The Effects of Postembryonic Receptor Cell Addition on the Response Properties of Electroreceptive Afferents

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The weakly electric fish *Sternopygus* detects electric fields with a receptor organ called a tuberous electroreceptor. Previous studies have shown that an electroreceptive afferent fiber innervates a single organ in small fish and that as fish grow some of these organs divide, giving rise to daughter organs; these divide in turn to produce a cluster of organs. All of the organs in a cluster are innervated by the original afferent, which sprouts new terminals to accommodate them. Other organs, however, seldom divide. Thus, the distribution of the number of tuberous organs per afferent becomes increasingly bimodal with fish body length (Zakon, 1984a).

In order to investigate the effect of organ addition on neural coding within the afferent fiber, activity was recorded from single units within the anterior branch of the anterior lateral line nerve in fish of a range of sizes. It was found that, as fish increase in body length, the best frequency, sharpness of tuning, and sensitivity increase in a subset of afferents, while others remain essentially unchanged. This results in an increasingly bimodal distribution of these physiological measures with increasing fish body length. These results suggest that the afferents that innervate multiple receptor organs are more sensitive and possess higher best frequencies than those that innervate 1 or 2 organs. This was confirmed by dye injections of electroreceptive fibers with Lucifer yellow. Stimulation of afferents with stimuli of the intensity produced during the normal discharge of the electric organ showed that the sensitive afferents that innervate multiple organs are T receptors (phase-coders) and those that innervate a single organ are P receptors (probability coders) (Bullock and Chichibu, 1965). Our results demonstrate for the first time a morphological difference between these 2 unit types.

The postembryonic addition of receptor cells has been demonstrated in the sensory systems of many anamniotes and seems to be correlated with indeterminate growth. Sensory cell addition occurs in the amphibian lateral line (Lannoo, 1985), the retina of amphibia (Straznicky and Gaze, 1971) and fish (Johns

and Easter, 1977), the inner ear of amphibia (Shofner and Feng, 1984; Corwin, 1985), skates, sharks (Corwin, 1981, 1983), and teleost fishes (Popper and Hoxter, 1984), and the electrosensory system of weakly electric fish (Zakon, 1984a, 1987). In the hair cell and electrosensory systems, receptor cells are added but afferents are not. This raises an interesting question: How does the increase in the number of receptor cells contacting an afferent fiber influence its responses to sensory stimulation?

The electrosensory system provides an excellent model in which to study cell addition, for several reasons. First, it is relatively simple, allowing easy access to the receptor cells and their afferent fibers both histologically and electrophysiologically. Second, the exact stimulus to the receptors can be accurately controlled and monitored, a task which is much more difficult in other sensory systems.

The weakly electric fish *Sternopygus* creates an electric field around its body by means of an electric organ located in its tail. This electric field creates a voltage drop across the skin which is sensed by specialized receptors in the skin termed tuberous electroreceptors. Groups of receptor cells are organized in single clusters within epithelial capsules in the dermis. A canal which penetrates the overlying epidermis allows current to enter the lumen of each capsule and stimulate the receptor cells. A capsule and its complement of receptor cells is called a *tuberous organ*. An afferent fiber from the anterior lateral line nerve (ALLN) innervates each organ and, in the epithelial base of the capsule, branches to contact each receptor cell. More than one organ may be innervated by the same fiber; the afferent and all of the organs that it innervates are referred to as a *tuberous unit* (Zakon, 1984a).

As fish grow, some tuberous organs divide, and their afferents branch to innervate the new daughter organs. In this way, some afferents may come to innervate 10 or more organs in a large (>30 cm) fish. However, other organs do not divide, or do so at a very low rate. Thus, with growth there is an increasingly bimodal distribution in the number of organs per unit: some afferents still innervate only 1 or 2 organs, whereas others can contact many more, depending upon fish size (Fig. 1) (Zakon, 1984a).

