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C. Bell · G. von der Emde

Electric organ corollary discharge pathways in mormyrid fish
II. The medial juxtalabar nucleus

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Abstract The electrosensory lobe of mormyrid fish *Gnathonemus petersii* is strongly affected by corollary discharge signals associated with the electric organ discharge (EOD) motor command. This study is a physiological examination of one important source of corollary discharge signals to the lobe, the medial juxtalobar nucleus.

Intracellular recordings from neurons of the medial juxtalobar nucleus show a single corollary discharge driven spike occurring at about 7.5 ms after the initiation of the EOD motor command with a temporal jitter in individual cells of less than 0.05 ms. Responses to intracellular current injection suggest the presence of a low threshold outward current that is similar to outward currents that have been identified in those neurons of the auditory system in which precise temporal information is preserved.

Results from stimulation and lesion experiments indicate that the medial juxtalobar nucleus is responsible for major corollary discharge effects in the mormyromast regions of the electrosensory lobe, including: 1) the gate-like excitation of granule cells; and 2) various excitatory effects on other cells of the lobe.

The medial juxtalobar nucleus transmits precise temporal information about the timing of the EOD motor command to the electrosensory lobe. This information is probably used in decoding electrosensory afferent latency as a measure of stimulus intensity.

Key words Efference copy · Electric fish · Timing · Electrosensory · Mormyridae

Abbreviations *Ad* antidromic · *bc* bulbocerebellar tract · *BCA* bulbar command associated nucleus · *CD* corollary discharge · *EGp* eminentia granularis posterior · *ELL* electrosensory lobe · *ELLga* electrosensory lobe, ganglion layer · *ELLgr* electrosensory lobe, granule layer · *ELLml* molecular layer of ELL cortex · *EOD* electric organ discharge · *JLl* lateral juxtalobar nucleus · *JLm* medial juxtalobar nucleus · *MCA* mesencephalic command associated nucleus *MO* medial octavolateral nucleus · *MOml* molecular layer of the medial octavolateral nucleus · *nALL* anterior lateral line nerve · *PCA* paratrigeminal command associated nucleus

C. Bell, G. von der Emde (✉)
R. S. Dow Neurological Sciences Institute,
Legacy Good Samaritan Hospital and Medical Center,
1120 N.W. 20th Ave,
Portland, OR 97209, USA

Introduction

Corollary discharge signals associated with the motor command that drives the electric organ to discharge are prominent in the electrosensory lobe of mormyrid electric fish and of great importance in the initial processing of electrosensory information. The corollary discharge signals interact in various ways with the reafferent input evoked by the fish's own electric organ discharge (EOD) in the primary afferent fibers from electroreceptors.

Previous anatomical work has identified many of the central structures and pathways that convey the corollary discharge signals to the electrosensory lobe (Bell et al. 1981, 1983). But little is known about the physiology of these structures or about the corollary discharge effects that they mediate in the electrosensory lobe. The previous paper in this series (Bell et al. 1995) examined one important structure in the corollary discharge pathway, the mesencephalic command associated (MCA) nucleus. This paper examines a second such structure, the medial juxtalobar nucleus.

The juxtalobar nucleus is located at the anterior ventral margin of the electrosensory lobe (Fig. 1A). Cells in the medial portion of the nucleus (the medial juxtalobar nucleus) project bilaterally to the cortex of the electrosensory lobe, whereas cells in the lateral half of the nucleus (lateral juxtalobar nucleus) project to the eminentia granularis posterior (Bell et al. 1981). The fibers to the electrosensory lobe cortex end mainly in those parts of the cortex that receive input from the mormyromast type of electroreceptors, i.e., the medial and dorsolateral zones. Within these cortical zones, the fibers from the medial juxtalobar nucleus terminate mainly in the intermediate and granule layers, where afferent fibers from electroreceptors terminate. Some terminals of medial juxtalobar axons are also present in the plexiform and ganglion layers (Bell, unpublished observations). Recent anatomical work (Bell, unpublished observations) indicates that the medial juxtalobar cells receive their input from the juxtalemniscal region which in turn receives its input from MCA.

The previous paper (Bell et al. 1995) showed that the corollary discharge-driven activity of MCA is responsible for many of the corollary discharge effects in the mormyromast regions of the electrosensory lobe. The anatomy indicates that the medial juxtalobar nucleus is well positioned between MCA and the electrosensory lobe to mediate these corollary discharge effects. The physiological experiments described in this paper strongly support such a role.

Corollary discharge inputs to the electrosensory lobe have several different roles in the central processing of electrosensory information (Bell 1989; Bell and Grant 1992). One role that may be mediated by the medial juxtalobar nucleus is the decoding of reafferent latency as a function of stimulus intensity. The latency of reafferent responses to the fish's own EOD in afferent fibers from mormyromast electroreceptors is a precise measure of the intensity of the self-generated current. In addition, behavioral experiments have shown that mormyrid fish can detect small shifts in afferent latency following the EOD motor command and that a corollary discharge signal is used as a temporal reference in detecting such shifts (Hall et al., in press). Corollary discharge input from the juxtalobar nucleus conveys information about the timing of the EOD motor command with better than 0.05 ms accuracy and could well provide the timing information that is necessary for accurate decoding of reafferent latency.

The preservation of timing information within sensory pathways has received some attention within the neuroscientific literature (Carr 1986; Konishi et al. 1988; Bell and Grant 1989). But relatively little attention has been paid to the preservation of timing information in motor pathways. The corollary discharge pathways in mormyrid fish form a motor command associated system. Several structures in these pathways, including MCA and the medial juxtalobar nucleus, are remarkable for the precision with which they signal the timing of the EOD motor command. Studies of the medial juxtalobar nucleus and other nuclei in the mormyrid corollary discharge pathway may help reveal the most general morphological and physiological requirements for the preservation of timing information.

Methods

General

These experiments examined the activity of cells in the medial juxtalobar nucleus and the effects of this activity on the electrosensory lobe. Cells were examined intracellularly for their corollary discharge responses, for responses to intracellular current pulses, and for responses to antidromic activation. As in the previous paper (Bell et al. 1995), the corollary discharge responses were determined in curarized fish by simultaneously recording: a) the activity of medial juxtalobar neurons: b) the EOD motor command signal over the electric organ; and c) the corollary discharge-evoked field potentials in the medial zone of the electrosensory lobe (a mormyromast zone). The effects of medial juxtalobar activity on the electrosensory lobe were assessed by electrical stimulation and by lesions.

Although the major focus of this study was on the medial juxtalobar nucleus, some experiments were also done to evaluate the contribution of nucleus praeeminentialis. Nucleus praeeminentialis projects to the lower molecular layer of the electrosensory lobe and the cells of the nucleus are affected by corollary discharge signals of the EOD motor command (G. von der Emde and C. Bell, unpublished). The contribution of nucleus praeeminentialis was evaluated by examining the potentials evoked in the electrosensory lobe by stimulation of nucleus praeeminentialis and by stimulation of its efferent axons. These potentials were compared with those evoked by the corollary discharge and by stimulation of the medial juxtalobar nucleus.

Eighteen mormyrid fish of the species, *Gnathonemus petersii*, were used. The basic methods were the same as those described in the previous paper of this series and only those methods which were unique to this study are described here.

Surgery

The caudal part of the skull was removed and the valvula was reflected anteriorly with a glass retractor. This exposed the eminentia granularis posterior (EGp), a granule cell mass that covers most of the electrosensory lobe. A small portion of the ventral molecular layer of the electrosensory lobe is not covered by EGp and is visible below the ventral margin of EGp. This exposed molecular layer was used as an external landmark in locating the medial juxtalobar nucleus.

Recording

The precise location of the medial juxtalobar nucleus was determined by monitoring corollary discharge-evoked field potentials with extracellular recording electrodes. The electrodes were directed at angles of about 45° with respect to the mid-sagittal plane and with a slight posterior to anterior direction. Entry points for the electrode tracks were just dorsal to the anterior tip of the exposed electrosensory lobe molecular layer. Corollary discharge-driven field potential characteristic of the medial juxtalobar nucleus were recorded in such tracks at depths of 800 to 1100 microns below the surface (see Results).

Micropipettes for intracellular recording were filled with 2% biocytin in 2 *M* potassium methyl sulfate. Depolarizing currents were injected into intracellularly recorded cells to label the cells morphologically. Three Hz positively biased sinusoidal currents of up to 2 nA maximum current were passed for 2 to 10 min.

Stimulation and lesion making

Tungsten microelectrodes, insulated except at the tip and plated with gold, were used for electrical stimulation of the brain and for making lesions. Placement of the electrodes at the intended sites was done by recording field potentials before using the electrode for stimulation or lesion making.

Cells of the medial juxtalobar nucleus were activated antidromically by two different methods: 1) stimulation of their axon terminals in the granule layer of the electrosensory lobe; or 2) stimulation of the contralaterally projecting axonal branches. For the first method, stimulating electrodes were placed in the granule layer of the ipsilateral medial zone of the electrosensory lobe. The electrode was placed in the caudal portion of the lobe where primary afferent fibers from the caudal body terminate. The characteristic corollary discharge-evoked field potential of the electrosensory lobe granule layer was used as an indicator of electrode location (Fig. 2F).

For the second method of antidromic activation, stimulating electrodes were placed in the bundle of contralaterally-projecting axonal branches of medial juxtalobar neurons. This bundle crosses the midline between the molecular layer of the medial octavolateral nucleus and the most anterior portion of the medial zone of the electrosensory lobe (Bell et al. 1981; Fig. 1A,D). Vertical tracks were made in the midsagittal plane through the most anterior portion of the medial zone of the electrosensory lobe. The granule layer in this region was first identified by the field potentials evoked by the corollary discharge and by electrosensory stimuli delivered to the tip of the lower lip appendage. Primary afferents from this skin region terminate in the anterior portion of the electrosensory lobe. The electrode was then lowered 100–200 microns to a point where antidromic responses in the medial juxtalobar nucleus could be obtained with low stimulus currents. A spike-like corollary discharge field potential was also recorded in this region at about 4.5 ms after the command signal, a potential which is probably due to impulses in medial juxtalobar axons.(The "time of the command signal" or "time 0 of the command signal" are defined as the time of the first large negative peak in the command signal as recorded at the tail over the electric organ in the curarized fish.)

