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MALCOLM, David Stuart, 1941-
STATIONARITY OF INDIVIDUAL RESTING
PATTERNS, US EFFECTS, AND DISCRIMINATED
PAVLOVIAN CONDITIONING OF THE ELECTRIC
ORGAN DISCHARGE IN MORMYRIDS.

City University of New York,
Ph.D., 1977
Psychology, experimental

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STATIONARITY OF INDIVIDUAL RESTING PATTERNS, US EFFECTS, AND DISCRIMINATED PAVLOVIAN CONDITIONING OF THE ELECTRIC ORGAN DISCHARGE IN MORMYRIDS

by

DAVID S. MALCOLM

A dissertation submitted to the Graduate
Faculty in Psychology in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy, The City University
of New York.

1977

This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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ABSTRACT

STATIONARITY OF INDIVIDUAL RESTING PATTERNS, US EFFECTS, AND DISCRIMINATED PAVLOVIAN CONDITIONING OF THE ELECTRIC ORGAN DISCHARGE IN MORMYRIDS

David S. Malcolm

Advisor: Professor Frank J. Mandriota

This series of experiments was designed to provide more extensive and systematic information on several aspects of the electric organ discharge (EOD) activity of mormyrid fish. In Experiment 1, the EOD activity of six *Gnathonemus petersii* was recorded for thirty-minute samples at various times during the light-dark cycle after the fish were adapted to isolation in individual tanks. Four *Brienomyrus niger* were recorded in a community tank and moved into isolation. Sixty-minute recordings were made at the same time daily for 20 consecutive days. It was found that even with the highly variable nature of the EOD activity that mormyrids, while not exhibiting stationarity as proposed by Bauer (1974), display a consistency in the distribution of their EOD activity over time. While individuals varied in the amount of stability shown, all of the fish showed a large degree of consistency. *G. petersii* had coefficients of concordance that ranged from .72 to .84 for the ten recordings in the light. Concordances were higher at night, .83 to .99, even though fish are most active then. This is largely due to the increase in burst activity which correlates with increased motor activity. The *B. niger* also showed a high degree of stability in EOD activity over days. When moved from the community tank to individual tanks all four fish had a decrease in burst activity over days. Five

fish from Experiment 2 which were placed into the experimental tank five times with 150-min adaptation showed only a slightly lower degree of stability than the fish that were undisturbed for 20 days in Experiment 1. This finding of stability of EOD activity can provide a stable baseline for experimental manipulations. The hypothesis of consistent individual differences proposed by Moller and Bauer (1973) was not totally substantiated. While the fish do show stable EOD patterns the differences are not great enough to allow all fish to be distinguished.

In Experiment 2, a systematic study of different levels of US found similarities and differences in individual responses to electric shock. Five levels of a 100 msec, DC pulse ranging from .0005 to 20 V/cm were presented in an ascending or descending order with one level of US per session. A reliable increase in EOD activity was seen to the higher levels of US while both increases and decreases were seen to the lowest levels. Of several response measures used the ratio of mean frequencies for the 1 sec pre- and post-US periods was the most sensitive and allowed reliable discrimination of a response to the four highest levels of US for the G. petersii.

Experiment 3 was an extension of the classical conditioning procedure of Mandriota, Thompson, and Bennett (1965) to a discriminated Pavlovian procedure. Changes in illumination were used for the CS + and CS -. Fish received one of the four levels of US that produced reliable responding in Experiment 2. Each fish received 40 CS only, 200 conditioning, 200 reversal, and 100 trials with a US of 20 V/cm. Dis-

criminated responding occurred in conditioning and reversal in only two of the eight fish. In most cases discriminated EOD behavior occurred when the CS + was the decrease in illumination. This CS also produced greater responding in the CS only phase and most of the instances of discriminated responding obtained would seem to be the result of an interaction between the light decrease CS and the US.

ACKNOWLEDGMENTS

This work was made possible by funds from the Biopsychology Program, Hunter College of the City University of New York.

