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THE NOVELTY RESPONSE OF PULSE-TYPE WEAKLY ELECTRIC FISH: A NEUROTHELOGICAL STUDY

The University of Oklahoma PH.D. 1985

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THE NOVELTY RESPONSE OF PULSE-TYPE WEAKLY ELECTRIC FISH: A NEUROETHOLOGICAL STUDY

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

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By

HAROLD JAMES GRAU

Norman, Oklahoma

1985

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By

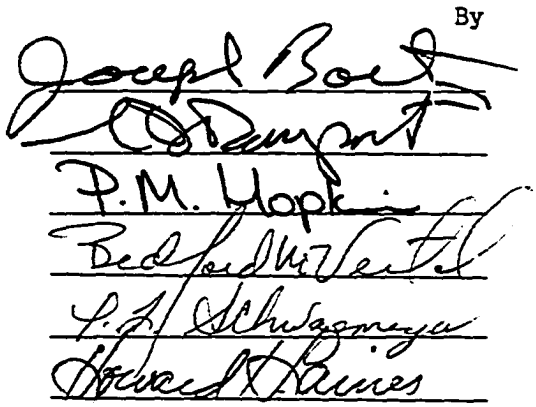

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

A series of experiments was performed to investigate various behavioral and neurophysiological aspects of the Novelty Response (NR) of pulse-type weakly electric fish. The NR is a reflexive behavior in which the fish momentarily increases the rate of Electric Organ Discharges (EODs) in response to a novel stimulus. The first set of experiments (Chap.I) examined the effects of stimulus modality and intensity, as well as curariform-drug immobilization on the magnitude and habituation of the NR in three gymnotiform species: Hypopomus artedi, H. occidentalis, and Gymnotus carapo. H. artedi gave higher responses and habituated more slowly to electric and light stimuli than to sound stimuli. H. occidentalis made higher responses and habituated more slowly to electric stimuli than to light or sound stimuli, and G. carapo made higher responses and habituated more slowly to sound stimuli than to electric or light stimuli. All three species habituated to electric stimuli at about the same rates. Stimulus intensity changes and immobilization affected response sizes, but not habituation rates. A series of neurophysiological experiments examined the Torus Semicircularis (TS) and other electrosensory-processing regions of the fish brain for neural activity that could be associated with the behavioral NR (Chap.II). Event-related potentials (EPs) and single unit recordings indicated that some cells of the TS respond only to electrosensory stimuli that are presented at intervals that are longer than the fish's EOD interval length, i.e., the activity is novelty related. The magnitude of this novelty-related activity in the

TS can be correlated with certain features of the behavioral NR, and may represent the activity of electrosensory novelty detectors that are necessary for recognition of sensory changes and the elicitation of the appropriate behavioral response. Bilateral lesions of the Torus Semicircularis (TS) did abolish electrosensory induced NRs, but not those elicited by light or sound stimuli (Chap.III). Gross ablation of the forebrain or the optic tectum of these fish did not eliminate the NR or its habituation.

THE NOVELTY RESPONSE OF PULSE-TYPE WEAKLY ELECTRIC FISH: A NEUROETHOLOGICAL STUDY

CHAPTER I

BEHAVIORAL STUDIES

Introduction

South American gymnotiform fish are weakly electric; they generate an electric field in the water by means of a neural or muscular electric organ. Weakly electric fish can be classified according to the nature of their Electric Organ Discharge (EOD). "Wave" species produce a continuous quasi-sinusoidal voltage and "pulse" species produce a series of voltage pulses separated by relatively longer inter-pulse intervals. The animal can evaluate the local intensity of the trans-epidermal EOD field voltage by means of specialized receptors (electroreceptors). Objects with conductivities differing from that of the background cause field distortions that can be measured by the receptor system and evaluated by higher brain centers (Heiligenberg 1977). This enables the animal to navigate and locate prey in the absence of other cues (Lissman & Machin 1958).

Pulse-species individuals commonly raise their EOD rate (i.e., decrease the inter-pulse interval) in novel situations, presumably to increase their temporal resolution (Heiligenberg 1977). This "Novelty Response" (NR) seems to be an example of Sokolov's "orienting reflex" (1960). The NR will occur in response to changes in electroreceptive afference (Larimer & MacDonald 1968), such as are caused by objects in the environment or by the EODs of other weakly electric fish. The recognition of such distortions of the electric field must result from recognition of changes in receptor afferent information. This implies that there exists a mechanism by which incoming information is compared with some centralized template or memory. Heiligenberg (1980) suggests that a response to local field distortions results from some integration of messages from successive EOD's, and notes that the nature of this central template which represents steady state spatial and temporal patterns of sensory afferences is of particular interest. To date, the nature of this template is unknown. In addition to electrosensory stimuli, visual and acoustic stimuli are effective in eliciting the NR (Lissman 1961, Hopkins and Heiligenberg 1978). Whether different sensory modalities have independent templates or not, and how these might interact to determine the fish's behavior are not known. Differences among species' response sizes and habituation to various stimulus types might indicate underlying differences in the behavioral ecology and neurophysiological processing among the species examined. The reliability of NR elicitation, and its relative ease of measurement make this simple behavior particularly amenable to experimentation. This chapter describes the results of an

investigation into the effects of various stimulus modalities and intensities on the magnitude and habituation of the NR in several gymnotiform species.

Methods

Procedures

Three species of gymnotiform fish were used as subjects: Hypopomus artedi (n=29), H. occidentalis (n=14), and Gymnotus carapo (n=14). Fish were obtained from commercial suppliers and maintained in a controlled environment (14:10 L/D cycle). Fish were generally tested in the light phase of the L/D cycle, but sometimes experiments ran into the dark phase. There were no apparent differences between results from experiments conducted in the two phases. Before testing, the fish's EOD was measured through electrodes placed near the head and tail and fed to a Grass P-15 pre-amplifier with filters open and a gain of 10. This signal was analog-to-digital converted at 40 KHz and Fourier analyzed. The EOD of a pulse-type fish has a broad power spectrum, and this was analyzed to determine the EOD's dominant frequency. Species' identifications were based on these EOD waveform analyses, and on morphological characteristics (Schultz 1949, Hoedeman 1962). Nine H. artedi and six G. carapo were tested intact; all others were tested after being immobilized with intramuscular injections of gallamine triethiodide solution (Flaxedil: 0.01-0.02 mg), a curariform drug. Intact animals were held loosely in a gauze sling; immobilized animals were held loosely in a sponge-lined clamp and their gills were aerated with bath water (ca. 0.2 ml/s) by a tube inserted into the mouth. The temperature of the water bath used in all experiments was held constant at 25°C (+/- 0.5), pH = 6 - 7, and resistivity ranged from 0.7 - 10.0 kohm·cm (mean = 1.8 +/- 0.06 kohm·cm).

The behavior studied consists of changes in the intervals between

successive EODs. In intact fish, the EODs were monitored with a pair of wires placed near the head and tail of the subject and delivered to a triggering device (Fig.1-1). In immobilized fish the EOD no longer occurs, but the spinal Pacemaker-related Signal (PS), which in intact fish triggers the EOD (Bennett 1968), is still present after Flaxedil injections. The PS was monitored with a suction electrode placed around the fish's tail, amplified, and delivered to the triggering device. The trigger pulse caused by the EOD or PS was sent to an on-line computer, which counted the signals and measured the intervals between (10 us resolution). When an artificial EOD was used with immobilized subjects, the PS triggered a single cycle of a modified sine wave of the appropriate frequency and intensity. This signal was delivered to a pair of electrodes via a stimulus isolation unit; one electrode was placed in the mouth of the fish through the aeration tube, the other formed a loop around the fish's tail. This configuration produced a reasonable mimic of the natural EOD occurring at a rate selected by the fish.

Subjects were tested in a series of experiments that measured the NRs that occurred when the fish was presented with a series of electrosensory, visual, or acoustic stimuli. These experiments were designed to assess the effects of Flaxedil immobilization, stimulus intensity, and stimulus modality on the magnitude and habituation of the NR. Most fish were tested with each of the three stimulus modalities, usually more than once. Thresholds for each subject were determined before any experimental trials began. Each stimulus modality was tested at three different intensities: 1X, 2X, and 4X

threshold. (H. artedi had high thresholds to electrosensory stimuli and thus could be tested at only 1X and 2X threshold intensities.) The order of stimulus modality and intensity presentation was informally randomized to avoid any systematic production of stimulus generalization or response fatigue for particular modality/intensity combinations.

Each experiment consisted of 32 pairs of trials. Each pair consisted of a stimulus trial preceded by a control trial (in which no stimulus was presented). A total of 64 EOD or PS intervals were measured in each trial (Fig.1-2). The first 16 intervals of a trial were averaged, yielding a baseline interval; the sum of the differences between each of the sixteen intervals following the stimulus presentation (intervals 33 - 48) and the baseline interval was calculated and used as a response measure. Thus, an EOD acceleration, such as occurs during the NR, results in a negative sum of interval differences. A larger negative sum indicates a greater EOD acceleration. As an additional control measure, the sum of the differences between each of the sixteen EOD or PS intervals preceding the stimulus (intervals 17 - 32) and the baseline interval was also calculated. Thus each response measure has three corresponding control measures: the pre-stimulus sum of interval differences for both the control and the stimulus trials, and the control response measure. The interval between the initiation of successive trials was selected by the experimenter and controlled by the computer. The interval selected was close to the minimum allowed by the EOD/PS interval length (i.e., there are 64 intervals in a trial, so the interval between trials must

be greater than 64 X EOD interval length). The minimum trial interval used was 5 s, and the greatest trial interval generally did not exceed 20 s. Thus, stimuli were presented every 10 - 40 s, depending on the resting EOD rate (median trial interval = 8 s). The control and response measures for each trial were then stored for later analysis.

Stimuli

Two different electrosensory stimuli were used: a short-circuit within the animal's electric field, which mimics the presence of an object, and a transversely applied square-wave pulse, which mimics the presence of another weakly-electric fish (Bullock 1982). The short-circuit stimulus was produced by a transistor switch, to which were connected a pair of electrodes in series with a potentiometer. With the switch open, the resistance between the electrodes is very high, and no effective stimulus is present. When the switch is closed, the resistance between the electrode tips decreases, the exact value depending on the conductivity of the water and the potentiometer setting. The resistance across the closed switch was approx. 0.15 kohm. Short-circuit stimulus intensity was adjusted by changing the potentiometer setting and measuring the resistance across the stimulus electrodes (range: 4.0 - 0.1 Mohm): a lower resistance represents a stronger stimulus. Short-circuit durations were long enough to ensure the occurrence of at least one EOD/PS cycle during stimulus presentation. Square-wave stimuli were generated by means of a Grass S-44 stimulator and a stimulus isolation unit, the output of which was delivered via two coiled wires on either side of the water bath.

Square-wave intensities were measured by monitoring the output of a paired field-measuring electrode (placed in the bath perpendicular to the long axis of the fish about 1 cm from the pectoral region) on an oscilloscope. Square-wave stimulus duration was 0.1 ms.

Visual stimuli were produced by a Grass PS-22 photic stimulator aimed at the bath. The intensity of the light flash was controlled by an intensity switch on the stimulator. Auditory stimuli were generated by sending a stimulus pulse to a Grass S-9 stimulator that was set to deliver a 0.5V, 200ms pulse to an audio-amp/speaker unit. The intensity of the sound stimulus was controlled by an intensity control dial on the speaker. The speaker was mounted approx. 12 cm from the edge of the tank. The intensity of the sound was measured by placing a microphone above the fish and reading the output (in mV) on an oscilloscope.

Data Analysis

Preliminary observations indicated that the largest response to a stimulus series occurs within the first six presentations of the stimulus, and also that the responses fully habituate by the end of the series. Therefore, two measures of NR size were used in the analyses: Rmax, the largest of the first six responses in an experiment (not always the first response if sensitization occurs), and Rt, the habituated response level in an experiment (mean of last five stimulus trial responses). These values were averaged for a given fish if more than one experiment with a given modality/intensity was performed. Treatment effects (Flaxedil immobilization, stimulus intensity,

stimulus modality, and subject species) were tested with a general linear matrix model, which is a modified ANOVA for unequal cell sizes (SAS 1982).

Habituation, a reversable decline in response size to repeated stimulation, was assessed in two ways. The amount of habituation was calculated as the ratio of Rt/Rmax. Because this measure indicates the proportion of the maximum response that remains after habituation, a larger value indicates a smaller amount of habituation. This is a measure of how much the response has declined, but not how rapidly that amount of decline occurred. To assess the rate of habituation, a time constant (Tau) of the response decline pattern was calculated from the best-fit curve fitted to a plot of response size (normalized to a standard range) as a function of stimulus presentation number (Appendix A). This value represents the stimulus number (or fraction thereof) at which the response has declined by 63%. A larger value indicates a slower habituation rate. Treatment effects on habituation were also tested with GLM analyses. When a main treatment was found to be non-significant, data from cells within that group were pooled, and the data were retested with lower-order analyses. Likewise, when a significant interaction between main treatments occurred, these groups were tested separately. Duncan's multiple range test (SAS 1982) was used for a posteriori comparisons of means for all measures.

Results

No statistical differences in response sizes or habituation rates or amounts between the two types of electrosensory stimuli were found; thus the data from both short-circuit and square-wave stimuli are pooled in the analyses. Some fish would not give reliable responses to a particular stimulus; therefore, cell sample sizes are not equal in all cases.

Significant correlations between a subject's response sizes and its EOD resting interval were found. To eliminate this correlation, response size data were normalized by dividing a subject's response size measures by its resting EOD interval. Both normalized response size measures (Rmax,Rt) were significantly correlated with each other ($r = 0.79$, $n = 346$, $P<0.0001$); Rt was significantly correlated with the amount of habituation ($r = 0.38$, $n = 346$, $P<0.0001$), but Rmax was not ($r = -0.05$, $n = 346$, $P=0.33$). Thus the amount of habituation may depend on the habituated response level, but not the initial or maximal response level. Habituation rate (Tau) did not correlate with any response size measures, or with the amount of habituation.

Main Treatment Effects

1. Flaxedil

Immobilization with this drug generally caused higher maximal responses (Rmax) to light stimuli in both *H. artedi* and *G. carapo*, particularly at threshold (1X) intensities (Fig.1-3). Both Rmax and habituated response sizes (Rt) to sound stimuli were higher for immobilized than intact *H. artedi* (Figs.1-3,4). Flaxedil did not

change response sizes to electric stimuli, with the exception of increasing G. carapo responses to threshold (1X) electric stimuli. The effects of Flaxedil on G. carapo responses to light and sound stimuli must be viewed with caution. Sample sizes for injected subjects were low, especially at higher intensities. In addition, one immobilized individual made an exceedingly high response to the threshold light stimulus (Rmax = 32.). In no case were the mean normalized responses of immobilized fish significantly lower than those of intact fish; typically, mean responses were higher, especially at lower intensities.

Relative to intact fish of the same species, the amount of habituation was significantly less for immobilized G. carapo responses to electric stimuli ($P<0.003$), and for immobilized H. artedi responses to sound stimuli ($P<0.002$: Fig.1-5). This was due mainly to higher habituated (Rt) responses in these cases. The amount of habituation did not always decrease when habituated responses were greater; with G. carapo responses to light stimuli, both Rmax and Rt increased such that the amount of habituation was the same. The rate of habituation (Tau) was not significantly affected by Flaxedil immobilization in any case. The data for both intact and immobilized fish were therefore pooled by species for finer analyses of effects on habituation rates.

2. Intensity

In general, both Rmax and Rt increased with increased stimulus intensity, though the effect was statistically significant in only a few cases (Table 1-1,2), such as intact G. carapo responses to electric stimuli (Rmax, $P<0.004$; Rt, $P<0.002$). Habituated response levels (Rt) showed more consistent intensity effects overall. The amount of

habituation was significantly lowered ($P<0.025$) by increased stimulus intensity only for intact G. carapo responses to electric stimuli; a nearly significant ($P<0.055$) increase in habituation amount with light stimuli also occurred (Table 1-3). The rate of habituation was not affected significantly by stimulus intensity, and thus the data for all intensities were pooled by species for further analyses.