Stimulation of nucleus praeeminentialis was accomplished by inserting the tungsten stimulating electrode directly into the nucleus. Anterior reflection of the valvula exposed a small portion of the nucleus praeeminentialis just anterior to EGp and the electrode was inserted into the nucleus at this point. A field potential with a sharply rising negative wave beginning 10–11 ms after the command signal is recorded in n. praeeminentialis (G. von der Emde and C. Bell, unpublished) and this potential was used to confirm the electrode placement.

Axons of nucleus praeeminentialis were also directly activated by stimulating the praeeminential-electrosensory tract which conveys the praeeminential fibers to the electrosensory lobe. This tract lies just external to the molecular layer of the electrosensory lobe. Stimulating electrodes were placed in the tract by lowering them about 1 mm below the surface in vertical tracks near the midline. Small movements of the electrode were then made to find the lowest threshold point for evoking the characteristic negative field potential in the molecular layer of the electrosensory lobe (see Results).

Electrolytic lesions were made to destroy the ipsilateral juxtalobar nucleus and the bundle of axons which cross the midline from the contralateral nucleus. Lesions were also sometimes made to confirm the sites of electrical stimulation.

Histology

Following the lesion experiments, the brains were sectioned and counterstained as described in the previous paper (Bell et al. 1995) to establish the location of the lesions. Following intracellular recording, the fish were perfused with saline followed by a mixture of 1.5% paraformaldehyde and 1.5% glutaraldehyde in 0.1 *M* PO_4 buffer. The tissue was sectioned and reacted to reveal the presence of biocytin (Horikawa and Armstrong 1988).

Results

Morphology

Ten cells were labeled by the intracellular injection of biocytin. All ten cells were within the medial juxtalobar nucleus (Fig. 1A,B). The cell bodies were round or ovoid in shape with a longest axis of about 25 μm. Dendritic branches were well stained in five of the cells (Fig. 1C). Two or three such branches emerged from the cell body, each in the form of a short initial segment terminating in a densely branched tuft (Fig. 1C, double arrow). The terminal tuft was itself only about 25 μm in diameter. Thus, the dendritic arbors of individual cells appeared to be restricted to the near neighborhood of the cell body and extended into only a small portion of the nucleus as a whole.

Rather thick axons of 3–5 μm in diameter also emerged from the cell bodies (Figs. 1B,C, single arrows). Branches of these axons could be traced into the bundle of contralaterally projecting fibers crossing over the top of the molecular layer of the medial octavolateral nucleus (crista cerebellaris; Fig. 1D, single arrow). Some fibers could be seen to enter the granule layer of the electrosensory lobe on the contralateral side. Labeled fibers could also be seen to enter the granule layer of the ipsilateral electrosensory lobe (Fig. 1D, double arrow), but the fine terminals in the granule layer were not stained.

Corollary discharge-evoked field potentials in the medial juxtalobar nucleus

Extracellular recordings in the region of the medial juxtalobar nucleus showed two spike-like events, one occurring at about 2.0 ms after the command signal and another at about 3.8 ms. The two potentials varied independently in amplitude and polarity. The first spike-like event at 2.0 ms was larger rostrally (Fig. 2A, B), whereas the second event at 3.8 ms was larger caudally (Fig. 2D, E). Extracellular biocytin injections (C. Bell, unpublished observations), intracellular biocytin injections, and electrolytic lesions at sites where the second potential was large all showed that this second potential arises in the juxtalobar nucleus.

Fibers which convey the corollary discharge signals from the juxtalemniscal region to the juxtalobar

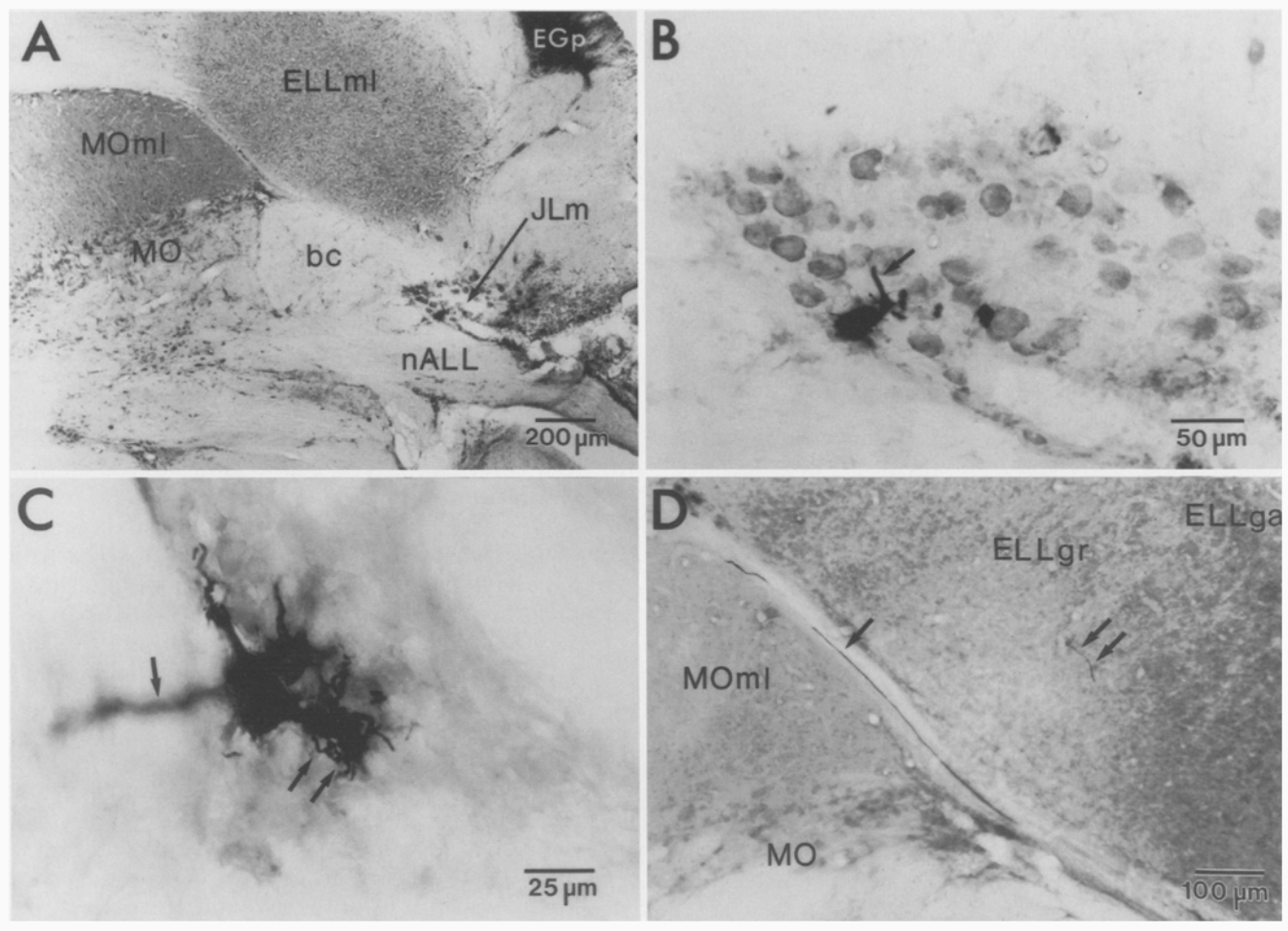

Fig. 1A–D Photomicrographs showing the location of the juxtalobar nucleus and biocytin filled neurons of the juxtalobar nucleus. **A** Transverse section at the anterior margin of the electrosensory lobe showing the location of the juxtalobar nucleus (*arrow*). The cell body of a biocytin filled cell is visible at medial margin of the nucleus. The section is at the same rostrocaudal level as that shown in Fig. 2C of the preceding paper (Bell et al. 1995). **B** Same section as the one in **A** but at a higher power. The cell body of the biocytin filled cell can be seen more clearly. The *arrow* points to the thick axon of the cell. **C** Another biocytin filled juxtalobar neuron with a bushy dendritic arbor (*double arrows*) and a thick axon (*single arrow*). **D** Axonal branch of a juxtalobar neuron entering the bundle of contralaterally projecting axons just dorsal to the molecular layer of the medial octavolateral nucleus (*single arrow*). An axonal branch in the granule layer of the ipsilateral electrosensory lobe can also be seen (*double arrows*). The juxtalobar neuron was stained by the intracellular injection of biocytin

nucleus enter the nucleus at its anterior pole (C. Bell, unpublished observations). The greater size of the first potential anteriorly suggests that this potential is due to afferent input from the juxtalemniscal region. The fact that the second potential is largest caudally and within the juxtalobar nucleus itself, suggests that this second potential is due to the responses of juxtalobar neurons. These hypotheses are supported by the intracellular recordings, as described in the next section.

Intracellular recordings from medial juxtalobar nucleus

Two distinct types of elements were commonly recorded from the region of the medial juxtalobar nucleus. Both elements appeared to be simple relays of information on the timing of the EOD motor command, since the activity in both types of elements occurred only at short fixed latencies after the command signal. One type of element showed a single corollary discharge-driven spike that arose directly from the baseline, indicating an axonal recording (Fig. 3B). The variation in latency for any one element was about 0.05 ms (note the superimposed traces of Fig. 3B). Such latency variation is similar to that which was observed in single MCA cells (Bell et al. 1995). The variation among different elements of this type was also quite small. Latencies of 17 different elements, recorded in 9 different fish, all fell between 2.0 and 2.2 ms after the command signal. The time of spike onset was the same as the time of the first spike-like component of the field potentials. These elements are presumed to be afferent fibers from the juxtalemniscal region that convey the corollary discharge signal to the juxtalobar nucleus.