The author wishes to thank his advisor, Professor Frank J. Mandriota, and the committee members, Professors Peter Moller and Robert L. Thompson for their guidance, thoughtful comments, and generous support throughout his graduate career. Additional thanks are given to Professors James Atz and Peter Gold for their helpful comments and advice while serving on the final examination committee.

Lastly, a special thanks to my wife and fellow graduate student Terry for her patient support and understanding.

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INTRODUCTION

The weakly electric fish have evolved a highly complex system of an electric organ and electroreceptors which is used for electrolocation and communication. Bullock (1973) and Scheich and Bullock (1974) have proposed a division of these fish into two groups based on differences in the type of electric organ discharge (EOD) they exhibit: wave species, characterized by an approximately sinusoidal waveform; and pulse species, where the duration of the EOD is brief compared with the interpulse interval. The wave species show great regularity in their discharge with only short variations in frequency of under 2%. The frequency of the EOD is usually high compared with the pulse species. Most wave species are gymnotoids such as Sternopygus macrurus (50 to 150 Hz), Eigenmannia virescens (250 to 600 Hz), and Sternarchorhamphus species (greater than 2000 Hz) (Bullock, 1973). Individual Gymnarchus niloticus have a frequency range of 300 to 400 Hz. All of the mormyrids and several gymnotoids, including Electrophorus, Gymnotus, and Hypopomus, are pulse species. In mormyrids the EOD is typically a brief, biphasic pulse. The pulse duration varies over a range of at least .3 to 5.0 msec. Bennett (1971a) reports a duration of .3 msec for Gnathonemus niger. G. petersii and Marcusenius species also have a pulse duration of .3 msec (Mandriota, Thompson, & Bennett, 1965). Moller (Note 1) has observed pulse durations of up to 5.0 msec duration in some species. In pulse species, particularly mormyrids, the highly variable nature of the EOD activity is a conspicuous feature. Changes in EOD activity are correlated with spontaneous movements of the fish, changes in illumination, and introduction of various stimuli. These include the onset of aeration, mechanical disturbances, sounds, food, electric currents, and the introduction of objects of different conductivity into the aquarium (Scheich & Bullock, 1974).

Electric Organs

In mormyrids the electric organ is modified from caudal muscle fibers (Szabo, 1961). It is located anterior to the caudal fin, in the caudal peduncle, and consists of four columns of cells containing 90 to 200 cells per column. The muscle which usually moves the caudal fin having been completely modified into electric organ, the only other structures found in the peduncle are the spinal column, skin, and the tendons connected to the caudal fin. The innervation of the electric organ is by spinal neurons. The electrocytes, the active cells of the organ, function in the same manner as ordinary muscle or nerve cells, by means of selective permeability and passive movements of ions down concentration gradients. The potentials are generated when the opposite faces of the electrocytes are at different levels. The current flows in a circuit that involves the two cell membranes and the cytoplasm, and is completed through the external medium of the water (Bennett, 1971a). Moller and Bauer (1973) measured 1.1 to 2.1 V for several G. petersii between the mouth and the tail with the fish out of water. In water of 300 to 100 µmho approximately 1 V was recorded at a distance of 5 cm from the fish and .1 V at 20 cm distance. Bell, Bradbury, and Russell (1976) have shown that the peak to peak value and the relative heights of the two phases of the biphasic pulse vary as a function of the conductivity of the water. The first phase, or head positive part of the pulse, shows a monotonic increase in voltage with decreasing conductivity, rising sharply to about 5 V at