3. Modality

Each species showed a 'preference' for one or two of the stimulus modalities over the other(s). 'Preference' is defined here as greater normalized response magnitudes. H. occidentalis made consistently higher responses to electric stimuli than to light or sound stimuli (Figs.1-3,4 & Tables 1-1,2). For H. artedi both response measures were lower for sound stimuli than for light or electric stimuli in intact fish. In immobilized H. artedi normalized Rt responses were greater for light and sound stimuli than for electric stimuli, but Rmax values did not differ among stimulus modalities. Intact G. carapo exhibited greater Rmax and Rt responses to sound than light or electric stimuli. When immobilized, the responses to sound stimuli were diminished. The maximal responses (Rmax), but not habituated responses (Rt) of immobilized G. carapo were greater to light stimuli than to electric stimuli (Fig.1-3).

H. artedi habituated less to light stimuli than to electric or sound stimuli when intact, but habituated less to sound when immobilized (Fig.1-5). Intact G. carapo habituated less to sound stimuli, which elicited greater responses (Rmax & Rt). But for immobilized G. carapo light stimuli caused greater responses than

electric or sound stimuli, and more, not less habituation. The amount of habituation of H. occidentalis did not differ among the three stimulus modalities.

The 'preference' for a given modality can also be illustrated by differences in habituation rates (Tau) to the three stimulus modalities (Fig.1-6, Table 1-4). H. artedi habituated to sound stimuli more quickly than to light or electric stimuli (sound stimuli evoked lower responses in intact H. artedi). G. carapo habituated more slowly to sound stimuli, which elicited the biggest responses in intact fish of this species, than to light or electric stimuli. H. occidentalis, which made the biggest responses to electric stimuli, also habituated more slowly to electric stimuli than to light or sound stimuli. Threshold stimulus intensities did not vary systematically among the species. For any stimulus modality the variation in intra-specific intensity thresholds was as great as the inter-specific variation.

4. Species

Normalized H. occidentalis responses (Rmax, but not Rt) to electric stimuli were greater than those of G. carapo and H. artedi, the latter having the lowest Rt values for electric stimuli. Light stimuli evoked the highest responses from immobilized G. carapo and the lowest responses from H. occidentalis (both Rmax & Rt). Mean normalized responses to sound stimuli were lowest for intact H. artedi, and greatest for intact G. carapo. The amount of habituation to electric stimuli did not differ among the species tested. G. carapo habituated least of all species when immobilized and most when intact. H. artedi habituated least and G. carapo most to light stimuli,

regardless of injection state. Intact H. artedi and H. occidentalis habituated more to sound stimuli than G. carapo or immobilized H. artedi. The rate of habituation to electric stimuli did not differ among species. H. artedi habituated slower to light stimuli than did the other species. For sound stimuli, G. carapo habituated more slowly than either Hypopomus species, which did not differ from each other.

Interactions

As mentioned above, Flaxedil caused increased responses to light stimuli, particularly at lower intensities. As a consequence, intensity effects were diminished with immobilization, due to a 'ceiling effect'. However, the 'ceiling effect' cannot explain the lack of intensity effects on H. occidentalis responses to light stimuli, as these responses were not nearly maximal for these fish (Figs.1-3,4). It is interesting that for all three species, there were no intensity effects on responses to light stimuli when Flaxedil-immobilized.

Because Flaxedil caused increased responses to light in G. carapo and H. artedi, the apparent 'preference' of these species for a given modality was different between intact and immobilized fish of the same species. Relative to intact fish, Flaxedil lowered the responses of G. carapo to sound and increased the responses to light stimuli. Likewise, intact H. artedi made the lowest responses to sound stimuli, but when immobilized their responses to sound were as large as the responses to light stimuli. Thus immobilized H. artedi show no clear 'preference' for any stimulus modality.

Other Analyses

A few experiments were performed to see if the time course of the NR was the same for all stimulus modalities and species, i.e., is the response the same? The data collection scheme was essentially identical to that in the above set of experiments, except that only one stimulus trial was given, and the actual EOD/PS intervals were stored on disk. With these I could analyze the pattern of EOD acceleration/deceleration that occurred after stimulus presentation. The results of this analysis indicate that the response was essentially the same with any of the three stimulus modalities tested. Differences in time course of the response depended on the magnitude of the response. A larger response would have a greater initial acceleration and a more exponential deceleration than a smaller response. Thus a response of given magnitude to an electric stimulus would not differ from a response of the same magnitude to a light or sound stimulus. I cannot unequivocally state whether there are species differences in the time course of the NR. Individual variation appeared to be as large as species variation. None of these additional analyses were evaluated statistically.

Discussion

The Novelty Response (NR) of pulse-type fish was found to habituate to electrosensory stimuli as well as to visual and auditory stimuli. Habituation may be simply defined as a reversible decline in response to repeated stimulation, and has been described as the most elementary and ubiquitous form of behavioral plasticity (Thompson and Spencer 1966, Pinsker et al 1970). Habituation of a wide variety of behavioral and neuronal responses has been studied in rats (Griffin and Pearson 1967, Hoffman and Stitt 1969, Buckland et al 1969, Korn and Moyer 1966), cats (Kileny et al 1980), rabbits (Horn 1967), birds (Petrinovich and Peeke 1973, Shalter 1975), non-electric fish (Russell 1967, Peeke et al 1979), turtles (Hayes et al 1968), frogs (Ewert and Ingle 1971, Farel et al 1973, Kimble and Ray 1965, Megela and Capranica 1983), polychaetes (Clark 1960, Dyal and Hetherington 1968), molluscs (Pinsker et al 1970, Castellucci et al 1970, Kandel and Schwartz 1982), and even protists (Wood 1970, Osborn et al 1973). Habituation is one of the means by which an animal can adapt to its environment and to changes in the environment (Griffin 1970). Assuming that the EOD rate of a species is determined and fixed by evolution and structure, an increase in EOD rate could present some costs to the fish, such as increased metabolic demand by the electric organ system or increased probability of signal interference; otherwise they may be expected to always fire at a faster rate if this acceleration does indeed increase the temporal resolution of incoming information. Thus, unnecessary NRs could be wasteful, and the habituation of the NR to repetitive, non-noxious stimuli could be adaptive.

The decline in response magnitudes seen in this series of experiments fits many of the criteria for establishing a phenomenon as habituation (Thompson and Spencer 1966). The response decline is specific to the stimulus modality tested, as can be shown by using a different stimulus modality to elicit a relatively large response after the putative habituation to the test stimulus has occurred (Fig.1-7), or by the observation that after a habituation experiment using one modality, the initial responses to the next experiment using a different modality are of normal (i.e., non-habituated) magnitudes. Spontaneous recovery of the NR to a specific stimulus type is indicated by observing increased NR magnitude following the omission of a stimulus during a series of stimulus trials (Fig.1-7). The decline in response is not due to fatigue of the neural elements involved with electrosensory processing; neurophysiological results (Chap.II) indicate no reduction in neural activity to stimuli presented under the paradigm used here.

The relationship between habituation and the frequency of stimulation was not tested in these experiments, nor was the effect of sub-zero habituation training assessed. Intensity effects on habituation were tested. Thompson and Spencer (1966) suggest that stronger stimuli should produce less rapid and/or a lower amount of habituation than weaker stimuli. In the present study habituation rates were not significantly affected by stimulus intensity changes. Some of the studies of habituation that have examined stimulus intensity effects (e.g., Davis and Wagner 1968, Wicklegren 1967) used different intensity training- and test-stimuli. This paradigm is

actually a test for stimulus generalization and not intensity effects, because a condition of habituation training is repetition of a stimulus, the properties of which remain unchanged during repeated presentations (Thompson et al 1973). In many of these studies that use various stimulus intensities, the effects of intensity on habituation rates are abolished or minimized if one uses relative response decline measures instead of absolute response sizes (Hinde 1970). In the present study the amount of habituation usually was not affected significantly by increased stimulus intensity, but when effects did occur the amount of relative habituation was less at higher intensities (Fig.1-5). Stimulus generalization of habituation was not formally tested in this study, but I observed that responses to a short-circuit stimulus were often reduced by prior exposure to a square-wave stimulus, and vice-versa. Two additional characteristics of Thompson and Spencer's model deal with what has been traditionally called 'dishabituation'. Groves and Thompson (1970) present evidence to support a dual-process theory of habituation that suggests that what has been called 'dishabituation' is actually a second, independent process they call 'sensitization'. In essence, an increased response following a 'dishabituating' stimulus is the result of a process that is distinct from habituation, and thus the ability to 'dishabituate' a response should no longer be considered a criterion for habituation. In the present study attempts to 'dishabituate' classically the NR generally failed. However, sensitization of the response often occurred in the first several trials of an experiment, resulting in a pattern of response increases followed by response decreases. This

sensitization pattern usually occurred in the earlier experiments of a day's testing of a given fish. Similar patterns of sensitization followed by habituation are often reported in habituation studies (see Groves and Thompson 1973, Thompson et al 1973 for reviews).

Habituation of the NR to electrosensory stimuli has been reported previously (e.g., Lissman 1958). Heiligenberg (1980) also noted that habituation of the NR will occur if stimuli are presented too closely in time to one another. He notes that curarized animals will habituate more readily than intact fish. In the present study this was not the case. The differences probably lie in differences in assessment of habituation rate, and generalizations about habituation rates can be misleading if different methods of calculating the rates are employed (Hinde 1970). Flaxedil is a curariform drug that competes with acetylcholine at cholinergic receptor sites (Waser 1961). It is a long-lasting, non-depolarizing substance that can increase heart rate and blood pressure (AMA 1973). In this study Flaxedil caused a general increase in response sizes, especially habituated response levels (Rt), and consequently often reduced the amount (but not the rate) of habituation. The enhanced responses seen in this study were generally to non-electroreceptive stimuli. Cholinergic drugs can have both inhibitory and excitatory effects on arousal and reticular formation responses (Kent 1973), and various effects on behavior and sensory perception (Seiden and Dykstra 1977). Paralysis may have significantly reduced the amount of sensory afference that the fish normally receives while mobile, and because of this partial sensory deprivation the immobilized fish were somehow 'primed' to respond. Habituation rates

of the NR to all stimulus types were not affected by this drug.

In this study I found that the NR varies in magnitude as a function of stimulus modality and intensity. As would be expected of a reflex response (Kandel 1976), increased suprathreshold stimulus intensity caused increased response sizes, up to a point. Species show characteristic response levels to various stimulus types. None of the few studies on the ecology of these fish (e.g., Lissman 1961, Hopkins and Heiligenberg 1978) give any clues as to why the species differences seen in this study might occur, e.g., why G. carapo makes larger NRs to sound stimuli. G. carapo are facultative aerial respirators (Liem et al 1984), but whether they use the air bladder for acoustic communication as do some other fish is unknown. This species is very aggressive, and may develop relatively complex social systems (Black-Cleworth 1970). Hypopomus are not as aggressive, and feed on smaller organisms (Ellis 1913) than do Gymnotus, which have larger mouths.

Rate may be the preferred measure of habituation and its associated processes. Habituation rate was determined in this study to be independent of such factors as stimulus intensity and presence or absence of Flaxedil. Under a variety of conditions the habituation rate of a fish appears to reflect faithfully that species' 'preference' for given stimulus modalities. The amount of habituation and other measures that directly depend on response size are influenced by stimulus intensity and immobilization state, and are apparently indicative of processes different from those assessed by habituation rate.

Even though the different species responded in various degrees to electrical stimuli and showed different amounts of habituation, the rate of habituation to electrosensory stimuli was the same (statistically) in all species. This suggests that the central processing of electrosensory information is the same (relatively) in all gymnotiform pulse species. This evolutionary conservation of learning may result from the relatively restricted variation in effective electrosensory stimuli, i.e., there are probably fewer parametric variables associated with electrosensory stimuli than with visual or acoustico-lateral stimuli. Neurophysiological results indicate that similar neural phenomena associated with novelty detection occur in all the species studied here (Chap.II).

Sudden increases of EOD rate followed by decreases have been reported in gymnotiform fish previously (Black-Cleworth 1970, Hopkins and Heiligenberg 1978). They occur in a variety of behavioral contexts, including predation, locomotion, and feeding (Black-Cleworth 1970), and possibly serve a communicative function (Westby 1975). The NR described here may be the same as these EOD rate-changing behaviors, or at the least represents a particular subset of them that occurs in certain contexts. The value of increased EOD rate apparently lies in increased environmental sampling. Heiligenberg (1980) demonstrated that the detection of electrosensory novelties is improved at higher EOD rates.

Like habituation, the NR requires the storage of information against which incoming signals are matched. The detection of changes in sensory afference may occur by excitation of a population of neurons

different from those excited by the previous state of afference, or the change in input might excite neurons that are no longer firing in response to the previous state of afference, i.e., have habituated to the previous state. It may thus be postulated that the novelty response utilizes an habituating pool of neurons. In these experiments the fish quickly adapted to steady afferences and responded to changes in that state. If an electrosensory stimulus of very long duration was presented, the fish responded to the onset of the stimulus, returned to some baseline EOD rate, and then usually responded to the offset of the stimulus. The response to both the 'on' and 'off' stimulus was the same, and habituation to the two stimulus 'types' was the same, implying similar or identical processing of the stimulus novelty in both cases. Heiligenberg (1980) reports similar results, and as a consequence suggests that the critical feature of an electrosensory novelty is that it causes a change in the electroreceptive afferences which prior to the stimulus were constant for some minimal time. The NR to electrosensory stimuli may thus be elicited by a generalized electrosensory difference detector. Gymnotus carapo are sensitive to electrical signals of almost any frequency (Watson and Bastian 1979). The NR occurs to electroreceptive stimuli even in the absence of an EOD or EOD-mimic (Chap.II). The next chapter reports on electrosensory neural responses, the activity of which is related to the degree of stimulus novelty. These general electrosensory "novelty detectors" may be responsible for the elicitation of the NR to electrosensory stimuli.

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Table legends

Table 1-1: Probability values for effects on normalized maximal response sizes (Rmax). Body of table indicates significance of intensity effects within that cell. Values in columns labelled Flax indicate significance of Flaxedil immobilization effects on response sizes within species. Far right-hand column, upper value indicates significance of modality differences within that species (all intensities pooled), lower value indicates significance of intensity effects on that species (all modalities pooled). Bottom-most row, upper value indicates significance of species differences for that modality (all intensities pooled), lower value indicates significance of intensity effects for that modality (all species pooled).

Table 1-2: Probability values for significant effects on normalized habituated response sizes (Rt). Labels, columns, etc., identical to Table 1-1.

Table 1-3: Probability values for significant effects on amount of habituation (Rt/Rmax). Labels, columns,etc., identical to table 1-1.

Table 1-4: Probability values for significant effects on habituation rate (Tau). No significant effects of intensity or Flaxedil were found. Values thus represent significance of effects of stimulus modality (all intensities and Flaxedil state pooled) on each species (upper portion), and significance of species' differences within each modality (lower portion).

Table 1-1
Rmax

Species	Electric	Flax	Light	Flax	Sound	Flax	modality- all intensities intensity- all modalities
H.artedi -NO FLAX	0.18	0.64	0.16	0.58	0.26	0.18	0.10 0.03
H.artedi - FLAX	0.14		0.75		0.41		0.87 0.11
G.carapo -NO FLAX	0.004	0.17	0.06	0.003	0.12	0.37	0.04 0.0005
G.carapo - FLAX	0.96		0.68		0.04		0.06 0.43
H.occident. - FLAX	0.09	—	0.88	—	0.14	—	0.16 0.002
species- all intensities	0.65		0.0001		0.05		
intensity- all species	0.004		0.53		0.12		

Table 1-2
Rt

Species	Electric	Flax	Light	Flax	Sound	Flax	modality- all intensities intensity- all modalities
H.artedi -NO FLAX	0.09	0.46	0.03	0.90	0.26	0.07	0.001 0.0003
H.artedi - FLAX	0.80		0.96		0.43		0.56 0.24
G.carapo -NO FLAX	0.002	0.009	0.05	0.25	0.18	0.40	0.0001 0.0002
G.carapo - FLAX	0.86		0.77		0.08		0.82 0.36
H.occident. - FLAX	0.31	—	0.36	—	0.04	—	0.0009 0.21
species- all intensities	0.24		0.04		0.0001		
intensity- all species	0.01		0.11		0.006		

Table 1-3
Rt/Rmax

Species	Electric	Flax	Light	Flax	Sound	Flax	modality- all intensities intensity- all modalities
H.artedi -NO FLAX	0.72	0.76	0.86	0.04	0.69	0.002	0.006 0.22
H.artedi - FLAX	0.08		0.41		0.53		0.005 0.69
G.carapo -NO FLAX	0.025	0.003	0.06	0.67	0.30	0.21	0.0001 0.0005
G.carapo - FLAX	0.95		0.85		0.74		0.15 0.89
H.occident. - FLAX	0.16	—	0.21	—	0.66	—	0.51 0.14
species- all intensities	0.03		0.01		0.0006		
intensity- all species	0.17		0.42		0.08		

Table 1-4
habituation rate (Tau)

species	effect of modality
Hypopomus artedi	$P < 0.0005$
Hypopomus occidentalis	$P < 0.015$
Gymnotus carapo	$P < 0.03$

modality	effect of species
Electric	N.S. ($P = 0.91$)
Light	$P < 0.004$
Sound	$P < 0.0001$

Figure Legends

Fig.1-1 Basic features of the experimental apparatus. The fish's Electric Organ Discharge (EOD) or spinal Pacemaker-related Signal (PS) was recorded and amplified by a pre-amplifier, and sent to an oscilloscope and a triggering device. Trigger pulses caused by the EOD/PS were sent to the computer, which counted events and intervals between. The computer controlled triggering of the electric, light, and sound stimuli that were presented to the fish.