Previous studies have suggested that there may be 2 distinct response types within the tuberous unit population in adult fish: P- and T-coding units (Bullock and Chichibu, 1965; Scheich et al., 1973; Hopkins, 1976; Bastian and Heiligenberg, 1980). The P-units, or probability coders, have high thresholds and broad tuning curves. When stimulated at natural stimulus intensities, they have long latencies, cannot be driven on every cycle, and phase-lock poorly. These units encode stimulus intensity as a function of probability of firing. The T-units, on the other hand, have low thresholds, sharp tuning curves, and short latencies;

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Figure 1. Schematic diagram of the change in the distribution of the number of tuberous organs per axon as fish grow (after Zakon, 1984a). Whereas small fish only have one organ per afferent fiber, large fish show a bimodal distribution due to the selective addition of organs to one group of afferents.

they phase-lock well with the stimulus and fire a spike on every cycle. These units encode the timing of the positive zero-crossings of each stimulus cycle. However, results from a study by Viancour (1979a) contest the existence of 2 distinct response types and suggest instead a gradient of response characteristics.

The purpose of this work is to reexamine whether there are 2 tuberous unit response types, the P- and T-coders, in this species and, if so, whether they correspond to the 2 morphological types of tuberous unit previously described (single or multiple organs) (Zakon, 1984a). We also address the extent to which increases in the number of organs innervated by a single afferent may influence its response properties.

Materials and Methods

Approximately 50 *Sternopygus macrurus*, ranging in size from 12 to 40 cm in length, were obtained through field collections in Venezuela (Estado Apure). The fish were kept in the lab in aquaria at $25 \pm 2^\circ\text{C}$, at a water conductivity of 1–2 kOhm-cm, and a 12/12 hr light/dark illumination cycle. The fish were fed frozen blood worms daily, and the larger fish were supplemented with live *Gambusia* or earthworms.

Single-unit recording and analysis. Afferent stimulation and recordings were made by standard procedures (Zakon, 1986). Briefly, a fish was placed in the recording tank and allowed to equilibrate for at least 10 min at $25.0 \pm 0.2^\circ\text{C}$. Electric organ discharge (EOD) frequency was measured, the fish was immobilized and electrically silenced with curare, and it was placed in a cushioned clamp at the water's surface with a tube in its mouth to flow aerated water over its gills.

Prior to exposing the nerve trunk for single unit recording, a 5% procaine solution was applied to the skin. The infraorbital component of the anterior branch of the anterior lateral line nerve (aALLN), located under the skin and just posterior to the eye, was exposed. This branch receives electrosensory input from the cheek and snout (Zakon, 1986).

Standard techniques were used to record afferent activity. For each unit we determined threshold and best frequency (BF). We used a 1:1 criterion for threshold, that is, one spike per stimulus cycle (Hopkins, 1976). We then increased the stimulus intensity 10 dB above threshold to determine the sharpness of tuning by calculating $Q_{10\text{dB}}$ (which is determined by dividing a unit's BF by the bandwidth of its tuning curve at 10 dB above threshold).

In addition, for some units, period histograms were taken using a window discriminator and a period histogram program on a DEC PDP "micro" 11 computer. The binwidth was 3.6° of the complete cycle (i.e., 1/100 of the cycle) and each histogram was a compilation of spikes from 400 successive cycles. In this manner, histograms of different units, with different BFs, could be compared since each bin represented the same portion of the stimulus cycle.

Records were also kept by the computer of the number of spikes that occurred during 400 consecutive stimulus cycles. From these records it was possible to calculate an average probability of firing for each unit and to generate probability-intensity curves.

Stimulus generation and measurement. The fish was presented with a sine wave stimulus from a function generator in series with 2 attenuators and an isolation transformer to 2 carbon rods in the recording

tank (Zakon, 1986). The stimulus rods were positioned underwater along the sides of the tank and parallel to the fish's long axis. This produced a so-called "transverse electric field" as current lines from the electrode ran in parallel in one side of the fish's body and out the other.

