

The Oscillatory Responses of Skate Electroreceptors to Small Voltage Stimuli

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ABSTRACT Tonic nerve activity in skate electroreceptors is thought to result from spontaneous activity of the luminal membranes of the receptor cells which is modulated by applied stimuli. When physiological conditions are simulated *in vitro*, the receptor epithelium produces a current which flows inward across the luminal surface. This epithelial current exhibits small spontaneous sinusoidal fluctuations about the mean that are associated with corresponding but delayed fluctuations in postsynaptic response. Small voltage stimuli produce damped oscillations in the epithelial current similar in time-course to the spontaneous fluctuations. For lumen-negative, excitatory stimuli, these responses are predominantly an increase over the mean inward current. For inhibitory stimuli they are predominantly a decrease. Increased inward current across the luminal membranes of the receptor cells increases depolarization of the presynaptic membranes in the basal faces leading to increased release of transmitter and an excitatory postsynaptic response. Decreased inward current decreases depolarization of the presynaptic membranes leading to a reduction in transmitter release and an inhibitory postsynaptic response. Clear changes in postsynaptic response are detectable during stimuli as small as $5 \mu\text{V}$ with saturation occurring at $\pm 400 \mu\text{V}$. The evoked oscillations in epithelial current are damped and the postsynaptic responses decline during maintained stimuli with large off-responses occurring at stimulus termination. The initial peak of the off-response is similar to the response produced by onset of an oppositely directed stimulus. These observations substantiate the role of receptor cell excitability in the detection of small voltage changes.

INTRODUCTION

Elasmobranch fishes are capable of detecting remarkably small potential changes in their environment. Dijkgraaf and Kalmijn (1966) demonstrated behavioral responses in dogfish to gradients as small as 10 nV/cm . Kalmijn (1971, 1974) showed that feeding rays and dogfish utilize low amplitude bioelectric potentials in locating prey. The ampullae of Lorenzini were first implicated as the organs responsible for electroreception by Murray (1962) who recorded from the afferent fibers. He found that most ampullary nerve fibers have a resting discharge which is modulated by small electrical stimuli. Lumen-

negative stimuli accelerate the resting discharge and lumen positive stimuli inhibit it. During prolonged stimuli of moderate amplitude, impulse frequency returns toward the resting level with a roughly exponential time-course, declining by half within ~ 5 s. The time-course of accommodation is similar for excitatory and inhibitory stimuli. The accommodation does not affect incremental sensitivity (Murray, 1965) because a superimposed stimulus produces a change in the impulse frequency of the accommodated receptor like that produced by application of the second stimulus from the base line. Thus, skate electroreceptors are sensitive to small changes in potential rather than its absolute magnitude.

In these receptors lumen-negative stimuli produced by passing current across the epithelium can evoke all-or-none action potentials lasting ~ 100 ms (Waltman, 1968; Obara and Bennett, 1972; Clusin and Bennett, 1977 *a*). These responses involve the receptor cells, because they are associated with synaptic transmission to the afferent nerve. Their polarity and voltage sensitivity suggest that they result from depolarizing excitability of the luminal faces.

To explain the stimulus response relation of the afferent discharge it was proposed that the receptor cells are tonically and asynchronously active, perhaps generating full-sized impulses (Obara and Bennett, 1972). In this model a stimulus which depolarizes the luminal membranes increases receptor cell activity, while a hyperpolarizing stimulus suppresses it. These changes modulate release of a chemical transmitter, which in turn regulates spike frequency in the postsynaptic fibers. The great sensitivity of the electroreceptors was attributed to the high gain of excitable receptor cells poised near threshold.

To study the excitability properties of the epithelium, we developed an electrically isolated preparation in which the epithelium could be voltage clamped (Clusin and Bennett, 1977 *b*). As in other excitable tissues an N-shaped current-voltage relation is obtained. For lumen-negative stimuli of intermediate strength, an early inward current across the luminal surface of the epithelium is followed by a late outward current. Voltage clamp of the epithelium can approximate membrane voltage clamp only when there is little active current because the excitable luminal faces are in series with the basal faces, whose resistance probably exceeds that of the luminal faces when the latter are excited. Experiments reported in the following paper (Clusin and Bennett, 1979) indicate that the basal faces also exhibit some electrical excitability. However, by perfusing luminal and basal faces with various solutions and by measuring postsynaptic responses, it can be inferred that the epithelial currents are primarily generated by the luminal faces of the receptor cells. The abolition of the early inward current when the lumen is perfused with a zero Ca EGTA solution implicates Ca as the predominant carrier of the inward current. During large stimuli, onset of the late outward current is slowed and delayed. When the stimulus voltage equals or exceeds the equilibrium potential for the early current, no late current is present. These results indicate that the late outward current is activated by the presence of calcium at the inner surface of the luminal membranes (Clusin and Bennett, 1977 *b*).

In many preparations the currents evoked by voltage displacements into the negative resistance region are complex. Instead of a single inward current peak,

there are damped oscillations at about 20 Hz (Clusin and Bennett, 1979). As noted above, the series resistance of the basal membranes represents a significant fraction of the total resistance across the receptor cells after activation of the early current. Because of the series resistance, this inward current across the luminal membrane depolarizes the receptor cells leading to regenerative excitation. In other excitable cells, damped oscillations are frequently observed when the membrane is voltage clamped through a significant series resistance (e.g., Keynes et al., 1973).