Fig.1-2 Examples of Novelty Response (NR) experimental trials . Points represent EOD/PS interval length (ordinate). Mean of first sixteen intervals is baseline interval. Intervals used for control and response measures indicated by labels. Electrosensory stimulus applied in **A** and **B**, no stimulus (control) in C.

Fig.1-3 Mean normalized maximal response (Rmax) sizes (ordinate) of 3 gymnotiform species to electric, light, and sound stimuli. Stimulus intensity indicated on abscissa (1X, 2X, & 4X threshold). NO FLAX = intact fish, FLAX = immobilized fish. Bars = +1 SE. Cells with only one replicate indicated (n = 1).

Fig.1-4 Mean normalized habituated response (Rt) sizes (ordinate) of 3 gymnotiform species to electric, light, and sound stimuli. Axes and labels same as Fig.1-3.

Fig.1-5 Mean amount of habituation (Rt/Rmax) of 3 gymnotiform species to electric, light, and sound stimuli. Axes and labels same as Fig.1-3.

Fig.1–6 Mean habituation rate (ordinate), given as time constant (Tau) of best-fit curve of 3 gymnotiform species to electric (E), light (L), and sound (S) stimuli (abscissa). Bars represent +1 SE.

Fig.1–7 Novelty Response size (non-normalized) to repeated electrosensory stimulus presentations (abscissa). Fish in this example habituated rapidly. Light stimulus added to electric stimuli #15 and #16 (arrow #1) caused increased response. Stimulus removed at #18 and #19 (arrow #2) caused partial recovery of response at stimuli #20 and #21. Light stimulus preceding (by a few ms) electric stimulus #23, and again at #28 (arrow #3) caused partial recovery of response that was smaller the second time.

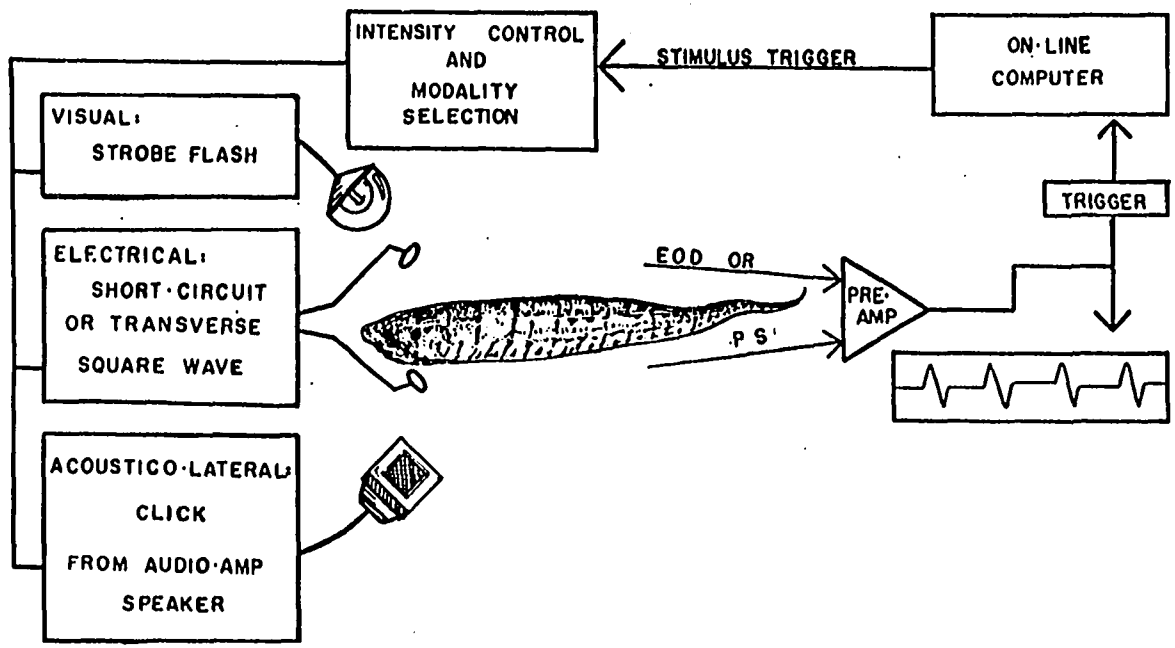
INTENSITY CONTROL AND MODALITY SELECTION
STIMULUS TRIGGER
ON-LINE COMPUTER
VISUAL: STROBE FLASH
TRIGGER
ELECTRICAL: SHORT-CIRCUIT OR TRANSVERSE SQUARE WAVE
EOD OR
PRE-AMP
P S
ACOUSTICO-LATERAL: CLICK FROM AUDIO-AMP SPEAKER

Fig. 1-1

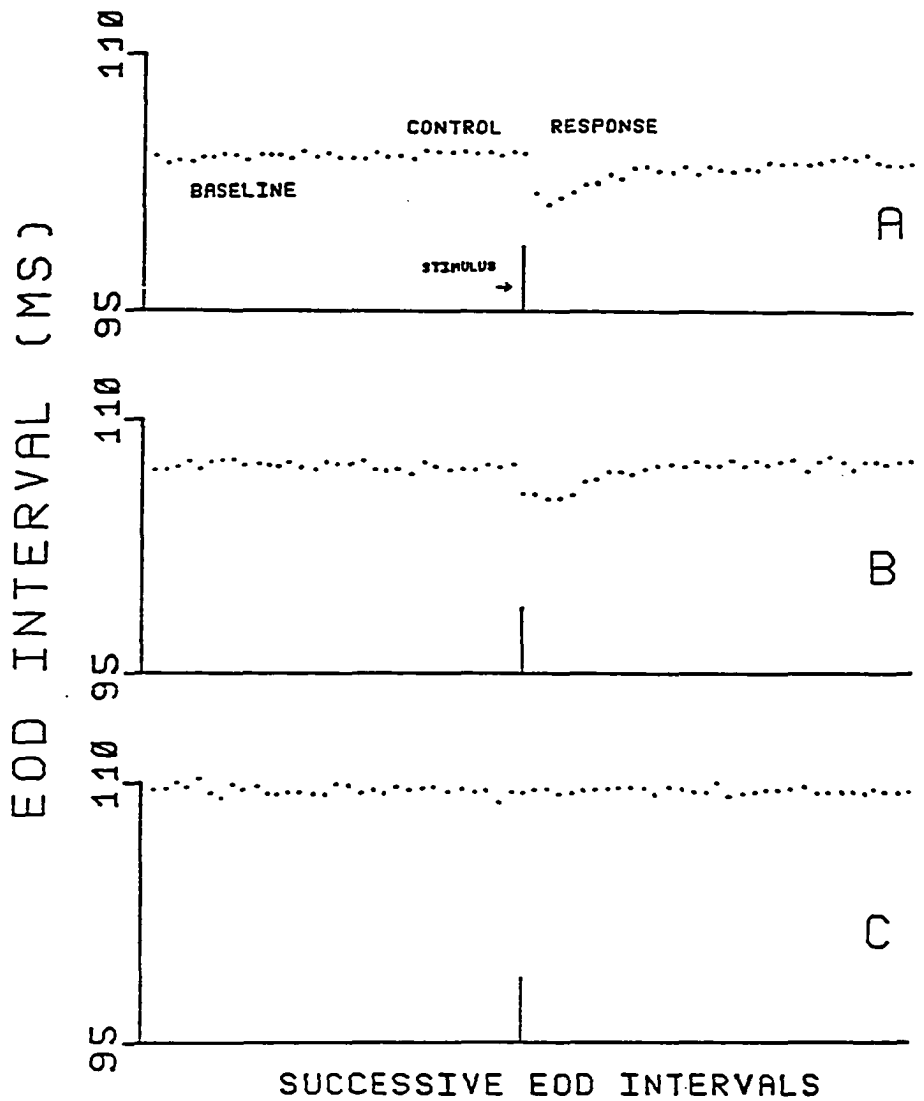
EOD INTERVAL (MS)
110
95
CONTROL
RESPONSE
BASELINE
STIMULUS
A
B
C
SUCCESSIVE EOD INTERVALS

Fig.1-2

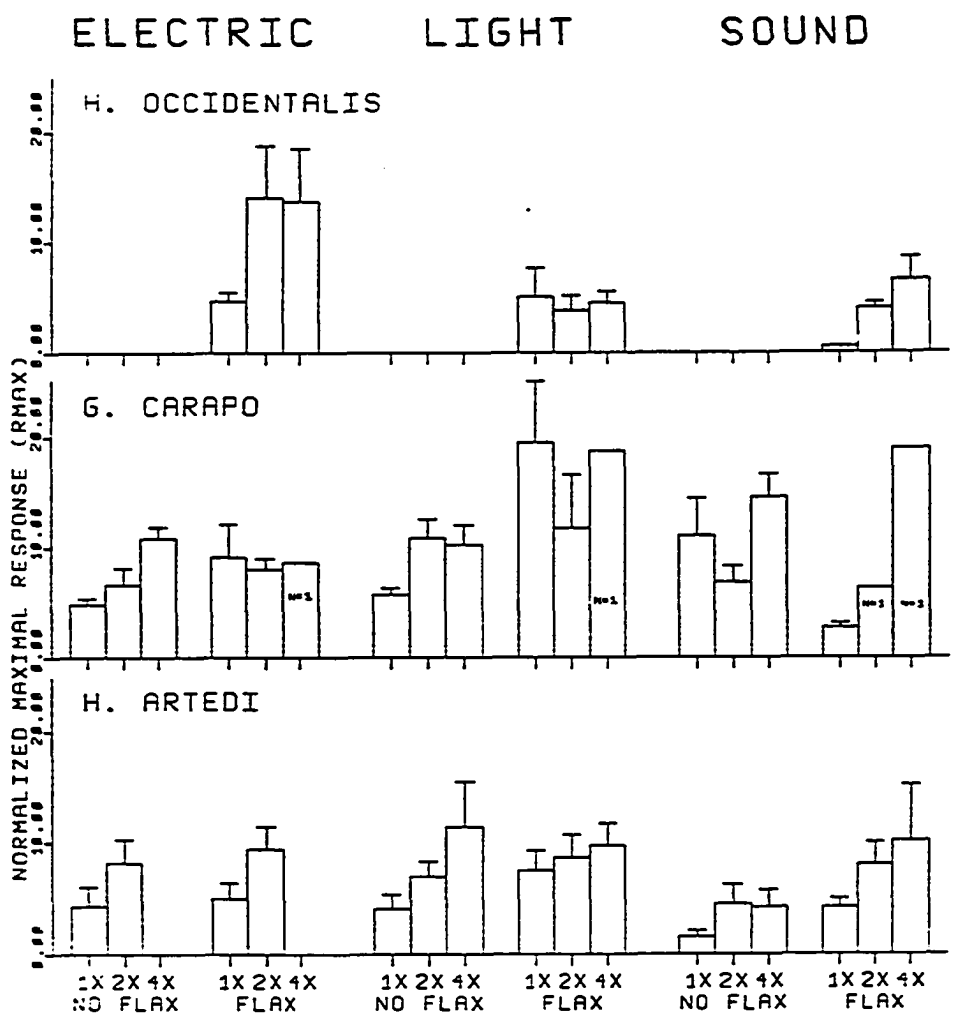
ELECTRIC
LIGHT
SOUND
H. OCCIDENTALIS
G. CARAPO
H. ARTEDI
NORMALIZED MAXIMAL RESPONSE (RMAX)
1X 2X 4X
NO FLAX
1X 2X 4X
FLAX
1X 2X 4X
NO FLAX
1X 2X 4X
FLAX
1X 2X 4X
NO FLAX
1X 2X 4X
FLAX

Fig.1-3

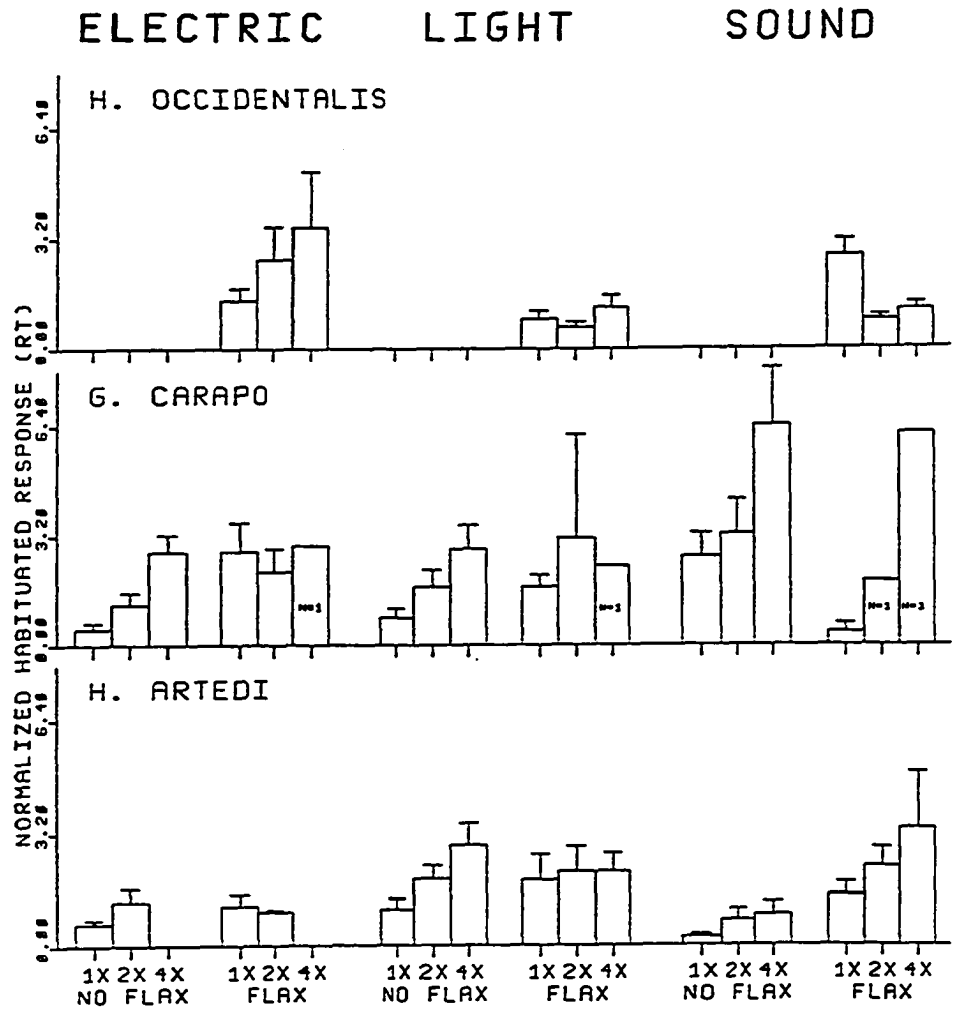
ELECTRIC
LIGHT
SOUND
H. OCCIDENTALIS
G. CARAPO
H. ARTEDI
NORMALIZED HABITUATED RESPONSE (RT)
1X 2X 4X
NO FLAX
1X 2X 4X
FLAX
1X 2X 4X
NO FLAX
1X 2X 4X
FLAX
1X 2X 4X
NO FLAX
1X 2X 4X
FLAX

