Descending Control of Electrosensation. II. Properties of Nucleus Praeeminentialis Neurons Projecting Directly to the Electrosensory Lateral Line Lobe

Bradford Bratton and Joseph Bastian

Department of Zoology, University of Oklahoma, Norman, Oklahoma 73019

The nucleus praeeminentialis projects to the electrosensory lateral line lobe via 2 distinct pathways. Neurons that project to the posterior eminentia granularis and therefore influence the electrosensory lateral line lobe indirectly are described in the preceding report. This report describes the physiological properties and anatomical characteristics, revealed with Lucifer yellow staining, of n. praeeminentialis neurons that project directly to the ventral molecular layer of the electrosensory lateral line lobe. The neurons studied were the stellate cells described by Sas and Maler (1983), and we found 2 physiological subtypes of these. These neurons typically had no spontaneous activity, but responded vigorously to either increased electric organ discharge amplitude on the contralateral side of the body (ST-E cells) or to decreased amplitude (ST-I cells). These neurons also responded to low-frequency sinusoidal electric organ discharge amplitude modulations (AM) but were inhibited by AMs having frequencies greater than about 16 Hz. These stellate neurons were unable to encode information about long-term changes in electric organ discharge amplitude, but they responded very well to moving electrolocation targets. The relatively long response latency of these neurons suggests that they receive inputs from higher centers in addition to those from the electrosensory lateral line lobe. It is suggested that these cells alter the sensitivity of restricted populations of output cells in the electrosensory lateral line lobe and process temporally and spatially restricted stimuli. They may act to increase the intensity of the neural representation of important stimuli.

The nucleus praeeminentialis dorsalis (NPd) is an electrosensory processing area thought to be primarily involved in the feedback or descending control of the first-order electrosensory nucleus, the electrosensory lateral line lobe (ELL). The NPd receives afferents from the ELL, from higher-order electrosensory nuclei including the torus semicircularis and the optic tectum, and from the caudal lobe of the cerebellum. The NPd projects primarily to the ELL and to a mass of cerebellar granule cells lying dorsal to the ELL, the posterior eminentia granularis (EGP), and it sends a minor output to the reticular formation (Sas and Maler, 1983, 1987; Carr and Maler, 1986). These efferent pro-

jections are bilateral. The direct projection from the NPd to the ELL, the tractus stratum fibrosum, terminates in the ventral molecular layer and makes synaptic contact with ELL interneurons as well as output neurons. The NPd projection to the EGP forms the indirect projection to the ELL; axons of the EGP granule cells, parallel fibers, project to the ELL forming its dorsal molecular layer. In addition to the massive descending electrosensory input, the EGP also receives spinal input via the lateral reticular nucleus and several other minor inputs (Sas and Maler, 1987). These 2 morphologically distinct descending pathways from the NPd to the ELL have been well defined anatomically, and the studies of Sas and Maler (1983, 1987) have identified the major neuron types that project via each of these. Additional information about the anatomical relationships among these nuclei and details of the anatomy of the ELL itself are included in the preceding report (Bastian and Bratton, 1990), in which the physiological and morphological characteristics of neurons projecting to the ELL indirectly via the EGP are described. This report focuses on the principal category of neurons projecting directly from the NPd to the ELL ventral molecular layer, the stellate neurons of Sas and Maler (1983).

Materials and Methods

The materials and methods used in this study are the same as described in the preceding paper (Bastian and Bratton, 1990).

Results

Probably the most common cell type found within the NPd, the stellate neuron described by Sas and Maler (1983), projects directly to the ELL via the tractus stratum fibrosum. Extracellular single-unit recordings were used to establish neuron categories based on physiological properties. Intracellular recording and single-cell marking via Lucifer yellow iontophoresis were then used to morphologically identify individual neurons representative of these categories.

Responses to stepwise changes in EOD amplitude

Unlike other NPd neurons and those found in lower-order electrosensory processing regions, the stellate neurons are typically silent unless presented with an appropriate change in electric organ discharge (EOD) amplitude. The spontaneous activity recorded from a sample of 37 stellate neurons averaged 0.54 ± 0.19 spikes/sec; of these 28 had no spontaneous activity at all, and the remaining 9 had resting discharges of less than 5 spikes/sec.

The stellate neurons respond well to brief (100 msec) stepwise changes in EOD amplitude, as shown in Figure 1, and these

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Correspondence should be addressed to Dr. Joseph Bastian at the above address. Copyright © 1990 Society for Neuroscience 0270-6474/90/041241-13\$02.00/0

Figure 1. Responses of 2 types of NPD stellate neurons to 100-msec stepwise changes in EOD amplitude. *A, B*, Raster displays and post-stimulus-time histograms of the responses of an ST-E cell to 45 replicates of a 0.375 mV/cm rms increase (*A*) and decrease (*B*) in EOD amplitude. *C, D*, Responses of an ST-I cell to 45 replicates of these same stimuli.

neurons fall into 2 subcategories depending on their responses to increases or decreases in EOD amplitude. These stimuli were presented transversely between electrodes lateral to each side of the fish. A stimulus polarity that causes an increase in amplitude on one side of the body, therefore, results in a simultaneous decrease in amplitude on the opposite side. Since the input to the NPD is predominantly, but not totally, from the

contralateral ELL, a stimulus polarity that increases the EOD amplitude contralaterally is expected to activate the electroreceptor population, and subsequently the ELL, that provides the major input to the NPD recorded from. The ipsilateral receptors and ELL will simultaneously receive decreased input.