In the present study, we have attempted to characterize receptor cell activity by measuring epithelial current when physiological conditions are simulated *in vitro*. *In situ* the receptor is shunted by the low resistance of the external environment and the skate's body, which only to a minor extent distorts an applied field (Murray, 1967). Because of the long space constant and low axial resistance of the canal (Waltman, 1966) the stimulus across the ampullary epithelium is close to that between canal orifice and the body interior adjacent to the ampulla (Murray, 1967). We find that the DC potential generated by the tissues between exterior and ampulla is small, and thus the receptor normally operates in the voltage range where oscillatory responses are observed in voltage clamp experiments. The *in-vitro* and shunted ampulla generates a mean current that is in the same direction as the inward calcium current across the luminal membranes of excited receptor cells. The mean current exhibits small sinusoidal fluctuations which we conclude arise from regenerative depolarizations in the receptor cells. Small stimuli produce oscillatory epithelial currents that are in the opposite direction to those expected for a passive system, indicating that the functioning electroreceptor operates in the negative resistance region of its current voltage characteristic. The ionic basis of the oscillatory responses is examined in the following paper (Clusin and Bennett, 1979). Some of our observations have appeared in preliminary form (Clusin and Bennett, 1973, 1974).

METHODS

Intact ampullary electroreceptors with attached canals were removed from skates (*Raja ocellata* and *R. erinacea*) and prepared as previously described (Clusin and Bennett, 1977a). Skates used in these experiments were obtained near Woods Hole, Mass., during the winter months and kept in running seawater at 1–10 degrees C. *In-vitro* sensitivity to small stimuli was rarely observed during the summer when the water temperature at Woods Hole was incompatible with prolonged survival of skates.

The recording apparatus used in most of the experiments is diagrammed in Fig. 1. The canal was suspended on thin pins across an air gap between two saline pools at 10°C and then washed with an isosmotic sucrose solution to prevent the flow of current along its external surface. The saline pools were connected by a low resistance bridge containing saline with 2% agar. The saline was modified from Fühner's formula (415 mM urea, 340 mM NaCl, 6 mM KCl, 215 mM MgCl₂, 1.8 mM CaCl₂, 2.5 mM NaHCO₃, and 5 mM Hepes at pH 7.4). Cerebrospinal fluid from the cranial cavity was sometimes used instead of saline in the pool bathing the ampulla with no difference in receptor responses.

Current flowing in the canal, which is the epithelial current, was measured by incising the canal 5 mm into the air gap and recording voltage between the incision and the canal

orifice using a Metamatrix M1 differential amplifier (Metametrics Cooperative, Inc., Carlyle, Mass.) and two chlorided silver wires. Zero current was established by removing the salt bridge after which only applied currents could flow in the canal. The recording was calibrated by measuring the voltage drop produced by a known current passed between the saline pools after removal of the salt bridge. Voltage across the ampulla was measured between the electrode at the incision and a chlorided silver wire in the bath containing the ampulla. Use of a low-resistance chlorided silver electrode reduced interference, allowing much higher voltage gains than were possible in previous studies where the canal was impaled with a microelectrode. Errors attributable to the incision were insignificant. The voltage drop produced by current flowing across the 5 mm of canal between the incision and the ampulla was estimated as $12 \mu\text{V}$ for the epithelial current evoked by a 1-mV stimulus (see Fig. 5). No significant leakage of current occurred through the incision, as indicated by the fact that placement of the incision did not significantly change the input impedance of the preparation (measured in the

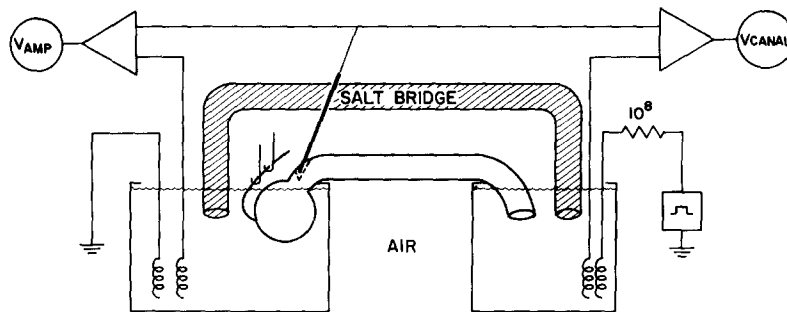


FIGURE 1. Arrangement for recording epithelial currents from the ampulla of Lorenzini during small stimuli. In the absence of stimulation, the ampullary potential is held near zero because of the low resistance of the canal. The low resistance of the salt bridge allows voltage stimuli to be applied by passing constant current between the saline pools. Epithelial current is measured as the voltage drop between the opening of the canal and a wire electrode inserted through an incision in the canal wall (dashed lines) near the ampulla. Ampullary voltage is measured between the incision and the pool containing the ampulla. Nerve activity is recorded with a pair of fine wires.

absence of the salt bridge). Voltage stimuli were generated by passing constant current between the saline pools with the $2\text{-k}\Omega$ salt bridge in place. Constant current was generated using an isolated voltage source in series with a $100\text{ M}\Omega$ resistor. Because of the high resistance of the ampulla and canal, almost all of the stimulus current that passed between the saline pools flowed across the salt bridge producing a voltage drop between the pore of the canal and the basal surface of the ampulla. Current generated by the epithelium produced little voltage drop across the salt bridge. The epithelial current evoked by the 1-mV stimulus in Fig. 5 produced a calculated voltage drop of $12 \mu\text{V}$. Voltage between the external orifice of the canal and the basal surface of the ampulla was therefore clamped at the stimulus voltage, simulating the physiological condition in which the receptor is shunted by the low environmental and tissue resistances.

In some experiments a voltage clamp was used instead of the salt bridge to achieve the same result. Application of the voltage clamp technique was as previously described

(Clusin and Bennett, 1977 *b*) with two important exceptions. First, the voltage in the bath containing the pore of the canal was clamped rather than the voltage at the neck of the ampulla. Thus, the series resistance of the canal was interposed between the ampulla and the voltage clamp circuitry. Second, the holding potential was set at 0 mV where the receptor cells produce fluctuating epithelial currents, instead of at the resting potential for the electrically isolated epithelium, where the receptor cells are inactive. Although the technique used in Clusin and Bennett (1977 *b*) was preferable for characterization of the variable conductances in the lumenal membranes, the above modifications give a better reproduction of the physiological condition.