Fig.1-4

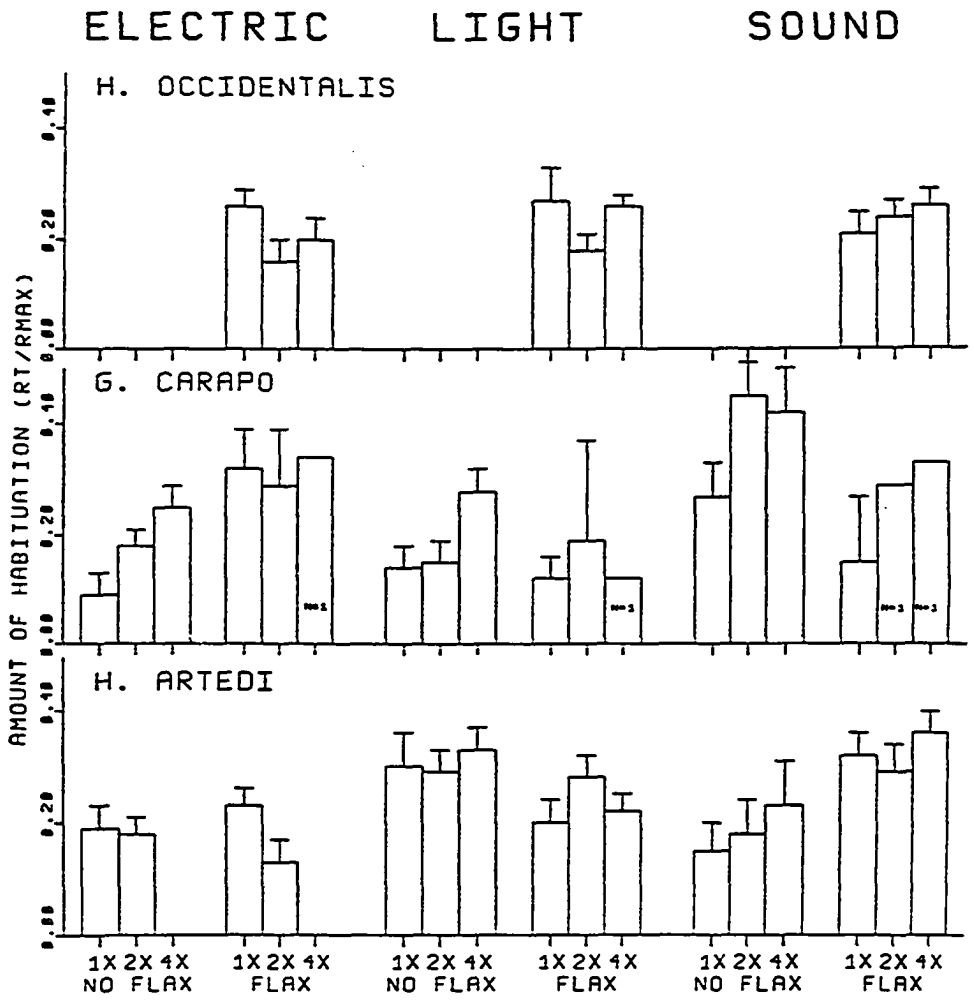
ELECTRIC
LIGHT
SOUND
H. OCCIDENTALIS
G. CARAPO
H. ARTEDI
AMOUNT OF HABITUATION (RT/RMAX)
1X 2X 4X
NO FLAX
1X 2X 4X
FLAX
1X 2X 4X
NO FLAX
1X 2X 4X
FLAX
1X 2X 4X
NO FLAX
1X 2X 4X
FLAX

Fig.1-5

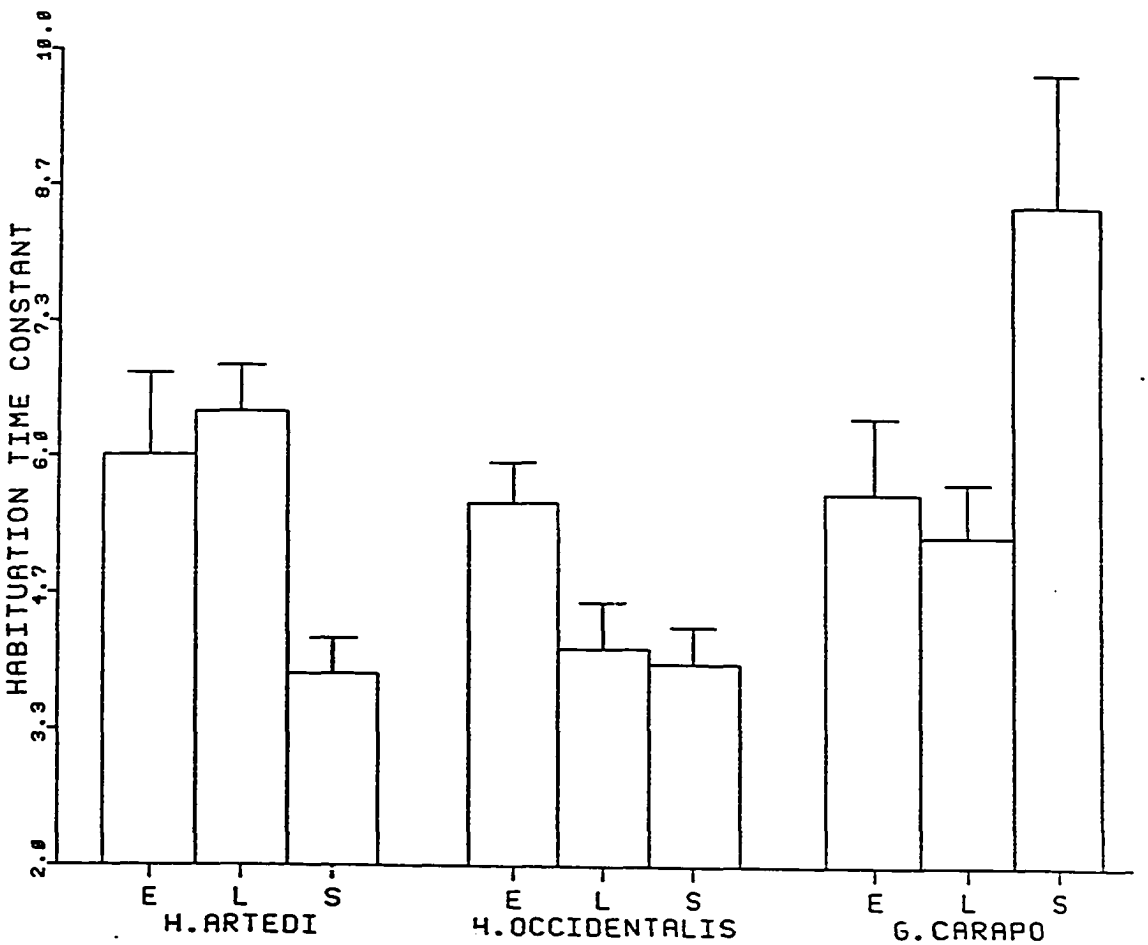
HABITUATION TIME CONSTANT
10.0
8.7
7.3
6.0
4.7
3.3
2.0
E
L
S
H.ARTEDI
E
L
S
H.OCCIDENTALIS
E
L
S
G.CARAPO

Fig.1-6

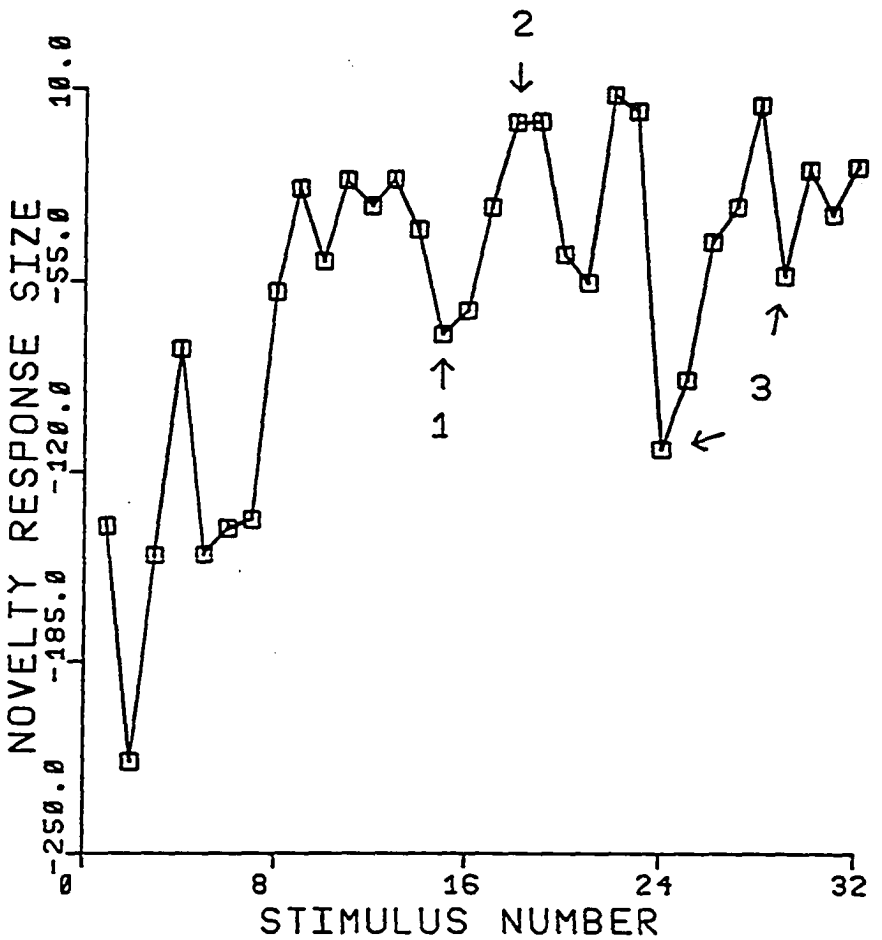
NOVELTY RESPONSE SIZE
10.0
-55.0
-120.0
-185.0
-250.0
0
8
16
24
32
STIMULUS NUMBER
1
2
3

Fig.1-7

Appendix A

CALCULATION OF HABITUATION RATE

Plots of response size in a given stimulus trial vs trial number for a given experiment indicated a possible exponential relationship between these parameters; various equations were tested to fit this relationship, and one equation that approximates the curve is:

$E(J) = (Rmax - Rt)e^{(-X)(J - 1)} + Rt$, where

E(J) = the theoretical point on the fitted curve,

Rmax = the largest of the first six responses in an experiment,

Rt = the baseline level of response in an experiment (= mean of last 5 responses),

X = an exponent related to the time constant of the curve, and

J = the trial number.

Because the novelty response is a decrease in the EOD interval, the responses measured have negative values. A computer program determines X via an algorithm that tries a value of X (beginning with 0.1), finds the differences between E(J) and R(J)(actual data values), computes the sum of squares of the difference and then tries a new value of X. The program continues searching for the minimum sum of squared differences by changing X until the smallest difference is found. The value of X used to generate this smallest difference represents the exponent of the best-fit curve. A small value X indicates slow habituation, a large X rapid habituation. The exponent X was used to calculate a time constant for the curve, TAU, which indicates the number of stimulus trials occuring before the response

declines by 63% of the maximum. After using this curve fitting equation for data from several sets of experiments, I determined that it was quite sensitive to Rmax values and the magnitude of the range Rmax - Rt. Further analysis indicated that these measures are not consistent across treatments. Thus the time constants being generated were being influenced by factors which did not vary in a predictable manner. I eliminated the influence of these factors by normalizing each response value in the series to a % of the span of that series, ie,

$$R(J)^* = (R(J) - Rt)/(Rmax - Rt).$$

These normalized values were then transformed to fit a standard span $R(J)^*(Rmax^* - Rt^*)$, where $Rmax^* = -1000$, $Rt^* = -100$. The equation now becomes:

$$E(J) = (-900)e^{(-X)(J - 1)} - 100.$$

Thus, the time constant is affected only by the degree of change in the response levels with repeated presentation, and not the actual values of the response. An example of a curve fitted to novelty response size data is shown in Fig. A-1.

Legend for Figure A-1: Novelty response sizes, normalized to a standard range, as a function of stimulus presentation number. Best-fit curve, generated as described above, also shown. In this example, the response declined by 63% after 5.2 presentations.

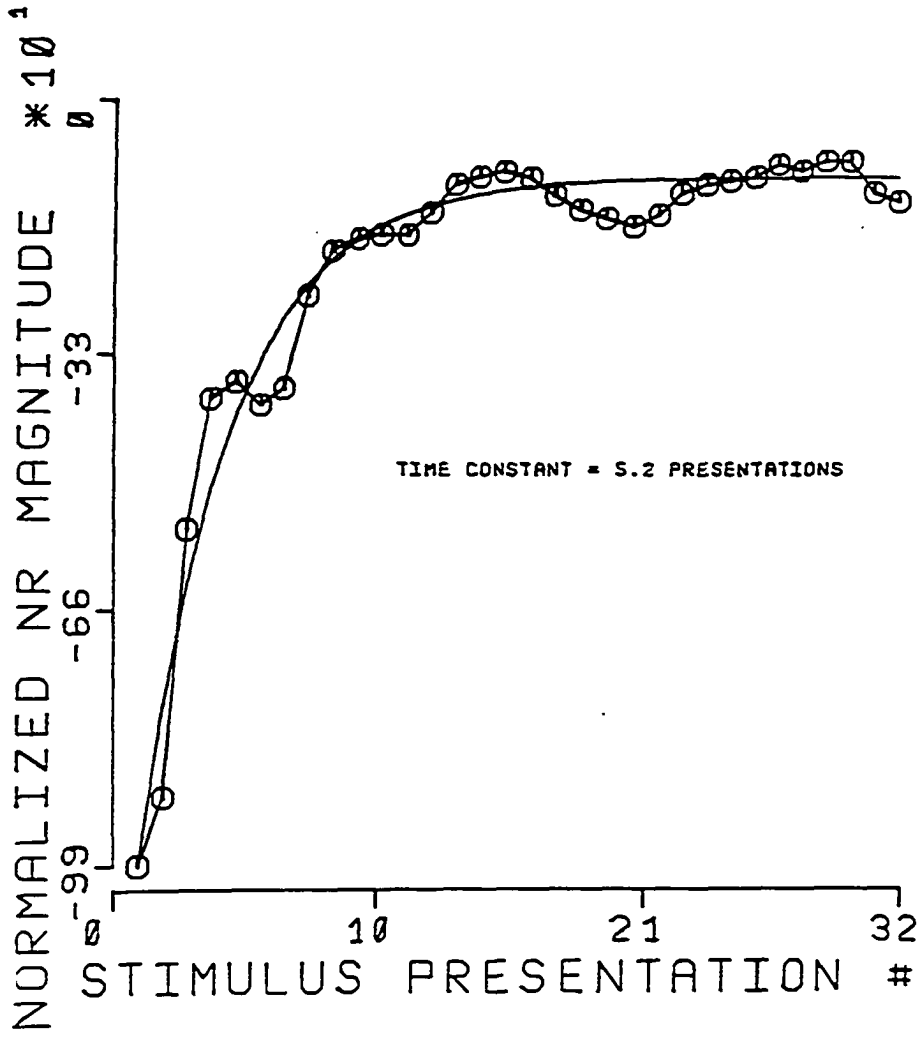
NORMALIZED NR MAGNITUDE *10¹
0
-33
-66
-99
0
10
21
32
STIMULUS PRESENTATION #
TIME CONSTANT = 5.2 PRESENTATIONS

Fig.A-1

Appendix B

Additional results

Included here are the complete set of mean (SE,n) values for Rmax, Rt, Rt/Rmax, and Tau that were discussed in Chapter 1. The initial response size (R1) was also measured and analyzed in this study. The effects of stimulus intensity, stimulus modality, Flaxedil and subject species on R1 were essentially the same as those on Rmax. R1 was highly correlated ($P<0.0001$) with Rmax, and in some cases was identical. Because there were no different effects found on R1, and because only Rmax and Rt were used for habituation measures, R1 was not included in the discussion of results in Chapter 1. The results are presented here for informational purposes.

Table Legends

Table B-1: Mean values for normalized maximal (Rmax) and normalized habituated (Rt) response sizes, amount of habituation (Rt/Rmax), and habituation rate (Tau: column 3) for each cell in experimental design. Column 1 indicates species (H. occid. = Hypopomus occidentalis), column 2 indicates Flaxedil state (NO FLAX = intact, FLAX = immobilized). Below each mean is SE and sample size (n).