Figure 1*A* shows the responses of a neuron that was strongly excited by an increase in EOD amplitude on the contralateral side of the body (ST-E Cell). Firing frequency averaged over the stimulus period was 231 spikes/sec, and the neuron's response adapted. Firing frequency during the first 25 msec averaged 304, while that during the last 25 msec averaged 170 spikes/sec, and the adaptation time constant, time at which firing frequency averaged over 5-msec epochs decayed to 37% of the peak firing frequency, was 88 msec. Responses to the opposite stimulus polarity, contralateral decrease in EOD amplitude, typically consisted of a brief burst of activity following the end of the stimulus, as shown in Figure 1*B*. Less frequently, longer-duration bursts of activity followed the end of the stimulus, and some neurons of this type showed no response to the contralateral EOD reduction.

The second subcategory of stellate neuron responded oppositely to these same stimuli, as shown in Figure 1, *C, D*. Increases in the EOD amplitude on the contralateral side of the body resulted in either no response or weak responses at the end of the stimulus, but these neurons gave strong responses to decreases in EOD amplitude on the contralateral side of the body (Fig. 1*D*). In this case spike frequency averaged over the stimulus period was 60 spikes/sec, the responses during the initial and final 25 msec averaged 120 and 25 spikes/sec, respectively, and the time constant of adaptation was 9 msec. The stellate neurons were also stimulated with changes in EOD amplitude presented with the symmetrical geometry which causes modulations of EOD amplitude of like sign over the whole body surface. No significant differences were seen in the stellate cell responses contingent on this stimulus geometry.

This dichotomy in the responses of these 2 types of neurons is similar to that of the responses of the 2 classes of ELL output neurons that encode EOD amplitude. One category of these, the basilar pyramidal cells or E-cells (Maler et al., 1981), respond to increased EOD amplitude with excitation (Enger and Szabo, 1965; Bastian, 1981b; Saunders and Bastian, 1984). The second category, nonbasilar pyramidal cells or I-cells, respond to this same stimulus with inhibition.

Response latencies were measured for 23 ST-E and 17 ST-I cells and these averaged 8.2 ± 0.76 and 13.8 ± 1.38 msec, respectively. These mean latencies are significantly different as judged by a *t* test ($p < 0.01$) and are longer than typical ELL E- and I-cell latencies of 3.6 and 6.8 msec, respectively. This relatively long latency suggests that a major input to the stellate neurons may come from higher centers rather than directly from the ELL. Time constants of adaptation were also measured for 23 ST-E and 17 ST-I cells, and these averaged 24.0 ± 4.2 and 17.2 ± 2.1 msec for the ST-E and -I cells, respectively. These means are not significantly different from each other, and the average ST-E adaptation time constant is not different than that of ELL E-cells, 24.4 ± 3.0 msec, measured in an earlier study (Bastian, 1981b). The ST-I cells may, however, adapt more rapidly than do the ELL I-cells. The time constant of adaptation for the latter averaged 31.4 ± 4.5 msec.

The stellate neurons' ability to encode brief changes in EOD magnitude was quantified by measuring their responses to various-sized 100-msec stepwise modulations in EOD amplitude.

Figure 2. Average responses ± 1 SE of stellate E- and I-cells to changes in EOD amplitude of various magnitudes. ST-E cells (*A*) were stimulated with 100-msec stepwise increases in EOD amplitude. Spike frequencies were computed over the first 25 msec of the response (dashed line), over the last 25 msec of the response (dotted line) and over the entire stimulus period (solid line). ST-I cells (*B*) were stimulated with 100-msec stepwise decreases in stimulus amplitude. Responses were calculated over the same periods as in *A*. Best-fit lines determined for responses measured over the 100-msec stimulus period for amplitudes ranging between -10 and -40 dB were $y = 190 + 3.51x$ and $y = 168 + 3.87x$ for ST-E and -I cells, respectively. Zero-dB corresponds to a stimulus field of 3.75 mV/cm rms.

Contralateral increases were used to stimulate ST-E cells and contralateral decreases were used with the ST-I cells. Figure 2, *A*, *B*, summarizes average firing frequency measured over the first 25 msec of the response (dashed line), over the entire 100-msec response period (solid line), and over the last 25 msec of the response (dotted line) for 23 ST-E cells and 17 ST-I cells. The ST-E cells were typically more sensitive than the ST-I cells. The threshold stimulus intensity for the former was less than 3.75 μ V/cm rms (-60 dB), while the latter typically showed no responses to stimuli less than about 12 μ V/cm rms, or about -50 dB. The difference in sensitivity between stellate cell types remained over the range of EOD modulations used; the mean ST-E cell responses measured at each stimulus amplitude ranged between 21 and 34 spikes/sec higher than the responses of the ST-I cells. The difference in sensitivity is particularly apparent when the initial responses, measured over the first 25 msec, are compared at high stimulus amplitudes. The ST-I cells' early responses plateau or are actually suppressed due to decreases in EOD amplitude greater than about 375 μ V/cm (-20 dB). The ST-E cells' responses also level off at the highest stimulus intensities used, but the average changes in activity of the ST-E cells are nearly 100 spikes/sec greater than those of the ST-I cells. The ELL I-cells also show a saturation or plateau effect similar to that of the ST-I cells with EOD amplitude reductions of similar sizes (Bastian, 1986a). The slopes of the best-fit lines determined for responses to -10 through -40 dB stimuli were 3.5 and 3.9 spikes/sec per dB for ST-E and ST-I cells, respectively. These are larger than the slopes describing the relationships between stimulus amplitude and change in firing frequency measured for ELL E- and I-cells. These were 1.5 and 1.4 spikes/sec/dB, respectively (Bastian, 1986a); hence these NPD neurons

are able to encode a given change in EOD amplitude with higher resolution than the amplitude encoding neurons of the ELL.

