

MOTOR COROLLARY DISCHARGE ACTIVITY AND SENSORY RESPONSES RELATED TO VENTILATION IN THE SKATE VESTIBULOLATERAL CEREBELLUM: IMPLICATIONS FOR ELECTROSENSORY PROCESSING

GREGORY O. HJELMSTAD, GEOFFREY PARKS AND DAVID BODZNICK*

Department of Biology, Wesleyan University, Middletown, CT 06459, USA

Accepted 20 November 1995

Summary

The dorsal granular ridge (DGR) of the elasmobranch vestibulolateral cerebellum is the source of a parallel fiber projection to the electrosensory dorsal nucleus. We report that the DGR in *Raja erinacea* contains a large percentage of units with activity modulated by the animal's own ventilation. These include proprioceptive and electrosensory units, responding to either ventilatory movements or the resulting electroreceptive reafference, and an additional population of units in which activity is phase-locked to the ventilatory motor commands even in animals paralyzed to block all ventilatory movements. A

principal function of processing in the dorsal nucleus is the elimination of ventilatory noise in second-order electrosensory neurons. The existence of these ventilatory motor corollary discharge units, along with other DGR units responsive to ventilatory movements, suggests that the parallel fiber projection is involved in the noise cancellation mechanisms.

Key words: electroreception, elasmobranch, skate, *Raja erinacea*, cerebellum, proprioception, efference copy.

Introduction

The electrosense of elasmobranch fish, like many other vertebrate sensory systems, is vulnerable to interference from stimuli created by the animal's own activity. Electrical signals that are byproducts of the movements of the fish modulate the activity of its electroreceptors; however, neural circuits of the medulla selectively remove the effects of this self-stimulation by mechanisms that we are interested in understanding.

Ventilation is the major source of self-generated noise (or reafference) for the elasmobranch electrosense. The transdermal electrical potentials created by ventilatory movements are typically tens of microvolts in amplitude and can drive the electroreceptive primary afferents through two-thirds or more of their dynamic range. These ventilatory potentials are created when the standing d.c. potential that exists across the skin of the fish is variably shunted to the surrounding water with the opening and closing of the gills, mouth and spiracle (Bodznick *et al.* 1992). Because the resistance of the skin is somewhat higher than that of the internal tissues, the ventilatory potential is nearly uniform throughout the internal tissues of the head, which serve as the reference point for all of the electroreceptors. A mechanism within an area of the dorsal octavolateralis nucleus (hereafter referred to as the dorsal nucleus), the medullary electrosensory

nucleus, utilizes this aspect of the ventilatory potential and, by differentially measuring inputs among receptors, selectively cancels out the common-mode noise. The result is that the second-order ascending efferent neurons (AENs), which are the projection neurons of the dorsal nucleus, are unaffected or less affected by ventilation than are the primary afferents (Montgomery, 1984; New and Bodznick, 1990; Bodznick and Montgomery, 1992; Bodznick *et al.* 1992). Common-mode rejection, as this mechanism is called, is a direct outcome of the opposing receptive fields of the AENs created by excitatory and inhibitory synaptic connections thought to be made entirely within the central neuropil of the nucleus.

While common-mode rejection circuits may have primary responsibility for cancelling ventilatory reafference in AENs, other neural circuits and mechanisms might also be involved. The dorsal nucleus has a close affinity to the cerebellum. The nucleus is bounded laterally by a broad molecular layer containing parallel fibers that originate in a granular area of the vestibulolateral cerebellum termed the dorsal granular ridge (DGR) (Fig. 1). The AENs are situated in the peripheral zone of the nucleus beneath the molecular layer, and each AEN has two distinct dendritic fields. One set of dendrites extends into the central neuropil (or central zone) and is involved in the

*Author for correspondence

common-mode rejection circuits already mentioned. A second set of apical dendrites extends in Purkinje cell fashion into the molecular layer, where it receives excitatory and inhibitory inputs from parallel fibers and stellate cells (Paul and Roberts, 1977; Paul *et al.* 1977). What is the nature of these molecular layer inputs? Earlier physiological studies have shown that the DGR contains proprioceptive units responsive to changes in body position and higher-order electrosensory units carrying descending feedback to the dorsal nucleus (Schmidt and Bodznick, 1987; New and Bodznick, 1990; Conley and Bodznick, 1994). These DGR units are somatotopically mapped, and their projection onto the AENs appears to be topographically organized (Schmidt and Bodznick, 1987; Conley and Bodznick, 1994). It is notable that, in these earlier studies, several DGR proprioceptive and electroreceptive units recorded in small samples from freely breathing skates were also modulated by ventilatory movements. This suggests that the parallel fiber projection to the dorsal nucleus might contribute in some way to ventilatory noise suppression. Furthermore, recent studies (Bell *et al.* 1993) suggest that a modifiable efference copy mechanism responsible for cancelling unwanted electrosensory reafference in second-order neurons of mormyrid fish is mediated by a comparable parallel fiber projection from the caudal cerebellum to the medullary electrosensory nucleus. These findings prompted us to examine the DGR further in terms of its potential link to ventilatory noise reduction in the dorsal nucleus.

We report here that, in single-unit recordings, the spiking activity of the great majority of proprioceptive and electroreceptive units in the DGR of the little skate are modulated by ventilation. Additionally, there is a prominent class of corollary discharge units with activity related to ventilatory motor commands. These results support the hypothesis that the DGR has a central role in an additive mechanism using available sensory cues and motor-command-related signals to further reduce the effects of the fish's own ventilatory movements on its electroreceptive system.

Materials and methods

Experiments were performed on specimens of the little skate (*Raja erinacea* Mitchell) obtained locally from Long Island Sound and maintained in seawater aquaria at a temperature of 10–12 °C. Animals were anesthetized by immersion in approximately 0.025% tricaine methanesulfate. The cranial roof was removed, exposing the brain, and the animal was decerebrated by transection of the forebrain at the optic chiasm. In some experiments, the skate was curarized by intravenous injection of 2.5–3.5 mg kg⁻¹ tubocurarine chloride or 0.6–1.0 mg kg⁻¹ pancuronium bromide, and artificially respired with a flow of sea water delivered through the mouth. In others, the spinal cord was transected, allowing natural ventilation, but otherwise immobilizing the animal. All procedures for the care and use of the animals followed NIH guidelines and were approved by the Wesleyan Animal Care and Use Committee.