A problem with many previous studies is the lack of accurate determination of unit thresholds due to the lack of good control of electrical stimulation. The fish's own EOD is produced as a radial field with current lines emanating in all directions from the body surface (Heiligenberg, 1975; Knudsen, 1975). Thus, when the fish discharges, receptor cells on all parts of the body surface will be equally stimulated. Many studies have utilized externally generated electric fields that do not produce an even voltage drop across the skin. If units with receptive fields all over the body are recorded without knowledge of the local voltage drop across the skin, values of threshold can be misleading. Since a major difference between P- and T-receptors is in threshold, and differences in apparent threshold can be artifactually produced by electric field geometry, this is an important consideration. In our study, we eliminated this problem by recording only from receptor units in the cheek region, where they are densely packed and therefore sense the same voltage.

We measured the actual voltage drop across the skin rather than the electric field in the water. This was done with electrodes made from surgical needles soldered onto shielded wires. One electrode was placed intramuscularly in the back of the fish, which was held out of the water such that the bare metal was not directly in contact with water. The other electrode, which was insulated except for its tip, was held touching the fish's cheek. This gives an accurate measure of the voltage drop across the skin since most of the IR drop is across skin rather than the interior of the fish (Scheich and Bullock, 1974; and personal observations). The signal was amplified and measured on an oscilloscope. The peak-to-peak voltage of the stimulus across the cheek skin was calibrated to 10 mV at -15 dB by adjusting the Wavetek output.

Intracellular injection. Medium-sized fish (approximately 25 cm long) were used for the injections. The nerve was exposed, and recordings made in the manner previously described. Pipettes were back-filled with 3% Lucifer yellow in double-distilled water, and the remainder of the pipette was filled with a 0.1 M LiCl solution. Their resistances were measured at approximately 100 M Ω .

Many units were sampled from a fish to determine the range in thresholds before making any injections. Units with either high or low thresholds (less than -60 dB and greater than -40 dB, respectively) were chosen for injection in a given fish. Injections were performed iontophoretically with 1 Hz hyperpolarizing current pulses of approximately 20 nA (modified from Sento and Furukawa, 1987, and Lewis et al., 1980). Cell spiking was checked every 5 min, and the injection was continued for 20–30 min, or until the cell no longer showed spike responses.

Five to ten units were filled in each individual. Two or more hours were allowed for diffusion of the dye. Cheek skin was removed and fixed in formalin. The experimental cheek was fixed for over 12 hr in 10% unbuffered formalin, dehydrated, cleared in methyl salicylate, whole-mounted, and viewed using epifluorescence on a Zeiss microscope with the appropriate filter. The distribution of receptor organs per unit was measured in the Lucifer yellow-injected piece of skin by counting the number of receptor pores in a cluster (Zakon, 1984a).

Results

Tuning properties

The EOD frequencies of *Sternopygus* vary from 50 to 200 Hz, with mature males discharging below and mature females discharging above 100 Hz (Hopkins, 1974). Figure 2 shows histograms of BF distribution in large (>30 cm) and small (<20 cm) fish. The BFs of individual fish show a range of approximately 50 Hz. However, regardless of EOD frequency, the mean BFs of small fish (top row) are distributed below the EOD frequency, whereas those of large fish (bottom row) are above their EOD frequency. Note that, especially in the large fish, there is a bimodal distribution of BFs.

The relationship between relative BF and body length is shown in Figure 3, where average BFs for 12 fish were normalized with respect to each fish's EOD frequency and plotted against head-

Figure 2. Best frequency histograms. Triangles along the abscissa show the frequency of the EODs, asterisks give the mean BF. The upper pair represent 2 small fish and the lower pair, 2 large fish. Histograms on the left show fish with high-frequency EODs and those on the right, low-frequency EODs. Note differences in the distribution of BF's relative to the EOD between the large and the small individuals.