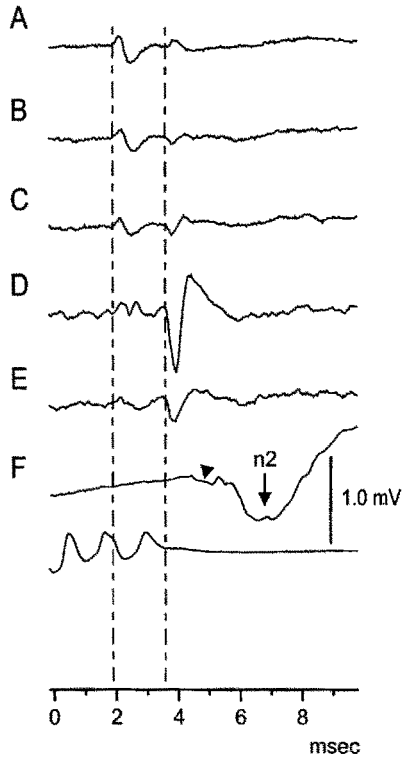

Fig. 2 Corollary discharge-evoked field potentials in the region of the juxtalobar nucleus. All sweeps were triggered by the command signal shown in the bottom trace and the records are averages of 6 sweeps. The records in *A–E* were obtained in a rostro-caudal series of electrode tracks at depths of 800 to 1100 μm from the surface. The recording in *A* was most rostral and the one in *E* was most caudal. The tracks were separated by 50–100 μm and the entry points were in the region just dorsal to the anterior tip of the portion of electrosensory lobe molecular layer that is exposed below the EGp. The record in *F* is from the granule layer of the electrosensory lobe. The *arrow* points to the n2 component of the corollary discharge-evoked field potential. The *arrowhead* points to a small potential that probably reflects the arrival of input from the juxtalobar nucleus in the electrosensory lobe. The gains of records *A–F* are given by the *scale bar* below *F*. The gains of the command signals in this and subsequent figures are not shown. The amplitudes of command signals in different fish were between 100 and 200 μV. The *dashed lines* show the onset times of the two potentials described in the text

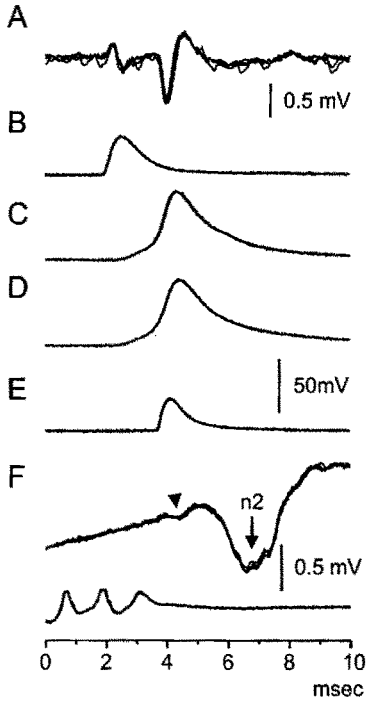

Fig. 3 Corollary discharge responses in the juxtalobar nucleus. The records were all taken from the same fish. Each record shows four superimposed sweeps to illustrate the minimal variability in the responses. *A* Field potential in the juxtalobar nucleus. *B* Intracellular recording, presumed to be from a fiber afferent to the juxtalobar nucleus. *C* Intracellular recording from a juxtalobar neuron. *D* Intracellular recording from a second juxtalobar neuron. *E* Intracellular recording from a presumed axon of a juxtalobar neuron. The action potential in this fiber begins at about the same time as the action potential recorded from the cell bodies (*C* and *D*), but is shorter in duration and reaches a peak much earlier. *F* Field potential in the granule layer of the electrosensory lobe. Amplitudes of the intracellular recordings are indicated by the *scale bar* between *D* and *F*

The second type of element also showed a single corollary discharge-driven spike. The spike occurred at a longer latency, however, and arose from a ramp-like depolarization. Once again, the variation in latency for any one element was very small, about 0.05 ms (note the superimposed traces of Fig. 3C and D). The variation in latency among different elements of this type in different fish was small, although somewhat larger than that of the presumed afferent fibers. The departure of the ramp-like depolarization from the baseline occurred at about 2.5 ms (range 2.1–2.8 ms across all elements and all fish) and the inflection point where the ramp-like potential gave rise to the spike occurred at a latency of about 3.5 ms (range 3.3–4.0 ms). The latency of the peak of the spike ranged between 4.0 and 4.6 ms. Twenty-seven elements of this type were recorded.

Elements of the second type could be morphologically identified as cells of the medial juxtalobar nucleus by the intracellular injection of biocytin. Such identification was possible even though both types of elements were sometimes recorded with the same biocytin-filled electrodes and in the same electrode tracks. Elements of the first type, those assumed to be afferent fibers, were recorded for only a few minutes, with low membrane potentials (10–50 mV) and spike heights (5–40 mV). The second type of element, however, was often recorded for more than an hour with good membrane potentials (50–70 mV) and spike heights (40–65 mV) in the absence of hyperpolarizing current. Depolarizing currents of up to 2 nA were injected into the second type of element for labeling purposes (see Methods), but no such currents were injected into the first, or afferent, type of element.

Ten cells of the medial juxtalobar nucleus were labeled histologically but no afferent fibers to the

nucleus were labeled. The higher quality of recordings from the second type of element, the longer duration of the recordings, and the injection of current into these elements all indicate that this second type of element that was the one that was labeled morphologically and that the second type of element was therefore a postsynaptic medial juxtalobar cell, not an afferent to the nucleus. Moreover, in one fish, 3 elements of the second type were recorded for long periods and were injected with additional current to pass biocytin into the cells; but no elements of the first, or afferent, type were recorded. Histologically, three well stained cells were found in the medial juxtalobar nucleus of this animal. Thus, elements of the second type, with spikes arising from a ramp-like depolarizing potential, will be referred to as 'medial juxtalobar neurons' in what follows.

The apparent latency of spike onset in medial juxtalobar neurons at the end of the ramp-like depolarizing potential (about 3.5 ms), was close to the latency of the second spike-like potential in the extracellularly recorded field potential (about 3.8 ms). Thus the second component of the field potential is probably due to spikes in medial juxtalobar cells. In addition, six elements with spikes arising directly from the baseline at latencies of 3.7–4.1 ms were recorded just dorsal to the caudal limit of the medial juxtalobar nucleus (Fig. 3E). These elements were presumed to be the axons of medial juxtalobar neurons on their way to the electrosensory lobe. The peak of the spike in these presumed axons of juxtalobar neurons occurs before the peak of the spike in the cell itself (compare Fig. 3E with C and D). Lower thresholds and earlier occurrence of axon spikes has also been observed in other cells (Stuart and Hauser 1994).

The spikes of medial juxtalobar neurons occur about 1.5 ms before the sharp n2 potential in the granule layer of the electrosensory lobe (arrows in Figs. 2F and 3F). Spikes in medial juxtalobar axons could therefore be responsible for the n2 wave, and the results from stimulation and lesions of the medial juxtalobar nucleus support this conclusion (see below). The n2 potential probably reflects a strong corollary discharge-driven excitation of granule cells (Bell 1990a; Bell et al. 1992). A smaller negative wave often precedes the n2 wave in the granule layer of the electrosensory lobe (arrowheads in Figs. 2F and 3F). This smaller negative wave probably reflects the arrival of impulses at the terminals of medial juxtalobar axons in the electrosensory lobe cortex.

The ramp-like potential that gives rise to a spike in juxtalobar cells is presumed to be an excitatory synaptic potential evoked by the afferent input. For individual fish, the delays between spikes in the presumed afferent fibers and the beginning of the ramp-like potentials in medial juxtalobar neurons were only 0.2 to 0.6 ms. The shorter delays are very short for chemical synaptic transmission at 25°C and suggest the possibility of electrical transmission (see Discussion).

An attempt was made to examine the synaptic input in isolation from the spike by blocking the spike with a brief pulse of hyperpolarizing current during the time of the ramp-like potential and the spike. Current pulses of up to 5 nA were passed, but the spike was never blocked by such currents, and the only effect was to increase spike height. The corollary discharge-driven spike could be blocked by prior antidromic activation of the cell, however, and this procedure appeared to reveal a part of the underlying synaptic potential (see below).

Small corollary discharge-driven prepotentials of unknown significance were observed in 20 of the 37 recorded medial juxtalobar cells. These prepotentials began 1.5 ms *before* the command signal. The prepotentials could be absent (Fig. 4C), negative going (Fig. 4B), or positive going (Fig. 4A) at different times during the recording of a single cell. These prepotentials were not caused by an extracellular field, since the baseline was flat after leaving the cell. The prepotentials were generally positive-going at more negative (hyperpolarized) membrane potentials and negative at more positive (depolarized) membrane potentials. This relationship suggests an inhibitory synaptic input with a reversal potential near the resting potential. But artificial polarization by current injection during a period when the prepotentials were absent did not make them appear. The potentials were small, usually less than 1 mV and never more than 2 mV. The appearance of a prepotential

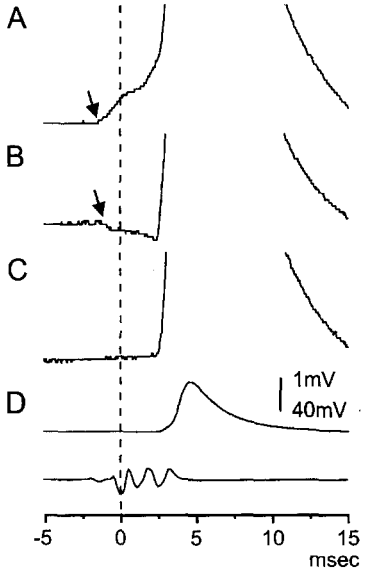

Fig. 4 Intracellular recordings from a juxtalobar neuron showing small early potentials that begin at negative delays with respect to the command signal. The recordings were taken at different times from the same cell. *A* High gain recording showing positive going early potential. *B* High gain recording showing negative going early potential. *C* High gain recording showing absence of early potential. *D* Low gain recording of trace in *C*. A *dashed line* is drawn through the first large peak of the command signal to show time 0 of the command signal