In curarized animals, the motor branch of the hyomandibular nerve (seventh cranial), which contains the motor signal to the branchial muscles, was exposed, and its activity was recorded with a suction electrode. This multiunit motor discharge was integrated by passing the signal through a low-pass filter, and it was then used as a trigger for histograms of unit activity recorded from the medulla. As a result of this filtering, the histogram trigger (time=0) was delayed and in some cases, depending on the exact setting of the histogram trigger level, followed the onset of motor command activity by several hundred milliseconds. Single or multiple units from the DGR or dorsal nucleus were recorded extracellularly using platinum-black-tipped indium electrodes with resistances ranging from 2 to 6 MΩ. In some cases, afferent units from the contralateral anterior lateral line nerve were simultaneously recorded intracranially using glass microelectrodes (20–30 MΩ) filled with 4 mol l⁻¹ NaCl.

In experiments with freely breathing animals, histograms were triggered from ventilatory movements detected with an isometric force transducer coupled to the gill region. The ventilatory potentials in these fish were measured with a AgCl-tipped silver wire inserted under the skin on the head of the fish and referenced against a similar electrode in the sea water. The amplitude and polarity of the ventilatory potential could be modified as previously described (Bodznick *et al.* 1992) by passing a d.c. current through a monopolar seawater–agar electrode made from 1 mm i.d. polyethylene tubing and inserted into the gut of the animal *via* the cloaca.

Electroreceptive units were identified by their response to either a 1–20 μV cm⁻¹ uniform field or a 2–100 μV dipole field measured at a distance of 1 cm from the closest electrode along the dipole axis and relative to a distant reference. When presented, the dipole was also positioned at the same distance (1 cm) from the fish along the dipole axis. Fields were presented *via* a constant-current stimulus isolation unit and salt-bridge electrodes as 500 ms d.c. steps or as 2 Hz sinusoids. In some cases in spinalized but unparalyzed fish, sensory stimuli induced 'coughs' or extra ventilatory movements. For this reason, histograms triggered by the stimulus were made, and putative electrosensory units were assayed for a consistent short onset latency. This ensured that they were direct responses to the external sensory stimulus and that they were not due to a behavioral artifact, i.e. a proprioceptive response or motor corollary discharge related to the induced movements.

Proprioceptive units were identified by applying a 1 cm lift or depression to the ipsilateral pectoral fin edge. This is known to be an effective stimulus for most DGR proprioceptive units (Conley and Bodznick, 1994).

All spike data were collected and averaged over 40 sweeps using IBM clone personal computers and RHIST software produced by Run Technologies. Data were analyzed statistically using the Student's *t*-test (Sokal and Rohlf, 1981). Sensory responses were evaluated by comparing the average spike rate during the stimulation period with the rate during a comparable prestimulus interval. The modulation of activity during ventilation or fictive ventilation was evaluated by

comparing the peak-to-peak modulation in spike rate during the active ventilation period with a comparable period just preceding it.

Results

Recordings from freely breathing fish

In recordings from freely breathing skate, the majority of both single units and multiple units in the DGR showed modulation of spiking activity tied to the ventilatory movements. In 13 ventilating animals, 73% (47 of 64) of the units recorded showed a distinct correlation between unit activity and ventilatory movements. The DGR records often showed several temporal peaks (Fig. 2), roughly correlating with the different movements of the ventilatory cycle (mouth and spiracle closure, contraction and then expansion of the branchial chamber). This 'multi-phasic' pattern is evident in some single-unit as well as multi-unit recordings.

There are several potential sources for the responses seen in the DGR. The responses may be (1) proprioceptive, caused by the ventilatory movements themselves; (2) electroreceptive, caused by the induced transdermal potential changes which modulate the activity of the electroreceptors of the fish during ventilation; or (3) centrally originating corollary discharge signals related to the ventilatory motor commands. We find that all three of these are clearly present in the DGR.

Proprioceptive single units were positively identified by their distinct response to a 1 cm lift to the ipsilateral fin. This

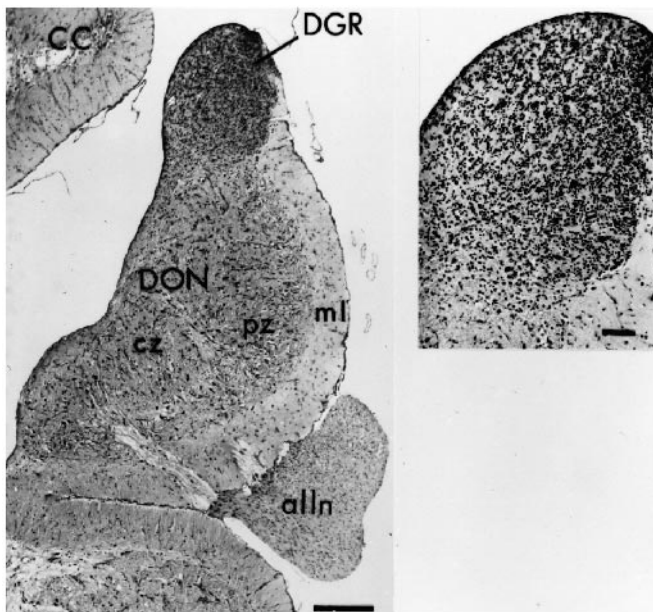


Fig. 1. Cresyl-stained transverse section of the dorsal nucleus and dorsal granular ridge in the skate medulla. The inset (upper right) is a higher-power view of the dorsal granular ridge showing dense granule cells interspersed with much larger, darkly staining Golgi cells. CC, corpus of the cerebellum; DRG, dorsal granular ridge; DON, dorsal octavolateralis nucleus; ml, molecular layer; cz, central zone; pz, peripheral zone; alln, anterior lateral line nerve. Scale bars, 500 μ m (left), 100 μ m (right).