Table B-2: Mean normalized initial response values (R1) for each cell in experimental design. Columns, labels, etc. identical to those in Table B-1.

Table B-3: Probability values for significant effects on normalized initial response sizes (R1). Body of table indicates significance of intensity effects within that cell. Values in columns labelled Flax indicate significance of Flaxedil immobilization effects on response sizes within species. Right-hand column, upper value indicates significance of modality differences within that species (all intensities pooled), lower value indicates significance of intensity effects on that species (all modalities pooled). Bottom-most row, upper value indicates significance of species differences for that modality (all intensities pooled), lower value indicates significance of intensity effects for that modality (all species pooled).

Table B-1
means

1	2	3	Electric 1X	Electric 2X	Electric 4X	Light 1X	Light 2X	Light 4X	Sound 1X	Sound 2X	Sound 4X
H		Rmax	4.34	8.21	-	4.12	6.98	11.33	1.63	4.56	4.26
y	N		0.6,9	2.0,6		1.3,8	1.3,8	4.0,8	0.5,6	1.8,6	1.6,4
o	O	Rt	0.62	1.25	-	1.00	1.89	2.85	0.22	0.70	0.86
p	F		0.1,9	0.4,6		0.3,8	0.4,8	0.6,8	0.1,6	0.3,6	0.4,4
o	L	Rt	0.19	0.18	-	0.30	0.29	0.33	0.15	0.18	0.23
p	A	Rmax	.04,9	.03,6		.06,8	.04,8	.04,8	.05,6	.06,6	.08,4
o	X	Tau	4.55	5.08	-	8.73	5.38	6.11	2.94	3.32	3.13
m			0.8,9	2.1,6		1.8,8	1.0,8	0.8,8	0.3,6	0.7,6	0.6,4
u											
s		Rmax	5.06	9.38	-	7.52	8.66	9.68	4.23	8.03	10.17
			1.4,13	2.0,4		1.7,11	2.0,15	2.0,16	0.8,12	2.0,10	4.9,12
a	F	Rt	1.12	0.95	-	1.86	2.10	2.09	1.44	2.24	3.31
r	L		0.3,13	.06,4		0.7,11	0.7,15	0.5,16	0.4,12	0.6,10	1.6,12
t	A	Rt	0.23	0.13	-	0.20	0.28	0.22	0.32	0.29	0.36
e	X	Rmax	.03,13	.04,4		.04,11	.04,15	.03,16	.04,12	.05,10	.04,12
d		Tau	7.03	7.40	-	5.35	6.47	6.72	3.78	5.85	3.14
i			1.1,13	4.6,4		1.2,11	0.9,11	0.9,16	0.5,12	1.3,11	0.3,12
H		Rmax	4.61	14.12	13.76	5.00	3.75	4.44	2.71	3.93	6.55
.			0.8,14	4.7,11	4.8,10	2.7,11	1.4,12	1.1,14	0.5,10	0.5,14	2.1,13
o	F	Rt	1.39	2.59	3.55	0.84	0.61	1.18	0.49	0.82	1.10
c	L		0.4,14	1.0,11	1.6,10	0.2,11	0.2,12	0.4,14	0.1,10	0.1,14	0.2,13
c	A	Rt	0.26	0.16	0.20	0.27	0.18	0.26	0.21	0.24	0.26
i	X	Rmax	.03,14	.04,11	.04,10	.06,11	.03,12	.02,14	.04,10	.03,14	.03,13
d		Tau	5.53	4.94	6.20	3.37	4.02	4.89	3.32	4.11	4.32
.			0.7,14	0.6,11	0.8,10	0.3,11	0.6,12	1.0,14	0.5,10	0.7,14	0.6,13
		Rmax	4.80	6.60	10.82	5.69	10.80	10.19	11.00	6.81	14.57
G	N		0.6,6	1.5,6	1.0,6	0.6,6	1.8,6	1.8,6	3.4,3	1.4,3	2.1,5
y	O	Rt	0.48	1.19	2.72	0.79	1.69	2.80	2.58	3.24	6.41
m	F		0.2,6	0.4,6	0.5,6	0.3,6	0.5,6	0.7,6	0.7,3	1.0,3	1.6,5
o	L	Rt	0.09	0.18	0.25	0.14	0.15	0.28	0.27	0.45	0.42
t	A	Rmax	.04,6	.03,6	.04,6	.04,6	.04,6	.04,6	.06,3	.06,3	.08,5
u	X	Tau	6.30	5.74	4.58	4.88	6.40	4.84	9.27	4.98	8.48
s			1.2,6	1.0,6	0.8,6	0.5,6	1.4,6	1.0,6	3.7,3	0.7,3	0.7,5
		Rmax	9.11	8.02	8.62	19.45	11.70	18.70	2.66	6.30	18.87
c			3.0,6	0.9,4	-,1	5.4,4	4.9,2	-,1	0.4,2	-,1	-,1
a	F	Rt	2.74	2.15	2.90	1.70	3.12	2.30	0.36	1.85	6.17
r	L		0.8,6	0.7,4	-,1	0.3,4	3.0,2	-,1	0.3,2	-,1	-,1
a	A	Rt	0.32	0.29	0.34	0.12	0.19	0.12	0.15	0.29	0.33
p	X	Rmax	.07,6	0.1,4	-,1	.04,4	0.2,2	-,1	0.1,2	-,1	-,1
o		Tau	5.07	7.66	2.42	5.59	3.49	4.15	7.55	4.03	22.49
			1.4,6	4.7,4	-,1	2.0,4	0.5,2	-,1	1.1,2	-,1	-,1

Table B-2
R1 means

1	2	3		Electric			Light			Sound	
			1X	2X	4X	1X	2X	4X	1X	2X	4X
H.	N										
a	O	R1	3.89	6.57	-	3.53	5.60	10.30	0.95	4.38	2.52
r	F		1.7,9	1.7,6		1.3,8	1.6,8	4.1,8	0.2,6	1.8,6	1.4,4
t	L										
e											
d	F	R1	3.74	8.61	-	7.05	7.05	8.41	3.46	6.41	8.81
i	L		1.0,13	2.6,4		1.8,11	2.0,15	2.0,16	1.0,12	1.1,10	5.0,12
H.	F	R1	3.90	12.62	12.80	4.53	3.66	3.60	2.42	3.11	5.80
o	L		0.7,14	4.8,11	4.8,10	2.7,11	1.4,12	1.0,14	0.5,10	0.4,14	2.2,13
c.											
G.	N										
o	O	R1	2.67	5.42	8.89	4.35	8.45	7.09	10.47	6.04	12.56
c	F		0.5,6	1.0,6	1.8,6	0.7,6	2.2,6	1.3,6	3.9,3	2.0,3	1.6,5
a	L										
r											
a											
p	F	R1	6.53	4.81	8.62	18.11	11.70	18.70	2.04	1.15	16.50
o	L		2.4,6	0.5,4	-,1	5.8,4	4.9,2	-,1	0.2,2	-,1	-,1

Table B-3
R1

Species	Electric	Flax	Light	Flax	Sound	Flax	modality- all intensities intensity- all modalities
H.artedi -NO FLAX	0.29	0.73	0.20	0.64	0.19	0.23	0.13 0.07
H.artedi - FLAX	0.04		0.85		0.48		0.83 0.17
G.carapo -NO FLAX	0.01	0.29	0.20	0.002	0.21	0.18	0.02 0.006
G.carapo - FLAX	0.73		0.77		0.02		0.02 0.25
H.occident. - FLAX	0.13	—	0.92	—	0.20	—	0.004 0.24
species- all intensities	0.06		0.0005		0.10		
intensity- all species	0.004		0.73		0.04		

CHAPTER II

NEUROPHYSIOLOGICAL STUDIES

Introduction

The Electric Organ Discharge (EOD) of pulse-type weakly electric fish consists of brief pulses separated by larger time intervals; hence these fish sample the environment for brief periods that are separated by relatively longer intervals. When a detectable change in electroreceptive afference occurs, gymnotiform pulse fish will commonly raise the rate at which their EODs occur, presumably to increase sampling frequency (Heiligenberg 1980). This reflexive behavior, which can be elicited by stimuli of various sensory modalities, is called the Novelty Response (NR), and has been described in numerous studies (e.g., Lissman 1961, Hagiwara and Morita 1963, Westby 1975, Hopkins and Heiligenberg 1978). Larimer and MacDonald (1968) suggested that these fish must compare incoming electroreceptive afference with some "built-in reference" based on preceding afferences in order to detect novelties. This concept was further developed by Heiligenberg (1980), who suggested that these fish maintain a 'template' or central state of electroreceptive afferences against which novelties are detected. When the novelties persist (i.e., the change in afference occurs for several successive EODs), the fish adapts to this new steady state and-updates its 'template'.

It is possible that this 'template' consists of a set or sets of rapidly habituating neurons. (Habituation is used here in the most general sense, referring only to response decrement induced by repeated stimulation, akin to Horn's "self-generated depression of sensitivity" concept (1967), and is not meant to imply any specific mechanism or to satisfy the requirements of habituation proposed by Thompson and Spencer (1966).) Changes in electroreceptive afference could excite neurons not excited by the previous afference, or neurons that have habituated to the previous afferences, or both. Activity in these neurons would thus represent a change, or novelty in the environment. If the change in electroreceptive afference persists, the neurons would rapidly habituate and novelty-signalling activity would decline. Recordings from electrosensory nerve fibers do not exhibit habituation per se (Suga 1967), but Hypopomus electroreceptor afferent neurons reportedly will cease responding for up to 30s following a brief, strong stimulus (Baker 1980). If a stimulus causes a large number of spikes in an electroreceptor afferent neuron, there will be fewer spikes elicited by an immediately-following stimulus (Hagiwara and Morita 1963). It is thus possible that electroreceptor neurons could code for the occurrence of a stimulus novelty by exhibiting a response to the first occurrence of a stimulus that is stronger than the responses to subsequent occurrences of the stimulus that immediately follow. Other electrosensory processing regions that receive inputs from these adapting units could exhibit some degree of rapid habituation, and thus could act as electrosensory 'novelty detectors'.

This set of experiments was designed to examine the electrosensory

processing regions of pulse-type gymnotiform fish for neural activity that is associated with the detection of electrosensory novelties. Differences in neurophysiological responses to novel and non-novel or persistent stimuli indicated the possible presence of novelty-detecting units within the region examined. Additional experiments were performed to relate the activity of novelty-detecting regions with certain aspects of the behavioral novelty response observed in these species.

Procedures

The basic features of the experimental apparatus were described earlier (Chap.I). Gymnotus carapo (n=26), Hypopomus artedi (n=6), H. beebei (n=9), and H. occidentalis (n=20), purchased from commercial suppliers, were used as subjects. Fish were immobilized with intramuscular Flaxedil injections, given topical applications of Xylocaine to the skull region, and then were prepared for neurophysiological recording. In most instances, the spinal Pacemaker-related Signal (PS) was monitored with a suction electrode placed over the tail, amplified, and delivered to a triggering device. In some subjects the PS would disappear after immobilization and preparation; in these instances, a Grass S-9 stimulator, set at a pulse frequency approximately the same as the subject's resting EOD rate, was used to provide artificial PS pulses to the triggering device. The trigger pulse was used to control the timing of stimulus presentations. Using an electronic counter with the trigger allowed a stimulus (0.1 ms square wave) to be presented every n-th PS cycle (= various degrees of novelty), or at every PS cycle (= persistent stimulus). For instance, a stimulus presented every 16 PS cycles (1/16) represents a novel stimulus, one presented every 4 PS cycles (1/4) is less novel, and one presented every PS cycle (1/1) is least novel (after the first presentation of the series). PS cycle rates varied among subjects from 5 - 25 Hz; thus a 1/1 stimulus could have intervals ranging from 40 - 200 ms, depending on the subject. For comparisons among subjects having different rates, stimulus intervals are given in PS cycles, along with time references for these intervals. Stimuli were usually

applied transversely via two coiled electrodes on either side of the fish, but could also be applied longitudinally via electrodes in the mouth and around the tail (viz, an EOD mimic). The effects of stimulus geometry were tested by applying stimuli transversely or longitudinally and noting any differences in response properties. Stimuli were presented either in the absence or presence of an artificial EOD, and with or without a delay (between PS trigger and stimulus onset) to test for possible gating requirements of the phenomenon. Other stimulus modalities were used (light flashes, auditory stimuli) to test for stimulus specificity. Electrosensory stimulus intensity was measured by monitoring the output of a paired field electrode, oriented perpendicularly to the long axis of the fish and placed approx. 0.5 cm from the fish's body just behind the pectoral region.

In one set of experiments, Event-related Potentials (EPs) were recorded from a number of the known electrosensory-processing regions (see Bullock 1982 for review) in the same fish with 3.0 M NaCl - filled glass microelectrodes (tip diam. 5-15 um). The anterior lateral line ganglion, Electrosensory Lateral Line Lobe (ELLL), Torus Semicircularis (TS), and the Lobus Caudalis (LC) of the cerebellum were examined for EPs that differed between novel stimulus presentation and persistent stimuli (novelty-related EPs). EPs were generally averaged over 10, 20, or 30 stimulus presentations, and the sampling rate was 40 Khz. Qualitative comparisons of EPs were made by visual inspection of EP traces. Quantitative analysis consisted of comparing the magnitude of the EP voltages measured at particular latencies. Horseradish peroxidase injections were made in many cases to verify the areas of

the brain from which recordings were made.

Indium-filled microelectrodes (tip diam. 5 - 15 um) were used to record single-unit spike activity in response to novel vs persistent stimuli. Data were recorded on an FM tape-recorder and processed in real time by an on-line computer program that generated post-stimulus time histograms of the responses. Stimulus interval effects were quantified by analyzing total spike activity that occurred immediately following stimulus presentation (for up to 50 ms maximum) as a function of stimulus interval length. Comparisons among different units within the same fish as well as between fish were made by normalizing all total spike activities as a multiple of the activity of that unit in response to persistent (1/1) stimuli. Alcian blue was injected by standard techniques to identify some of the single-unit recording sites.

The relationship between neural activity and the behavioral NR was examined in several sets of experiments. In one set of experiments, the subject's PS signal was monitored and the intervals between them were measured and stored by computer (Chap.I - Methods). Simultaneously, neural activity was recorded and also stored. Secondly, novelty-related EPs were also recorded from subjects undergoing behavioral NR habituation (Chap.I - Methods). A third set of experiments examined the sufficiency of the novelty related areas for eliciting an electrosensory-induced NR by directly stimulating these regions with bipolar microelectrodes (25ms train of 0.5ms, 1-4uA pulses at 200 pps), and observing the subsequent behavioral activity.

Results

The results from all species examined are summarized together. The neurophysiological phenomena described below appeared to be the same for all fish studied; limited sample sizes precluded any statistical analyses of possible species differences. In the discussion of EPs below, the letter and numeral in parenthesis ( ) refer to the labelled potentials in Figure 2-1.

Neural responses to novel vs persistent stimuli

A. Ganglion of the anterior lateral line (electrosensory) nerve: Field potentials (n1) due to electroreceptor afferent activity were recorded and responses to persistent stimuli (1/1) were essentially no different from those to novel stimuli (e.g., 1/16: Fig.2-1a). In some cases, the latency of the response was about 1 ms longer to persistent stimuli than novel stimuli. This same change in latency was seen in single-unit recordings made from the ganglion. Latencies ranged from 1 ms (stim. = 140 mv/cm) to 4 ms (14 mv/cm). At high stimulus intensities (70 mv/cm or more), the EP exhibited 'ringing' responses, presumably due to synchronized burst duration coder activity. The duration of this ringing was consistently shorter when stimuli were presented more frequently, as is the duration of the burst of spikes of an individual receptor afferent neuron (Hagiwara and Morita 1963, Suga 1967, Heiligenberg 1980). Both pulse-marker and burst duration coder receptor activity were recorded from the ganglion. Total spike activity recorded from most of the units found was slightly greater at

larger stimulus intervals (Fig.2-2a). The mean total response to 100 consecutive novel (1/16) stimuli was about 50% greater than that to persistent (1/1) stimuli (Fig.2-2b). This difference resulted largely from a decline in number of spikes per burst when stimuli were presented in rapid series.