There was no important difference between the receptor responses measured with the voltage clamp and the salt bridge techniques. The voltage clamp eliminated the small errors attributable to the finite resistance of the salt bridge. However, the voltage clamp had the disadvantage that drift in the recording electrodes, which sometimes reached 1 $\mu\text{V/s}$, could produce significant changes in the holding potential during application of a series of stimuli. Similar electrode drift occurred with the salt bridge technique and prevented the study of responses to stimuli longer than a few seconds. However, drift in the recording electrodes had no effect on the true potential across the electroreceptor when the salt bridge technique was used.

To establish the appropriate DC holding potential for the receptor, it is important to determine whether there are significant DC potentials between the exterior and the inside of the capsules in which the ampullae lie. In two experiments a skate was spinalized by a small middorsal incision. The skate was placed in a shallow plastic tray and seawater was added to cover the body up to the level of the incisions that were made on the dorsal surface and over the hyomandibular capsules. The capsules were exposed and a small cut made in them to expose the ampullae. A broken 3 M KCl-filled microelectrode was moved back and forth between the bath and test site in the body to measure potential levels. No potential differences greater than 1 mV were observed between exterior and just subcutaneous tissue or capsule interior. (Of course, much larger negative demarcation potentials could be observed at sites where muscles were deliberately cut.) The observed potential differences should be within the range over which the receptor accommodates without loss of sensitivity (Murray, 1965). Thus, the salt bridge or voltage clamp techniques should satisfactorily reproduce the *in situ* condition in respect to DC level as well as shunting.

Postsynaptic responses were recorded extracellularly by draping the nerve over a chlorided silver hook that was then retracted into an oil-filled glass capillary. Differential DC recordings were usually obtained with a Metametrix amplifier and a second wire electrode placed near the opening of the capillary. The ampullary nerve in skates contains 5–7 afferent fibers which ramify profusely and innervate the several thousand receptor cells in each ampulla. In some experiments, 10^{-7} M tetrodotoxin (TTX) was used to abolish the sodium-dependent action potentials (Steinbach, 1974). The remaining responses were presumably distorted by the variable potassium conductance in the nerve fibers, but were well correlated with presynaptic responses as shown below.

RESULTS

Evidence for Spontaneous Activity of the Receptor Cells

If the tonic nerve discharge in skate electroreceptors results from tonic and asynchronous electrical activity of the receptor cells, this activity might be detectable in high gain recordings. When the receptor is shunted by the salt bridge technique, the epithelial voltage exhibits small sinusoidal fluctuations of

variable amplitude (Fig. 2 A). The maximum amplitude of these fluctuations is $\sim 35 \mu\text{V}$ and the frequency is $\sim 20 \text{ Hz}$. In the absence of an applied stimulus, the voltage across the epithelium is equal to the voltage drop between the two ends of the canal (Fig. 1). Thus, the epithelial oscillations can be calculated from the canal resistance. In Fig. 2, the fluctuations in epithelial current are as large as 1.2 nA.

The oscillations produced by the shunted receptor represent small fluctuations in the magnitude of a larger mean epithelial current. This larger current can be measured by recording the change in voltage along a segment of canal

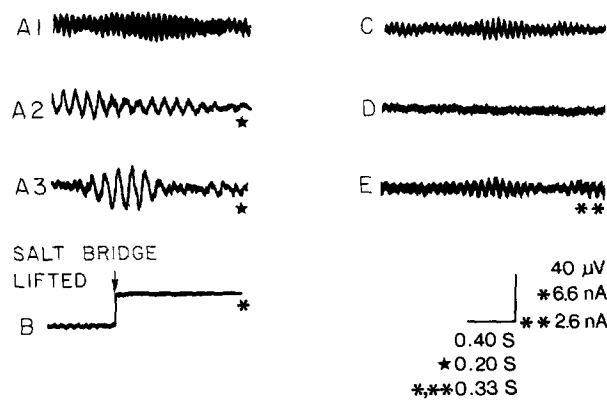


FIGURE 2. Spontaneous epithelial currents produced by an ampulla of Lorenzini whose canal is shunted by a salt bridge. Spontaneous oscillations in potential across the epithelium are shown in A1-A3 (lumen-negative up). In (B) the total receptor current is measured as the change in voltage along the canal produced by lifting the salt bridge, which reduces current in the canal to zero. Inward current through the luminal membranes is shown down. Spontaneous oscillations are discernible in the epithelial current recorded before the salt bridge is lifted. Parts C and D illustrate suppression of the spontaneous oscillations by a strong inhibitory stimulus. Spontaneous oscillations in (C) are abolished by a maintained 4-mV inhibitory stimulus in part D (high gain AC-coupled recordings). In (E) the epithelial currents are measured with a voltage clamp. Voltage is measured at an incision in the canal near the ampulla, and positive series feedback is used to compensate for the series resistance of the small segment of canal between the incision and the ampulla. Spontaneous oscillations in epithelial current as large as 1.1 nA are observed.

with known resistance when the salt bridge is lifted, which interrupts the current path (Fig. 2 B). The mean current in Fig. 2 B is 4.0 nA, inward across the luminal surface of the epithelium and outward across the basal surface. Small sinusoidal fluctuations of about 5% of the mean current are also apparent in Fig. 2 B.

The experiments in Figs. 2 C-D and 3 D suggest that the fluctuations in epithelial current involve excitation of the luminal membranes of the receptor cells. In Fig. 2 C, spontaneous receptor oscillations were recorded with a Tektronix AC coupled amplifier (Tektronix, Inc., Beaverton, Ore.) interposed

between the output of the Metamatrix amplifier and the oscilloscope. A sustained 4-mV lumen-positive stimulus, which hyperpolarizes the luminal membranes, abolishes the oscillations (Fig. 2 D). Receptor cells are involved in generating the spontaneous fluctuations because there are corresponding post-synaptic responses in the TTX-treated afferent nerve (Fig. 3 D). The sustained current in the canal and the small superimposed fluctuations are consistent with summation of asynchronous regenerative responses in a large number of excitable cells.