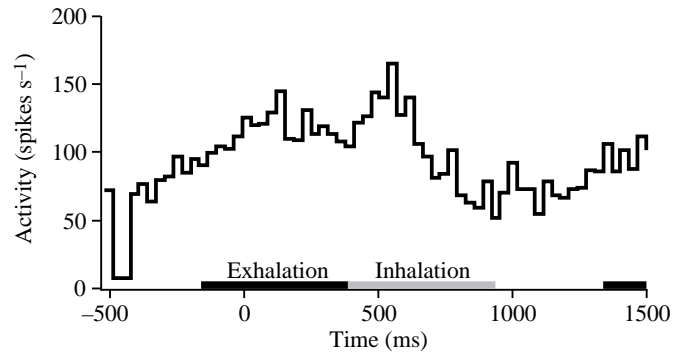


Fig. 2. Histogram of multiunit activity recorded from the DGR in a freely ventilating skate. The histogram was triggered by ventilatory movements at time zero. Separate activity peaks were associated with both exhalation and inhalation. In this and subsequent figures, approximate times of exhalation and inhalation are indicated by black and gray bars, respectively. Following inhalation, there is an interval without movement, the duration of which is variable and depends on the overall ventilatory period (which can range from 1.5 s to more than 5 s). The increased firing rate at the end of the histogram is due to the onset of the next ventilatory cycle.

is a stimulus previously shown to be effective for the great majority of DGR proprioceptive units (Conley and Bodznick, 1994). Of those ascertained to be proprioceptive, 58% (7 of 12) responded to ventilation (Fig. 3). Surprisingly, in this small sample, the receptive field of a proprioceptive unit for fin movements was not a clear predictor of whether the unit would be affected by ventilation. Units sensitive to movements of the caudal portion of the pectoral fin were as likely to exhibit ventilatory modulation as those with rostral receptive fields nearer the level of the branchial chamber.

Electroreceptive units in the DGR were also modulated by ventilatory movements. In 85% (22 of 26) of the electroreceptive units recorded, spiking activity was significantly modulated by ventilation (t -test; $P < 0.05$), usually quite strongly so. When presented as histograms, the responses of the units usually reflected the profile of the ventilatory potential, with the modulation of the units being largely or totally predicted by the modulation pattern of the electroreceptive primary afferents (Fig. 4). However, this was not always the case. Occasionally, an electroreceptive unit exhibited additional activity that was not linked to the ventilatory potential or to the activity of the primary afferents. This was further demonstrated in the following experiments in which the ventilatory potential was experimentally altered.

As already described, the measured ventilatory potential of the animal is actually the fluctuation in a transcutaneous d.c. potential resulting from the variable shunting of the d.c. potential with opening and closing of the mouth, spiracle or gill openings. The driving current for the ventilatory potential appears to be linked to the ionic gradient between the animal and its external milieu. By changing this driving force, we can effect either an increase or a decrease in the ventilatory potential, even though the amount of shunting remains unchanged. Completely offsetting the driving current by

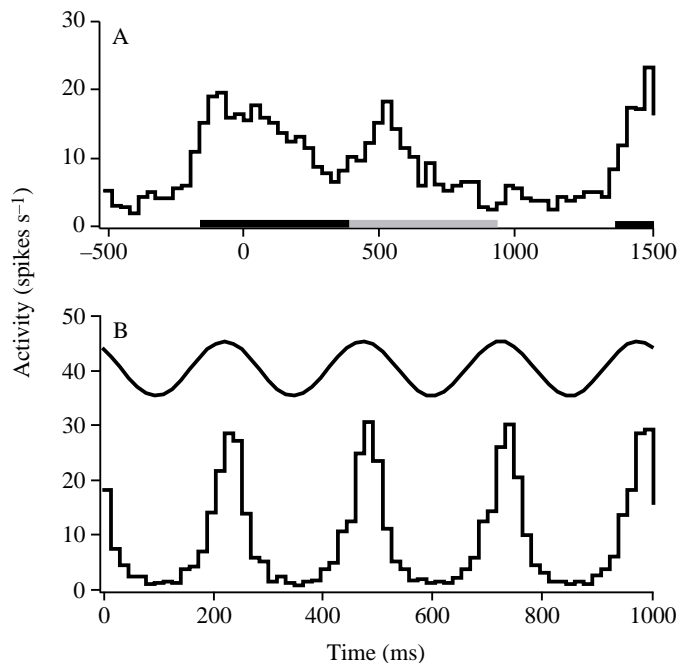


Fig. 3. (A) Example histogram of a DGR proprioceptive unit responsive to the movements of both exhalation and inhalation. (B) The response of the same unit to a 1 cm, 4 Hz sinusoidal lift to the ipsilateral fin (shown above the histogram).

passing a constant current of an appropriate magnitude into the gut of the animal entirely negates the ventilatory potential. This potential can be monitored with a silver wire embedded under the skin or, more effectively, by monitoring the activity of electrosensory primary afferents which are all affected in the same way by the transdermal ventilatory potentials (for a more detailed explanation, see Bodznick *et al.* 1992).

Fig. 5A demonstrates an example of a DGR electrosensory unit with two parts to its response. An excitatory phase occurs just before the onset of exhalation, when there is no corresponding change in firing of the primary afferents. The unit also displays a burst of activity associated with the decline in afferent activity (perhaps a post-inhibitory rebound) following inhalation. By passing a constant current into the gut of the skate, we were able to cancel completely the ventilatory potential modulation (Fig. 5B; the primary afferent has a flat response). The burst in the DGR unit associated with inhalation disappears, but the early excitatory phase is untouched. Enhancement of the ventilatory potential by the same means (Fig. 5C) causes an excitation of the unit correlated with the inside-negative phase of the ventilatory potential, again with the apparently non-electroreceptive phase unchanged. The source of the non-electroreceptive phase in these units is unclear from these data alone, but further experiments indicate that it is due to a centrally originating corollary discharge of ventilatory motor commands.

Recordings from curarized fish

In order to measure corollary discharge activity of the motor

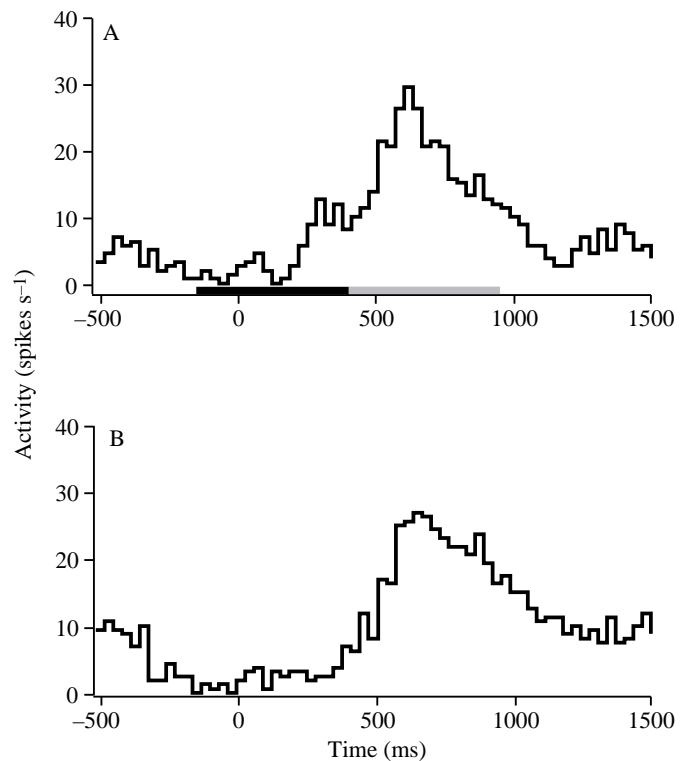


Fig. 4. Example of a DGR electroreceptive unit. The unit shows a response (A) to the ventilatory potential very similar to that recorded simultaneously from a single electroreceptive primary afferent (B), which is indicative of the uniform modulation of the primary afferents. In this fish, the ventilatory potential slightly inhibits the electroreceptive primary afferents during exhalation and excites them during inhalation.