Figure 3. Mean BF as a function of body length. Each point on this plot represents one fish. Best frequencies for the units in each fish were first averaged and then normalized with respect to the EOD frequency. The dotted line indicates a perfect match between mean BF and EOD frequency. The solid line is the regression line ($r = 0.693$).

to-tail length. The horizontal dotted line represents an equivalence between BF and EOD frequency. The graph shows that the mean BF's of fish less than 25 cm are, on the average, tuned below the EOD frequency, whereas those greater than 25 cm are tuned above the EOD frequency. The relationship plotted in Figure 3 is independent of absolute EOD frequency as indicated from Figure 2. Thus, there is a growth-dependent increase in BF relative to EOD frequency.

Another response characteristic, Q_{10dB} , is a measure of the sharpness of tuning; a high Q_{10dB} value denotes a sharply tuned unit. As in a previous study (Zakon and Meyer, 1983), Q_{10dB} values of units tuned to frequencies below the EOD frequency were usually below 2.0, whereas those tuned to BF's above tended to be higher. Higher Q_{10dB} 's were common in large individuals since relatively more of their units were tuned above EOD frequency.

Threshold and probability of firing

In order to determine the sensitivity of tuberosus units, the average probability of firing was calculated at various stimulus intensities for each of several units in randomly chosen individuals. Figure 4 shows curves for a large fish. It can be seen from these curves that each unit possesses a dynamic range of half of a log unit but that thresholds range over almost 3 log units.

In addition, many of the sensitive units (the curves towards the left on this plot) show less steep slopes at the very weak stimulus intensities. This was due to spontaneous activity of these cells. These units never reached zero probability of firing, although many of them came close. The unit represented by the curve on the far left in Figure 4, in fact, never showed a probability of firing of less than 0.65, even when the stimulus was turned off. This unit had a very high rate of spontaneous firing. It was also the most sensitive unit of those observed in that individual. Note that the most sensitive units show a change in probability of firing at a stimulus intensity of 10 μ V or less. These units are often spontaneously active and fire at a frequency that is at or close to its BF, suggesting that their receptor cells oscillate.

The probability curves for all units in a given fish have similar shapes regardless of the absolute sensitivity of each unit. There-

fore, any criterion for threshold can be used to make comparisons (actually, since the slopes of the low-threshold units are less steep than the high-threshold units, we slightly underestimate the sensitivity of the low-threshold afferents by using a 1:1 criterion).

Due to the care we took in stimulating the receptors, it is clear that the differences of threshold recorded in a given fish are real and not the result of different transepidermal voltage drops across the body as occurs when units are recorded from all over the body with a transverse field. Additional proof of this is that units with very different thresholds may be localized to the same small area of skin with a small dipole electrode (Zakon, unpublished observations).

Figure 5 shows threshold distribution histograms in 2 large and 2 small fish. The small individuals have a narrower range of thresholds and the maximum sensitivity is less than in large fish. The histograms generally show a bimodal distribution in threshold values, and the separation between peaks increases with fish size.

Figure 4. Probability of firing in tuberosus afferents as a function of stimulus intensity. These curves are from a large fish. Note the wide range of intensities which can be represented by these units (nearly 3 log units).

Figure 5. Threshold histograms. Each histogram represents threshold values collected from one fish. Threshold is plotted in dB units (0 dB = 60 mV across the skin). Top row, data from small fish; bottom row, from large fish. The body length and EOD frequency for each fish are indicated at the top of each plot.

In order to quantify the apparent differences in sensitivity between fish of various sizes, thresholds were averaged for the most sensitive and least sensitive units in each fish. This was done by ranking the units according to threshold and then averaging the top 25% and the bottom 25%. These averages are plotted against fish length for 17 individuals in Figure 6. The open circles denote the threshold average for the least sensitive units and the filled circles represent the most sensitive units of each fish. The linear regressions for the 2 sets of data show that the high-threshold units appear to be increasing only very slightly in sensitivity as the fish increase in size. In contrast, however, the low-threshold units increase in sensitivity, on average, by an order of magnitude. In fish of this size range, the maximum number of organs per afferent increases from 1 to about 12 (Zakon, 1984a, b).