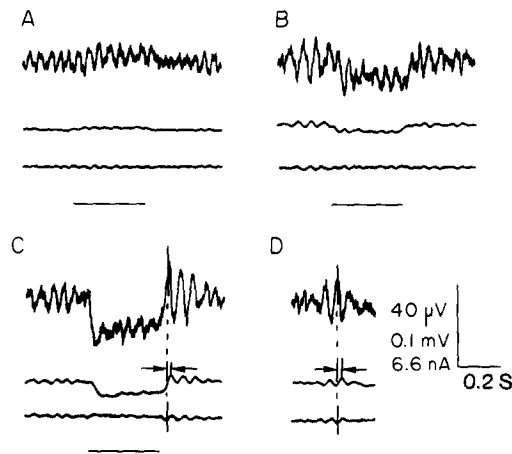


FIGURE 3. Responses of the shunted electroreceptor to small stimuli. The voltage across the ampullary epithelium is shown in the upper trace, excitatory (lumen-negative) displacements shown up. The recording from the afferent nerve is shown in the middle trace with depolarizing responses shown up. Postsynaptic action potentials have been abolished by $0.1 \mu\text{M}$ TTX. Epithelial currents, measured as the voltage drop along the canal, are shown in the lower trace. Current flowing inward across the luminal membranes of the receptor cells is shown down. Applied stimuli are indicated by bars beneath each record. In (A) an excitatory stimulus of $\sim 5 \mu\text{V}$ produces a clear depolarization of the nerve. In (B) an inhibitory stimulus of $\sim 10 \mu\text{V}$ produces a hyperpolarization ascribable to decreased release of excitatory transmitter. In (C) a $50\text{-}\mu\text{V}$ inhibitory stimulus produces a much larger inhibitory response. Termination of the stimulus produces $60\text{-}\mu\text{V}$ oscillations in epithelial voltage and corresponding oscillations in current. There are also associated peaks in the postsynaptic response which are delayed by ~ 20 ms. The presence of similarly delayed fluctuations in the postsynaptic response during spontaneous fluctuations in epithelial voltage is shown in (D).

Our earlier voltage clamp experiments demonstrated a shunt pathway through the epithelium in parallel with the receptor cells (Clusin and Bennett, 1977 *b*). The resistance of the shunt pathway is some 10-fold greater than that of the canal so that the oscillations in current through the receptor cells would be $\sim 10\%$ greater than those recorded along the canal.

If the receptor is electrically isolated by removing the salt bridge, the ampulla develops a 10–30-mV lumen-positive resting potential, which our calculations

indicate is primarily generated in the shunt pathway through the epithelium (Clusin and Bennett, 1977 *b*). In an experiment similar to the one shown in Fig. 2 B, a short-circuited electroreceptor produced a tonic current of 12 nA. When isolated, this ampulla had a resting potential of 18 mV and a leakage resistance of 485 Ω . This resistance lies mainly in the shunt pathway in parallel with the receptor cells. If there were no conductance changes, loading of the ampulla by the 30-k Ω resistance of the canal would produce a current of almost 35 nA flowing outward across the luminal surface of the epithelium. The observed epithelial current in the opposite direction must therefore have resulted from excitation of the receptor cells. The total inward current across the luminal faces of the receptor cells is the sum of the (net) epithelial current, which flows along the canal, and the current flowing through the shunt pathway. In this case the sum would have approached 47 nA. Thus, the spontaneous fluctuations in current constitute a smaller fraction of the total current generated by the receptor cells than they do of the current flowing along the canal.

Responses of the Shunted Electroreceptor to Voltage Stimuli

Fig. 3 shows the responses of an electroreceptor to small stimuli applied while it is shunted by the salt bridge. In (A) a 5- μ V lumen-negative stimulus is applied between the pore of the canal and the luminal surface of the epithelium. A postsynaptic depolarization (middle trace) is clearly discernable. In (B) a 10- μ V lumen-positive stimulus is applied and the nerve is hyperpolarized. Neither of these small stimuli produces a noticeable change in the epithelial currents as displayed in the bottom traces. However, in (D) fluctuations in current can be seen which correspond to the larger fluctuations in epithelial voltage. A 50- μ V inhibitory stimulus is applied in (C), which produces a large postsynaptic hyperpolarization. There is also a sustained outward deflection in epithelial current. This deflection is opposite to that expected for a passive response, and represents a reduction in inward current generated by the receptor cells. After stimulus termination, there is an oscillatory off-response in which there are several peaks of inward current. The off-response produces an excitatory postsynaptic response, which also has several peaks. The peaks in postsynaptic response follow those in epithelial current with a delay of ~ 20 ms. Fluctuations in postsynaptic response also follow the spontaneous oscillations in epithelial potential with a similar delay between the peak inward current and the peak nerve depolarization (arrows). The delays demonstrate that the postsynaptic responses are not due to electrical pickup from the epithelium.

Responses of the electroreceptor to larger stimuli are shown in Fig. 4. Lumen-negative stimuli, which are excitatory, are defined as positive and displayed upward. Stimulus amplitudes, ranging from -0.63 to 0.52 mV are indicated on the left. Transepithelial voltages produced by these stimuli are shown in the traces in the first column and are a nearly linear function of stimulus strength. Small damped oscillations occur at the onset and termination of the larger stimuli. The epithelial currents are shown in the second column, inward currents through the luminal membranes displayed downward. Immediately after stimulus onset, there is a large transient current, which is upward for excitatory stimuli and downward for inhibitory stimuli. Equal and opposite

deflections occur when the stimuli are terminated. These deflections result from charging of the ampullary capacities (Clusin and Bennett, 1977*b*), but are further prolonged by the additional series resistance of the canal.