commands, it was necessary to eliminate both proprioceptive and electroreceptive modulation during ventilation. Paralyzing the skate with curare effectively eliminates all ventilatory movements and associated sensory signals. However, the rhythmic ventilatory motor discharge which was recorded from the seventh cranial nerve continues with a normal period of 2–3 s for many hours and is still present, though usually with a longer period, after 3 or more days.

In 25 cases where the animal was curarized, 43% (71 of 164) of the units recorded in the DGR were modulated with the fictive ventilation. The ongoing activity of a unit, the onset latency of its response relative to the motor discharge and the duration of that response are all highly variable between recorded units (Fig. 6), even in a single animal. Additionally, the shape of the response histogram varies among units. Some units (Fig. 6A) show a multi-phasic response similar to the response shown in Fig. 2. The onset latencies of unit activity with respect to the motor discharge are spread throughout the ventilatory cycle (Fig. 7), with the activity in the majority of units falling within the period of the fictive ventilatory activity, i.e. during the period when ventilatory movements would normally be taking place rather than in the intervals between.

Of the units recorded in paralyzed animals which could be classified as having corollary discharge activity, 44% (31 of

71) also responded to extrinsic electric fields and 4% (3 of 71) responded to both electric fields and proprioceptive stimuli. Thirty-one of the units (44%) showed only corollary discharge activity. Electroreceptive and proprioceptive responses in the remaining six units were not determined. In two units where it was examined, the effects of sensory stimuli appeared to show simple summation with the command-related activity. No synergistic interactions were found when the electric or proprioceptive stimuli were presented at different phases of the fictive ventilatory cycle. We did not thoroughly characterize the electroreceptive or proprioceptive responses of motor corollary discharge units, but several observations can be noted. The electrosensory receptive fields of the units were large and poorly defined like those of the DGR electroreceptive units recorded earlier (Conley and Bodznick, 1994), some of which may also have been corollary discharge units. Consistent with their large receptive fields, these DGR units generally responded well to large-scale uniform electric fields or to

whole-body, common-mode electric field stimuli similar to ventilatory potentials but created in this case by delivering current through an electrode in the gut of the fish.

Throughout this study, units were characterized as proprioceptive only if they responded in a specific phase-locked fashion to fin movements or passive displacements of the body wall, mouth or spiracle. Most of the corollary discharge units were not proprioceptive by these criteria. However, in many of the corollary discharge units, spiking activity was clearly altered during the proprioceptive stimulation but in a nonspecific way, not linked to any particular aspect of the sensory stimulus. The altered firing rates of the units in these cases was not accompanied by any detectable change in the ventilatory command signal.

Although curarizing the animal blocked all visible movements and reduced the ventilatory potential below measurable levels, the possibility remained that the apparent motor corollary discharges were actually highly amplified