Because average BFs and unit sensitivities both increase with fish length, one might ask if these parameters are correlated. Figure 7 shows size-dependent changes in plots of threshold versus BF. Small fish show no significant relationship between threshold and BF; however, large fish demonstrate strong negative correlations ($p < 0.05$) which are independent of EOD frequency. Also note that there are 2 distinct "clusters," or subpopulations, in the data points from the large fish, in contrast to the small fish, for which the data points lie on a continuum. Together with the previous data (Fig. 6), these plots suggest that 2 subpopulations of tuberous units develop as fish grow: one population includes low-threshold units with relatively high BFs, and the other includes sensitive units with relatively lower BFs.

Figure 8 shows period histograms from units of the 2 subpopulations in the same fish. Each histogram was taken while the unit was being stimulated at 66 Hz (the fish's EOD frequency) and at an intensity of 0.25 mV across the cheek (a normal EOD intensity). The top row represents the high-threshold units, and the bottom row the low-threshold units. These histograms show that the low-threshold units fire 1:1 and are highly phase-locked, while the less sensitive units respond less reliably and with more jitter. These 2 populations correspond to the P- and T-units as defined in this species by Bullock and Chichibu (1965), Scheich et al. (1973), Hopkins (1976), and Bastian and Heiligenberg (1980).

Figure 6. Range of thresholds as a function of body size ($N = 17$). The top 25% (high-threshold units; open circles) and the bottom 25% (low-threshold units; closed circles) were averaged and then plotted for each individual as a function of body length. The regression lines for each set show that, as a fish grows from approximately 10 to 40 cm in length, the thresholds of the high-threshold units do not change significantly, while those of the low-threshold units decrease even further ($r = -0.203$ for less sensitive units; $r = -0.765$ for highly sensitive units).

Intracellular labeling

We were struck by the correlations between growth-related changes in the physiology and morphology of the tuberous units. The gradual increase in sensitivity with growth observed in the low-threshold units corresponds well with the gradual accumulation of tuberous organs of some units. Similarly, the lack of shift in sensitivity in the least sensitive organs corresponds with the lack of addition of organs in the other subgroup. These results suggest that those organs that have added organs the most rapidly are the most sensitive units and that those that have added very slowly or not at all correspond to the least sensitive units. In order to investigate this relationship more rigorously, individual afferent fibers were filled with dye.

A total of 9 dye-filled units were recovered from 7 fish. The afferent illustrated in Figure 9A innervated only a single organ, had a high threshold (i.e., -40 dB or more), and was from a 28.5 cm fish. The afferent illustrated in Figure 9B that innervated 5 organs, had a low threshold (i.e., threshold of -60 dB or less), and was from a 24.5 cm fish. This result is shown more quantitatively in Figures 10 and 11. These histograms give the number of organs per cluster in a piece of skin containing 1 or 2 labeled fibers. The asterisks, placed above the appropriate bin, each denote a filled cell, and they are according to the number of organs innervated by the filled afferent. Figure 10 represents low-threshold units that were labeled in 3 individuals. Note that only units with 4 or more organs were filled when highly sensitive units were targeted for dye injection, although these fish had units with 1–6 organs. Figure 11, on the other hand, shows that high-threshold units correspond to afferents innervating only 1 or 2 organs. Thus, these histograms clearly show a correspondence between unit threshold and the number of organs per axon.

