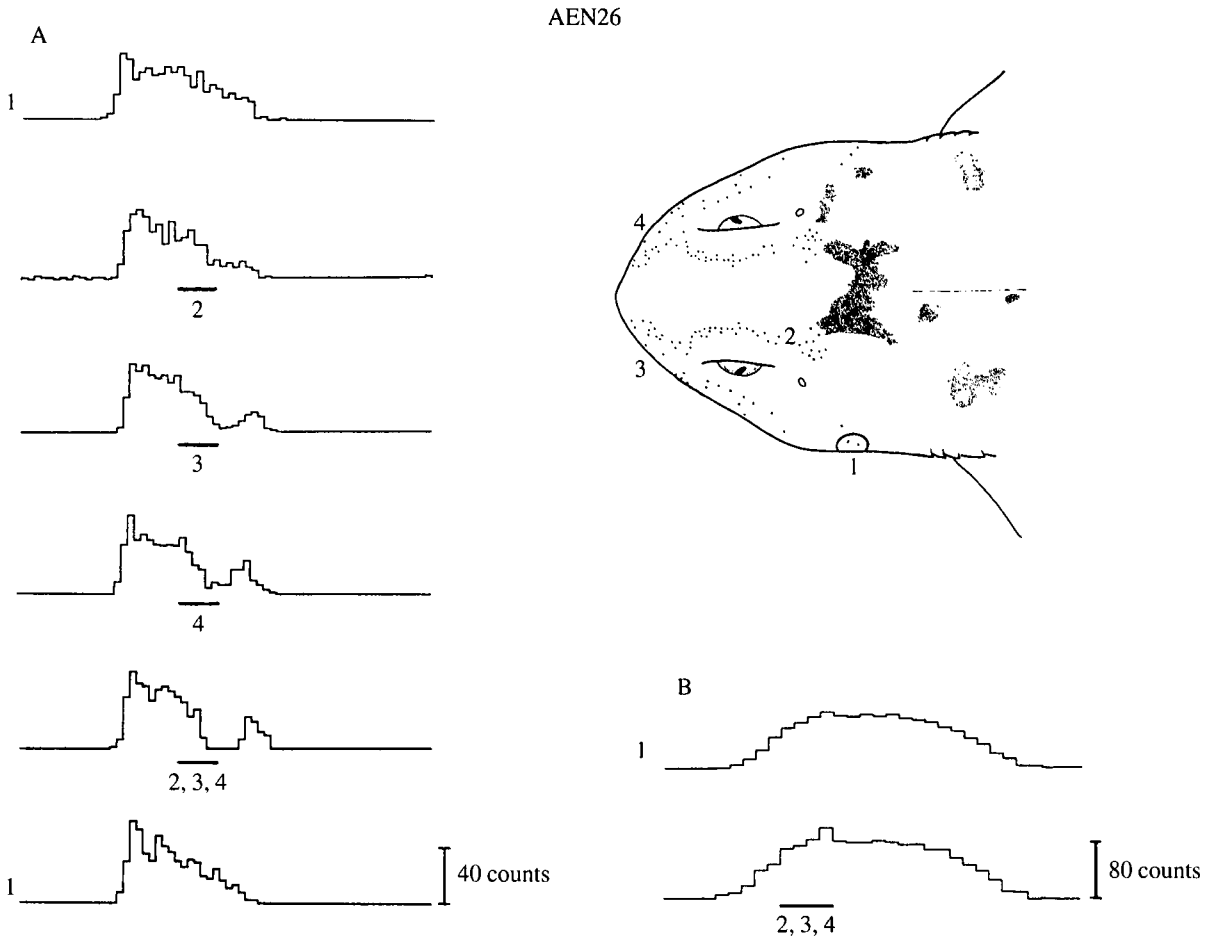

Fig. 2. (A) Receptive field organization for one ascending efferent neuron (AEN26). Dipole 1 is located in the excitatory receptive field and activated by a 1 Hz sine wave and a peak-to-peak intensity of 5 μV at the skin surface. The locations of the other dipoles are indicated on the diagram of the fish's head. Dipoles 2, 3 and 4 were activated with a 2 μV square wave of 100 ms duration (indicated by a solid line below histograms) timed to coincide with the excitatory portion of the response to activation of dipole 1. Response records are firing rate histograms of 50 stimulus presentations. Numbers below each response record indicate the active dipoles. Activation of dipoles 2, 3 or 4 results in a small inhibitory response, activation of dipoles 2, 3 and 4 together produces strong inhibition. 16 ms bins. (B) Responses of a primary electrosensory afferent from the same fish and with the same excitatory receptive field as AEN26. Stimulation is as in A except that dipoles 2, 3 and 4 are at an intensity of 5 μV at the skin surface. 32 ms bins.