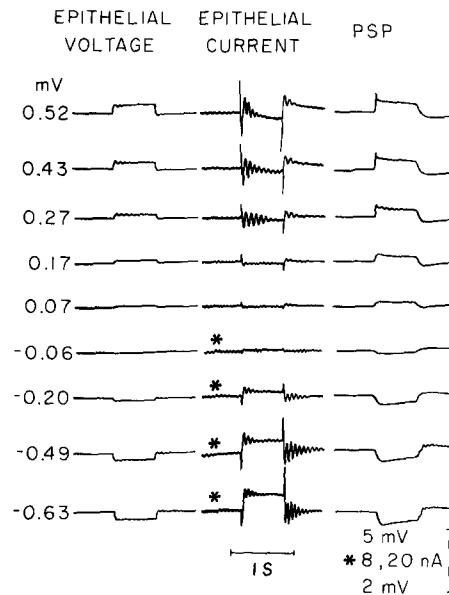


FIGURE 4. Responses of the shunted electroreceptor to moderate stimuli. Stimulus strength in millivolts is indicated on the left. Excitatory stimuli which are lumen-negative are defined as positive. The resulting change in epithelial voltage is displayed in the left column, excitatory displacements shown up. Epithelial current is in the middle column, inward current across the luminal membranes shown down. Postsynaptic responses are in the right column, depolarization shown up. Excitatory stimuli evoke damped oscillations in epithelial current which stabilize at a steady inward level. There are large depolarizing postsynaptic responses which show an initial peak and then a more gradual decline. Inhibitory stimuli evoke damped oscillations in epithelial current which stabilize to a steady outward level. There are corresponding hyperpolarizations in the postsynaptic responses which show a gradual decline back toward the resting level. The prominent oscillations in epithelial current produce small oscillations in postsynaptic potential (PSP) during moderate excitatory stimuli, but no postsynaptic oscillations are observed during inhibitory stimuli. After strong stimuli of either polarity, off-responses are seen, which resemble the responses produced by application of opposite stimuli from the base line. The large transient currents at stimulus onset and termination are capacitive.

During excitatory stimuli, the capacitive transient is followed by damped oscillations at a frequency of ~ 20 Hz. For each downward peak the current relative to the base line is inward across the luminal surface of the epithelium and outward across the basal surface. The oscillatory peaks are greatly damped during the 660-ms stimulus. The current at the end of each excitatory stimulus

is slightly inward relative to the base line representing an increase in excitation of the receptor cells.

During inhibitory stimuli, there are similar oscillations whose initial peak is in the outward direction. For a given stimulus these oscillations are somewhat smaller and more rapidly damped than those evoked by excitatory stimuli. Current throughout the stimulus is outward relative to the base line, representing a diminution in excitation of the receptor cells.

The changes in epithelial current during small stimuli are always in the opposite direction to those expected for a passive response. When the peak of the first deflection in epithelial current after the capacitative transient is plotted as a function of stimulus voltage, a linear plot with negative slope is obtained (Fig. 5 A; ○: data obtained from the same ampulla as used in Fig. 4; later records cover a wider voltage range). When currents at the end of the pulses are plotted against stimulus voltage, a linear plot with negative slope is obtained over the same voltage range (Fig. 5 B). The persistence of the negative slope indicates that the stimuli produce a sustained change in receptor cell activity. The regions of negative slope extend from at least 1 mV (excitatory) to ~ -2 mV (inhibitory). Beyond -2 mV, increasingly strong inhibitory stimuli change the slope of the current voltage relations until they are again linear with a positive slope of $360 \text{ k}\Omega$ in each case. If strong inhibitory stimuli completely abolish currents produced by the receptor cells, then the slope of the current-voltage relations should represent epithelial leakage resistance plus the $30\text{-k}\Omega$ resistance of the canal. Although the epithelial leakage resistance was not directly measured in this experiment, a value of $330 \text{ k}\Omega$ is near the median for electroreceptors used in the present experiments. Thus, as predicted from previous work, the shunted ampulla operates in the negative resistance region of its current-voltage relation.

The postsynaptic responses of the TTX-treated nerve are shown in the third column of Fig. 4. Excitatory stimuli cause depolarization of the nerve and inhibitory stimuli cause hyperpolarization. The brief deflection at the beginning of the larger excitatory responses may result from onset of delayed rectification in the postsynaptic fibers. After the brief initial peak, there is a sustained plateau, which gradually declines toward the base line. Damped oscillations can be superimposed on the plateau phase of the excitatory postsynaptic response. These oscillations correspond to the oscillatory epithelial currents generated by the receptor cells. Inhibitory stimuli cause hyperpolarization of the nerve. In that these responses are associated with diminished excitation of the receptor cells, they presumably result from decreased liberation of excitatory transmitter.

The maximum deflection in postsynaptic response during each stimulus is plotted in Fig. 5 A (■). The synaptic response is a sigmoidal function of stimulus voltage and is very steep near the origin. In the region of maximum sensitivity a deflection equal to 1% of the total range of the postsynaptic response would be produced by a $4\text{-}\mu\text{V}$ stimulus. For excitatory stimuli, the postsynaptic response is saturated by an excitatory stimulus of $\sim 0.3 \text{ mV}$. Inasmuch as evoked epithelial currents are still increasing at this stimulus strength, saturation of the excitatory postsynaptic response may reflect maximal activation of the vesicle