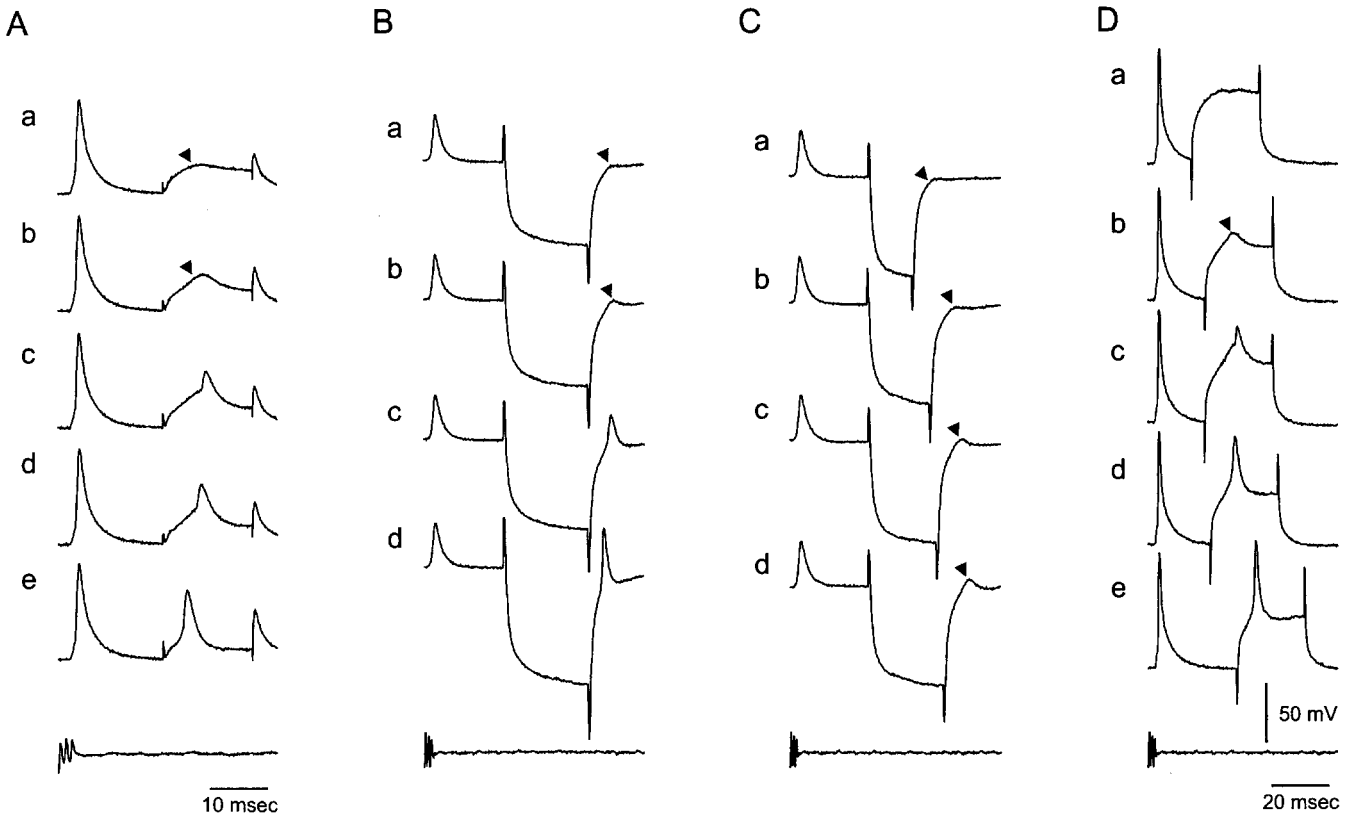

Fig. 5A–D Low threshold responses in juxtalobar neurons evoked by intracellular current injection. All intracellular current pulses were delivered at delays of 10 to 30 ms following the command signal. The corollary discharge response appears at the start of each sweep. *A* Responses to depolarizing current pulses. Current strength increases from *a* to *e*. Only low threshold hump-like responses are evoked in *a* and *b* (*arrowheads*). A spike appears in an all-or-none manner in *c*. The 'spike' grows in amplitude with further increases in current strength in *d* and *e*. *B* Anodal break responses at the off of hyperpolarizing current pulses. Current strength increases from *a* to *d*. Only low threshold responses are evoked in a and b (arrowheads). A spike appears in an all-or-none manner in *c*. The 'spike' grows in amplitude with further increases in current strength in *d*. *C* Effects of changes in the duration of the hyperpolarizing current pulse. The pulses are of progressively longer duration in a through *d*. The amplitude of the low threshold response (*arrowhead*) depends on pulse duration. *D* Refractoriness of responses to depolarizing current following the corollary discharge-evoked response. Current pulses of the same amplitude and duration were given at different delays following the corollary discharge response. The delays increase from *a* to *e*. No response is evoked at the shortest delay in *a*. A low threshold response is evoked at a longer delay in *b*. An all-or-none spike can occur on top of this low threshold response at the same delay (*c*). The spike increases in amplitude in a graded manner as delays are increased, as shown in *d* and *e*. Gains are the same in all sweeps and are indicated by the *vertical scale bar* in *D*. The time bases for **B**, **C**, and **D** are all the same and are indicated by the *horizontal scale bar* in **D**

or a change in its polarity did not affect the latency of the ramp-like potential leading to a spike or the latency of the inflection between the ramp-like potential and the spike. These small prepotentials indicate some type of synaptic input to the juxtalobar cells at a very short delay after the initiation of the EOD motor command, but the functional significance of this input is obscure due to the inconsistency, small size, and apparent lack of effect on spike generation.

Injection of depolarizing current pulses into medial juxtalobar cells evoked a graded, low-threshold depolarizing response at current levels of a few tenths of a nanoampere (small humps indicated by arrowheads in Fig. 5A). The response increased with increasing current strengths (Fig. 5Aa and Ab) until a small all-or-none spike was elicited at currents of 2 to 4 times the threshold of the low threshold response (Fig. 5Ac). Further increases in current strength lead to a graded increase in the size of the 'spike' (Fig. 5Ad and Ae; see Discussion for interpretations of the low threshold depolarizing response and of the graded increase in spike height).

The low threshold active response could also be evoked as an anodal break response at the end of a hyperpolarizing current pulse (Fig. 5Ba and Bb). As the strength of the hyperpolarizing current increased, the size of the low threshold active response also increased until an all-or-none spike was evoked. As with depolarizing pulses, the amplitude of this spike continued to increase in a graded manner with higher intensities of hyperpolarizing current (Fig. 5Bc and Bd). The amplitude of the low threshold active response depended on the duration of the hyperpolarizing current (Fig. 5C) as well as its amplitude . These responses to hyperpolarizng current pulses suggest that the voltage sensitive channels responsible for the low threshold response are partially inactivated at rest and can be

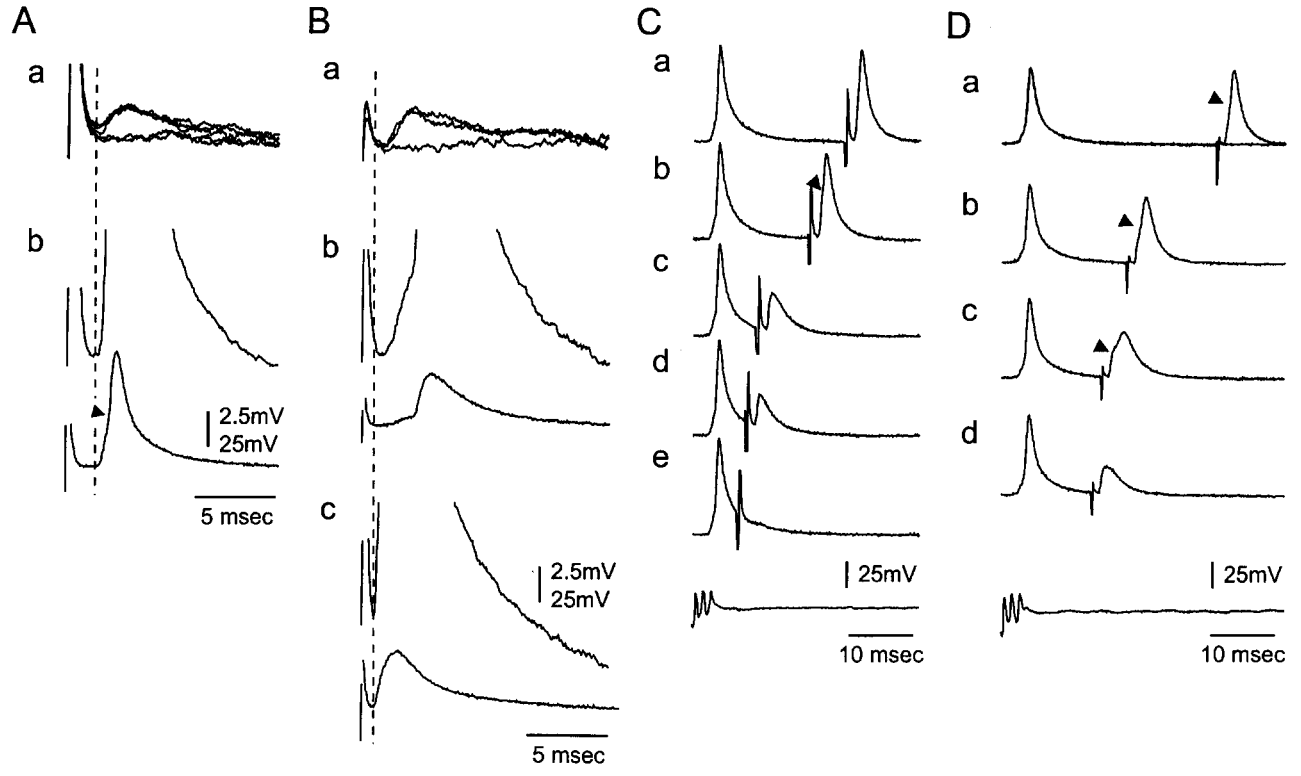

Fig. 6A–D Antidromic responses of juxtalobar cells. *A* Responses to stimulation of terminals of juxtalobar axons in caudal electrosensory lobe. *a*) A small all-or-none potential of 2.5 mV is evoked at the lowest stimulus intensity. *b*) Further increases in stimulus intensity evoke a full sized spike at the same latency as the small potential. The *arrowhead in the lower trace* (low gain) indicates an inflection point on the rising phase of the antidromic spike. The onset of the responses is shown with a *vertical dashed line* in *a* and *b*. The *vertical scale bar* gives the gains. The *upper number* gives the gain for a and for the upper (high gain) trace in *b*. The *lower number* gives the gain for the lower (low gain) trace in *b*. **B** Responses to stimulation of axonal branches of juxtalobar cells that cross to the contralateral side. *a*) A small all-or-none potential is evoked at the lowest stimulus intensity. *b*) As stimulus intensity is increased the small potential grows in amplitude and a spike is elicited at its peak. *c*) Further increases in stimulus intensity evoke a full sized antidromic spike at a short latency. Gains are given by the *vertical scale bar* as in **A**. **C** Refractoriness of antidromic responses following the corollary discharge response. Stimuli given at progressively shorter delays in *a* to *e*. *a*) A full-sized spike is evoked. *b*) A smaller spike with an inflection on the rising phase (*arrowhead*) is evoked at a shorter delay. *c* and *d*) An abrupt change in amplitude occurs when the stimulus is given at still shorter delays. *e*) The smaller response disappears at the shortest delay. **D** Refractoriness of antidromic response following corollary discharge response. Stimuli given at progressively shorter delays in *a* to *d*. *Arrowheads* point to an inflection on the rising phase. The two components of the antidromic response are shown more clearly in this cell than in the cell shown in **C**