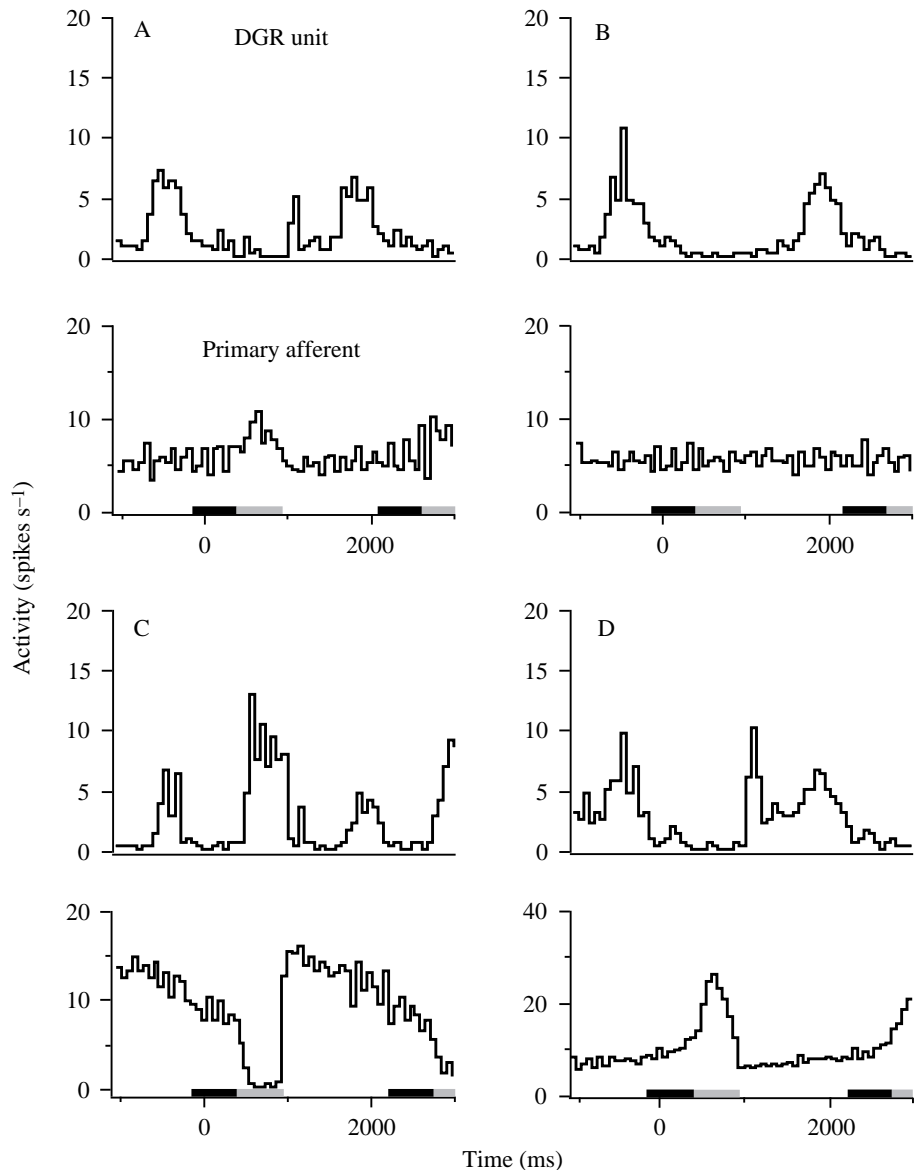


Fig. 5. Ventilatory modulation of an electroreceptive DGR unit (upper traces in A–D) and simultaneously recorded electroreceptive primary afferent (lower traces). (A) The DGR unit shows (1) a sign-inverted response to the ventilatory potential, i.e. the DGR unit responds with a burst of activity to the decline in afferent firing, and (2) excitation just prior to exhalation when there is no corresponding primary afferent response. (B,C) Constant current applied through the gut of the animal alters the ventilatory potential, as shown by the primary afferent response. (B) Cancelling the ventilatory potential eliminates the electroreceptive response in the DGR unit, but not the activity prior to exhalation. (C) Increased current reverses the ventilatory potential. The DGR unit is now excited during afferent inhibition. (D) Return to baseline (note the changed scale for activity).

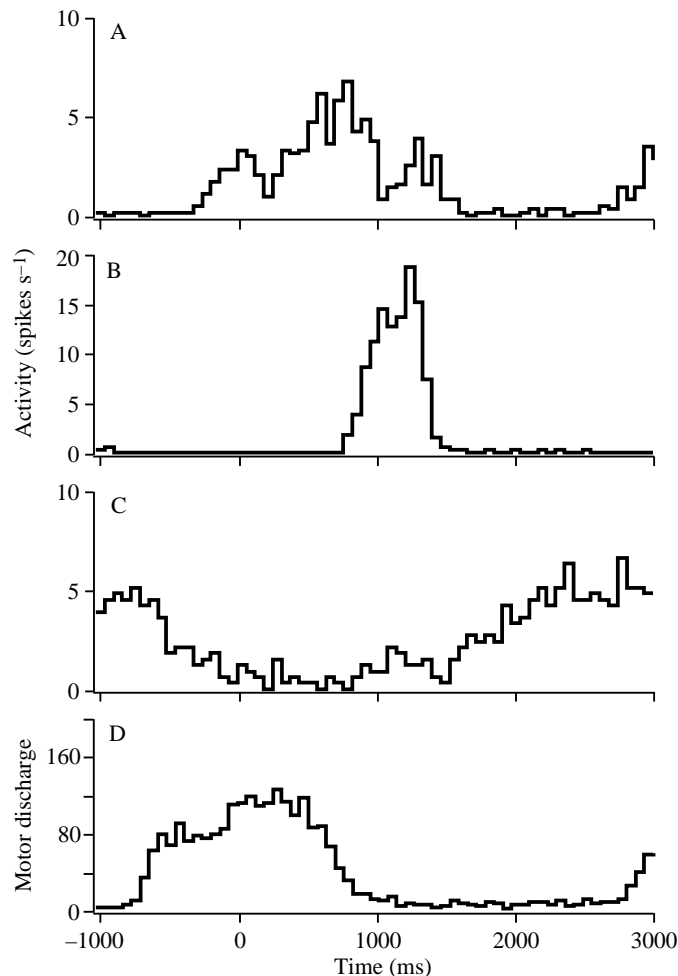


Fig. 6. (A–C) Histograms showing the activity of three corollary discharge units from one animal illustrating the variety of response patterns observed. Motor discharge (D) was recorded with a suction electrode from the motor root of the seventh cranial nerve. Histograms were triggered by the onset of the motor discharge as described in the Materials and methods section.

responses to imperceptible ventilatory movements. To control for this possibility, we bilaterally transected the lateral line nerves, cranial nerves V, VII, IX and X, and the spinal cord, thus completely eliminating the possibility of ventilatory movements and also isolating the brain from proprioceptive or electrosensory inputs. The motor discharge of the seventh cranial nerve was recorded intracranially, medial to the transection of the seventh nerve. Multiple-unit recordings made in such a nerve-transected animal continued to show a clear ventilatory modulation. Fig. 8 shows multi-unit corollary discharge activity in a curarized animal (Fig. 8A) and in an animal with cranial nerves and spinal cord transected (Fig. 8B). Both multi- and single-unit recordings demonstrated that the corollary discharge signals were present throughout the DGR. The traces of Fig. 8A suggest a gradual shift in onset latency of the corollary discharge responses along the length of the DGR. However, while there was variation in the amplitude and timing of multi-unit responses at different

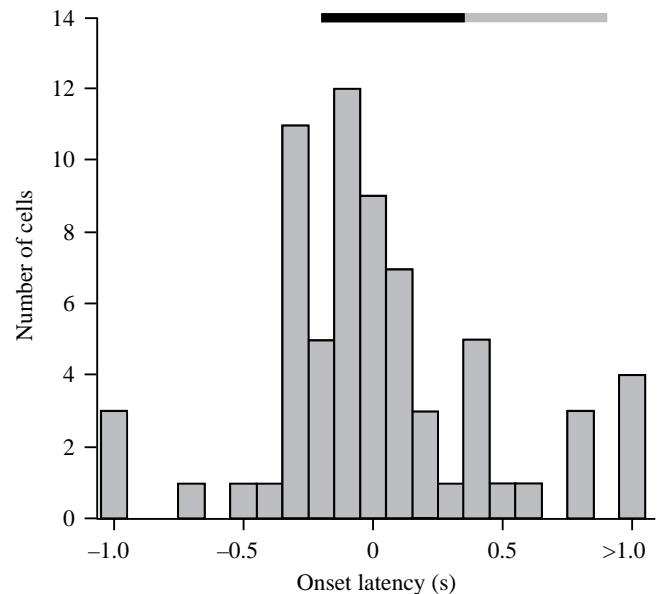


Fig. 7. Distribution of onset latencies of corollary discharge activity in 68 DGR units measured in relation to the motor discharge recorded from the seventh cranial nerve. Three units did not have discrete onsets and were not included in this histogram. The labels indicate estimated times of ventilatory movements and are subject to error due to the variable phase delays in the trigger as indicated in the Materials and methods section.

recording locations, our additional records did not show systematic changes either along the rostro-caudal axis or with depth. Similar multi-unit discharges related to the ventilatory motor commands were recorded from the lateral granular area just rostral to the DGR, but these were generally smaller in amplitude and more difficult to find.