B. Electrosensory Lateral Line Lobe /Eminentia Granularis Posterior (ELLL/EGP): EPs recorded from this area exhibited several components (Fig.2-1b,c,d). The shorter latency component (1.5 - 4 ms) was found throughout and was essentially the same as the EP found in the ganglion. This potential did not change with increased stimulus frequency. In electrode tracts penetrating the lateral portions of the ELLL, a second potential (p1) followed the first, non-adapting component (n1: Fig.2-1b). The latency of this response varied from 5 - 7 ms, depending on stimulus intensity. This EP did not change with increased stimulus frequency, but did vary with different recording depths. Recordings were also made from more medial electrode tracts, which, owing to the angle of penetration, entered the areas of the lobe ventral to the externally-visible praeeminential electrosensory tract descending from the nucleus praeeminentialis (Fig.2-1c,d). In these areas the short latency afferent potential often had positive polarity (p1); a reversal of the polarity occurred deeper in the rostral portion (Fig.2-1c). As in the lateral ELLL recordings, this first potential did not change with stimulus frequency changes. However, a second potential (p2), which in the rostral portion of the area had a latency shorter (range: 3.0 - 5.5 ms, mean (se) = 4.6 (0.6) ms, n=5) than the p1 from the lateral ELLL, exhibited novelty-related properties, ie, a

decrease in response with increased stimulus frequency. This novelty-related EP was stronger at shallow recording depths; the focus (area yielding the greatest response) was generally within 300 um of the brain surface. Deep in the rostral-medial tracts this novelty-related EP (p2) was not found; a longer latency (12 - 14 ms) p2 that did not exhibit novelty-related properties could sometimes be found (Fig.2-1c, 950 um). In more caudal electrode tracts ventral to the EGP, which lies over the ELLL, the short latency afferent potential (p1) was very small, and the novelty-related p2 had a greater latency (7.6 ms) than the novelty-related p2 from more rostral regions (5.0 ms). This caudal p2 did not occur at depths greater than 200 um, and at depths of 500 um or more, all potentials became very small. Very shallow recordings in this area sometimes resulted in a third, longer latency (8 - 12 ms) component (not shown) that was not found deep or lateral, and which also exhibited novelty-related properties.

When the size of the EP response was estimated by measuring voltage differences from baseline (0 v) at particular latencies or latency ranges, these novelty-related EPs were about twice as large to stimuli presented 1/16 PS cycles (720 - 3200 ms) than to stimuli presented every PS cycle (45 - 200 ms). These novelty-related EPs could reflect a change in ELLL E-cell burst duration (see below), or, because they were only found in shallow areas they may represent activity due to one or both of the descending inputs to the ELLL, namely the tractus stratum fibrosum and the praeeminential electrosensory tract (Maler 1979), or the response of dorsal ELLL components to such activity.

Both Inhibitory (I) and Excitatory (E) single units were recorded in the ELLL, the latter found more commonly in these experiments. Most of the units were recorded at depths ranging from 300 - 600 um, more shallow laterally than medially. I cells, which exhibit decreased activity upon stimulation, showed very slight novelty-related properties, i.e., the amount of inhibition increased with stimulus frequency, resulting in lower responses at higher stimulus frequencies. E cells exhibited various degrees of novelty-related properties, if any at all. At near-threshold stimulus intensities (10-30 mv/cm), each stimulus presentation typically evoked a single spike; there was no difference in response to novel vs persistent stimuli. At higher intensities, E cells typically fired a burst of spikes, the duration of which was often inversely related to stimulus frequency, as seen in the ganglion (Fig.2-2a). For instance, one unit responded to several series of 100 25-mv/cm stimuli with a mean total of 81 spikes at 50 ms intervals (n=3), and 105.5 spikes at 3200 ms intervals (n=2); increasing stimulus intensity to 50 mv/cm elicited a mean total of 214 spikes at 50 ms intervals (n=3) and 406.5 spikes at 3200 ms intervals (n=2). ELLL E cells also exhibited some latency increases (of up to 2.0 ms) as stimuli became more persistent. The mean increase in total activity recorded from 22 cells in response to 100 consecutive novel stimuli is 60-70% greater than that to 100 consecutive persistent stimuli (Fig.2-2b).

C. Lobus Caudalis of the cerebellum (LC): EPs were recorded from more dorsal and medial portions of the LC in three animals. Novelty-related EPs were found, having latencies about 1 ms longer than

those from the ELLL/EGP area (eg., 6.4 ms (stim = 14 mv/cm), compared with 5.5 ms for the ELLL/EGP novelty-related EP from the same subject). More commonly, negative polarity EPs (arrow in Fig.2-1e) were found that were larger in response to persistent stimuli than to novel stimuli ('negative' novelty-related EPs). The latencies of these EPs were 8.2 - 14 ms (mean (se) = 10.7 (1.7) ms, n=3). The latency decreased with increased stimulus intensity, and with increased electrode recording depth. The onset of these 'negative' novelty-related EPs often matched the end of the ELLL ringing in the same subject. The ringing seen in Figure 2-1e was commonly seen in the LC when high stimulus intensities were used; it was essentially the same as the ringing seen in the ELLL. No single-unit recordings were made from this area.

D. Torus Semicircularis (TS): Many negative polarity novelty-related EPs were found in the dorsal TS (n1 in Fig.2-1f), mostly, but not exclusively, in the mid-posterior portion, at depths ranging from 400 - 1200 um. On a few occasions EPs that were larger in response to persistent stimuli ('negative' novelty-related EPs) were found in the TS, the latency of which (range 9.2 - 12.3 ms, mean (se) = 10.2 (1.0) ms, n=3) was comparable to those of the LC 'negative' novelty-related EPs. The common situation was to find a strong, 'positive' novelty-related potential. There were readily observable differences in responses to novel vs persistent stimuli. For a given fish, the biggest EP was found in a specific area roughly 100-200 um in diameter, but potentials could often be recorded throughout a range of depths as great as 1000 um. Latencies of the TS novelty-related EPs

were longer (mean (se) = 6.2 (1.2) ms, n=4) than the ELLL/EGP novelty-related EPs (mean (se) = 4.6 (0.6) ms, n=5), but shorter than those of 'negative' novelty-related EPs (see above). The latencies of the TS novelty-related EPs decreased with increased stimulus intensity (eg, 9.7 ms @ 14 mv/cm, 4.4 ms @ 100 mv/cm). The magnitude of the EPs to novel stimuli (1/16) were 300 - 800% greater than those to persistent stimuli. These response magnitudes are graded as a function of stimulus interval (Fig.2-3). In some cases, the novelty-related EP appeared to be superimposed on a smaller, longer duration potential that did not change with stimulus frequency.

The change in total spike activity that occurred with increased stimulus intervals was much more pronounced in the TS units than in the ELLL or ganglion (Fig.2-2a). The average activity of TS single units exhibiting novelty-related properties was 400-500% greater in response to novel vs persistent stimuli (Fig.2-2b). This average does not include data from one unit that exhibited extreme novelty-related properties, i.e., response increases of 3000% or more for stimuli presented 1/32 PS cycles (every 6400 ms) relative to responses to stimuli presented every PS cycle (200 ms). This unit exhibited a 1090% increase in activity to stimuli presented 1/4 PS cycles (800 ms). Aside from its very steep response/stimulus interval relationship, this unit exhibited no characteristics (i.e., overall activity level, threshold) dissimilar to others found in the TS. The magnitude of the novelty-related response (i.e., the amount of activity increase) depended at least in part on the intensity of the stimulus. At lower stimulus intensities, the effect was greater. For example, for one TS

unit, using a stimulus intensity of 70 mv/cm, the ratio of total spike activity to novel (1/16) stimuli over persistent (1/1) stimuli was 3.36:1 (567 spikes/222 spikes). Lowering the stimulus intensity to 40 mv/cm decreased the responses to both stimulus types, but the decrease was greater for persistent stimuli (332 spikes/12 spikes, 27.7:1), and reversing the stimulus polarity (which in this case reduced the stimulus' efficacy on the electroreceptors on that side of the fish) at 70 mv/cm resulted in an even greater difference in decreased activity (222 spikes/2 spikes, 111:1).

The time course of TS single unit novelty detection was examined by analyzing single unit activity recorded on FM tape. The responses to persistent stimuli showed a significantly ($P<0.0001$) greater decline in response to consecutive presentations than did responses to more novel stimuli (Fig.2-4). If the stimulus was presented every PS cycle (every 45-200 ms), there was (on average) a 50% decline in spike activity by the fifth consecutive stimulus, whereas responses to stimuli presented every 16 PS cycles (720-3200 ms) declined by only 18% by the fifth consecutive stimulus. The latency of the first spike often increased with consecutive 1/1 stimuli, but not to 1/16 stimuli. Units exhibiting low activity levels (1-2 spikes per stimulus) showed a decrease in spike probability when stimuli were persistent. Quite frequently, TS units would not respond at all after 5-10 consecutive persistent (1/1) stimuli.

Only one inhibitory TS unit was found; there were no clear novelty-related properties exhibited. Occassionally, TS units exhibiting increased activity in response to stimulation were

encountered that showed no novelty-related properties. Thus, novelty detection is not a feature of all TS electrosensory cells.

Not only do Toral units differ from ELLL and ganglion units in the degree of increased response to increasingly novel stimuli, but they also differ in the rate of the increase. Time constants, which describe the stimulus interval at which the response is 63% of the maximum value, and which varies with the rate at which this maximum is achieved, were calculated for individual units. TS units had a mean time constant for increased response to increasingly novel stimuli of 1383 ms (range: 676 - 3209 ms, se = 282, n = 9), which is significantly greater ($P<0.01$, Student's t-test) than the mean of 424 ms (range: 185 - 1110 ms, se = 142, n = 6) for ELLL units, and greater ($P<0.02$) than the mean of 570.9 ms (range: 463 - 848 ms, se = 93, n = 4) for ganglion units. There was a significant correlation between percent response increase and the corresponding time constant among all units studied ($r = 0.52$, $P<0.05$, $n = 19$). However, this was apparently due to the fact that TS units exhibited both the highest percent increases and time constants relative to ELLL and ganglion units; correlations between these measures within a group were not significant in any case. Within a group, there was no apparent relationship between the amount of increased activity and the time constant of that increase. For example, one ELLL unit was tested at two different stimulus intensities, and a time constant of 237.8 ms was calculated for a 64.4% response increase, compared with a time constant of 256 ms for an increase of 103% at the higher stimulus intensity. Two ganglion units

had the same time constant (463.3 ms), but one exhibited a 19% increased response to novel stimuli, the other a 57% increase.

Because the TS had the strongest novelty-related EPs and unit activity, additional experiments were performed to determine the characteristics of these responses. Stimulus geometry had no effect on the detection of electrosensory novelties; the novelty-related EPs occurred whether the entire series of stimuli was presented transversely or longitudinally, and it also did not matter if an artificial EOD was present during testing. It was not necessary for the stimulus to be presented in any temporal relationship to the fish's own PS signal; introducing a delay between the PS signal and stimulus onset did not affect the elicitation of a novelty-related EP. In fact, the response could be elicited using the free-running timing signals from the Grass S-9 stimulator, set at any pulse frequency. The necessary feature of the stimulus necessary to elicit a novelty-related EP was that it be presented after some minimum interval following the preceding stimulus.

Horseradish peroxidase and Alcian blue injections into the midbrain recording sites showed some variation in location, but in all cases were contained within the dorsal portion of the TS. Most injections were centered within the mid-posterior portion of the TS, including lamina VI and adjacent layers. Transport of peroxidase indicated that this area communicates with the ELLL, nucleus praeeminentialis, optic tectum, and some diencephalic structures, as shown by Carr et al (1981).

Neural activity & behavioral responses

Activity in the novelty-related regions of the TS was correlated with certain features of the behavioral Novelty Response (NR) of these fish. EPs were recorded from the TS during presentation of stimulus pulses that occurred every PS cycle (1/1). Simultaneously, the fish's PS intervals were measured to assess the magnitude of any behavioral responses concurrent with the recorded neural activity (Fig.2-5a). The onset of the stimulus train always induced an EP that declined rapidly in magnitude with successive stimulus presentations (a novelty-related EP). The novelty-related EPs recorded in these experiments were identical to those recorded from the TS in the experiments discussed above. The fish also consistently accelerated its PS rate at the onset of the stimulus train. In some cases, the magnitude of the PS interval change could be strongly correlated with the magnitude of the novelty-related EP in the TS for the entire stimulus series (Fig.2-5b). In other instances, only the initiation of the behavioral response could be associated with TS novelty-related EP activity. These fish exhibited a range of PS rate-changing behaviors under this stimulus regime, similar to those reported by Westby (1975) for Gymnotus carapo. For instance, some fish maintained the higher EOD rate during the entire stimulus series, i.e., did not decelerate until the stimulus was removed, and, while some fish decelerated at the end of the stimulus train, others accelerated after the stimulus was removed. In none of the experiments reported in this paper was an EOD acceleration observed in the absence of a TS novelty-related EP; however, novelty-related potentials could be recorded in the absence of any clear behavioral

response. This indicates that the TS may signal the presence of an electrosensory novelty, but the fish's behavior is controlled by some 'higher' center that utilizes this information supplied by the TS. In some experimental series, two sequential stimulus trains were presented to the fish with a variable number of non-stimulus PS intervals between to allow for recovery. The neural response (novelty-related EP) generally recovered more quickly and to a greater degree than the behavioral NR. When the novelty-related areas of the TS were directly stimulated, a 'normal' NR was elicited. If this direct stimulation was repeated at rates identical to those used in the previous paper (Chap.I), the response habituated at the same rate as the habituation to external electrosensory stimuli (mean (se) habituation rates : electrosensory stim. = 5.6 (0.75), direct stim. = 5.8 (1.30)).

That novelty-related activity in the TS does not directly control PS acceleration is further supported by some additional evidence. Presentation of stimuli at rates sufficiently 'novel' to elicit a novelty-related EP elicit behavioral responses (NRs) that decline with repeated presentations (habituate), i.e., the TS continues to signal the presence of an electrosensory novelty after it has ceased to be behaviorally relevant. When several consecutive series of 100 novel (1/16) stimuli were presented at these same rates, the total spike activity elicited by the tenth series was essentially the same as that elicited by the first series (Fig.2-6); the behavioral response habituated rapidly within the first series and did not occur to any of the others.

Discussion

It is apparent from these results that there exists within the electrosensory-processing regions of pulse-type gymnotiform fish neurons that respond preferentially to stimuli presented less often, i.e., novel stimuli. Both Event-related Potential (EP) and single unit activity indicate sensitivity of response size to the frequency of stimulation. This sensitivity is greatest in the Torus Semicircularis (TS). Whereas units of the electrosensory afferent ganglion and the Electrosensory Lateral Line Lobe (ELLL) show only a partial response decrement, and only at relatively rapid stimulation rates (1/0.4 - 0.6 s), TS units exhibit very strong "habituation" to stimuli presented as slowly as 1/1 s (Fig.2-2). The reduction in activity that occurs in TS single units when stimuli are presented frequently (1/1 PS cycle) occurs very quickly, usually within 3 - 5 stimulus presentations (Fig.2-4). The magnitude of the response decrement that occurs in the TS depends on the inter-stimulus interval (Fig.2-3). These TS novelty-related responses will occur to electrosensory stimuli that are applied either transversely or longitudinally with respect to the fish's body axis, i.e., stimulus geometry is not critical for the phenomenon to occur. Nor is the timing of the stimulus with respect to the fish's PS signal critical for the novelty-related response. Thus, the putative "novelty detection" that occurs in the TS is general with respect to specific stimulus features.

Single unit activity recorded from the electrosensory ganglion afferents indicated that these burst duration coders respond to stimuli presented at rapid rates with fewer spikes per burst than when

stimulation is less frequent, as has been previously reported (e.g., Hagiwara and Morita 1963, Suga 1967). Because descending input to the electrosensory ganglion is not known to exist, the stimulus frequency-sensitive properties of the burst duration coders may be an intrinsic characteristic of the neurons or electroreceptors themselves. In high frequency weakly electric fish, these neurons synapse with granule cells, basilar pyramidal cells, and polymorphic cells of the ELLL (Maler et al 1981), and presumably the same situation exists in the pulse species studied here. In the present study single unit recordings from the ELLL may have been from any number of cell types (Maler 1979). These units exhibited at best a doubling of spike activity with changes in stimulus frequency, and were recorded at depths from 300 - 600 um. The response latencies of these units ranged from 3.5 - 20 ms. These units were most likely basilar pyramidal cells ('E' cells: Saunders and Bastian 1984). In a previous study, Enger and Szabo (1965) recorded from ELLL pyramidal cells (at depths of 950 - 1150 um) activity that doubled with increased intensity.