Responses to AMs of various frequencies

One of the most definitive characteristics of the NPD stellate neurons is their lack of responsiveness to sinusoidal EOD amplitude modulations of frequencies greater than about 16 Hz. AM tuning curves were produced for 14 ST-E neurons and for 11 ST-I cells, and the average responses of these cells are plotted as a function of AM frequency in Figure 3, *A*, *B*. Response was measured in 2 ways. The maximum change in spike frequency within the AM cycle was measured at each frequency (solid lines), and the average spike frequency over the entire stimulus presentation period was measured (dashed lines). The average spike frequency remains constant for these neurons up to AM frequencies of about 8 Hz. Beyond this frequency the cells' activity decreases and in many cases the cells are silent for AM frequencies greater than 32 Hz. Measures of the difference in spike frequency within the AM cycle are equal to the average peak spike frequency based on the 5 largest consecutive bins in the period histogram minus the minimum frequency determined in the same manner. These values increased with AM frequency up to about 8 Hz, and beyond this frequency they also fell. The increases in the difference measurement show that for frequencies up to 8 Hz the neurons' responses are becoming more clustered. That is, although the overall spike frequency is not changing, the cells' responses become grouped into higher-frequency bursts as AM frequency increases to about 8 Hz. The ST-E and -I neurons behaved similarly, except that the average responses of the latter type were lower than those of the former for most of the AM frequencies used. The responses of these

Figure 3. Average responses, ± 1 SE, of ST-E and ST-I cells to various frequencies of sinusoidal EOD amplitude modulation. **A**, Average peak change in firing frequency of ST-E cells within the AM cycle (solid line). This was determined from period histograms as the difference between the maximum frequency computed from the 5 consecutive largest bins, and the minimum frequency determined from the 5 consecutive smallest bins. The ST-E cells' average firing frequency (dashed line) was calculated over the entire stimulus presentation period. **B**, Average peak change in ST-I cell firing frequency within the AM cycle (solid line) and the average firing frequency computed over the stimulus presentation period (dashed line). **C**, The upper trace is a cumulative frequency distribution of an ST-E cell's responses to a series of sinusoidal EOD AMs of logarithmically increasing frequency. The lengths of the lines in the lower portion of the figure show the frequency of each AM cycle. **D**, Cumulative frequency distribution of the responses of an ST-I neuron. AM amplitude was 2 mV/cm peak to peak.

neurons differ markedly from those of most of the ELL E- and I-cells, which respond well to low- as well as high-frequency AMs, sometimes preferring AM frequencies as high as 64 Hz (Bastian, 1981a, 1986a). ELL output neurons having similar low-pass properties have been seen in the related fish, *Eigen-*

mannia virescens. These were usually found in a specific region of the ELL, the centromedial section, and showed reduced responses to higher-frequency AMs, but were not silenced by these (Shumway, 1989).

An alternative method was also used to gain information

Figure 4. Responses of stellate cells to metal and plastic electrolocation targets moved 2.5 mm lateral to either side of the fish. Responses of the ST-E cell to the metal target moved contralaterally in the tailward and headward direction are shown in *A* and *B*, respectively. Responses to the metal object moved ipsilaterally are shown in *C* and *D*, and responses of this same cell to the plastic target are shown in *E–H*. Responses of the ST-I cell to the metal target are shown in *I–L* and to the plastic object are shown in *M–P*. The outlines of the fish are scaled to the length of target movement, and the length of the arrows corresponds to a distance of 4 cm.

about the AM frequency selectivity of these cells. AM frequency was continuously varied and responses were displayed as cumulative frequency distributions (CFD), as shown in Figure 3, *C*, *D*. In these experiments the AM frequency was initially 1 Hz, and after a short control period frequency was swept logarithmically up to about 40 Hz. The cumulative spike frequency distribution is computed as the summation of the response to each successive AM cycle (upper curves). Each line in the lower portion of the figures indicates the instantaneous frequency of each AM cycle. A cumulative frequency distribution of uniform slope indicates responses that do not change as a function of AM frequency, increases in slope indicate increased responsiveness, and decreases in slope reduced responsiveness. This method was used in addition to standard AM tuning curves because it is very fast—AM frequency selectivity can be assessed in less than 1 min. Hence, this method is better suited to the intracellular studies to be described later where time does not permit the production of a typical AM tuning curve.

Figure 3, *C*, *D*, shows results from an ST-E and -I cell, respectively. The pattern shown in Figure 3*C* is the more typical for stellate neurons. The number of spikes per AM cycle stays relatively constant until the cutoff frequency is approached. The cell is quickly silenced by AMs above this frequency, as indicated by the slope approaching zero. The pattern shown in Figure 3*D* is seen less often. In these cases, increasing AM frequency evokes increasing numbers of spikes per AM cycle (note the increasing slope of the CFD). A similar cutoff or inhibition of the cell's response also occurs at higher frequencies. This type of experiment also provides a high-resolution estimate of a neuron's preferred AM frequency and of the frequency at which the neuron stops responding. The "best" AM frequency averaged 4.09 ± 1.6 Hz and 5.9 ± 1.2 for ST-E and ST-I cells, respectively. These means are not significantly different, and these estimates of best AM frequency are less than the peak frequencies shown in Figure 3, *A*, *B*. The frequencies at which the

neurons ceased to respond were also very similar for both types of stellate neurons. These averaged 13.3 ± 1.8 and 13.5 ± 1.2 for the ST-E and ST-I cells, respectively. This loss of responsiveness to higher AM frequencies is not simply due to filter effects at the periphery or within the ELL. As will be shown in the next section, the high-frequency AMs mediate an inhibitory process which reduces the sensitivity of the stellate cells to other types of electrosensory stimuli. These estimates of best frequencies and cutoff frequencies were not corrected for the latencies of the neurons' responses, which are expected to alter these measures by less than 0.1 Hz.