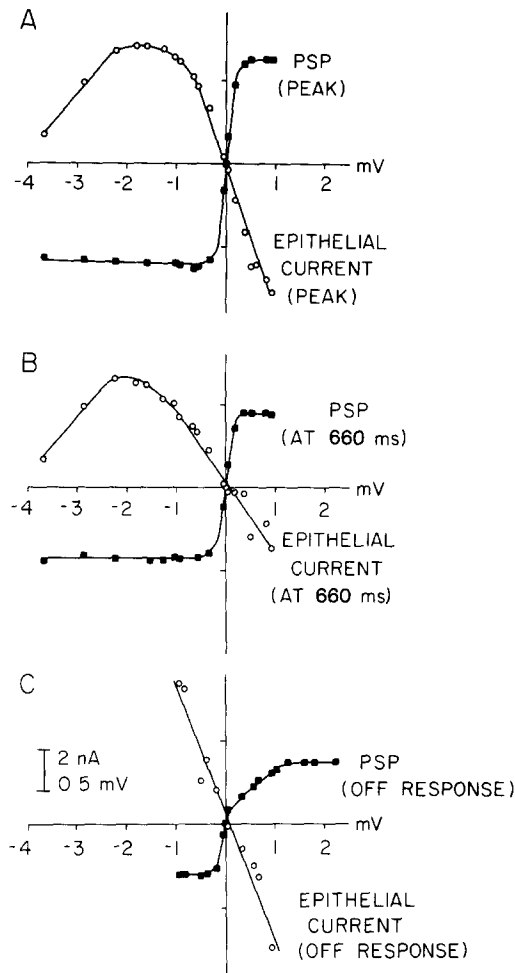


FIGURE 5. Current-voltage relations of the shunted electroreceptor. Same experiment as Fig. 4. In (A) current at the initial peak (○) is plotted as a function of stimulus voltage. The relation is linear between -0.6 and 1.0 mV and has a negative slope. For large inhibitory stimuli (shown negative) the I - V relation is linear with a positive slope. Peak postsynaptic response is plotted as (■). The stimulus response relation is quite steep, saturating within ± 400 μ V. In (B) epithelial current (○) just before stimulus termination is plotted. The linearity of the relation and the negative slope feature are preserved for moderate stimuli, but the plot is somewhat less steep. The relation between stimulus and postsynaptic response (■) also remains steep at this time, but the maximum deflections are smaller. In (C) off-responses are plotted as a function of the voltage change at stimulus termination; circles indicate the initial peak of the epithelial current after stimulus termination, relative to the original base line. The peak current is a linear function of the voltage change, with a fixed negative slope resistance close to that obtained at stimulus onset. Thus, the absolute current at the initial peak after termination of a 660-ms stimulus is the same as that produced by onset of an equal stimulus of opposite polarity. Solid squares in (C) indicate the amount by which the postsynaptic response undershoots or overshoots the base line, after stimulus termination.

release process or of the postsynaptic membrane. For inhibitory stimuli, saturation of the postsynaptic response occurs at -0.5 mV. This effect can be ascribed to complete suppression of transmitter release by partial inhibition of electrical activity in the receptor cells.

During a prolonged stimulus, the postsynaptic response gradually declines, falling to $\sim 70\%$ of its maximum value within 660 ms after stimulus onset. When the postsynaptic potential at 660 ms is plotted as a function of stimulus voltage (Fig. 5 B), the resulting stimulus-response relation is a sigmoid that is symmetric about the origin, but somewhat less steep than the plot of postsynaptic responses shown in Fig. 5 A. The decline in the postsynaptic response is responsible for at least part of the accommodation of impulse frequency reported by Murray (1962, 1965). A corresponding reversion in the mean epithelial current toward the base line is not seen in the records of Fig. 4 although there is a decline in the response to the inhibitory stimulus in Fig. 3 C. When the relations between epithelial current and stimulus strength are compared for the initial peak and the end of the stimulus, the slope for the latter is reduced by 60% (Fig. 5 A and B). Although the potential in individual receptor cells during prolonged stimuli has not been established, it is possible that the reduction in the evoked epithelial oscillations is due to changes in receptor cell activity that also tend to reduce transmitter release.

The last step in transduction by the receptor is initiation of afferent discharges. Recordings from a short-circuited receptor in the absence of TTX demonstrate modulation of postsynaptic impulse activity by applied stimuli (Fig. 6). Inhibitory stimuli decrease the afferent discharge and excitatory stimuli increase it. The nerve impulse activity increases the noise level in the epithelial current.

Off-Responses and Accommodation

As noted in the introduction, skate electroreceptors accommodate without loss of sensitivity, and the impulse discharge produced by termination of a prolonged stimulus is comparable to the response occurring at initiation of an equal and opposite stimulus applied from the base line (Murray, 1962, 1966). A further indication of involvement of the receptor cells in accommodation is obtained by examining epithelial currents at stimulus termination. When a 660-ms stimulus is terminated, the epithelial current does not merely return to the base line, but exhibits an off-response. This off-response is comparable to that produced by onset of an oppositely directed stimulus of equal amplitude. Fig. 5 C shows the relation between the change in voltage at stimulus termination and current at the largest subsequent peak, measured relative to the original base line. The resulting plot is linear and has a negative slope equal to the corresponding slope in Fig. 5 A. Thus, the stimulus-response relation for the peak is shifted along the voltage axis during prolonged stimuli. This shift, which is no longer present several hundred ms after stimulus termination, may be related to the observed accommodation in the postsynaptic response.

The oscillations in the off-response after termination of prolonged excitatory stimuli are superimposed on a long-lasting outward component that slowly decays toward the base line. The time-course of this outward current is similar

to that of the tail currents associated with turn-off of the calcium-activated outward current in voltage clamp (Clusin and Bennett, 1977 *b*). The presence of this slow component suggests involvement of the calcium-activated outward current in accommodation. The relative constancy of the mean epithelial currents during small stimuli that produce some accommodation in the postsynaptic response may reflect a summation of components changing in opposite direction.