Discussion

By recording large numbers of afferents from many individuals, we have found that in the weakly electric fish *Sternopygus*, re-

Figure 7. Threshold versus BF. The BF is plotted against threshold value for approximately 30 units in each of 4 different individuals.

sponse properties of electroreceptive afferents undergo several gradual changes with growth: their mean BFs increase relative to the EOD frequency, tuning sharpness increases, and the low-threshold units become even more sensitive. This gradual change of physiological properties with growth also correlates with the

age-related addition of receptor organs that occurs in this species (Zakon, 1984a). This suggests that the low-threshold afferents with sharp tuning curves and BFs higher than EOD frequency innervate multiple end organs, while high-threshold units with broader tuning curves and lower BFs innervate single or paired

Figure 8. Period histograms. These histograms give the timing of firing of each spike throughout the stimulus cycle (on the abscissa), using a stimulus of EOD frequency and natural stimulus intensity. Each histogram is the response of one unit over 400 consecutive cycles. Upper pair of histograms are from less sensitive units, which behave as P-coders. Lower pair are from sensitive units, which behave as T-coders. Phase is arbitrary. Although the T-units appear to be phase-delayed with respect to the P-units in these plots, they are actually phase-advanced by about 90°.

Figure 9. Sketches of 2 Lucifer yellow-filled afferent terminals. The dye did not completely fill the terminal branches leading from the axon to the base of each organ. The end organs are depicted by the elliptical profiles, and their pores are indicated by dashed lines. A, High-threshold unit; B, low-threshold unit.

end organs. We have confirmed this with dye-injection of afferent fibers. In addition, our results support the previous classification of tuberous receptor afferents into two distinct classes: T- and P-type. However, we emphasize that the differences between these 2 unit types are difficult to discern in small fish and become accentuated with age.

Figure 10. Distribution of the number of organs per cluster in skin containing dye-filled afferents. Each histogram is from a different fish. Only low-threshold units (thresholds greater than -60 dB) were injected with Lucifer yellow. The asterisks indicate the number of organs innervated by each dye-filled afferent.

Figure 11. Same as Figure 10 except that less-sensitive units (thresholds greater than -40 dB) were injected with Lucifer yellow in these 3 fish.

Effect of growth on tuning and sensitivity

Afferent tuning curves may be influenced by the frequency response of the receptor cell membrane, synaptic mechanisms, or at the afferent fiber's postsynaptic membrane. For example, the refractory period of the postsynaptic membrane may act as a low-pass filter. If the afferent fiber were unable, due to refractoriness, to follow each burst of transmitter release with a spike, the high-frequency slope of that unit's tuning curve would be influenced (i.e., the highest frequencies would be attenuated). By the same logic, afferent tuning curves may also be affected by processes occurring at the receptor-afferent synapse. However, these mechanisms are unlikely to play a role in the tuning of the electroreceptive units of *Sternopygus*. The absolute refractory period in these units, determined by driving them at high frequency and intensity, is approximately 2 msec or less (Zakon, 1986). This indicates that afferents ought to be able to fire 1:1 to stimuli at least as high as 500 Hz, which is higher than the frequency range of the tuberous receptors in this species.

In *Sternopygus*, the BFs of regenerating tuberous units start out low and increase to match the EOD frequency (Yialamas and Zakon, 1984; Zakon, 1986). The tuning of electroreceptors may be monitored by recording impulse-induced receptor oscillations (Bennett, 1971; Meyer and Zakon, 1982). Receptor oscillations during receptor regeneration also start out low and gradually rise to match the EOD frequency. Since receptor cell oscillations correlate with unit tuning, this result suggests that it is the receptor cell properties that most strongly influence the tuning recorded from the electroreceptive afferents of *Sternopygus* (Zakon, 1986).

Electroreceptor cells show electrical resonance of their receptor potentials (Viancour, 1979b; Meyer and Zakon, 1982; Zakon, 1984b). The resonant frequency closely matches the BF of the afferent tuning curve and is presumed responsible for the frequency tuning of the afferents. A similar resonance has been

demonstrated in hair cells in turtle and frog, where they have been shown to be due to the interaction between an inward calcium current and an outward calcium-dependent potassium current (Crawford and Fettiplace, 1981; Lewis and Hudspeth, 1983; Art et al., 1986). Thus, we suggest that differences in electroreceptor tuning could correspond to differences in the properties of ion currents in the receptor cells and that these ion currents change as fish grow. We suspect that the age-related shift in receptor tuning depends on changes in the membrane of the receptor cell, and, although it is correlated with increases in the number of organs per axon, it is not directly dependent on it.