Responses to moving electrolocation targets

Conducting or nonconducting (12-mm metal or plastic) cylinders were used as stimuli to determine the stellate neurons' ability to respond to moving targets, the laterality of electrosensory input to these cells, and the neurons' receptive field sizes.

Typical responses of ST-E and ST-I cells to targets moving parallel to the long axis of the fish at a lateral distance of 2.5 mm are summarized in Figure 4, *A–H* and *I–P*, respectively. The ST-E cells respond most strongly to a metal target moving along the contralateral side of the body, and the responses are not typically different for opposite movement directions except for small shifts in the position of the peak (Fig. 4, *A*, *B*). Similar shifts in the position of the peak of a response contingent on movement direction are also seen in ELL recordings, but not when recordings are made from single electroreceptor afferents (Bastian, 1986c). The ST-I cells responded best to plastic objects moved along the contralateral side of the body (Fig. 4, *M*, *N*) and these responses were also independent of target movement direction. Typically, targets moving along the ipsilateral side of the fish evoked very weak increases in firing (Fig. 4, *C*, *D*, *G*, *H*, *K*, *L*), less often ipsilateral stimuli caused no response at all (Fig. 4, *O*, *P*), and in a few cases ipsilateral targets caused responses nearly as large as those caused by the contralateral tar-

Figure 5. Responses of a stellate cell to electrolocation targets with and without simultaneous 32 Hz 2 mV/cm p-p sinusoidal EOD AMs. *A, B*, Responses to a metal target moving 2.5 mm lateral to the contralateral side of the fish. *C, D*, Responses to the metal target in the presence of continuous 32-Hz EOD AM. *E, F*, Responses of this same neuron to the plastic target moved contralateral to the NPd recorded from.

get. The stellate neurons probably receive their main excitatory input from the contralateral ELL, and the responses due to ipsilateral stimulation may be due to the small changes in the contralateral electroreceptors' activity evoked by stimuli moving near the opposite side of the body. ELL neurons, which only receive inputs from ipsilateral receptors, also respond weakly to targets moving along the contralateral side of the body even when the contralateral receptor input is eliminated by sectioning the anterior lateral line nerve ganglion (Bastian, 1986a). Targets other than those most effective for a particular cell type, plastic for ST-E cells and metal for ST-I cells, also often evoked responses when moved along the contralateral side of the fish. These responses were usually small, as shown in Figure 4, *E, F*, and *I, J*, but occasionally were nearly as large as a cell's response to its preferred target type. These responses were also typically displaced rostrally or caudally to the position of the preferred response, and in some cases 2 smaller peaks appeared bordering the position of the main response, as seen in Figure 5, *E, F*. These smaller responses to the nonpreferred stimulus type suggest the presence of inhibitory regions of each neuron's receptive field.

These neurons do not respond to high-frequency EOD AMs as shown in the preceding section. The idea that the loss of

Figure 6. Responses of an ST-E cell to the metal target moved at the indicated distances lateral to the fish. *A, B*, Responses to contralateral target movement in the headward and tailward directions; *C, D*, responses to ipsilateral movements in the headward and tailward directions. Proceeding from the outermost inward, contours below the outlines of the fish in *A* and *B* enclose areas within which at least 20, 40, 60, and 80% of the peak increase in firing frequency occurred.

Figure 7. Average responses, ± 1 SE, of stellate neurons to electrolocation targets moved at various distances lateral to the fish contralateral to the NPD recorded from. The metal target was used with ST-E cells (*A*), and the plastic target was used with ST-I cells (*B*). Solid lines connect average responses to tailward movements, and dashed lines connect average responses to headward movements. Best-fit lines based on pooled responses to tailward and headward moving objects between distances of 2.5 and 25 mm are $y = 115.3 - 27.3 \ln x$ and $y = 112.0 - 29.3 \ln x$ for ST-E and -I cells, respectively.

responsiveness is due to inhibition rather than solely a consequence of low-pass filtering somewhere in the antecedent circuitry is strengthened by the observation that the responses of these neurons to moving targets are reduced due to the presence of high-frequency AMs. Figure 5, *A, B*, shows the responses of an ST-E neuron to a 12-mm metal cylinder moving 2.5 mm lateral to the fish. The peak frequency evoked by this target, averaged for both movement directions, was 165 spikes/sec. Simultaneous stimulation with a 32-Hz AM reduced the cell's response to the moving target to 102 spikes/sec, or to about 60% of its normal value. In this case the AM stimulus also evoked a low rate of continuous activity of about 2.9 spikes/sec.