The sole output of the DGR is *via* the parallel fiber pathway terminating on the AENs in the dorsal nucleus. We looked to see whether the activity of AENs was affected by the ventilatory signals in the DGR by bilaterally transecting the anterior lateral line nerve in freely breathing animals to remove all electroreceptive input. In five of eight AENs, the activity was robustly modulated during ventilation and, as expected, none was responsive to applied electric fields. Two examples are provided in Fig. 9. Of the remaining three AENs, two showed some modulation, while the third was silent.

Discussion

It is apparent from these experiments that the activity of most DGR units is modulated by ventilatory movements. Earlier studies of the DGR also showed that the majority of proprioceptive units (59%, New and Bodznick, 1990) and electrosensory units (53%, Conley and Bodznick, 1994) responded to ventilation. The percentage of electrosensory units showing ventilatory modulation in the current studies (85%) is higher than those previously found, but this difference might be attributable to the small sample sizes. The

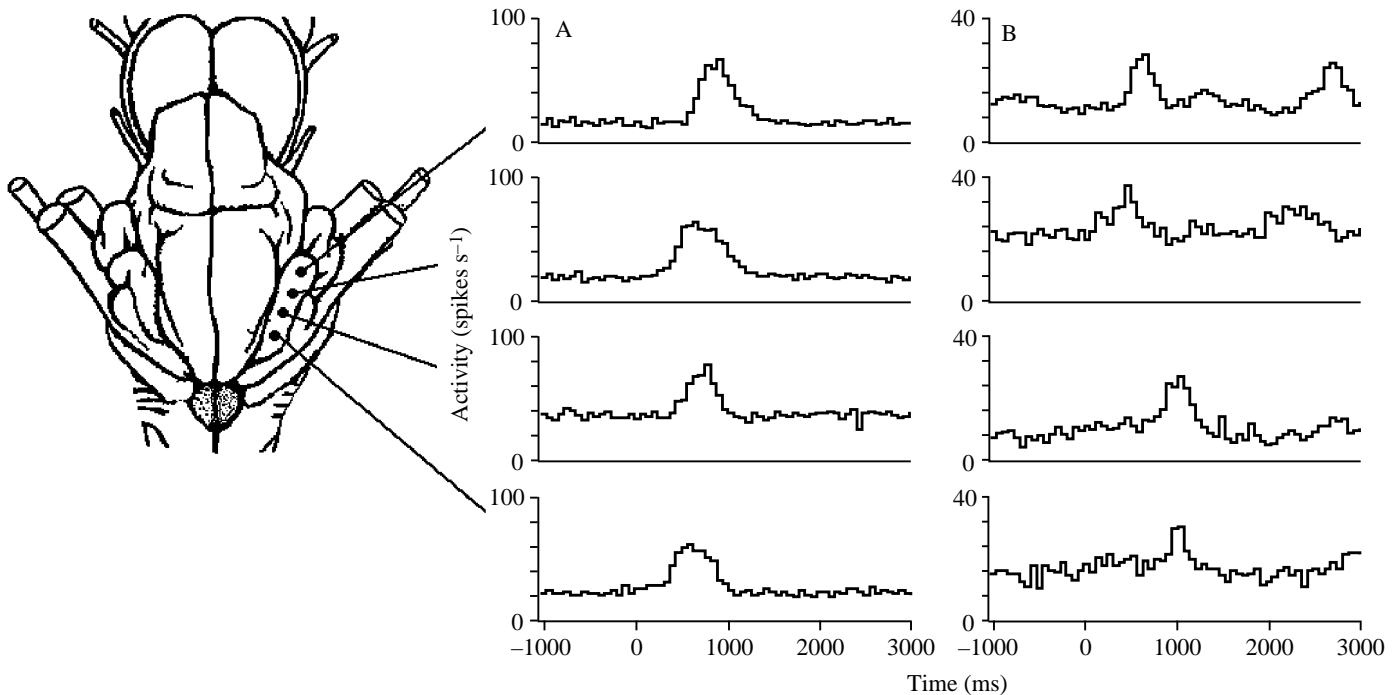


Fig. 8. Multiunit recording in two paralyzed skate from the DGR sites indicated on the dorsal view of the hindbrain (on the left). Response strength and delays varied within single recording tracks and among tracks, but corollary discharge activity was present throughout the DGR. Animal A was curarized. In animal B, the spinal cord was transected at the obex and cranial nerves V, VII, IX and X, and the lateral line nerves were bilaterally transected within the cranium. Hindbrain drawing is courtesy of R. G. Northcutt.

major new finding of the current study is that a substantial proportion of DGR units carry centrally generated corollary discharge signals of the ventilatory motor commands.

Among the DGR corollary discharge units, there is a great variation in temporal firing patterns and onset latencies relative to the start of the ventilatory cycle. A portion of the apparent variability in onset timing is certainly due to variation in the exact timing of the histogram trigger relative to the actual onset of the ventilatory motor command in cranial nerve VII. However, this does not appear to be responsible for most of the variation as a wide range of onset times are seen in even single animals (see Fig. 6) where the timing of the histogram trigger relative to the motor command is constant. The great variation in the onset times of the DGR corollary discharge units is not unexpected if the motor commands for different component movements of the ventilatory cycle each send their own distinct corollary discharge signals to the DGR. The ventilatory cycle comprises several different movements (mouth, spiracle and branchial musculature). Activity that appears to be coincident with each of these different movements can be seen in DGR unit responses. The mouth movements are driven by a motor discharge in cranial nerve V, and nearly synchronous bursts in nerves VII, IX and X elicit contractions in the branchial adductor muscles (Robert and Ballintijn, 1988). Our recordings confirmed this pattern; the discharge from the motor root of nerve V preceded those of nerve VII by the same period as those two phases of ventilation (data not shown).