The novelty-related properties of the ELLL single units recorded in the present study do not differ much from those of the ganglion afferents, and therefore, as in the latter case, these properties probably reflect some intrinsic adaptation of the ELLL units. But, unlike the ganglion receptor afferents, ELLL units do receive descending feedback signals, from both the nucleus praeeminentialis and the Lobus Caudalis (LC) of the cerebellum (Sas and Maler 1983). It is possible that this feedback loop plays an important role in the novelty-related phenomena described here. Recordings from the dorsal

region of the ELLL/EGP area, which includes fiber tracts of this feedback system (Maler et al 1982) show strong novelty-related patterns of activity not seen in deeper layers of the ELLL. The output of the ELLL cells could be attenuated by increased inhibitory feedback from the LC or nucleus praeeminentialis. Tong (1982) reports that some nucleus praeeminentialis unit activity in the electroreceptive bullhead catfish will rapidly decline when stimuli are presented frequently. Bastian (1985) has recently reported that descending input to the ELLL is responsible for attenuating the responses of ELLL units in some high frequency gymnotiform fish.

The strongest novelty-related properties were found in the TS. The TS receives electrosensory input via both a rapid- and a slow-conducting pathway (Szabo et al 1975). The results presented here suggest that the novelty-related activity of the TS is mediated by the slow system. The rapid-conducting system is characterized by very short response latencies, and essentially no neurophysiological activity recorded in the TS had latencies that correspond to those reported for the rapid system. (This may be due to stimulus artifact, which in some instances lasted more than 1 ms, possibly obscuring such responses.) Also, Szabo et al (1975) reported that the fast-conducting system has a relatively long (3 ms) "unresponsive" period following each response to electroreceptor input. Similarly, Schlegel (1977) found a refractory period of up to 18 - 20 ms following strong stimulation. In the present study when an EOD-mimic (S-1) pulse was used which caused a response in this area (EPs were recorded in response to the S-1), an S-2 stimulus pulse occuring within this

"unresponsive" period would still elicit a novelty-related EP. This indicates that the fast system cannot account for all of the novelty-related properties observed in the TS. The single-unit activity recorded from the TS may be from units previously mentioned by Schlegel (1977). He reports that some units in the TS had latencies of 5 ms or more, bursts of 1 - 4 spikes which increased in number (up to 7 - 8 spikes/burst) with changes in stimulus intensity or frequency, and that these responses were rare and short-lasting.

Because the ELLL input to the TS is direct, the differences in the magnitudes of the novelty-related properties of these two areas is probably not due to a change in the information flow between them, but instead is due to some property of the TS itself. It is possible that some of the TS units provide an inhibitory feedback that is dependant on the temporal relationship between successive inputs. It is also possible that some other brain structures might inhibit TS responses to frequent stimuli. LC EPs were inversely related in magnitude to those of the TS (Fig.2-1), and could represent feedback potentials. Crispino (1983) found that cerebellar stimulation will reduce TS electroreceptor responses in the catfish.

Relationship to behavior

It has been generally suggested that EOD rate-changing behavior is a response to detected changes in electroreceptive afference (Larimer and MacDonald 1968, Black-Cleworth 1970, Heiligenberg et al 1978, Heiligenberg 1980, Baker 1980,1981). The detection occurs by comparing successive EOD afferences, and rapidly adapts to the change. The

novelty-related activity described in the present study rapidly adapts to changes in electroreceptive afference, and could represent the afference difference-detection that precedes these electrosensory behaviors. All behavioral EOD accelerations to stimulus onset were preceded by or simultaneous with a novelty-related EP in the TS (Fig.2-5). The neural response could be evoked with stimuli applied either longitudinally or transversely (relative to the fish's principle body axis). Similarly, stimulus geometry is not critical for evoking a behavioral Novelty Response (NR: Heiligenberg 1980, chap.I this volume), or the jamming avoidance response (Heiligenberg et al 1978). In a majority of the EOD rate-changing behaviors that these fish perform, the initial response is a rapid EOD acceleration. This is possibly a reflexive response to the change in afference. This reflex would be adaptive, in that it increases information flow to the fish (Heiligenberg 1980), allowing the fish to follow with the appropriate response (EOD cessation, jamming avoidance, etc.).

Several lines of evidence suggest that although TS electrosensory novelty detection may be necessary for a behavioral response to electrosensory novelties to occur, some higher center is responsible for the control of the fish's behavior. Other sensory modalities can elicit a behavioral NR (Lissman 1961). If the novelty-related area of the TS is ablated, electrosensory-induced NRs no longer occur, but responses to visual and auditory stimuli remain intact (Chap.III). This indicates that the NR is controlled by multiple, independent sensory systems, as suggested by different habituation rates (Chap.I). When the TS was directly stimulated, thereby simulating "novelty

detection" responses, a behavioral NR occurred to only the first several stimuli, i.e., the behavior habituated. Also, novelty-related activity recorded from the TS did not habituate at stimulation rates that produce behavioral habituation (Fig.2-6). Thus it seems that TS novelty detection is not sufficient for elicitation of a behavioral response. Similarly, Megela and Capranica (1983) found that while lower auditory-processing regions in the frog do not habituate to stimuli presented at rates as fast as 1/1 s, the dorsal thalamus habituates to stimuli presented at rates as slow as 1/20 - 30 s, and does not respond at all to stimuli presented 1/3 s. The TS of the frog provides input to the dorsal thalamus and habituates to stimuli presented at 1/1 - 2 s rates, but not at slower rates. The behavioral response to these auditory stimuli, evoked calling, will also habituate to stimuli presented 1/20 - 30 s. In another study of neural habituation effects on behavior, Kileny et al (1975) correlated the novelty-related responses of vestibular nuclei in the cat with the behavioral vestibular reflex that occurs with rotational stimulation. Perhaps novelty detection is a general requisite for a host of reflexive behaviors observed in animals.

Comparison with other systems

In the present study, neurons were found that habituated to stimuli presented at rates ranging from 1/0.1 s - 1/1.5 s. Other neural "novelty detectors" and rapidly-habituating neurons have been

described in various animal groups. In the squid, the number of action potential spikes transmitted across the stellate ganglion synapse will decline if the stimulus train is presented at rates as low as 1/10 s (Horn and Wright 1970). Other neural responses will habituate at stimulation rates almost as low, including invertebrate (e.g., land snail ganglion, 1/5 s: Holmgren and Frenk 1961) and vertebrate preparations (e.g., cat caudate nucleus, 1/5 s: Vinogradova and Sokolov 1975). Some of the neural systems demonstrating habituation properties are monosynaptic, and some form of homosynaptic depression is indicated as a mechanism (e.g., frog ventral root response, 1/5 s: Farel et al 1973). Aljure et al (1980) suggest post-synaptic depression is responsible for the habituation of the Mauthner cell-mediated startle response in the hatchetfish. It is fairly certain that the habituation of the gill withdrawal reflex in Aplysia is mediated by modulation of the pre-synaptic calcium current that is crucial for transmitter release across the synapse (Kandel and Schwartz 1982). Novelty detection by cells of the cat nerve cord dorsal horn is eliminated if descending brainstem impulses to the dorsal horn are blocked by freezing (Wall 1967), indicating a role of feedback in this system.

The mechanism(s) of neural habituation or novelty detection in the electrosensory processing system of pulse-type weakly electric fish is not known. The results presented here indicate that this system could be a fruitful model for investigations of the mechanisms of neural habituation and of the phenomenon of sensory afference-difference detection in vertebrates.

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Figure Legends

Fig.2-1. Event-related Potentials (EPs) recorded from various electrosensory processing regions of *H. occidentalis* in response to stimuli presented once every sixteen (1/16) PS cycles, or once every (1/1) PS cycle. Total duration of traces is 25 ms. Stimulus is indicated by artifact at beginning of each trace. Recording depth is indicated by values (in um) at beginning of each trace. Horizontal bar = 10 ms. Polarity is indicated in **A**. EPs discussed in the text are indicated by arrows. Traces in **A - D, F** from same fish, using same stimulus intensity (24 mv/cm). Trace **E** from another fish of same species, stimulus intensity = 100 mv/cm. **A.** anterior lateral line ganglion. **B.** lateral Electrosensory Lateral Line Lobe (ELLL). **C.** rostral portion of medial ELLL/Eminentia Granularis Posterior (EGP). **D.** caudal portion of medial ELLL/EGP. **E.** Lobus Caudalis (LC) of cerebellum. **F.** Torus Semicircularis (TS). Stippled area represents the externally - visible praeeminential electrosensory tract.

Fig.2-2 **A.** Total single-unit spike response to 100 stimuli (ordinate) presented at various interstimulus intervals (abscissa, log scale). A typical TS unit (▯) shows a strong increase in spike activity at intervals greater than 500 ms, whereas the average ELLL (○) or ganglion (△) unit shows minor changes. Bars indicate +/- 1 SE for those intervals at which the unit was tested more than once. Stimulus used was a 0.1 ms square pulse, intensity = 30 mv/cm (TS), 25 mv/cm (ELLL), and 75 mv/cm (ganglion).

B. Mean normalized single-unit activity (ordinate) in response to 100 consecutive stimuli presented at different intervals (abscissa). Stimulus intervals are normalized to PS cycles. Means derived from data similar to those in A, normalized to the response at 1/1 PS cycle for each unit. Bars indicate 95% confidence limits. Mean intervals per PS cycle = 176 ms (TS - □), 58 ms (ELLL - ○), and 166 ms (ganglion - △).

Fig.2-3. Torus Semicircularis (TS) novelty-related EP magnitude (ordinate) increases with increased stimulus interval. Shown are H. occidentalis TS responses to stimuli presented every (1/1) PS cycle (40 ms), every fourth (1/4) PS cycle (160 ms), every sixteenth (1/16) PS cycle (640 ms), every sixty-fourth (1/64) PS cycle (2560 ms), and every two-hundred fifty sixth (1/256) PS cycle (10240 ms). Trace begins at 1.2 ms following stimulus onset (part of stimulus artifact shown at left), and ends at 25 ms after stimulus onset. Horizontal bar = 10 ms.

Fig.2-4. Mean percent change in Torus Semicircularis (TS) unit activity with repeated stimulation is greater with persistent (1/1 PS cycle) stimuli (□) than with novel (1/16 PS cycles) stimuli (○). PS cycle intervals varied from 45 - 200 ms. Bars represent +/- 1 SE. Data are arcsine transformed. Slopes of regression lines (not shown) fitted to these points are significantly different ($P << 0.001$). Equations for regression lines: 1/1, $y = -26.6 - 0.99x$; 1/16, $y = -9.2 - 0.20x$.

Fig.2-5 A. Simultaneous neurophysiological and behavioral records of activity in response to a series of electrosensory stimuli in H. beebei. In upper part of figure, PS intervals are plotted (numbers) in order of occurence. Horizontal line = value of first interval measured (not shown). Small vertical bars above interval numbers indicate when stimuli were presented. Lower part of figure contains EP traces recorded from the TS. Number at end of each trace indicates the PS interval within which the EP was recorded. Stimulus artifact shown in traces 11 - 31. Small vertical bars indicate latency at which the magnitudes of the EPs were measured. Polarity is indicated in first trace. Note the decline in EP magnitude after the first two stimuli. Baseline interval (#1) was 250 ms.

B. Correlation of data from A. Each PS interval was subtracted from the first interval, and plotted against the magnitude of the corresponding EP, measured at the points indicated in a and normalized by subtracting the potential magnitude in trace #1 from each. A significant correlation ($P<0.001$) exists between PS interval length and TS EP magnitude.

Fig.2-6. Post stimulus time histograms of G. carapo TS single unit activity in response to several series of 100 consecutive stimuli presented at novel (1/16 PS cycle) intervals. Height of each bin indicates total single unit spikes recorded at that latency in response to 100 stimulus presentations. Vertical calibration = 25 spikes. Each bin = 0.4 ms, and each histogram (except control) contains 128 bins. Response persists after the 10th series. Novelty-related activity is also maintained; response to stimuli presented at persistent intervals (1/1 PS cycle) is considerably reduced (control), but activity returns when the interval is again increased to 1/16. Because the PS interval was about 40 ms, only 100 0.4ms bins could be captured in the control run. First seven bins in each histogram were zeroed to remove stimulus artifact.

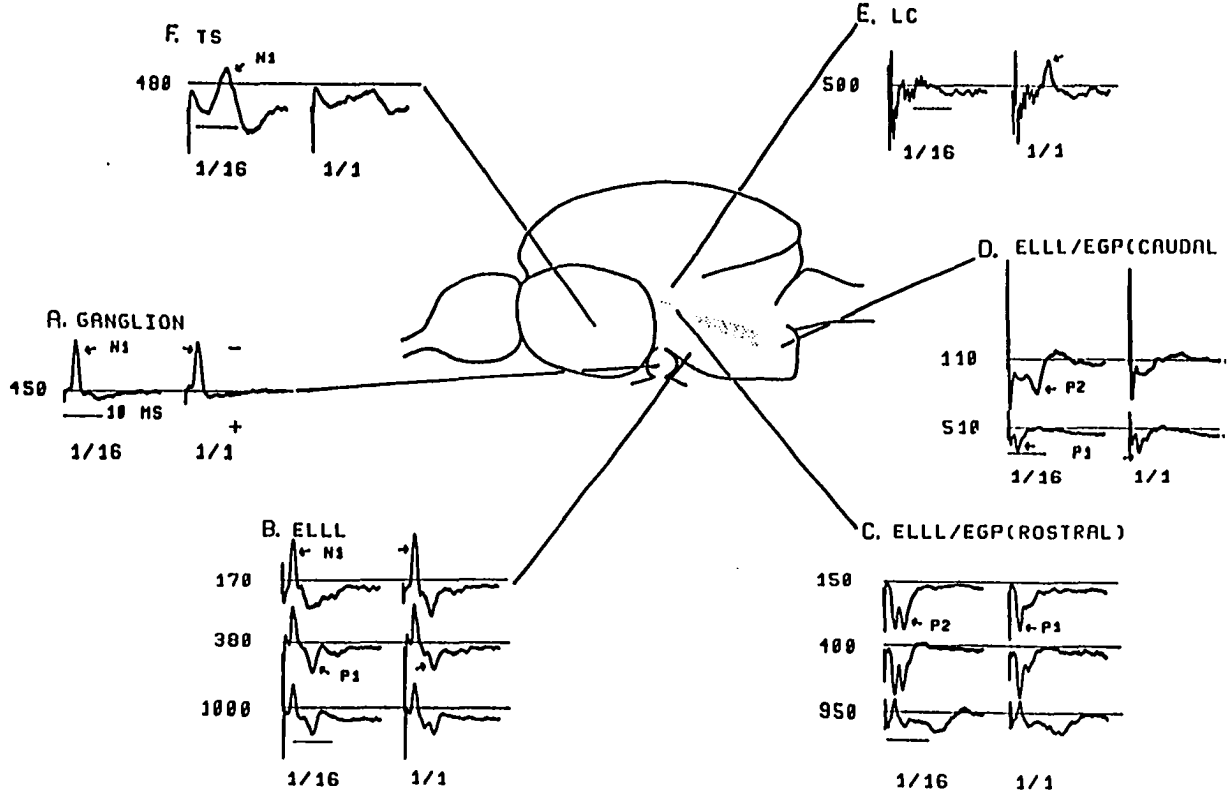

F. TS
100
N1
1/16
1/1
E. LC
500
1/16
1/1
A. GANGLION
N1
450
10 MS
1/16
1/1
D. ELLL/EGP(CAUDAL
110
P2
510
P1
1/16
1/1
B. ELLL
N1
170
380
P1
1000
1/16
1/1
C. ELLL/EGP(ROSTRAL)
150
P2
P1
100
950
1/16
1/1