Responses to electrolocation targets moving parallel to the fish at a series of increasing lateral distances were also studied in order to determine the range at which and the areas within which stellate cells can detect moving targets. The family of response profiles of Figure 6, *A, B*, summarizes the responses of an ST-E neuron to tailward and headward target movements, respectively. Response amplitude decreases approximately linearly with the logarithm of target distance, and responses are clearly present out to a fish-target distance greater than 37.5 mm. Figure 6, *C, D*, shows the responses of this same neuron to the metal target moving along the side of the fish ipsilateral to the NPD recorded from. A low rate of spontaneous activity was present during this phase of the experiment and this activity was inhibited as the object passed certain regions of the fish. Precisely the same types of responses as shown in Figure 6 were recorded from ST-I neurons when nonconducting electrolocation targets were moved past the contralateral side of the fish.

The average peak responses of 21 ST-E and 12 ST-I neurons to their preferred electrolocation targets moving contralaterally at sequentially greater distances lateral to the fish are shown in Figure 7, *A, B*, respectively. Responses to the tailward target movement (solid lines), are not significantly different than those to headward movements (dashed lines). Best-fit lines were computed for the pooled headward and tailward responses for distances between 2.5 and 25 mm lateral to the fish. Responses at 2.5 mm averaged 81.8 and 78.5 spikes/sec for the ST-E and -I cells, respectively, and the slopes of these lines, -27.3 and -29.3 , indicate that these average responses will decay to 50% of their peak values at distances of approximately 15 and 12 mm lateral to the fish. The responses of 21 ELL output neurons, basilar pyramidal or E-cells, were analyzed in a similar fashion (Fig. 7*A*; Bastian and Bratton, 1990) and the average response of these neurons to a 12-mm metal target moving 2.5 mm lateral to the fish was 87.9 spikes/sec. The slope of the best-fit line to the ELL E-cell data, -30.8 , predicts that responses will decrease to 50% of their peak value when the object moves at a distance of about 10 mm lateral to the fish. Hence, these stellate neurons show a very similar electrolocation range as do the output neurons of the ELL, and both the stellate neurons and the ELL output cells show nearly twice the electrolocation range as compared to the NPD multipolar cells (Bastian and Bratton, 1990).

The areas lateral to the fish within which the 12-mm metal or plastic targets can evoke responses were estimated by computing iso-spike-rate contours for each data set such as shown below the outlines of the fish in Figure 6. Proceeding from the outermost, these contours show the areas within which the target caused increases in activity of 20, 40, 60, and 80% of the unit's

Figure 8. Anatomy and intracellularly recorded responses of an ST-E cell. *A*, Reconstruction of a Lucifer yellow fill; the neuron's axon (*ax*) can be seen leaving the NPd medially. *B*, *C*, Three superimposed records of this neuron's responses to 0.375 mV/cm rms increases and decreases in EOD amplitude, respectively. *D*, Cumulative frequency distribution of the neuron's responses to sinusoidal EOD AMs of frequencies ranging from 1 to 40 Hz.

peak response. The areas of the 50% contour, determined for both headward and tailward moving targets, were measured for 23 ST-E cells and 12 ST-I cells. No differences were seen for headward and tailward responses, so these data were pooled. The average areas of the 50% response contours were 255 ± 29.8 and 329 ± 82 mm² for the ST-E and ST-I cells, respectively, and these are not significantly different as judged by a *t* test. One of the ST-I cells studied had an extraordinarily large receptive field, nearly 4-fold greater than the mean area. Elimination of this measurement reduces the average area of the ST-I cells' 50% contour to 226 ± 44.7 mm².

A similar analysis was performed on the sample of 21 ELL E-cells. These are spontaneously active, and since responses to metal targets typically include both increases and decreases in firing frequency, contour areas for 50% of the peak increase and decrease relative to the cells' spontaneous firing frequency were determined separately. The average area of the 50% increase contour, 96.0 ± 8 mm² was significantly smaller than that of either type of stellate neuron ($p < 0.001$). The area of the 50% decrease contour averaged 247.2 ± 22 mm², and the total area of both the increase and decrease contour for the ELL neurons averaged 343.2 ± 24.4 mm². This was significantly larger than

the 50% increase contours for both types of stellate neuron ($p < 0.05$).

Intracellular responses of stellate neurons

Six ST-E and 6 ST-I cells were recorded from over periods long enough to determine their physiological characteristics and were sufficiently filled with Lucifer yellow to allow morphological identification. Reconstructions of filled ST-E and I cells are shown in Figures 8*A* and 9*A*, respectively. These neurons closely match the descriptions of stellate neurons given by Sas and Maler (1983). The somas are round or elliptical, their diameter or length of the major axis averaged 7.2 μm, and they typically gave off 5 major processes. In 1 of the 12 cases studied only 3 processes were seen, but this fill was weak, and therefore less conclusive. The soma size measured in this study is somewhat less than the 9 – 15 μm reported by Sas and Maler, but this may be due to tissue shrinkage in our preparations. As was described by Sas and Maler, we also noted some variability in the organization of these neurons' dendritic arbors. When the soma was located well within the body of the nucleus, the dendritic arbor was nearly circular. When the soma was near one of the borders of the nucleus, the dendritic field was very asymmetrical, being

Figure 9. Anatomy and intracellularly recorded responses of an ST-I cell. *A*, Reconstruction of a Lucifer yellow fill; *ax* indicates the neuron's axon. *B*, *C*, Three superimposed records of the cell's responses to 0.375 mV/cm increases and decreases in EOD amplitude, respectively. *D*, Cumulative frequency distribution of this neuron's responses to EOD AMs ranging from 1 to 40 Hz.

compressed against the border and extending into the body of the nucleus. The maximum dimension of the dendritic arbor seen in well-filled neurons was on the order of 200–250 μm , which is within the range of 180–270 μm reported earlier (Sas and Maler, 1983). In most cases the axon was visible, even when the dendritic arbor was not filled completely, and the axon always coursed medially, entering the tractus stratum fibrosum. The axon branched upon entering this tract and, in well-filled cases, could be traced bilaterally to the ELL ventral molecular layers. None of our fills was strong enough to allow reconstruction of the terminal fields of these neurons. It was clear, however, that the stellate neurons do not arborize through large areas of the ELL, since the axons could be traced for long distances within the ventral molecular layer with no evidence of branching.