obtained. These ratios were 8.3, 3.3 and 24.1. The latency to onset of the inhibition ranged from 50 to 125 ms but was typically about 70 ms. Four DN cells were also tested for inhibitory inputs; two with buccal receptive fields showed inhibition from both ipsilateral and contralateral dipoles. Of two with superficial

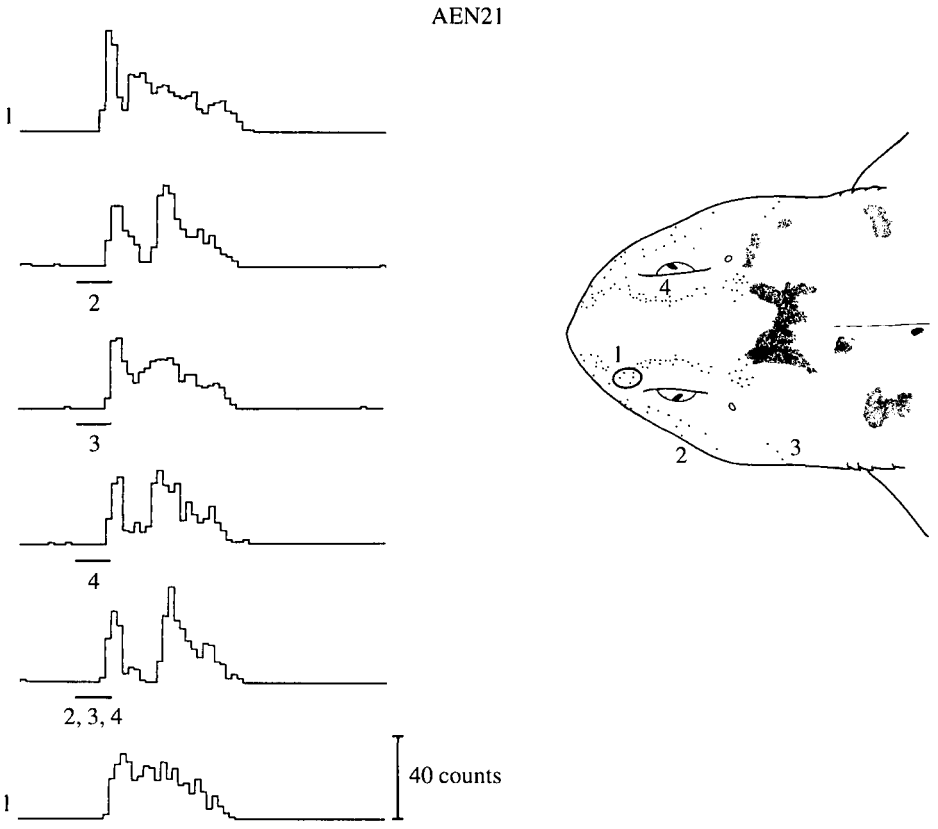

Fig. 3. Receptive field organization for AEN21. Details as for Fig. 2. 16 ms bins.

ophthalmic receptive fields, one showed no inhibition from the dipoles tested and the other was inhibited only from dipole 4 on the caudal dorsal superficial ophthalmic pore group.

Intensity–response functions determined for a small sample of primary afferents and four AENs, two with uniform and two with dipole field stimulation, are shown in Fig. 5. These few data suggest that the gain and saturation level of AENs may be lower than those of primary afferents for uniform fields but equal to or higher than those of primary afferents for dipole fields.

Discussion

The suppression of ventilatory reafference has now been demonstrated in three species of elasmobranchs: the two previously studied batoids, *Platyrhinoidis triseriata* (Montgomery, 1984*a*) and *Raja erinacea* (New and Bodznick, 1990), and the galeoid shark *Cephaloscyllium isabella*. It is a reasonable assumption that it will turn out to be a universal feature of elasmobranch electroreception. The