50 μmho and then showing a more gradual increase to approximately 8 V as the conductivity is lowered to 5 μmho. The second phase does not show a monotonic function of conductivity but rises to a peak of 8 V at approximately 33 μmho and then declines to less than 4 V as the conductivity decreases toward 5 μmho. The biphasic discharge of the electrocytes is produced by the action of both the anterior and posterior faces of the cell (Bennett, 1971a). The posterior face is innervated and produces the initial head positive phase of the EOD except in *Mormyrops*. The uninnervated, anterior face of the electrocyte produces the head negative phase of the EOD when stimulated by the current flow of the first phase (Bell et al., 1976). Bell et al. calculated the average open circuit voltage per electrocyte for several *G. petersii* and obtained values of 100 to 116 mV for the first phase or posterior face, and 108 to 129 mV for the second phase or anterior face. Bennett and Grundfest (1961) reported values of - 60 mV and 20 mV for the resting and overshoot potentials from intracellular recordings in mormyrids. These values give a sum close to, but smaller than, the values determined by Bell et al. who conclude that the difference is due to damage of the electrocyte following microelectrode penetration. The finding of maximal values for the total output voltage per electrocyte also supports the idea that these must be a barrier to local current flow between the anterior and posterior faces of the cell as this current flow would reduce the total output of each cell. Because of "tight junctions" with a relatively high resistance the current generated at the posterior face must return through the other electrocytes, the skin, and the external medium. When the external medium has a high resistance (low conductivity) there will be little current flow and, therefore, little excitation

of the anterior face of the cell giving rise to a reduced second phase of the EOD spike as was found in their experiments.

A high degree of synchrony is needed in mormyrids to produce the short duration EODs. This is obtained when all regions of the face of the cells fire at the same time. In mormyrids this is obtained by a reduction, through a fusion, of the number of innervating fibers each of which ends on a stalk on the cell face. In mormyrids there are differing numbers of stalks in different species and this number correlates with the duration of the EOD pulse (Bennett, 1971a). In Mormyrus which has up to ten stalks per electrocyte, the discharge duration is .6 msec (Kramer, 1974) while in G. petersii and Marcusenius where the number of stalks is reduced to one through fusion the duration is .3 msec (Mandriota et al., 1965). In some species of mormyrids (e.g., G. petersii) there is a penetration of the innervated face by some stalks which then have their final stage of fusion on the anterior side of the electrocyte. It is in these species that a small, head negative component preceeding the biphasic EOD is observed (Bennett, 1971a).

There are two important aspects of the neural control of the electric organ in mormyrids; the decision to fire the electric organ and the mechanism required for the synchronous firing of all the electrocytes of the organ which is necessary to produce the brief EOD. The command center for control of the electric organ in mormyrids is more complex in function than in other electric fish (Bennett, 1971a). A bilateral pacemaker in the midbrain receives excitatory and probably inhibitory inputs which affect its decision to fire. A command signal from either side is passed to a medullary relay. This single spike generates a two spike discharge in the medullary relay. As the neurons involved have electrotonic junctions the second spike is propagated backward to the half of the pacemaker that initiated the spike and both spikes propagate to the other half. This causes both halves of the nucleus to be placed in the hyperpolarized state that follows an EOD command and both halves are therefore reset, assuring a uniform initial state for the pacemaker after each EOD. The double spike from the medullary relay propagates to the spinal relay where a triple spike is generated leading to a triple spike discharge at the stalks innervating the posterior face of the electrocytes, each of which has a cumulative effect in the generation of the spike discharge of the electrocyte. While one function of this system seems to be to guarantee an "all or none response" of the EOD the full significance of this sequence of events is not known (Bennett, 1971a).

The second important aspect of the neural control of the electric organ is the mechanism involved in the near synchronus firing of the several hundred electrocytes. Because of the different distances involved in the neurons needed to reach the electrocytes some means must be found to equalize the conduction times. One method that occurs in most electric fish is an equalization of the path length by not taking the most direct route to the electrocyte to be innervated. In mormyrids the neurons innervating the more anterior electrocytes appear to have a smaller diameter which gives a lowered conduction time, serving as an additional means to assure that the spikes arrive at the innervated face of all electrocytes simultaneously (Bennett, 1971a).