deinactivated by hyperpolarizing current pulses. The amount of deinactivation depends on both the amplitude and the duration of the hyperpolarizing pulse as is true for many voltage sensitive channels, including ones responsible for outward currents and ones responsible for inward currents (Hille 1984). The low-threshold active response and the spike that it evokes were refractory immediately following the corollary discharge evoked response (Fig. 5D), indicating that the voltage sensitive channels responsible for the low threshold response are activated during the the corollary discharge response.

Cells of the medial juxtalobar nucleus could be driven antidromically by stimulating their axonal terminals in the electrosensory lobe or by stimulating the contralaterally projecting axonal branches, as described in Methods. The latencies following stimulation of the electrosensory lobe were 1.5–2 ms. The latencies following stimulation of the contralaterally projecting bundle of medial juxtalobar axons were 0.8–1.2 ms.

In some cells, the lowest threshold response was a full sized antidromic spike arising directly from the baseline, at a short fixed latency following the stimulus. In other cells, the lowest threshold response was a brief depolarizing potential of only a few millivolts. This small potential could be evoked in an all-or-none manner (Fig. 6Aa and Ba) and could also be graded with increasing stimulus strength. When the small potential was evoked at threshold (Fig. 6Ba), further increases in stimulus intensity usually resulted in graded increases in the small potential that could lead to a full sized spike at the peak of the small potential (Fig. 6Bb). Further increases in stimulus intensity could also result in a full sized spike arising directly from the baseline (Fig. 6Ab and Bc). The latency of the small event was either the same as that of the full sized spike recorded in the same cell and arising directly from the baseline (Fig. 6A), or only a few tenths of a millisecond longer

(Fig. 6B). These small events are probably the antidromic spikes of other medial juxtalobar cells that are electrically coupled to the recorded cell (see Discussion).

As might be expected, the antidromically evoked spike was refractory following the corollary discharge-evoked spike (Fig. 6C). The antidromically evoked spike became progressively smaller as the delay between the corollary discharge spike and the antidromic stimulus was reduced below 20 ms. At delays of about 10 ms the amplitude of the antidromic spike suddenly fell to about 1/3rd of the original amplitude, suggesting the presence of two components to the antidromic response (Fig. 6Cc). The smaller component finally disappeared in an all or none manner at delays of less than 5 ms (Fig. 6Ce). The presence of two components to the antidromic response was also suggested by the occurrence of a slight inflection on the rising phase of the antidromic response. This inflection and its exaggeration when the antidromic stimulus was given following a corollary discharge response was more clear in some cells (arrowheads in Figs. 6D and 7B) than in others. The two components of the antidromic response are probably due to spikes in different regions of the cell such as the axon and soma.

Most of the corollary discharge response in juxtalobar cells was refractory following an antidromically elicited spike. When an antidromic spike was elicited before the corollary discharge response, the response almost disappeared (Fig. 7C). A small component remained as a depolarizing hump on the descending phase of the antidromically elicited spike, however (arrow in Fig. 7C). This remaining component that was not refractory following the antidromic spike could be the underlying synaptic potential that normally elicits the active response of the cell.

Figure 7 shows that the rising phase of the antidromically elicited spike is quite different from the rising phase of the corollary discharge evoked response (compare Fig. 7A and B). The antidromically elicited spike rises more steeply from the baseline and lacks the ramp-like potential that precedes the spike of the corollary discharge response. The absence of a ramp-like potential in the antidromic response supports the hypothesis that the ramp-like potential is a synaptic input to the juxtalobar cell and not an active response of the postsynaptic cell. The ramp-like potential and the hump on the descending phase of the antidromic spike when the antidromic spike precedes the corollary discharge response could therefore be part of the same, large synaptic potential. This hypothesis is illustrated in Fig. 7D by the dotted line which shows the hump as a continuation of the ramp-like potential.

In conclusion, the intracellular recordings suggest the presence of three components to the corollary discharge response of medial juxtalobar cells – an initial synaptic potential followed by a spike with two components. The two components of the spike are clearest in the antidromic responses. But a second inflection point (upper arrowhead in Fig. 7A), in addition to the inflection point at the end of the ramp-like potential (lower arrowhead in Fig. 7A) appeared to be present in the corollary discharge response of some cells. The second inflection occurring at a slightly more depolarized level and reflecting a transition between the two components of the spike.

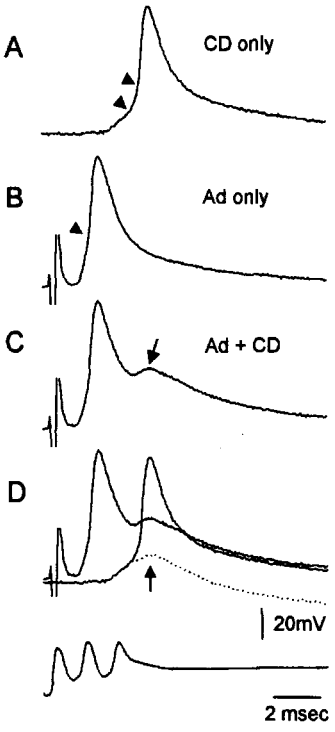

Fig. 7 Effect of an antidromic spike on the corollary discharge response of a juxtalobar cell. *A* Corollary discharge response only ('CD only'). The *two arrowheads* point to two different inflection points on the rising phase of the response. *B* Antidromic response only ('Ad only'). The *arrowhead* points to an inflection on the rising phase. *C* Antidromic response elicited just before the corollary discharge response ('Ad + CD'). Note that the spike of the normal corollary discharge does not occur due to refractoriness following the antidromic spike. The *arrow* points to a hump on the descending phase that is probably due to a corollary discharge-driven synaptic input. *D* Superimposition of trace *A* and trace *C*. The hump from the descending phase of the antidromic response is superimposed as a *dotted line* on the corollary discharge response to show that the hump could be a continuation of the initial, probably synaptic, component of the corollary discharge response

Stimulation of medial juxtalobar nucleus.

Electrical stimulation of the juxtalobar nucleus evoked field potentials in the medial zone of the electrosensory lobe that were similar to the corollary discharge-evoked field potentials in the same structure (Fig. 8). The potentials evoked by stimulation of juxtalobar nucleus were also very similar to those evoked by

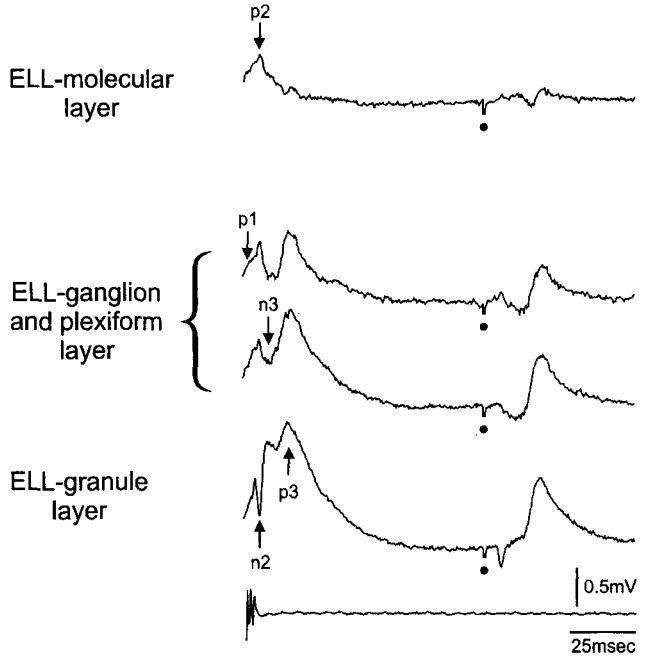

Fig. 8 Field potentials in the different layers of electrosensory lobe cortex that are evoked by stimulation of juxtalobar nucleus. Field potentials evoked by the corollary discharge in the different layers appear at the start of the sweeps. Stimuli are given at constant delays following the corollary discharge responses. A *dot below the trace* shows the time of the stimulus. Different components of the corollary discharge field potentials are indicated by *arrows* at the start of the sweeps

stimulation of MCA, as described in the preceding paper of this series.

Responses to juxtalobar stimuli recorded in the granule layer of the electrosensory lobe consisted of an initial sharp negative wave followed by a broader positive wave. The initial negative wave was similar in its duration and spatial distribution to the n2 component of the corollary discharge potential in the same layer, although smaller in amplitude; and the positive wave was similar to the p3 component of the corollary discharge-evoked potential. (The previous paper, Bell et al. 1995, should be consulted for a description of the different components of the corollary discharge-evoked potentials in the electrosensory lobe and the presumed origins of these components within the circuitry of the electrosensory lobe.)