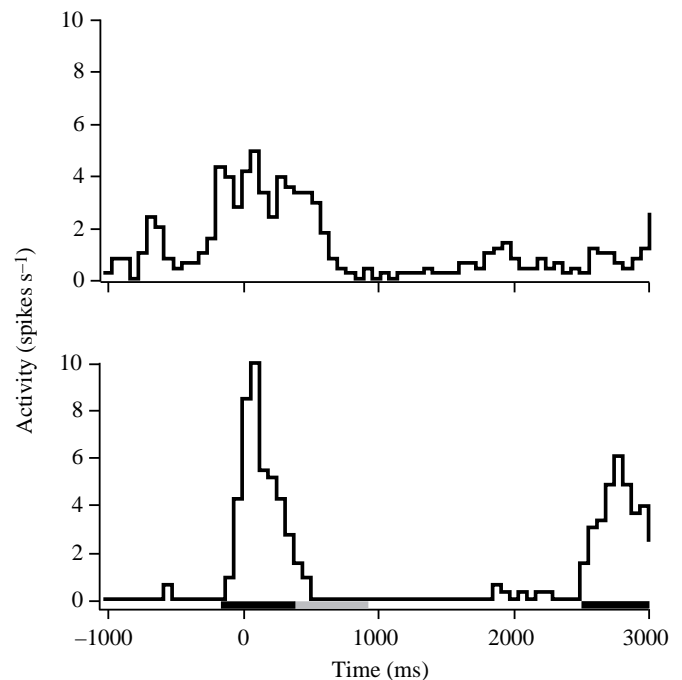


Fig. 9. Two examples of ascending afferent neuron activity in a freely breathing animal in which the anterior lateral line nerves were transected bilaterally to eliminate all electroreceptive inputs. Units were unresponsive to electric fields, as expected, but continued to be clearly modulated by ventilatory movements, presumably driven by proprioceptive and corollary discharge inputs from the DGR.

Almost half of the motor corollary discharge units we studied in curarized skate also respond to electrosensory stimuli and a much smaller proportion are also proprioceptive, so the ventilatory modulation recorded from some DGR electrosensory and proprioceptive units in freely breathing skate may be in part motor-command-related activity rather than sensory responses. This was clearly shown to be the case in several electrosensory units when the transdermal potentials associated with ventilatory movements were experimentally reduced to zero. However, the electrosensory units in the DGR are in general also quite responsive to the common-mode ventilatory potentials themselves, and this accounts for most of the ventilatory modulation in these units. This seems particularly significant in view of the fact that in most lower-order units, including many AENs, responses to ventilatory potentials and other common-mode stimuli are actively suppressed (Bodznick *et al.* 1992), and it also raises the question of just what is the source of the responses to common-mode stimuli in the DGR. The DGR receives no direct input from primary afferents (Schmidt and Bodznick, 1987); its electrosensory inputs appear to descend from isthmial or midbrain nuclei (Bodznick and Boord, 1986; Conley and Bodznick, 1994). Responses to common-mode stimuli in the DGR therefore, must be either (1) reassembled by summation of inputs from neurons that are themselves only weakly responsive to common-mode signals, or (2) relayed indirectly from a fraction of AENs that do not exhibit common-mode rejection.

The recordings from curarized animals show that proprioceptive units that also show motor corollary discharge activity are rare. Thus, in most cases, the ventilatory modulation in proprioceptive units recorded in freely breathing animals is presumed to be in response to the actual movements of mouth, spiracles or branchial chambers. In one unit it was possible to confirm this by eliciting proprioceptive responses to passive movements of the mouth and spiracle. However, in most units the proprioceptive nature of the ventilatory modulation was not confirmed experimentally because of the difficulty of creating passive movements that were similar to the actual ventilatory movements. For this same reason, our small sample may also have missed proprioceptive units that are responsive only to ventilatory and not to fin movements. The presence of motor-command-related activity in some proprioceptive units in freely breathing skate might account for the apparent discrepancy between some of the receptive fields of some units for fin movements and their likelihood of responding to ventilatory movements.

In summary, we have identified three kinds of units in the DGR with activity related to ventilation, those carrying a corollary discharge of the ventilatory motor commands, and two types of purely sensory units: proprioceptive and electroreceptive (although we cannot rule out the possibility that these sensory units might exhibit corollary discharge activity related to movements other than ventilatory movements). About half of the ventilatory corollary discharge units also respond to the sensory stimuli related to ventilation. Because of the difficulty of identifying corollary discharge

units in freely breathing animals, the relative proportions of these unit types is uncertain, but together it appears that they represent nearly 75% of all recordable DGR units.

It should be noted that the anatomical identity of the cells corresponding to the units we have recorded is not known. Given the extremely small size, high packing density and notorious difficulty of recording from granule cells, it seems unlikely that they are the source of any of the unit recordings. Some proprioceptive units may represent fibers afferent to the DGR as Conley and Bodznick (1994) argued on the basis of the distinctive short-duration spikes of the units and the fact that they are recorded selectively by relatively high-resistance glass microelectrodes. A possible source of most other proprioceptive units is the Golgi cells, which are much larger than granule cells and are sparsely distributed throughout the DGR (Fig. 1). Most DGR units have relatively large spikes in the extracellular records. The recordings are generally quite stable, and individual units are sometimes held even while advancing the electrode tip several tens of micrometers. The packing density of the Golgi cells is also more consistent with the frequency with which the DGR units are encountered. If the recordings are from Golgi cells, then, while they serve to establish the kinds of information that reach the DGR, they do not allow us to know exactly what form this information takes in the parallel fiber projection to the dorsal nucleus. This is important information for any model of the role of the parallel fiber system in electrosensory processing, but it must await future studies and perhaps also the development of more advanced recording methods.

The indication from our results is that the DGR is supplied with virtually all of the sensory and motor-command-related information available to the animal concerning its own ventilatory movements. As noted above, the only significant output of the DGR is through the parallel fiber axons of the granule cells that form the molecular layer of the dorsal nucleus (Schmidt and Bodznick, 1987). The AENs, which are the projection neurons of the dorsal nucleus, have apical dendrites in this molecular layer and the measurements made in the present study in animals with electroreceptive afferents transected show that the AENs in fact do receive proprioceptive and/or command-related signals associated with ventilation. What is the use of these signals to neurons whose role is to process and relay electrosensory information to higher brain centers? We believe that the molecular layer inputs may be part of an additive mechanism to cancel reafference that is not entirely removed by the common-mode rejection circuits of the central zone of the dorsal nucleus.

Earlier workers (New and Bodznick, 1990) did not discount a role for the DGR in ventilatory noise cancellation by the AENs, but argued against it on the basis of the fact that, among the DGR units showing ventilatory modulation, there was a wide range of phase relationships between the activity modulation and the ventilatory movements; many were not in phase with the common-mode ventilatory reafference in the electroreceptive afferents recorded from the same animal. The same observations apply to the motor-command-related signals recorded from the DGR units in the present study; most are not of the same phase

or same temporal pattern of activity as the ventilatory reafference. Because the activity in most DGR units was not a good match to the electrosensory reafference caused by ventilation, it was argued that they could not mediate any simple additive (subtractive) cancellation mechanism (New and Bodznick, 1990). This requirement of a close match between DGR activity and reafference would be vital if the descending signals were hard-wired onto the AENs. However, it can be discounted if the molecular-layer synapses onto the AENs are labile and if a mechanism exists to adjust synaptic weightings in favor of those inputs that are effective in specifically reducing ventilatory reafference in the AENs (Montgomery and Bodznick, 1994).