The peak postsynaptic response after stimulus termination measured from the original base line is plotted in Fig. 5 C (■). The resulting stimulus-response relation is a somewhat distorted sigmoid compared to that of the on-responses (Fig. 5 A), and the off-responses are smaller for equal voltage steps. Further changes in receptor cell excitability, changes at the synapse, or changes in the afferent nerve may produce more complete accommodation during longer stimuli. Murray (1962) showed that the change in afferent discharge declines by one-half in 1 s and that accommodation is virtually complete in 7 s.

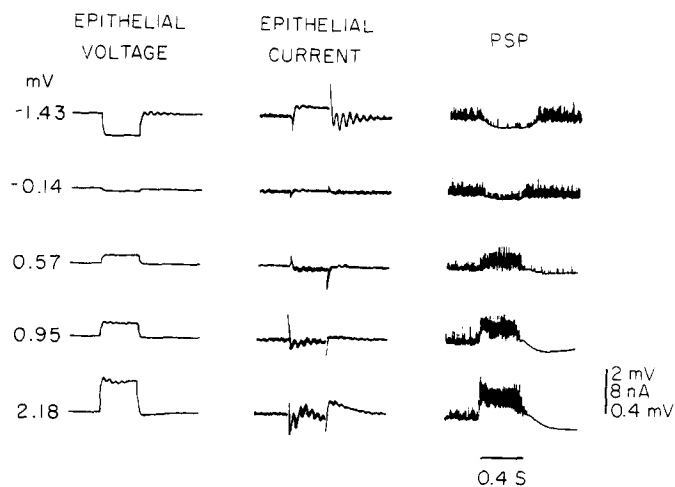


FIGURE 6. Nerve responses of the shunted electroreceptor in the absence of TTX. Recording conventions are as in Fig. 4. There is a tonic impulse discharge in the nerve fibers, which is modulated by the applied stimuli. Excitatory stimuli increase the impulse discharge, while inhibitory stimuli suppress it. Off-responses at stimulus termination are similar to the responses seen at onset of opposite stimuli. Nerve impulses produce some high frequency noise in the epithelial current.

DISCUSSION

The foregoing data provide strong support for the model of electroreceptor function previously proposed (Obara and Bennett, 1972, Clusin and Bennett, 1977 *a* and *b*). In the *in situ* (shunted) electroreceptor, the receptor cells produce spontaneous and asynchronous regenerative responses, giving rise to a noisy epithelial current. The mean current is inward across the luminal faces and outward across the basal faces of the receptor cells. Outward current across the

basal faces depolarizes them, causing secretion of excitatory transmitter onto the afferent nerve fibers. Applied stimuli affect the rate of transmitter release by modulating activity of the receptor cells. Excitatory stimuli increase receptor cell activity, thereby augmenting the mean epithelial current and increasing the rate of excitatory transmitter release. Inhibitory stimuli decrease receptor cell activity diminishing the epithelial current and reducing the rate of transmitter release.

Without intracellular recordings, the contribution of individual receptor cells to the spontaneous inward currents is uncertain. All the current could result from summation of asynchronous oscillatory responses of individual receptor cells. Alternatively, there could be a steady inward component produced by cells not undergoing regenerative responses. Involvement of many receptor cells in the production of oscillations can be inferred from previous voltage clamp data (Clusin and Bennett, 1977 *b*). The epithelium can generate a maximum net inward current of $\sim 1 \mu\text{A}$. Because the ampullae contain 10^4 receptor cells, the maximum inward current that can be produced by a single cell is $\sim 0.1 \text{ nA}$. Spontaneous oscillations produced by the short-circuited ampulla can be > 10 times larger and must therefore arise from many cells either through electrical interaction or random summation.

One possible mechanism for synchronization of receptor cells is interaction due to the voltage changes of the oscillations themselves (up to $35 \mu\text{V}$) which are within the sensitivity range for DC stimuli. This possibility has been investigated by voltage clamp in which the clamping point was a chlorided silver wire placed at a canal incision 5 mm from the ampulla. This reduced the series resistance from the $\sim 30 \text{ k}\Omega$ of the entire canal to $\sim 2 \text{ k}\Omega$ as measured by transection of the ampulla, which would reduce ~ 15 -fold the voltage across the epithelium produced by the oscillatory currents. The effect of the remaining $2 \text{ k}\Omega$ was reduced by series resistance compensation using positive feedback (see Clusin and Bennett, 1977 *b*). Feedback was set just below the level that produced oscillation of the amplifier. The adequacy of the series resistance compensation was verified by microelectrode impalement of the canal at the neck of the ampulla in which it was shown that large steps were clamped with negligible distortion. It can be concluded that in comparison to the shunted receptor the voltage at the neck of the ampulla was reduced well over the 15-fold that would be produced by voltage clamp alone without the compensation. The resulting epithelial currents with (Fig. 2 E) or without compensation showed spontaneous oscillations indistinguishable from those obtained with the clamping point at the canal opening.

Of course, the series resistance of individual alveoli within the ampulla is uncompensated with this technique. If each alveolus is a cylinder $150 \mu\text{m}$ across and $300 \mu\text{m}$ in length with an internal resistivity of $25 \Omega/\text{cm}^2$, then the maximum resistance along its length is $\sim 2 \text{ k}\Omega$. A 1-nA oscillatory response arising entirely within the apex of one alveolus would therefore produce a maximum voltage change of $2 \mu\text{V}$ which is somewhat smaller than the DC sensitivity of receptors in the present study. Also, the constancy of the spontaneous oscillations in the face of a 15-fold or greater reduction in the associated voltage changes suggests that they do not result from interaction of the receptor cells through the series

resistance of the canal, but the possibility of some electrical interaction cannot be excluded.