The medial juxtalobar stimulus evoked a longer latency and broader negative wave in the ganglion and plexiform layers that was similar to the corollary discharge-evoked n3 wave. Responses in the molecular layer consisted of a small, short latency positive wave and a small negative wave at a longer latency. The initial positive wave corresponds to the p2 component of the corollary discharge evoked potential, but the later negative wave was variable in occurrence and did not clearly correspond to any component of the corollary discharge field. Note that juxtalobar stimulation *did not* evoke any potential corresponding to the initial positive going p1 component of the corollary discharge response.

The lowest threshold responses were evoked by medial juxtalobar stimuli consisting of single negative-going pulses of less than 5 μA in amplitude and 0.3 ms in duration (Fig. 8). The sharp n2-like component of the of the granule layer response to such low threshold stimuli had the rather long latency of 4 ms. Inverting the polarity of the stimulus to a positive-going pulse and increasing stimulus intensity to between 10 and 20 μA brought a sudden reduction in the latency of the n2-like response to 1.5 ms (not illustrated). The time course and laminar distribution of the short and long latency responses were essentially the same. The lowest thresholds for both responses appeared to be within the confines of the juxtalobar nucleus as judged by the corollary discharge field potentials recorded with the same electrode. The long latency response was probably due to excitation of afferent terminals within the medial juxtalobar nucleus, whereas the short latency response was probably due to excitation of the juxtalobar cells themselves. Afferent terminals in some other neural systems have been shown to have a lower threshold for electrical stimulation than the postsynaptic cells on which they terminate (Jankowska et al. 1975; Gustafsson and Jankowska 1976).

Responses to juxtalobar stimuli were refractory when stimuli were given immediately after the corollary discharge responses (not illustrated), indicating that the same cellular elements are activated in both cases. The pattern of refractoriness was similar to that seen with MCA stimulation. As with MCA stimuli, the n3-like and p3-like components of the juxtalobar responses were markedly affected at long delays of 70 ms whereas the n2-like component was only affected at much shorter delays (6 or 7 ms for the juxtalobar stimuli).

Lesions of medial juxtalobar nucleus and its efferent axons

The effects of lesions of the medial juxtalobar nucleus and its efferent axons on corollary discharge field potentials in the electrosensory lobe were consistent with the effects of electrical stimulation (Fig. 9). Lesions were made in the ipsilateral juxtalobar nucleus and in the bundle of axons from the contralateral juxtalobar nucleus to the ipsilateral electrosensory lobe. The effects of such lesions were the same in all three fish in which they were carried out. Unilateral lesions (not illustrated) to either the juxtalobar nucleus or to the bundle of axons from the contralateral side caused a reduction in the n2 component of the corollary discharge potential, but had little effect on the n3 or p3 components. Bilateral lesions, however, resulted in the elimination of the n3 and p3 components, as well as the elimination of the remaining n2 component. Such elimination of the n2, n3 and p3 components by lesions

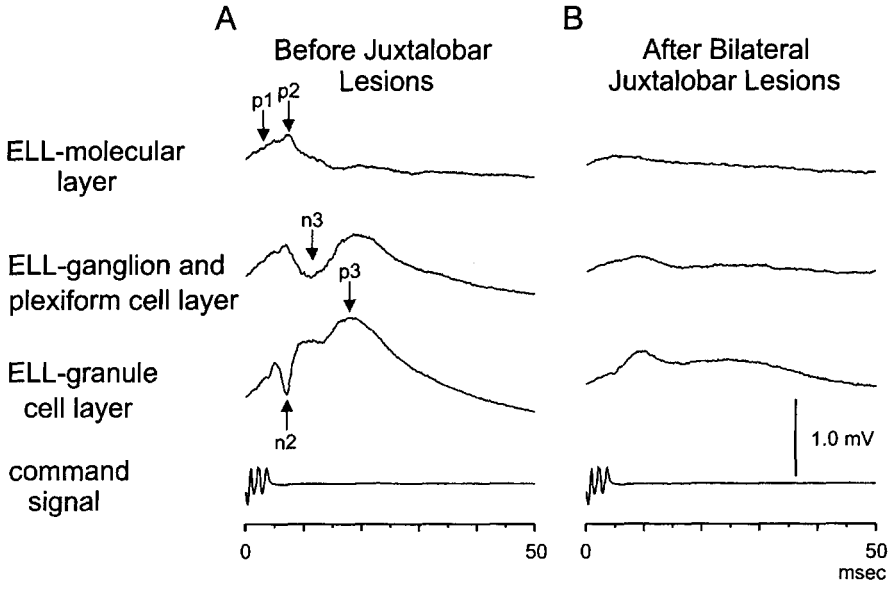

Fig. 9A, B Effects of removing the input from ipsilateral and contralateral juxtalobar nucleus on corollary discharge-evoked field potentials in the electrosensory lobe. **A** Corollary discharge-evoked field potentials before the lesions. The *arrows* point to the different components of the potentials that are discussed in the text. **B** Corollary discharge-evoked field potentials after lesions of the ipsilateral juxtalobar nucleus and of the axons from the contralateral juxtalobar nucleus. Note the disappearance of the n2, p2, n3 and p3 components. Also note reduction in the size and slope of the initial ramp-like p1 component. This reduction is probably due to damage of axons of passage rather than to removal of juxtalobar input

is consistent with the finding that electrical stimulation of the juxtalobar nucleus evokes responses that are very similar to these components in spatial distribution, latency, and amplitude.

The effects of unilateral and bilateral lesions of the medial juxtalobar input to the electrosensory lobe were quite similar to the effects of unilateral and bilateral MCA lesions (Bell et al. 1995), except for an effect of ipsilateral juxtalobar lesions on the initial positive wave, p1. This wave was not affected by MCA lesions, but was reduced following ipsilateral lesions of the juxtalobar nucleus (note the lower slope and size of the p1 component in Fig. 9B in comparison to the same component in Fig. 9A). This reduction in the early p1 component was probably due to damage of axons of passage rather than to damage of the juxtalobar neurons themselves. This is indicated by the fact that the reduction in p1 was not observed after lesions of the axons from the contralateral side but only after lesions of the ipsilateral juxtalobar nucleus. Moreover, the p1 component begins very early, 2.5 ms before the command signal, and could not therefore be evoked by input from juxtalobar neurons since these only discharge at 3.6–4.0 ms after the command signal.

The hypothesis is presented elsewhere (Bell et al. 1992) that the p1 wave in the electrosensory lobe is due to an early excitation of the eminentia granularis posterior (EGp) by corollary discharge input from the paratrigeminal command associated nucleus (PCA). A negative wave (n1) that is presumed to reflect such excitation is present in the EGp at the time of p1. The positive wave p1 is presumed to be due to the parallel fibers in the molecular layer acting as current sources for the excitation of the parent granule cell somas in EGp. The negative wave in the ipsilateral EGp was also reduced or eliminated by lesions of the juxtalobar nucleus (not illustrated). The fibers from PCA to EGp pass close to the juxtalobar nucleus and could easily have been damaged by the lesions.

Note on the lateral juxtalobar nucleus

The lateral juxtalobar nucleus which sends its axons to EGp is located laterally and slightly anteriorly to the medial juxtalobar nucleus (Bell et al. 1981). The cells of the lateral juxtalobar nucleus are smaller than those of the medial juxtalobar nucleus. No distinct set of field potentials or unit recordings that could be clearly attributed to the lateral nucleus were obtained in this study. It might be that most tracts did not pass through the nucleus or that the cells were too small to record. It is also possible that cells of the lateral nucleus behaved so similarly to cells of the medial nucleus that they were not distinguished.

It is clear, however, that the results of this study describe the medial nucleus. All ten of the intracellularly labeled cells were clearly within the medial juxtalobar nucleus. Most cells tested could be antidromically driven by stimulating the bundle of contralaterally projecting axons just dorsal to the molecular layer of the medial octavolateral nucleus. Cells of the medial but not the lateral juxtalobar nucleus send axons into this bundle. Stimulation in the region evoked short latency negative field potentials in the granule and ganglion-plexiform layers of the electrosensory lobe, a result which is readily explained by the connections of the medial nucleus but not by the connections of the lateral nucleus. Lesions probably damaged both medial and lateral portions of the nucleus and this must be considered in interpreting the results (see Discussion).

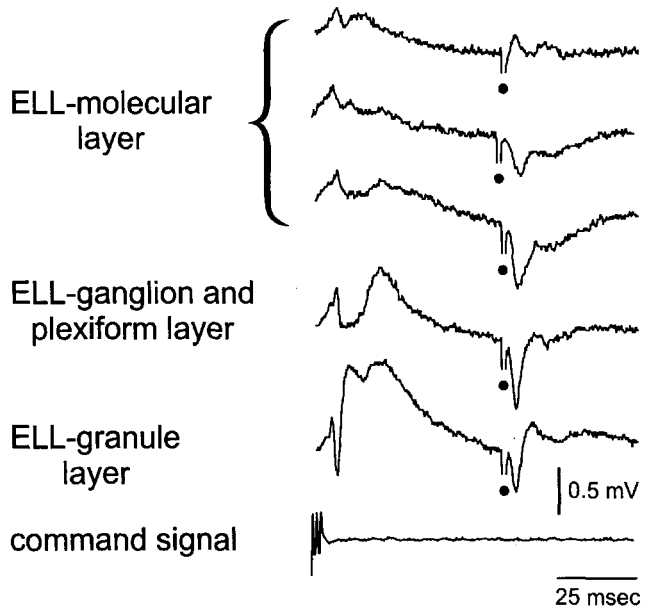

Fig. 10 Field potentials evoked in electrosensory lobe cortex by stimulation of nucleus praeeminentialis. The stimulus was of constant amplitude and was given at a fixed delay after the corollary discharge response. The time of the stimulus is indicated by a *filled circle*. Note the prominent early negative wave evoked by the stimulus that is present in the granule layer and reaches a maximum size in the ganglion and plexiform or lower molecular layers

Stimulation of nucleus praeeminentialis and the praeeminential-electrosensory tract

Stimulation of nucleus praeeminentialis or the praeeminential-electrosensory tract evoked responses in the electrosensory lobe that were quite different from the corollary discharge responses or from the responses evoked by juxtalobar stimulation (Fig. 10). Stimulation of either the nucleus or the tract evoked a short latency wave that was negative throughout the granule, plexiform, ganglion and lower molecular layers. The wave reached its largest size in the ganglion and lower molecular layers and inverted to a positive wave only in the outer molecular layer of the electrosensory lobe. The spatial distribution of this wave is thus quite different from that of the n2 component of the corollary discharge-evoked potential or the n2-like potential evoked by juxtalobar stimulation. Stimulation of nucleus praeeminentialis or of its efferent axons did not elicit n3-like or p3-like responses either. The lack of correspondence between the corollary discharge-evoked and praeeminentialis-evoked field potentials means that the field potentials are of little help in understanding the corollary discharge role of nucleus praeeminentialis (see Discussion).