It is likely that the increases in sensitivity are, however, directly due to the addition of organs. One way to enhance the sensitivity of an afferent is by bringing together several axon terminals, each capable of spiking, onto a common axon. In the electrosensory system, we presume that a spike-initiation site exists at the base of each organ as in the amphibian lateral line system (Murray and Capranica, 1973; Pabst, 1977). Using a potassium ferrocyanide stain, which has been shown to label spike-initiating membrane in other systems (Quick et al., 1980), Saidel (personal communication) has shown that the whole post-synaptic axon terminal at the base of each organ is stained. Thus, assuming that the whole terminal is effectively isopotential and inputs from the boutons average passively, a spike will occur in the axon terminal at the base of an organ. In a unit composed of more than one organ, every organ would be capable of generating an action potential. Therefore, increasing the number of organs in any unit would serve to increase the sensitivity of the unit by increasing the probability that a spike will occur in the axon at a given stimulus intensity.

Are there distinct P- and T-units?

Our data support the findings of Bullock and Chichibu (1965), Scheich et al. (1973), Hopkins (1976), and Bastian and Heiligenberg (1980) in that we are also able to classify tuberous units into 2 classes. Our results, however, appear to contrast with those of Viancour (1979a) in *Eigenmannia*, for which he proposed, rather than 2 subcategories of response, a gradient of response types without a bimodal distribution. This discrepancy can at least partially be attributed to the fact that thresholds, BF's, and Q_{10dB} 's are all size-dependent characters and that Viancour pooled data from fish ranging from 8 to 16 cm in length. Receptor organs are also added in *Eigenmannia*, and there is an increasingly bimodal distribution of the number of organs per axon in larger fish, although it appears to be less pronounced than in *Sternopygus* (Zakon, 1987). In addition, Viancour had a small number of units from each fish. Pooling of data over a range of body lengths with such a small sample from each fish would have the effect of blurring the distinction between response classes. The experiments performed in our study included between 30 and 40 units for each fish, and we did not pool the data across fish.

There are 2 additional reasons for proposing that P- and T-units are distinct. First, these physiological differences correlate with 2 morphologically recognizable subtypes of receptor unit. One type adds receptor organs as fish grow and possesses thin spindly terminal branches. The other type does not add receptor organs, or does so only at a low rate, and has thick and occasionally varicose terminal branches (Zakon, 1984a). We must emphasize, however, that while the greater number of organs per axon may explain differences in threshold between these 2 receptor

types, other reported differences as those in BF, adaptation rates and Q_{10dB} (Hopkins, 1976), must be explained by additional processes. The central connections of P- and T-units are also distinct within the electric lateral line lobe (ELLL) (Maler, 1979). In a recent study, physiologically identified P-units and T-units were filled with intracellular injection of HRP and their terminals in the ELLL were examined using electron microscopy (Mathieson et al., 1987). This study revealed that P- and T-units terminate on different neuron types.

The distinction between P- and T-connections in the CNS brings up an interesting developmental question. Are P- and T-units distinct in the periphery early in development when all tuberous afferents contact only a single organ (Kirschbaum and Denizot, 1975; Zakon, 1984a; Vischer, 1989) and before they make contact with the appropriate cells in the brain or are the receptor units identical until they make their central contacts? In other words, are the factors that differentiate T- and P-receptor types intrinsic to the receptor organ and/or afferent or their precursors or are they instructed by the postsynaptic target cells?