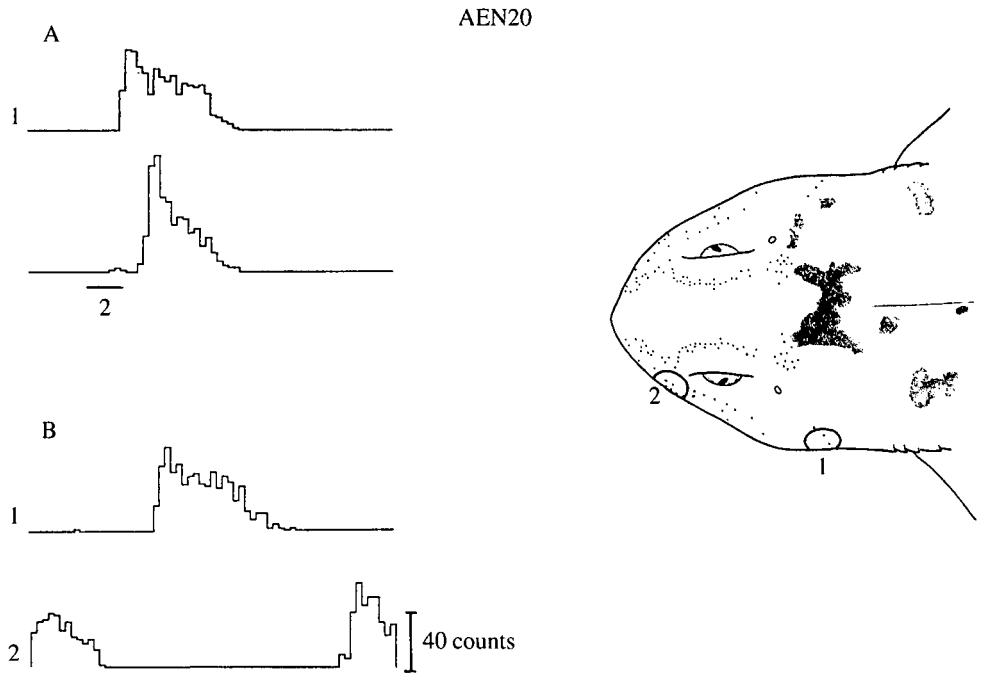

Fig. 4. Receptive field organization for AEN20. (A) Activation of dipole 2 during the excitatory phase of activation by dipole 1 produces a strong inhibition. (B) Activation of dipole 2 alone with a 5 μV, 1 Hz stimulus produces an out-of-phase response. 16 ms bins.

degree of suppression differs among the three species studied. In *Raja* and *Cephaloscyllium* the degree of suppression is variable, with some central units showing little S/N improvement and others showing virtually complete suppression of ventilatory modulation. Responses in *Platyrhinoidis* were not analyzed with the same S/N protocol as the other studies but, given the eightfold increase in sensory gain in central cells over the primary afferents (Montgomery, 1984*b*) and the virtual absence of ventilatory responses in central neurons, it is apparent that ventilatory suppression is more effective in this species. We have no explanation for this difference (but see Discussion in Bodznick *et al.* 1992).

Observations of the common mode nature of ventilatory reafference within one ampullary group (Montgomery, 1984*a*) and even between ampullary groups (New and Bodznick, 1990) indicated the possibility of a common mode suppression mechanism. The suppression of artificial common mode signals unrelated to ventilation provided the best evidence to date for the existence of a common mode mechanism (Bodznick *et al.* 1992). The observations presented here of a diffuse inhibitory component of the receptive field in AENs provides strong support for the hypothesis that ventilatory suppression in AENs is achieved by a common mode mechanism, each AEN receiving strong excitatory input from one group of afferent fibers that defines the focal excitatory field and weak inhibition from a

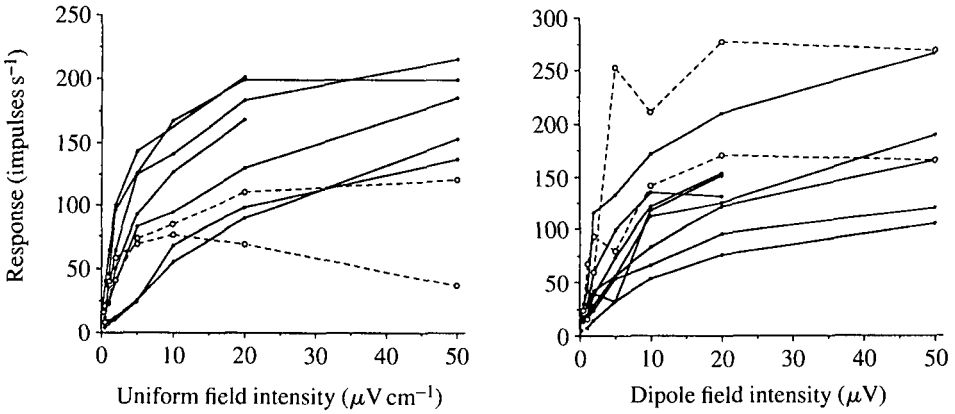

Fig. 5. Intensity–response functions for AENs (---) and primary electrosensory afferents (——) in response to uniform field and dipole field stimulation.

wide range of afferents from outside that area. The combined effect of the inhibitory inputs is to cancel a portion, or all, of the ventilatory drive being received from the excitatory inputs.