The responses to praeeminentialis stimulation are consistent with anatomical work showing that fibers from nucleus praeeminentialis terminate most densely in the lower molecular layer, just above the ganglion cells (Bell et al. 1981) – the sharp negative wave being due to synaptic activation of dendrites in the lower molecular layer and of cells in the ganglion layer. The smaller negative wave in the granule layer and below is presumably due to volume conduction. The positive wave in the outer molecular layer is presumably due to the dendrites in this region acting as passive sources for current sinks in the lower molecular and ganglion layers.

Stimulation of nucleus praeeminentialis elicited the same potentials both before and after the combined lesions of the ipsilateral juxtalobar nucleus and of the axons from the contralateral juxtalobar nucleus. This result shows that the strong effects of the lesions on corollary discharge-evoked field potentials were *not* due to the interruption of input from nucleus praeeminentialis.

Discussion

Preservation of timing information in the corollary discharge pathway

All of the activity in medial juxtalobar cells, as in MCA cells, occurs at short fixed delays after the initiation of the EOD motor command in the command nucleus. These nuclei thus appear to be simple relays of timing information about the EOD motor command. The measured variation in the latency of single medial juxtalobar neurons was only 0.05 ms. Such consistency or precision is remarkable given that there are at least 4 synapses and approximately 8 mm of axon trajectory (from the medulla to the mesencephalon and back to the medulla) between the command neurons and the juxtalobar neurons. In addition, the command signal used for the latency measurements reflects the activity of electromotoneurons in the spinal cord which are themselves 2 synapses and about 100 mm away from the command nucleus. Moreover, the command signal as we measured it includes some noise. The measured precision in the timing of juxtalobar activity would probably be better than 0.05 ms if intracellular recordings from the command nucleus were used for the latency measurements.

Temporal precision in the corollary discharge pathway provides the electrosensory lobe with exact information about the time of the EOD, because the time between command signal and EOD is fixed. The corollary discharge could be used as a temporal reference in measuring the latency of the reafferent input in mormyromast electroreceptors evoked by the fish's own discharge. This latency is a precise function of stimulus voltage at the receptor, and a latency code for stimulus intensity has been suggested for mormyromast afferents (Szabo and Hagiwara, 1967; Bell 1990b). By sensing the pattern of self-induced transcutaneous current,

the fish can detect external objects that distort the pattern, a process known as active electrolocation.

Recent behavioral experiments strongly support the hypothesis of a latency code in the active electrolocation system of mormyrid fish and the use of a corollary discharge signal in decoding latencies (Hall et al. 1995). The EOD was blocked by curare in these experiments and an artificial EOD was given at a short delay following the EOD motor command signal. The command signal was emitted spontaneously under these conditions ('fictive discharging'). A small shift in the delay of the artificial EOD evoked a vigorous novelty response from the fish, i.e., an acceleration in the emission rate of the command signal. This novelty response was entirely similar to the response evoked in the curarized fish by a change in intensity of the artificial EOD or in a discharging fish by a change in nearby conductances. Latency shifts as small as 0.1 ms in the delay of the artificial EOD were effective.

Measurement of such small changes in the latency of afferent input means that the time of the corollary discharge signal must be locked to the time of the EOD with great precision. The experiments described here and in the preceding paper indicate that such precision is indeed present in the corollary discharge signals that are sent to the electrosensory lobe.

The actual decoding of afferent latency probably occurs within the granule cells of the electrosensory lobe. Intracellular recordings from primary afferents that make electrical synapses on granule cells reveal synaptic potentials that are probably due to synaptic input to the granule cells (Bell 1990a). The recordings show corollary discharge-driven epsps that are temporally locked with great precision to the EOD motor command as well as epsps evoked by impulses in mormyromast afferents. The level of depolarization in the granule cell, and the spike output, depends on temporal summation of the corollary discharge epsp (with a fixed latency) and the afferent epsp (with a latency dependent on stimulus intensity).

The corollary discharge driven epsp in electrosensory lobe granule cells is reflected extracellularly in the n2 component of the corollary discharge-driven field potential (Bell et al. 1992). The results reported here indicate that the n2 wave and the corollary discharge-driven epsps which it reflects are evoked by corollary discharge input from the juxtalobar nucleus.

Specializations for the preservation of temporal information in the corollary discharge pathway

Neurons in the electrosensory systems of electric fish (Maler et al. 1981; Carr et al. 1986; Mugnaini and Maler 1987; Szabo et al. 1983; Bell and Grant 1989) and in the auditory system of barn owls (Carr 1986; Konishi et al. 1988) that are specialized for the transmission of precise temporal information often have either no dendrites or very small, short dendrites. Such morphology yields minimal electrotonic delays and maximal rise times for the postsynaptic response to synaptic input. In contrast, the dendrites of medial juxtalobar neurons are quite prominent. Two or three short and thick dendrites emerge from the cell body and terminate in spatially restricted bushy arbors. In this respect, the medial juxtalobar cells are like the bushy cells of the mammalian ventral cochlear nucleus, which are also specialized for the preservation of timing information. These cells have a single short thick dendrite ending in a bushy arbor (Smith and Rhode 1987). Similar morphology is also seen in the electrosensory lobe cells responsible for timing information in one group of gymnotid fish, the Sternopygidae (Losier and Matsubara 1990). The bushy arbors at the end of the dendrites allow for many terminals and a large synaptic input. Perhaps the large synaptic input can compensate for the longer time constants of cells with significant dendritic arbors.

A large synaptic input is probably an important specialization for the preservation of timing information because it assures a rapid and consistent postsynaptic response. Evidence was provided here for a large synaptic input of 15 to 20 mV in juxtalobar cells (Fig. 7D). Further work will be needed, however, to separate clearly this synaptic potential from the electrogenic responses that it evokes in the postsynaptic cell.

Low threshold hump-like responses to injection of depolarizing current similar to the ones in juxtalobar neurons have been observed in neurons that are specialized for the preservation of timing information in the avian (Zhang and Trussell 1994; Reyes et al. 1994) and mammalian (Wu and Oertel 1984; Manis and Marx 1991) auditory systems. Each of these studies has shown that the low threshold hump-like responses in their cells are due to the activation of a persistent 4-aminopyridine-sensitive outward current. The hump-like responses are explained as follows: Injection of depolarizing current causes a rapid depolarization due to the passive membrane properties of the cell. Depolarization turns on the persistent outward current at a slight delay causing the membrane potential to fall from its initial peak, thus causing a voltage hump at the onset of the current. Hyperpolarizing current pulses close channels that are open at rest and deinactivate inactivated channels. At the off of hyperpolarizing current, the potential will fall to a level that is more depolarized than resting potential because of the closed outward current channels. Activation of the outward current then returns the membrane to the resting voltage, thus causing a voltage hump at the off of hyperpolarizing current.

No attempt was made determine the cellular current responsible for the low threshold responses in medial juxtalobar neurons. Activation followed by inactivation of an inward current cannot be ruled out as

a cause. A few observations are more consistent with activation of an outward current in juxtalobar neurons, however. In Fig. 5D, note that the rate of rise of the membrane potential in response to a current step is slower when the low threshold response is evoked (Fig. 5Db) than when it is not evoked (Fig. 5 Da), suggesting that a current has been activated that opposes the depolarizing step. Note also that the voltage at the end of identical current steps is lower when the low threshold response occurs (Fig. 5Db) than when it does not (Fig. 5 Da), suggesting a persistent outward current or a persistent conductance change.

Some of the physiological functions that have been suggested for the persistent outward current in time preserving neurons of the auditory system (Manis and Marx 1991; Reyes et al. 1994; Wu and Oertel 1984; Zhang and Trussell 1994) may also be relevant for the time preserving neurons of the medial juxtalobar nucleus. For example, an increased conductance in the depolarizing direction reduces membrane charging time and allows a more rapid rate of rise for a large epsp. In addition, such a conductance helps insure that only a single spike will be generated by a discrete synaptic input.

Sensory systems that are specialized for the transmission of temporal information in electric fish often have electrical synapses between pre- and postsynaptic elements (Maler et al. 1981; Carr et al. 1986; Mugnaini and Maler 1987; Szabo et al. 1983; Bell and Grant 1989). Electrical transmission assures rapid transmission with minimal variability. Electrical transmission is not a universal feature of temporally specialized systems, however. Electrical transmission is not present, for example, in those parts of the bird auditory system that are specialized for temporal transmission (Konishi et al. 1988; Jhaveri and Morest 1982). Similarly, the long delay between afferent input to MCA and the response of MCA cells suggested that chemical transmission was present in that nucleus.

This study could not provide a clear answer to the question of electrical coupling between pre- and postsynaptic elements in the juxtalobar nucleus. On the one hand, delays as short as 0.2 ms were sometimes observed between the arrival of corollary discharge-driven input to juxtalobar neurons (as indicated by field potentials and recordings from presumed afferents) and the postsynaptic responses of juxtalobar cells. This delay is short for chemical transmission at 250°C. On the other hand, this delay is still not zero and most cells showed somewhat longer delays.