In theoretical accounts of electroreception the electric field generated by the electric organ was assumed to be a head to tail dipole by Lissmann and Machin (1958) and a dipole located at the ends of the electric organ by Frey and Eichert (1972). Knudsen (1975) made accurate measurements of the fields of four species of gymnotids and found that the field deviated substantially from a dipole in the area around the fish. The field was asymmetrical with the equipotential lines projecting further forward in the lead region, a greater current density at the tail, and a headward bending of the zero potential plane which intersected the fish's body at a point 20 to 30% of the body length ahead of the tail. The caudal pole is a point source but the rostral pole is distributed. These deviations from a dipole are due to the body of the fish. Knudsen found that deviations from a dipole field are increased by increasing fish size, decreasing water resistivity, and decreasing distance from the fish. A comparison of the available electrolocation data with his obtained field data lead him to the conclusion that the fish are electrosensitive only in the near field, the region where the field does not resemble a dipole field. For example the field of a 22 cm long <u>Apteronotus</u> in water of 260 µmho conductivity does not approach within ± 5% a dipole field even at a distance of 40 cm from the fish. Heiligenberg (1975) has studied the electric field and electrolocation by numerical computer simulation. The obtained results agree well with the available behavioral and neurophysiological data. He has determined that the "intensity of the electrical image" caused by the distortion of the fish's electric field by an object on the fish's body decays as a negative power function of the distance between the fish and the object. This finding would agree with the short effective distances for electrolocation obtained in experimental studies. Belbenoit (1970) found that the maximal distance for object location in G. petersii was approximately 4 cm. The field of mormyrids differs in some particulars from that of gymnotid and other electric fish with an elongated body but is basically the same (Heiligenberg, 1975). While the field is not a dipole at the distances involved in electrolocation it does approximate one more closely at the distances involved in communication between two fish. Moller and Bauer (1973) found the communication distance to vary between 12 and 27 cm for different pairs of G. petersii, with greater effective distances occurring when the pair of fish were more closely matched in size and voltage. The effective distances for both communication and electrolocation will depend on the conductivity of the water.

Electroreceptors

Weakly electric fish have a system of specialized receptors which combine with the electric organ to complete the electrosensory and electrocommunication system. Bennett (1971b) reviewed the anatomy and function of the electroreceptor organs. All the specialized electroreceptor organs are modified from normal lateral line organs. In weakly electric fish these are distributed along all or most of the body as would be necessary if they were to serve as part of an electrosensory system. In G. petersii they are found on the head and the dorsal and ventral areas of the body, while in Mormyrus they are found on the entire body except for the area over the electric organ (Fessard and Szabo, 1974). Mormyrids have three distinct types of receptors which are classified by the size of the pore, or opening in the skin. The large and medium types, also called Knollenorgane and

Mormyromast respectively (Fessard and Szabo, 1974), are tuberous organs and do not have an open canal connecting with the surface of the skin, the connection being made via intercelluar clefts. The small type are ampullary organs and have an obvious canal from the receptor cavity to the exterior of the skin. The receptors may also be divided into two classes, tonic and phasic, on a functional basis (Bennett, 1971b). Tonic receptors show long lasting responses to low frequency or maintained DC stimuli. Phasic receptors respond to high frequency stimuli with a brief response and show a small or no response to low frequency or DC stimulation. In mormyrids the large and medium types are phasic receptors and the small type are tonic receptors. All receptor types are innervated by the anterior or posterior lateral line nerves (Bennett, 1971b).

The tonic receptors have a canal open to the exterior which enlarges at its base to form an ampula. In mormyrids a single receptor cell is embedded in the wall of the ampula which is innervated by a single nerve fiber. The skin around the receptors contains a zone of specialised flattened cells with tight junctions which exclude the extracelluar space and therefore serve to channel the current flow through the receptor cell, increasing its sensitivity. Tonic receptors are rhythmically, spontaneously active. An anodal DC stimulus causes an increase in the rate of firing which is proportional to the magnitude of the applied stimulus. A silent period follows the termination of the stimulus. With the application of a cathodal stimulus the reverse occurs, a decrease in firing rate proportional to the magnitude of the stimulus and a burst at the termination of the stimulus (Bennett, 1971b). The sensitivity and range of effective frequencies for small receptors in mormyrids is not known (Kalmijn, 1974). Szabo (1970) has shown that the EOD activity has no effect on ampullary receptors of *G. petersii*. Fessard and Szabo (1974) suggest that they are 60 to 100 times less sensitive than Ampullae of Lorenzini which would place the threshold value in the range of 1 to 10 µV/cm. Suga (1967) measured a threshold of 60 µV/cm in *Gmynotus carapo* with recordings from the nerve fibers and Kalmijn (1973) found a behavioral threshold of 5 µV/cm in *Sternopygus*. As the structure of the ampullary receptors is similar in gymnotids and mormyrids (Bennett, 1971b), the mormyrids would be expected to have threshold values in this range.