Fig.2-1

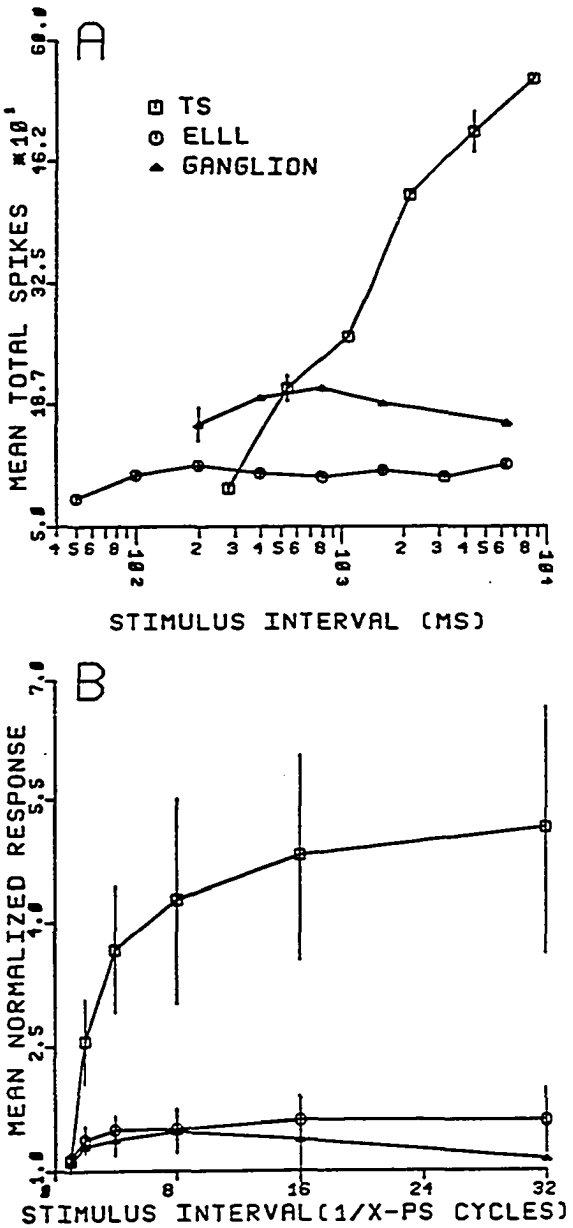

A
TS
ELLL
GANGLION
MEAN TOTAL SPIKES
STIMULUS INTERVAL (MS)
B
MEAN NORMALIZED RESPONSE
STIMULUS INTERVAL(1/X-PS CYCLES)

Fig.2-2

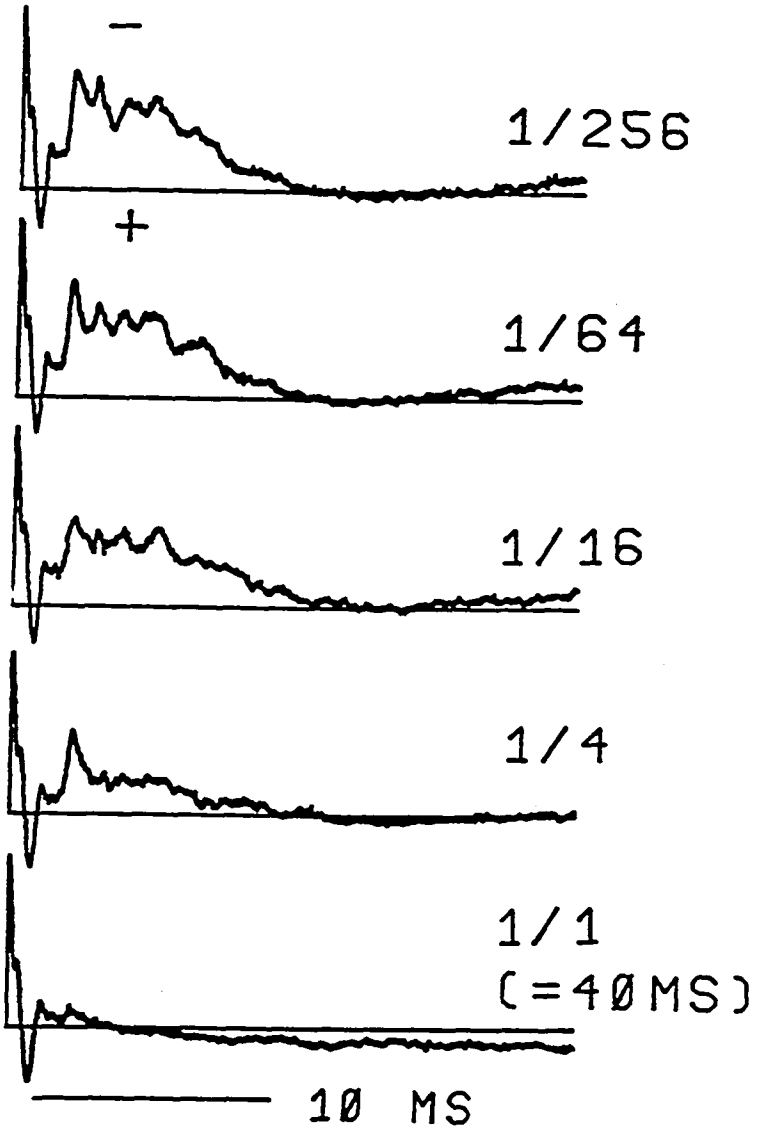


Fig.2-3

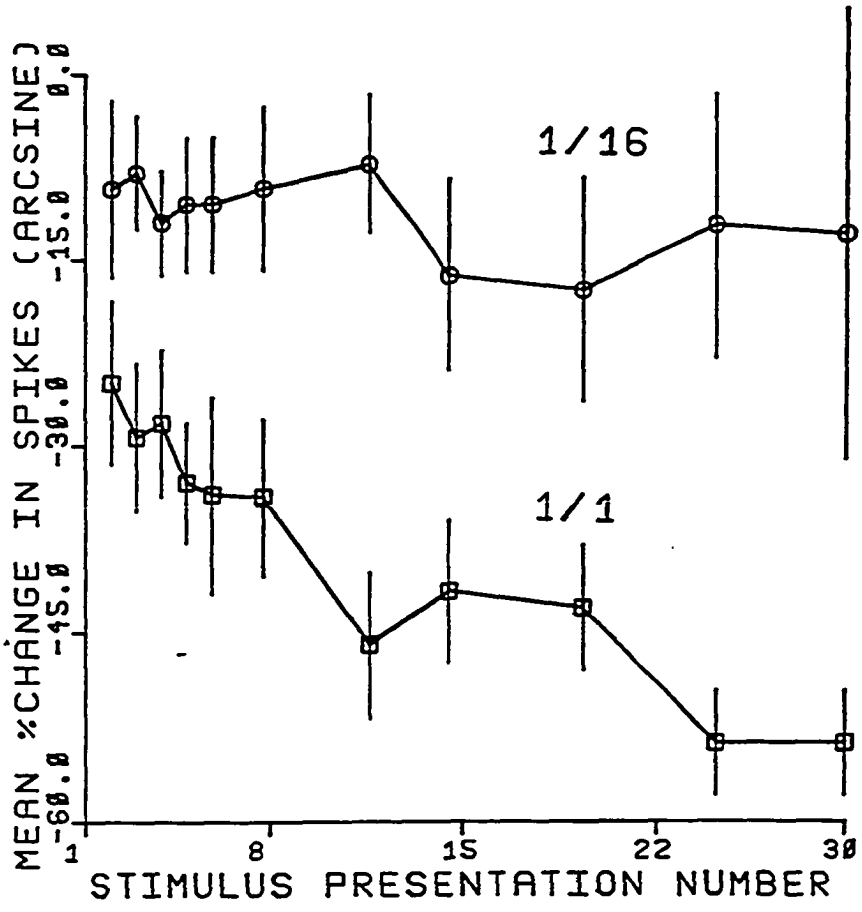
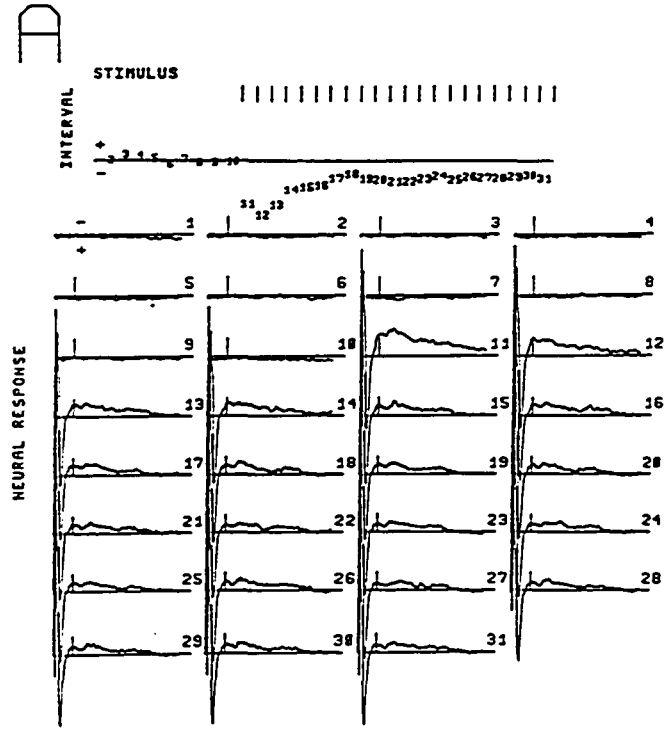

MEAN %CHANGE IN SPIKES (ARCSINE)
0.0
-15.0
-30.0
-45.0
-60.0
1/16
1/1
1
8
15
22
30
STIMULUS PRESENTATION NUMBER

Fig.2-4


A
STIMULUS
INTERVAL
NEURAL RESPONSE

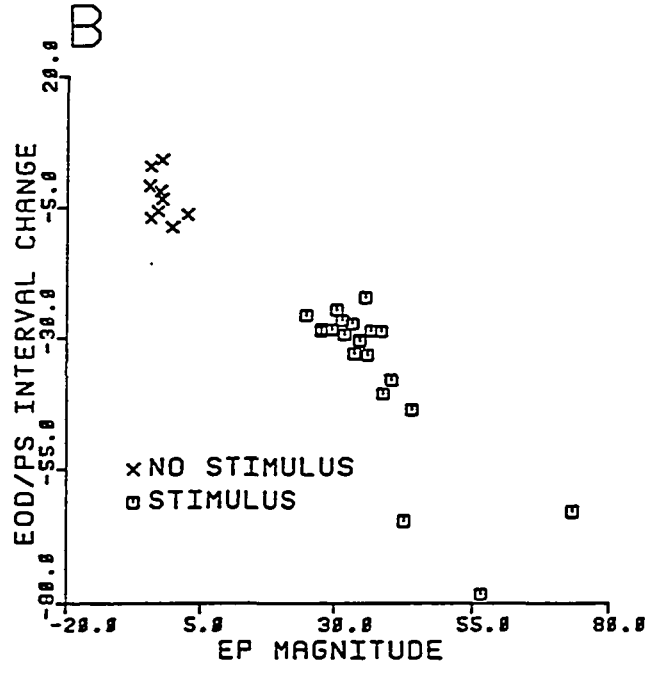

B
EOD/PS INTERVAL CHANGE
EP MAGNITUDE
× NO STIMULUS
□ STIMULUS

Fig.2-5

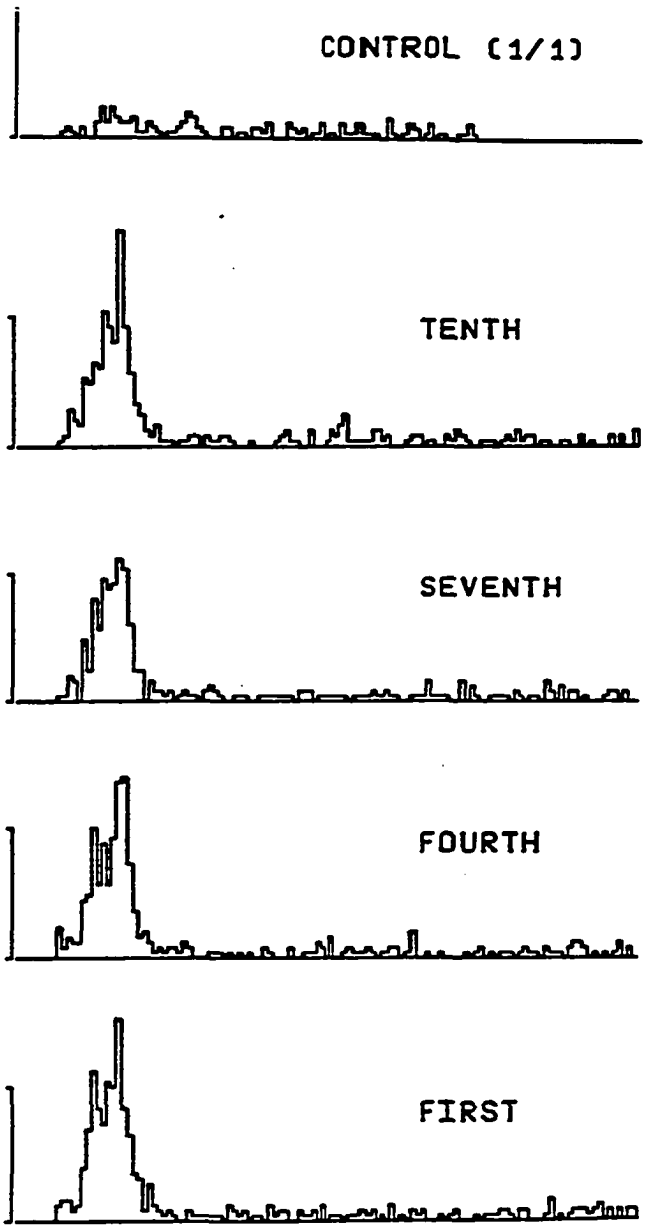


Fig.2-6

CHAPTER III

ABLATION STUDIES

Neurophysiological results (Chap. II) indicated that the Torus Semicircularis (TS) contained neurons that behave as putative electrosensory novelty detectors, and the activity of these neurons could be correlated with the behavioral Novelty Response (NR). To show that elimination of this area would cause elimination of the electrosensory-induced NR, two H. occidentalis were tested for normal electrosensory NRs, and then were given large bilateral lesions of the TS by passing a large current through the recording electrodes. The NR to electrosensory stimuli no longer could be elicited from these fish, although at least in one case a normal NR would still occur to acoustico-lateral stimuli. Histological verification of the lesion sites confirmed that the "novelty-detecting" regions of the TS were included in the lesions.

An additional set of experiments was performed to show that activity in other major brain regions is not necessary for the NR to occur, and to demonstrate that the loss of electrosensory-induced NRs in TS-ablated fish results from that particular region being damaged and not from general ablation effects. The basic features of these experiments, including immobilization of the fish, were described in Chapter I. Removal of the lobes was accomplished by aspiration of the

appropriate tissue with a glass pipette attached to a vacuum line. Fish were generally allowed one to two hours of recovery before testing. The NR could still be elicited in H. artedi after removal of both lobes of either the forebrain or tectum. Nine fish were forebrain-ablated and seven were tectum-ablated. Forebrain-ablated subjects showed slightly lower responses to light stimuli and habituated to it slightly faster (mean Tau: normal = 6.4, ablated = 5.6) than non-ablated fish. The most consistent effects of forebrain ablation were on responses to sound stimuli. Forebrain ablation enhanced the size of the NR to sound, relative to intact fish, and decreased the habituation rate (mean Tau: normal = 3.9, ablated = 4.9). None of these effects are statistically significant because of limited sample sizes. The significant effect of increased intensity on response size is normally reduced by Flaxedil immobilization. After forebrain ablation, the effect of stimulus intensity on response sizes was significant. There was no significant difference in the rate of habituation to the three stimulus modalities in the forebrain-ablated fish. Removal of the forebrain did not affect the responses to electric stimuli much at all. The rate of habituation was slightly faster (mean Tau: normal = 6.0, ablated = 5.0), but not significantly so. The startle response to acoustic stimuli of forebrain-lesioned Siamese fighting fish habituates more rapidly than that of intact fish, and exhibits a higher frequency of occurrence (Marino-Neto & Sabbatini 1983).

Other studies of the effects of lesions on habituation, done mostly on rats (Jordan & Leaton 1982, Capps & Stockwell 1968), implicate the reticular system as the effective locus. The results of

this study are far from conclusive, but do indicate that gross ablation of the forebrain alters the fish's state of arousal, and thus the reticular system may be involved in the habituation of the response examined. The effect of tectum ablation was mostly to reduce response size. Habituation rates to electric (mean Tau: normal = 6.0, ablated = 6.4) and sound (mean Tau: normal = 3.9, ablated = 5.6) stimuli were slower than normal. Responses to light stimuli did not occur regularly in tectum-ablated fish. As with forebrain-ablated fish, habituation rates to the stimulus modalities did not differ.

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