Nevertheless, some features of juxtalobar neurons suggest that the neurons are electrically coupled to each other. Such coupling could be by way of afferent fibers that make electrical contact with different cells in the nucleus (Bennett 1977) or by electrical contacts among the postsynaptic cells. Evidence for electrical coupling among juxtalobar neurons included the following: 1) Antidromic activation of the axons or axon terminals of juxtalobar cells evoked small potentials (Fig. 6Aa, Ba) at a latency that was close to that of full-sized antidromic spikes. The short latency indicates that the potentials are not due to chemical synapses, and there is also no known anatomical projection from the electrosensory lobe back to the juxtalobar nucleus. The small potentials were probably not axon spikes that failed to invade the somas of juxtalobar neurons since they were often graded with stimulus intensity. The best explanation is that the small potentials are antidromic spikes in other juxtalobar neurons that are electrically coupled to the recorded cell, as has been argued for similar potentials in other systems (Baker and Llinas 1971; Bennett 1977; Llinas et al. 1974). 2) The spikes of juxtalobar cells appeared to be graded in amplitude (Fig. 5). One possible explanation for such 'graded spikes' is that they are spikes in other juxtalobar neurons which are electrically coupled to the recorded cell. Further morphological and physiological work will be needed to fully resolve the issues of electrical coupling among juxtalobar cells and the site or sites of such coupling.

Overview of the timing of corollary discharge responses in MCA, the juxtalobar nucleus, and the electrosensory lobe

The timing relationships are illustrated in Fig. 11. To summarize: The EOD motor command is initiated in the command nucleus of the medulla, with the first spike in that nucleus beginning at about -3.5 ms (Grant et al. 1986; times are given with respect to the 'time of the command signal', i.e., the first large negative peak of the command signal as recorded over the electric organ in the tail). The output from the command nucleus evokes a response in BCA that reaches the terminals of BCA axons in MCA at about -2.5 ms. The input from BCA elicits a two spike response in MCA cells with the first spike occurring at -1.0 ms. Connections from MCA to cells of the juxtalemniscal region result in a corollary discharge driven input to the juxtalobar nucleus at $+2.0$ ms. This input elicits a spike in the juxtalobar neurons at about 4.0 ms. Axons from the juxtalobar neurons evoke the n2 component of the corollary discharge field potential in the electrosensory lobe at about $+5.5$ ms. The EOD, evoked by the pathway from the command nucleus through the medullary relay nucleus to the electromotoneurons of the cord, occurs at about $+4.5$ ms. Finally, the EOD elicits reafferent input in the primary afferents from electroreceptors that arrives at the electrosensory lobe between $+6$ and $+18$ ms – the precise latency depending on the location of the electroreceptor and on stimulus intensity. The corollary discharge and reafferent inputs interact within the electrosensory lobe.

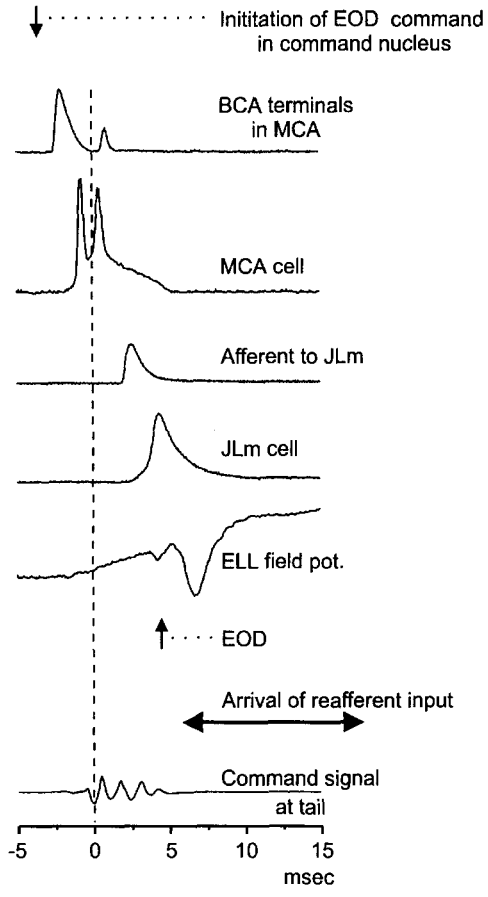

Fig. 11 Summary of timing relations among corollary discharge responses of various structures. The *vertical dashed line* is at time zero of the command signal. The *downward pointing arrow* at the top is at the time of onset of the first spike in the command nucleus. The *upward pointing arrow* at the bottom is at the time of onset of the EOD. All records have appeared in previous figures of this paper or the preceding paper (Bell et al. 1995). The previous figures should be consulted for the gains. Intracellular recordings are shown for the BCA terminals, the afferent to juxtalobar nucleus and the cell of juxtalobar nucleus. The smaller size of the second spike in the BCA terminals may be an artifact due to electrode penetration. An extracellular recording is shown for the MCA cell because the intracellular recordings did not show the correct timing of the second spike

The different sources of corollary discharge input and their effects on the cortex of the electrosensory lobe

As described in the previous paper (Bell et al. 1995) and as illustrated in Fig. 3 of that paper, at least four different central structures appear to send corollary discharge related signals to the mormyromast regions of the electrosensory lobe. The four structures are: EGp, nucleus praeeminentialis, the medial juxtalobar nucleus, and some neurons of the juxtalemniscal region. Physiological studies of EGp (Bell et al. 1992), nucleus praeeminentialis (G. von der Emde and C. Bell, unpublished) and the medial juxtalobar nucleus (this paper) show that these structures do indeed exhibit strong corollary discharge signals. Physiological studies have not been done on the juxtalemniscal neurons that project to the deeper layers of the electrosensory lobe and they are grouped among the corollary discharge sources simply because of their location within the area of termination of fibers from MCA.

EGp receives input from three different sources which are either known to, or are likely to, convey corollary discharge signals: PCA, the lateral juxtalobar nucleus and nucleus praeeminentialis (see Fig. 3 of the previous paper). EGp is so tightly linked to the electrosensory lobe as the source of parallel fibers that its inputs can be considered as inputs to the electrosensory lobe for discussion purposes. Thus, there appear to be five sources of corollary discharge input to the electrosensory lobe medial juxtalobar nucleus, PCA, nucleus praeeminentialis, lateral juxtalobar nucleus and some neurons of the juxtalemniscal region. The contributions of these different structures to the corollary discharge effects in the mormyromast region of the electrosensory lobe are discussed next.

The results of this study indicate that the medial juxtalobar nucleus makes a major contribution to the corollary discharge effects in the mormyromast regions of the electrosensory lobe cortex. Major components of the corollary discharge-evoked field potentials were duplicated by stimulation of the juxtalobar nucleus, and these same components disappeared after bilateral lesions of the nucleus or its efferent connections. The effects on the different components of the field potentials in the cortex indicate that juxtalobar activity is responsible for the corollary discharge driven excitation of granule cells and for much of the corollary discharge driven excitation of other cell types also (as described above and in the previous paper on MCA; Bell et al. 1995).

Corollary discharge effects of PCA are also clear. Prominent corollary discharge effects remained after bilateral lesions of MCA (Bell et al. 1995). If the anatomy is correct, then these effects that are independent of MCA must be mediated by PCA. The PCA effects are the very earliest ones, n1 in EGp and p1 in the electrosensory lobe. Individual PCA cells have not been recorded from directly, but field potentials have been recorded in PCA that begin at -2.9 ms (G. von der Emde and C. Bell, unpublished). In addition, fibers in EGp have been recorded that are probably the terminals of axons from PCA (Bell et al. 1992). These fibers show stereotyped bursts at short and fixed times with respect to the command signal. The first spike of the burst in different fibers ranges between -2.7 and $+11$ ms relative to the command signal. The timing of the bursts means that they could be responsible for the early n1 and p1 components of the corollary discharge potentials. The timing of many of the bursts also indicates that they do not originate from MCA, because the first spike in MCA cells only occurs at -1.0 ms, i.e., after the beginning of many of the bursts.

The juxtalobar nucleus together with PCA appear to account for a very large proportion of the corollary

effects in the electrosensory lobe, as judged by the field potentials. The present experiments, in fact, only point to corollary discharge effects that are due to either the juxtalobar nucleus or PCA and do not indicate any effects that can be clearly traced to the other known sources of corollary discharge input: nucleus praeeminentialis, the lateral juxtalobar nucleus, or cells of the juxtalemniscal region. The anatomy indicates that corollary discharge input to these latter structures passes through MCA (see Fig. 3 of the preceding paper). But the effects of stimulation and lesions of the juxtalobar nucleus were so similar to the effects of stimulation and lesions of MCA, as to suggest that most of the corollary discharge effects of MCA on the mormyromast regions of the cortex are mediated by the juxtalobar nucleus. Thus, the contributions of the other three sources of corollary discharge input remain obscure.

The contribution of the lateral juxtalobar nucleus may not have been noticed because lesions of the ipsilateral medial juxtalobar nucleus may well have disrupted the input from the more lateral nucleus to EGp. In addition, the few cells of the juxtalemniscal region that project to the cortex of the electrosensory lobe may not make a major contribution to the corollary discharge-driven field potentials. The lack of any clear indication of corollary discharge effects from nucleus praeeminentialis is more difficult to explain, however. The input from nucleus praeeminentialis was not disrupted by the medial juxtalobar lesion and nucleus praeeminentialis has massive projections to both the electrosensory lobe and EGp.

One would expect the nucleus praeemientialis to make an important contribution to the corollary discharge-evoked field potentials in the electrosensory lobe beginning about 11 ms after the command signal. Cells of the nucleus praeeminentiialis are excited by the corollary discharge, with action potential latencies ranging from 10 to 30 ms (G. von der Emde and C. Bell, unpublished). This corollary discharge activity should have a strong influence, given the heavy anatomical projection and the large field potentials evoked by stimulation of nucleus praeeminentialis in the cortex of the electrosensory lobe (Fig. 10). One is surprised therefore by the apparent lack of contribution from nucleus praeeminentialis as suggested by the similar effects of juxtalobar and MCA lesions, and by the lack of obvious correlation between corollary discharge-evoked responses and responses to stimulation of nucleus praeeminentialis. Perhaps field potentials are not a good way of observing the corollary discharge effects of nucleus praeeminentialis. It might be, for example, that the activity in nucleus praeeminentialis is spread out over time and only poorly synchronized, leading to a reduced effect on the field potentials.

In summary: the results have indicated a particular importance for inputs from the juxtalobar nucleus and PCA in determining the corollary discharge responses of cells in the electrosensory lobe. The contributions of other sources, and in particular that of nucleus praeeminentialis, remain to be established.

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