When physiological responses were recorded prior to penetrating these neurons, their behavior was identical to that seen with extracellular recording techniques. The main difference between extracellular and intracellular recordings was that in the latter case the cells often exhibited a higher degree of spontaneous firing. This spontaneous activity could often be abol-

ished by hyperpolarizing the cell, and this technique was frequently used to prolong the time during which data could be gathered and to begin filling the cell. Figures 8 and 9, *B*, *C*, show 3 superimposed responses of these ST-E and ST-I cells, respectively, to contralateral increases (*B*) and decreases (*C*) in EOD amplitude. The contralateral EOD increase evoked an initial burst of spikes and a depolarization that lasted for the duration of the stimulus in the ST-E cell (Fig. 8*B*), and the contralateral decrease in EOD amplitude caused a similar response in the ST-I cell (Fig. 9*C*). The opposite or ineffective stimulus polarity sometimes evoked a distinct hyperpolarization, as shown in Figure 9*B*, but this was not always seen, as was the case for the ST-E cell of Figure 8. In some cases this hyperpolarization may have been reduced or masked by the negative current injection during the recording.

Spike frequencies measured over the 100-msec stimulus period averaged 82.2 ± 27.4 spikes/sec for the 6 ST-E cells studied and 64.7 ± 12.9 spikes/sec for the 6 ST-I cells. These average responses were lower than those measured with extracellular techniques, 127.8 ± 15.4 and 93.8 ± 13.1 spikes/sec for ST-E and -I cells, respectively, but these means are not significantly

Figure 10. Diagrammatic illustration of the major connections of the NPD with its afferent and efferent nuclei. DML, Dorsal molecular layer; EGP, posterior eminentia granularis; ELL, electrosensory lateral line lobe; LL, lateral lemniscus; NP, nucleus praeminentialis; PECB, praeminential cerebellar tract; REC. AFF., receptor afferents; TS, torus semicircularis; TSF, tractus stratum fibrosum; VML, ventral molecular layer.

different as judged by a *t* test. The hyperpolarization used to prolong the recording sessions may have also reduced the responsiveness of these cells. The intracellularly recorded responses of these neurons to sinusoidal AMs of the EOD were also very similar to those recorded extracellularly, as shown in Figures 8D and 9D. The AM frequencies at which the intracellularly recorded responses ceased averaged 10.77 ± 1.01 Hz for the pooled samples of ST-E and -I cells, which is not significantly different from the average cutoff frequency of 13.4 ± 1.05 Hz for the pooled ST-E and -I cell results from extracellular studies. Likewise, the best AM frequencies determined from extra- and intracellular experiments were similar, averaging 5.09 ± 0.97 and 4.16 ± 1.6 Hz, respectively.

Discussion

The NPD stellate neurons, which project to the ELL directly, differ from the NPD multipolar neurons described in the preceding report (Bastian and Bratton, 1990), with regard to most physiological properties measured. The stellate cells typically show no spontaneous activity but the multipolar cells are spontaneously active at high rates. The earlier study of the NPD of catfish (Tong, 1982) also noted that the neurons fell into 2 groups based on the presence or absence of high rates of spontaneous

activity. Both cell types respond to brief stepwise changes in EOD amplitude, but the latencies for the ST-E and ST-I cells are longer than those of the multipolar cells. These averaged 8.2 ± 0.76 and 13.8 ± 1.38 msec for the ST-E and -I cells, respectively, while the multipolar cells' latency averaged 5.7 ± 0.41 msec (both stellate cell means are significantly longer as judged by a *t* test, $p < 0.005$). The relatively long latency of the stellate cell responses raises the possibility that important inputs to these cells may come from a higher center, probably the torus semicircularis, but the close association of these neurons with the incoming lateral lemniscal fibers also suggests that these cells receive input from the ELL (Sas and Maler, 1983). The latency of the multipolar neurons is similar to that of the ELL output neurons (Bastian, 1981b) and these probably receive their input predominantly from the ELL.

The stellate cells occur in clusters (Sas and Maler, 1983) and we often encountered both ST-E and -I cells very close together along the same electrode track; often both types were recorded together at a single locus. The dendritic arborizations of these neurons must overlap; hence, the presence of neighboring stellate cells of opposite response type indicates a very high degree of synaptic specificity within the NPD.

The initial response of both the stellate and multipolar cells is a phasic burst which adapts, but the mean adaptation time

constant of the multipolar cells' initial burst, 4.5 ± 0.47 msec is much shorter than those of the ST-E and -I cells, 24.0 ± 4.2 and 17.2 ± 2.1 msec, respectively; the time constant differences are significant at the 0.1% level as judged by a *t* test. After the initial response, the multipolar cells adopt a new stable firing frequency in response to prolonged changes in EOD amplitude, but the stellate neurons usually adapt completely and become silent or fire only a few spikes/sec.