In weakly electric mormyrid fishes, unwanted reafference in ampullary electroreceptors is created by the fish's electric organ discharge. This reafference is removed in the second-order medullary neurons by summing it with a centrally generated corollary discharge of the electric organ discharge command, which is a negative image (or inverse) of the reafference. The form of this efference copy signal is modifiable and constantly updated to match the form of the expected reafference (Bell, 1981, 1982). Recent studies (Bell *et al.* 1993) suggest that the immediate source of this efference copy signal is the granule cells of the caudal cerebellum, and that the modifiability of the signal may be due to plasticity in the synapses between fibers of the parallel fiber system carrying the EOD command and the second-order electrosensory neurons. The same basic mechanism probably acts to reduce the effects of body movements on the medullary electrosensory neurons in gymnotid electric fishes (Bastian, 1995). The electrosensory systems of the electric fishes and elasmobranchs are not homologous (Bullock *et al.* 1982). Nevertheless, the basic anatomical organization of the medullary nuclei in the groups is the same, and the parallels between the processing requirements of the systems are also striking. The current finding of corollary discharge signals of the ventilatory motor commands in the DGR is consistent with the suggestion that the parallel fiber projection in elasmobranchs could be similarly involved in generating a cancellation signal that is the inverse of the expected reafference in the AENs and which could sum with and cancel the reafference. Furthermore, the ventilatory modulation in DGR proprioceptive and electroreceptive cells suggests that they contribute to the formation of this negative image or cancellation signal. Evidence of such an adaptive mechanism for ventilatory noise reduction has recently been obtained, and a model for how it might be accomplished through anti-Hebbian plasticity of molecular layer synapses has been presented (Bodznick, 1993; Montgomery and Bodznick, 1994). For such a mechanism, it seems that the wide variation we have found in the phase and temporal pattern of ventilatory modulation among DGR proprioceptive and motor corollary discharge units would not be detrimental but instead would add to the variety of descending signals from which an effective cancellation signal could be constructed.

The authors thank the staff of the environmental laboratory of Northeast Utilities, who kindly provided the skate. We also

thank Dr Allison Doupe for helpful comments on the manuscript. This work was funded by an NSF grant to D.B.

References

- BASTIAN, J. (1995). Pyramidal-cell plasticity in weakly electric fish: A mechanism for attenuating responses to reafferent electrosensory inputs. *J. comp. Physiol. A* **176**, 63–73.
- BELL, C. C. (1981). An efference copy which is modified by reafferent input. *Science* **214**, 450–453.
- BELL, C. C. (1982). Properties of a modifiable efference copy in an electric fish. *J. Neurophysiol.* **47**, 1043–1056.
- BELL, C. C., CAPUTI, A., GRANT, K. AND SERRIER, J. (1993). Storage of a sensory pattern by anti-Hebbian synaptic plasticity in an electric fish. *Proc. natn. Acad. Sci. U.S.A.* **90**, 4650–4654.
- BODZNICK, D. (1993). The specificity of an adaptive filter that suppresses unwanted reafference in electrosensory neurons of the skate medulla. *Biol. Bull. mar. biol. Lab., Woods Hole* **185**, 312–314.
- BODZNICK, D. AND BOORD, R. L. (1986). Electroreception in Chondrichthyes. In *Electroreception* (ed. T. H. Bullock and W. Heiligenberg), pp. 225–256. New York: Wiley & Sons.
- BODZNICK, D. AND MONTGOMERY, J. C. (1992). Suppression of ventilatory reafference in the elasmobranch electrosensory system – medullary neuron receptive fields support a common mode rejection mechanism. *J. exp. Biol.* **171**, 127–137.
- BODZNICK, D., MONTGOMERY, J. C. AND BRADLEY, D. J. (1992). Suppression of common mode signals within the electrosensory system of the little skate *Raja erinacea*. *J. exp. Biol.* **171**, 107–125.
- BULLOCK, T. H., NORTHCUTT, R. G. AND BODZNICK, D. A. (1982). Evolution of electroreception. *Trends Neurosci.* **5**, 50–53.
- CONLEY, R. A. AND BODZNICK, D. (1994). The cerebellar dorsal granular ridge in an elasmobranch has proprioceptive and electroreceptive representations and projects homotopically to the medullary electrosensory nucleus. *J. comp. Physiol. A* **174**, 707–721.
- MONTGOMERY, J. C. (1984). Noise cancellation in the electrosensory system of the thornback ray; common mode rejection of input produced by the animal's own ventilatory movement. *J. comp. Physiol. A* **155**, 103–111.
- MONTGOMERY, J. C. AND BODZNICK, D. (1994). An adaptive filter that cancels self-induced noise in the electrosensory and lateral line mechanosensory systems of fish. *Neurosci. Lett.* **174**, 145–148.
- NEW, J. G. AND BODZNICK, D. (1990). Medullary electrosensory processing in the little skate. II. Suppression of self-generated electrosensory interference during respiration. *J. comp. Physiol. A* **167**, 295–307.
- PAUL, D. H. AND ROBERTS, B. L. (1977). Studies on a primitive cerebellar cortex. I. The anatomy of the lateral-line lobes of the dogfish, *Scyliorhinus canicula*. *Proc. R. Soc. Lond. B* **195**, 453–466.
- PAUL, D. H., ROBERTS, B. L. AND RYAN, K. P. (1977). Comparisons between the lateral-line lobes of the dogfish and the cerebellum: an ultrastructural study. *J. Hirnforsch.* **18**, 335–343.
- ROBERTS, B. L. AND BALLINTJN, C. M. (1988). Sensory interaction with central 'generators' during respiration in the dogfish. *J. comp. Physiol. A* **162**, 695–704.
- SCHMIDT, A. W. AND BODZNICK, D. (1987). Afferent and efferent connections of the vestibulolateral cerebellum of the little skate, *Raja erinacea*. *Brain Behav. Evol.* **30**, 282–302.
- SOKAL, R. R. AND ROHLF, R. R. (1981). *Biometry*. San Francisco: W. H. Freeman and Company.