Several factors combined have made demonstration of such inhibitory areas in the AEN receptive fields difficult. Typically, AEN spontaneous impulse rates are very low, making inhibition difficult to detect. More importantly, the skin of elasmobranchs is of a relatively low resistance and even local dipole electric fields can penetrate the body and affect the activity of primary afferents by acting directly on the internal face of the receptors in the ampullary clusters. A rather weak cathodal stimulus, which is excitatory at the receptor pore, can, by acting through the skin, directly inhibit afferent firing when presented outside the excitatory field, particularly in the region of the ampullary cluster. Lastly, as is clear from the present study, the inhibitory receptive fields of many AENs are diffuse and so the electroreceptors in any one area of the skin surface have only a weak inhibitory effect on the AEN. In the present studies we have circumvented these problems by delivering weak dipole field stimulation simultaneously from up to four dipoles located outside the AEN's excitatory receptive field and coincident with the presentation of a cathodal stimulus in the AEN's excitatory receptive field. Control tests carried out in an identical way with many primary afferents served to demonstrate that the AEN inhibition is mediated by circuitry within the brain.

Initially it was suggested that common mode suppression worked by a pairing of the inputs from opposite sides of an ampullary cluster (Montgomery, 1984*a*). However, it was subsequently shown in *Raja* that contralateral inputs made a contribution to cancellation and the results reported here further indicate that pairing of inputs from opposite sides of an ampullary cluster is not the typical pattern of inputs found in the AENs of *Cephaloscyllium*. In fact, one can argue

that a focal excitatory and focal inhibitory receptive field would create difficulties for the animal in that the receptive fields of AENs for small dipole stimuli would be inherently ambiguous. However, one receptive field of this type was found in *Cephaloscyllium* and the possibility remains that differences exist among species in the degree to which cancellation is achieved by a focal or diffuse inhibitory receptive field organization.

Indication of species differences may be seen in the different degrees of ventilatory suppression seen in the three species and in a comparison of intensity–response curves of *Cephaloscyllium* and *Platyrhinoidis*. In *Platyrhinoidis*, uniform field stimulation revealed that central neurons had an increased gain and reduced dynamic range in comparison with electrosensory afferents (Montgomery, 1984*b*). This result would not be predicted by a focal excitatory and diffuse inhibitory receptive field organization. Inhibitory inputs from canals with the same basic orientation as the canals providing the excitation would reduce the gain of central neurons to uniform fields, but not to dipole fields. The intensity–response functions shown in Fig. 5 are consistent with this expectation, though the data are few and thus only suggestive. Inhibitory field organization may depend on the signal of interest, a diffuse inhibitory receptive field being more suited to the localization of small dipole sources and opposing focal inhibitory and excitatory receptive fields being more suited for the detection of uniform electric fields such as motion-induced electric fields that might serve as cues for orientation in the marine environment (Kalmijn, 1988).

The pathway subserving the common mode rejection mechanism is as yet unknown. The AENs are large multipolar neurons concentrated in a band beneath the molecular layer in the peripheral zone of the dorsal nucleus (Bodznick and Boord, 1986; Paul and Roberts, 1977; Paul *et al.* 1977). They have one set of dendrites, which penetrate the molecular layer and have an appearance similar to Purkinje cell molecular layer dendrites. It is unlikely that the molecular layer system provides for common mode rejection since the major source of inputs to the molecular layer carries proprioceptive and descending electroreceptive information (Conley and Bodznick, 1989). In addition to the molecular layer system, the AENs have a ventral dendritic field, which extends into the neuropile in the central zone of the nucleus. Input from electrosensory afferents onto AENs is *via* the ventral dendrites. Within this neuropile is a variety of interneurons that probably includes inhibitory neurons mediating the ipsilateral common mode rejection pathway. The somata of neurons with projections to the contralateral dorsal nucleus are found in both the central and peripheral zones. At least a subset of these commissural cells must mediate the contralateral inhibitory component of AEN receptive fields. However, the details of both ipsilateral and contralateral common mode rejection circuitry remain to be established.

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