Both the stellate neurons and the multipolar cells respond to low-frequency amplitude modulations of the EOD, but while the latter are most sensitive to AM rates of about 64 Hz, the stellate cells are unresponsive to AMs of frequencies in excess of about 16 Hz. This loss of sensitivity probably represents an active inhibition of stellate responses since high-frequency AMs reduce a stellate neuron's responses to other excitatory stimuli such as moving electrolocation targets. The lack of spontaneity of stellate cells and the effect of continuous AMs on their responses to moving targets is somewhat similar to the responses of neurons found in the optic tectum of these fish. These also show no spontaneous activity and respond well to moving electrolocation targets. AMs of the EOD presented along with a moving target also reduce the responses of these tectal neurons (Bastian, 1982). Unlike the stellate neurons, however, these tectal cells are not driven by any frequency of AM that stimulates the entire fish, and the best frequency for reducing responses to moving targets is low, approximately 4 Hz. High-frequency AMs, e.g., 40 Hz, which reduce the responses of stellate neurons to moving targets have no effect on the object-evoked tectal responses.

The stellate neurons respond well to moving targets, as do the multipolar cells, and the receptive field area measurements, areas lateral to the fish within which 50% increases and decreases in activity occur, were not significantly different for these 2 cell types as judged by a *t* test. The combined areas of the excitatory and inhibitory regions of multipolar cells averaged 308 ± 34 mm² and the area of the stellate neurons' 50% increase contours averaged 245 ± 25 mm². The average area measured for stellate neurons only included excitatory regions since the cells had no spontaneous activity that would allow detection of inhibitory responses. That inhibitory regions of the receptive fields do exist is indicated by the occurrence of responses of either ST-E or -I cells to the nonpreferred stimulus types which usually occur bordering the preferred response region.

The electrolocation ranges, or distances lateral to the fish at which targets evoke significant responses, are far greater for the stellate cells. The absence of spontaneous activity in these neurons ensures that even weak responses caused by objects in excess of 37.5 mm lateral to the fish will be detectable. The high spontaneous activity of the multipolar cells, however, essentially masks any responses to targets moving at distances further than about 15 mm lateral to the fish. Also, the strongest responses of the multipolar cells, which averaged 53 spikes/sec and occurred with the objects moving close to the animal, are much smaller than the average responses of either the ELL output neurons or NPd stellate neurons, which gave average peak responses of 88 and 80 spikes/sec, respectively. These differences become even greater when peak responses are considered relative to the cells' background activity since the stellate cells have little or no spontaneous activity. The stellate cells seem to be specialized for detecting electrolocation targets in the environment, but the multipolar neurons are not. Rather, this latter category of NPd output neuron seems to be involved in mea-

suring the steady-state amplitude of the animal's electric organ discharge, as described in the preceding paper (Bastian and Bratton, 1990).

Figure 10 summarizes the anatomical relationships between the NPd output neurons described in this and the preceding report (Bastian and Bratton, 1990), the ELL, and the torus semicircularis. The multipolar neurons that project to the EGP via the praeminential cerebellar tract (PECB) encode EOD amplitude in a tonic manner and with high resolution. These neurons may provide the signal needed to optimize ELL sensitivity or gain in response to changes in the state of the EOD. The ability of ELL output neurons to produce responses of approximately constant magnitude, even when the EOD amplitude changes by significant amounts, requires that the NPd-to-EGP connection be intact (Bastian, 1986a, b). The multipolar cells could ultimately alter the sensitivity of the ELL pyramidal cells by modulating the activity of the EGP granule cells to which they project. Increases in EGP granule cell activity could increase ELL pyramidal cell excitability if the granule cell axons' (ELL dorsal molecular layer parallel fibers) primary target was the apical dendritic trees of the ELL output neurons. The opposite effect is also possible; increased granule cell activity could depress the ELL output neurons' excitability if the primary target of these ELL parallel fibers was the apical dendritic trees of ELL inhibitory interneurons.

The electron microscopic data of Maler et al. (1981) shows that the dorsal molecular layer parallel fibers make synaptic contacts with both interneurons and ELL output cells, and these are excitatory (Mathieson and Maler, 1988). The physiological evidence, however, indicates that the dominant effect of activity in this circuit results in reduced excitability of ELL output neurons since interruption of the PECB connection or lesions of the EGP itself increase ELL output neuron excitability as if a tonic inhibition were removed (Bastian, 1986a, b). Both excitatory and inhibitory effects of PECB tract activity probably occur, but a predominant inhibitory effect will reduce the probability of dynamic instability associated with a purely positive feedback network. The inhibitory mechanism is probably GABAergic since microiontophoresis of the GABA antagonist bicuculline mimics many of the effects of the EGP lesions (Shumway and Maler, 1989). The multipolar cell projection to the EGP is also likely to be quite divergent, as indicated in Figure 10. Single multipolar axons have been recorded from within the EGP and filled with Lucifer yellow (see Fig. 11, Bastian and Bratton, 1990), and these axons ramify over the entire mediolateral extent of the EGP and over more than 300 μ m in the rostrocaudal direction. The extensive branching within the EGP suggests that large numbers of granule cells are contacted and the parallel fiber output of these cells is expected to contact large numbers of ELL neurons (Maler, 1979). The effects of changes in multipolar cell activity may, therefore, result in changes in the sensitivity of ELL circuitry processing inputs from large regions of the body.