Another possible basis for synchronization of receptor cell responses would be the presence of electrotonic synapses in the epithelium. Loewenstein et al. (1965) impaled unidentified cells in the ampullary epithelia of *M. canis* and *R. psammobatis* and observed electrotonic spread between neighboring cells over limited areas. However, because they passed their electrodes down the canal, their recordings were most likely obtained in the marginal zone where there are no receptor cells. No electrical activity was reported that would have indicated penetration of receptor cells. No gap junctions have been described involving receptor cells in the ampullary epithelium. However, our principle conclusions would be unchanged if small groups of receptor cells were coupled by electrical synapses.

The most satisfactory explanation for the form of the spontaneous epithelial currents is that they represent the sum of a large number of low amplitude responses whose phase relation is more or less random. In voltage clamp records in which the inactive epithelium is stepped into the negative resistance region, oscillatory currents are often obtained that are well fit by a damped sinusoid (Clusin and Bennett, 1979). Moreover, because the amount of damping between successive peaks is usually < 20%, the oscillations can be adequately represented for intervals of less than two cycles by a non-damped sinusoid. We show in the next paper that there is no net outward current across the epithelium during oscillatory responses evoked by large stimuli. Rather, it appears that the oscillations represent pulses of inward current generated by the lumenal membranes, which can be approximated by

$$I = K (1 + \sin 2\pi \omega t), \quad (1)$$

where K is amplitude and ω is ~ 20 Hz. If individual receptor cells in the spontaneously active ampulla produce asynchronous oscillatory responses of identical frequency but with random phase lag, then the current produced by the entire ampulla can be represented as

$$I = \sum_{i=1}^N k [1 + \sin(2\pi\omega t + \alpha_i)], \quad (2)$$

where k is the amplitude of the current produced by one receptor cell and α_i is a random phase lag. Inasmuch as the sum of sinusoids with identical frequency is a sinusoid with the same frequency, Eq. 2 reduces to

$$I = N \times k + k \times \beta (\sin \omega t + \bar{\alpha}), \quad (3)$$

where $\bar{\alpha}$ is the mean of α_i and β is a factor between zero and N whose value is given by

$$\beta = \left[\left(\sum_{i=1}^N \cos \alpha_i \right)^2 + \left(\sum_{i=1}^N \sin \alpha_i \right)^2 \right]^{1/2}. \quad (4)$$

If all values of α_i have equal probability and N is large, then the probability distribution of β will be peaked and strongly skewed toward zero. β is therefore

likely to be much smaller than N , but is not likely to be zero. Total epithelial current will therefore consist of a tonic current of magnitude $N \times k$ with smaller sinusoidal fluctuations superimposed. Variations in these fluctuations could result from variations in the population of active cells and their exact phases and frequencies as discussed in the following paper (Clusin and Bennett, 1979).

The oscillatory responses evoked by small voltage stimuli could be explained in several ways. Applied stimuli might affect the proportion of receptor cells that were active. Excitatory stimuli would then activate previously silent cells, whereas inhibitory stimuli would block excitation in cells that were near threshold. Alternatively, the mean frequency of responses in single cells could be increased. Synchronization of the responses would contribute to the oscillations initially evoked by voltage steps. Damping of the evoked oscillations could represent a gradual desynchronization due to small differences in the frequency of the oscillations, a gradual decrease in the number of active cells, or a gradual damping of the response within individual cells.

The Receptors and Behavioral Sensitivity

The extraordinary sensitivity of ampullary electroreceptors is well established from behavioral studies in which stimuli as small as 10 nV/cm were shown to cause an alerting response (slowing of the heartbeat) in free-swimming dogfish (Dijkgraaf and Kalmijn, 1966, see also Kalmijn, 1974). Three factors may contribute to this sensitivity: (a) the electrical sensitivity of the receptor epithelium itself; (b) the anatomical properties of the canal; and (c) integration of many receptor responses by the central nervous system.

The role of the canal was first appreciated by Waltman (1966) who showed that the canal and ampulla comprise a core conductor terminated by a high resistance. Because the length constant of the canal is long and the input resistance of the ampulla is high, little voltage drop occurs along the canal. The voltage developed across the ampullary epithelium is therefore equal to the voltage difference between the pore of the canal and the basal surface of the epithelium. Inasmuch as the canals are up to 20 cm long (in animals with 40-cm body length) while the receptor cells are $\sim 15 \mu\text{m}$ across, the voltage sensed by the receptor cells is $> 10^4$ times greater than would be sensed in the absence of the canal.

The absolute sensitivity of the single ampulla was first measured by Murray (1967) who found that stimuli as small as $2 \mu\text{V}$ produce changes in postsynaptic impulse frequency that are detectable by ear. Similar results have been obtained in the present study, where $5\text{-}\mu\text{V}$ stimuli produce obvious changes in postsynaptic potential. The behavioral threshold of 10 nV/cm implies a potential of $0.2 \mu\text{V}$ across the ampullae with the longest canals, which is 10–25 times more sensitive than observed in single receptors. The discrepancy might lie in failure to achieve *in situ* sensitivity experimentally. However, the presence of spontaneous fluctuations in receptor potential which are considerably larger than the minimum effective stimulus for the *in vitro* receptor and which produce corresponding fluctuations in postsynaptic potential suggests that much more sensitive receptors will not be found. When the spontaneous postsynaptic fluctuations are compared with the evoked responses they are found to

represent ~ 1 % of the postsynaptic response range. Interpolation from the input-output relation in Fig. 5 A shows that a 4- μ V stimulus produces a postsynaptic response which is also 1% of the total range. Responses to stimuli smaller than a few microvolts are therefore likely to be lost in the physiological receptor noise. Of course, the experimenter could use signal averaging to detect responses to smaller stimuli. Similarly, averaging of activity from many receptors by the skate central nervous system could account for the discrepancy between single receptor thresholds and behavioral data. The voltage sensitivity of the electroreceptors themselves is far greater in marine elasmobranchs than in other fishes. The role of regenerative activity in achieving this great sensitivity will be discussed in the next paper (see also Bennett and Clusin, 1979).

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