A similar distinction between classes of peripheral afferents occurs in the auditory system of goldfish, where saccular afferents are classified into S1 and S2 types. Physiologically, S1 fibers have higher thresholds to sound than S2 afferents and are not spontaneously active, while S2 fibers are. Morphologically, S1 fibers have relatively larger diameter terminal axons and innervate fewer hair cells than S2 fibers (Sento and Furukawa, 1987). Kyogoku et al. (1986) have shown that S2 fibers have greater amplitude generator potentials than S1 fibers. They attribute these increased potentials of S2 afferents to their greater input resistances (smaller fiber diameters). In addition, they suggest that the summation of a greater number of postsynaptic potentials may also contribute to larger generator potentials.

High-threshold units (P) in *Sternopygus* resemble S1 afferents in that they also have larger diameter terminals and innervate fewer receptors than low-threshold (T) units. Thus, a similar morphological basis may underlie the differences in sensitivity and spontaneous activity of the T- and P-receptor types. That is, the increased sensitivity of T-units may result from an increase in the number of receptor cells per axon, and these differences in sensitivity might be further enhanced by larger generator potentials due to the thinner axon diameters.

Comparison with other systems

Postembryonic receptor cell addition has been demonstrated in the sensory structures of many anamniotes, and it seems to be correlated with indeterminate growth. Together with many of these demonstrations of cell addition, there have been additional studies showing increases in sensitivity, either in the sensory afferent or in the animal's behavior.

Ampullary receptor organs of weakly electric fish are also added with growth. In fact, they are added at about twice the rate of tuberous organs (Zakon, 1984a). Addition of ampullary organs is also observed in the closely related catfishes (Liang, 1983; Peters and Ieperen, 1989). A recent study using a catfish (*Clarius gariepinus*) has also demonstrated that the sensitivity of the afferents increases with increasing convergence of receptors (Peters and Ieperen, 1989). They found that ampullary units composed of 3 organs were 4.8 times more sensitive than those composed of a single organ.

A profound case of receptor cell addition occurs in the macula neglecta of the ray (Corwin, 1981, 1983). Corwin showed that

the average ratio of hair cells to afferent axons increased from 5:1 in the newly hatched to 60:1 in the adult ray. He also showed that during this period, the thresholds decreased 500-fold in linear units of vibration velocity. He suggested that increased convergence of receptors onto afferents might result in enhancement of the signal-to-noise ratio that is analogous to the enhancement gained through signal averaging. In the ray this could account for an increase in sensitivity of about 3.5 times. The rest of the sensitivity increase may be due to mechanical changes in the organ or the hair cells or in hair cell synapses; additionally, nerves may undergo changes with growth.

Behavioral significance of changes in BF and unit sensitivity

As fish grow, the BFs of their electroreceptive units increase relative to the EOD frequency so that the BFs start out low in small fish and increase to about 5 Hz above the EOD frequency in 40 cm fish. This is odd because one might expect BF to stop changing once they become matched to the EOD. In this respect it is interesting to note that most of the large fish sampled in this study were low-frequency and presumably males (Hopkins, 1974). One might hypothesize that it is important for males to be sensitive to frequencies higher than their own since they need to detect, court, and mate with high-frequency females (Hopkins, 1974). However, even large fish with high EOD frequencies, presumably females, showed average BFs higher than their EODs, suggesting that this change in average unit BF may not relate to communication.

With regard to the significance of changes in receptor sensitivity, the determination of physiological stimulus intensity, that is the voltage drop across the skin, becomes the critical factor. Preliminary results for *Sternopygus* (Sanchez, unpublished observations; E. S. Olson, personal communication) indicate that the EOD voltage across the skin is approximately 0.1–0.5 mV and remains relatively constant with growth. The effect of cell addition, then, would be to increase sensitivity of the low-threshold (T) units, thus enhancing the detection of weak stimuli in larger fish. This would extend the effective range of detection with growth. However, because the P-units do not add organs significantly, the T-system becomes relatively more sensitive, and it must therefore be primarily responsible for long-distance detection of objects and foreign EODs. This idea is in agreement with behavioral tuning curves of *Sternopygus*, which closely resemble the frequency range and sensitivity of T-receptors (L. J. Fleishman and H. H. Zakon, unpublished observations).

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