Less detailed information is available concerning the projection pattern of individual stellate neurons within the ELL ventral molecular layer (VML). We have been able to follow the axons of filled stellate cells for long distances within the VML, and the extensive branching characteristic of the multipolar projection was not apparent. Rather, it seems as if the stellate neurons ultimately affect a much smaller region of ELL circuitry. This same conclusion was reached on the basis of extracellular HRP injections into the NPd which label small regions of the

ELL ventral molecular layer closely associated with retrogradely labeled ELL efferents (Maler et al., 1982). The synaptic targets of the stellate cells also include ELL interneurons and output neurons (Maler et al., 1981). The stellate neurons completely adapt to long-term changes in EOD amplitude and cannot, therefore, contribute to prolonged changes in ELL output neurons' excitability. The vigorous responses of these cells to moving targets and low-frequency AMs, along with the limited divergence of their axons within the ELL, suggest that these neurons may contribute to more spatially and temporally restricted changes in ELL physiology.

Perhaps these neurons are involved in heightening the relative responses of small sets of ELL output neurons to relevant stimuli such as prey or other electrolocation targets in the immediate vicinity of the fish. This would be accomplished by enhancing the sensitivity of a small group of ELL output neurons currently processing important information, or by suppressing the sensitivity of ELL circuitry surrounding these. This role proposed for the stellate neurons, although far simpler, is somewhat analogous to the "searchlight" mechanisms discussed by Crick (1984). Such a mechanism should depend on inputs from higher centers which ensure that the stellate cells are somewhat selective and respond to "relevant" stimuli, or at least render them insensitive to frequently encountered "uninteresting" stimuli. The long latency of stellate cell responses relative to other neurons within the same nucleus suggests that some excitatory inputs originate in a higher center, perhaps in the torus semicircularis. The torus sends a massive projection to the NPD (Sas and Maler, 1983), but the synaptic targets of these toral fibers are unknown.

The insensitivity of the stellate neurons to EOD amplitude modulations of frequencies greater than about 16 Hz is very interesting and may provide a clue as to the function of these neurons. The two principal sources of EOD amplitude modulations are moving electrolocation targets and the EODs of neighboring electric fish. The temporal characteristics of the EOD AMs caused by objects in the fish's environment are dependent on the target's size and shape as well as its distance from and velocity relative to the fish. The spectral characteristics of EOD distortions produced by objects large enough to be above threshold for the receptor system are dominated by low frequencies. Objects having diameters ranging from 5 to 50 mm produce distortions having bandwidths ranging from about 0.6 to 2.5 Hz given a relative velocity of 5 cm/sec, and bandwidth increases linearly with velocity (Bastian, 1981a, 1986c). The addition of the EODs of 2 fish in close proximity results in continuous amplitude and phase modulations of the animals' discharges, and the frequency of these modulations, or beat frequency, will be equal to the difference in the discharge frequencies of the animals involved. When this beat frequency is low, 1–10 Hz, the EOD modulations significantly degrade the animals' ability to electrolocate (Heiligenberg, 1973, 1977; Bastian, 1987a). Within this range of beat frequencies the AM due to an electrolocation target is similar to that of the beat pattern, and the system's ability to identify the target is degraded. As the beat frequency rises, as occurs when the animals actively alter their individual frequencies during the jamming avoidance response (Watanabe and Takeda, 1963; Bullock et al., 1972; Heiligenberg, 1986), the higher-frequency AMs due to the beats become less disruptive and electrolocation improves. The EOD AMs due to the target and those of the beat are now presumably sufficiently different to allow the system to distinguish one from the other.

Electrolocation ability improves in the presence of increased beat frequency despite the fact that receptor afferents and many ELL output neurons respond more strongly to higher frequencies of AM (Behrend, 1977; Bastian, 1981b, 1987b). Hence, electrolocation ability improves while the apparent signal-to-noise ratio of the target's representation decreases at these lower levels within the electrosensory system. Some sort of low-pass filtering has been suggested as a mechanism that could facilitate the detection of the low-frequency AMs associated with an electrolocation target in the presence of the higher-frequency AMs of the beat pattern (Heiligenberg, 1977). Recordings in the cerebellum of the closely related fish *Apteronotus albifrons* revealed neurons sensitive to EOD AMs which had an appropriate low-pass property (Behrend, 1977).

The stellate neurons also show a very striking low-pass characteristic. If their role is to selectively enhance the sensitivity of small populations of ELL output neurons, as suggested earlier, then this ability will be obscured when continuous low-frequency AMs are present. Since the stellate neurons respond vigorously to AMs below about 16 Hz, continuous low-frequency AMs will affect all of the ELL circuitry simultaneously; hence, any "searchlight" would be tremendously blurred. As the beat frequency increases during the jamming avoidance response, the stellate cells become unresponsive to the continuous AMs but they still respond to the frequency modulation caused by an electrolocation target, as shown in Figure 5. Electrolocation performance should, therefore, improve. The responses to targets under these conditions are significantly attenuated, so completely normal performance may not be attained. The results of behavioral experiments show that electrolocation behavior does improve for *Apteronotus leptorhynchus* as the frequency of the continuous AM increases, but performance does not return to normal even when continuous AMs as high as 128 Hz are used (Bastian, 1987a). Similar behavioral experiments with the related fish *E. virens* did show complete recovery of electrolocation performance when jamming signals producing high modulation rates were used (Heiligenberg, 1973).

The detailed knowledge of the neuroanatomical relationships of the electrosensory lateral line lobe and the sources of descending input to this structure greatly facilitate the formation of specific hypotheses concerning the roles of these feedback connections in the electrosensory system. The large differences in the physiological characteristics of NPD output neurons projecting to the ELL via the 2 separate pathways suggest that multiple aspects of the ELL's performance may be plastic and under the control of these descending systems.

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