The phasic receptors in mormyrids are of two distinct anatomical types (Bennett, 1971b). The large receptors have a canal and ampula surrounded by the zone of cells with tight junctions as do the tonic receptors. In the large receptors however the canal is not open to the surface but is filled with a plug of loosely packed epithelial cells which seem to have a low resistance. The single receptor cell in the ampula protrudes into the cavity. Experiment indicates that the face of the receptor cell behaves as a series capacity. This feature would account for the phasic nature of the response of the large receptors. The capacitance would block any DC, and attenuate AC signals of low frequency resulting in these receptors responding only to high frequency signals, those in the frequency range of the fish's own EOD pulse. The threshold for the large receptors is approximately .2 to .5 mV/cm for a 50% response. One afferent nerve impulse is produced for each applied stimulus pulse and no effect is found on the output if the amplitude of the stimulus is increased over threshold values. These receptors cannot therefore give much information about variations in the fish's electric field. The afferent impulse is produced with the onset of the stimulus for anodal stimuli and at the offset for cathodal stimuli.

The medium receptors are morphologically more complex than the small or large receptors (Bennett, 1971b). They consist of two cavities with a connecting canal. Neither cavity has an opening through the skin. The outer cavity however is outside the insulating zone of flattened, tight junction cells. Each cavity has receptor cells; in the outer they are located in the wall of the ampula, and in the inner they protrude into the ampula. The receptor cells show the series capacity effect as do the receptor cells of the large type. Three nerve fibers innervate each cell. As is to be expected from the more complicated structure of these medium cells the neural response is more complex. The nerve response is phasic, and as in the large type is produced at the onset of anodal and offset of cathodal stimuli. Medium receptors in addition show a graded response by means of reduced latency and increased number of afferent impulses as the stimulus increases in magnitude (Fessard and Szabo, 1974). The threshold of the medium receptors can be as high as 10 mV/cm (Bennett, 1971b). While this is much higher than other receptor types it is within the range of stimulation of the field produced by the fish's own electric organ at the distances involved in electrolocation. These medium receptors are probably the most important for active electrolocation as they have the capability of producing a graded response.

The effective stimulus for the electroreceptor organs is the potential gradient across the skin (Bennett, 1971b). Because the internal resistance of a freshwater electric fish is much smaller than that of the external medium, the inside of the anterior body of the fish is isopotential, serving as a zero reference potential, and the current associated with the EOD passes the skin in the anterior part of the body perpendicular to its surface, thus stimulating the electroreceptors.

Mormyrids have the potential for passive as well as active electrolocation. The tonic receptors can detect currents from many naturally occurring sources in the environment (cf. Kalmijn, 1974) while the field produced by the fish's electric organ allows active electrolocation. The passive detection of external electric fields has not been studied in mormyrids as it has in many other fish probably because of the greater interest in the active system.

Parameters of Electric Organ Discharge Rate

Research with mormyrids has used many different stimuli and experimental situations and all of them have been found to have an effect, often excitatory on the EOD activity. Typical resting, or undistrubed, frequencies in mormyrids range from about 2 to 22 Hz (Kramer, 1974; Mandriota et al., 1965), with the mean EOD frequencies in the range of 2 to 15 Hz. For G. petersii Moller and Bauer (1973) found that 98% of the interpulse times (IPTs) were between 10 and 210 msec (100 to 4.8 Hz). A difference in mean resting frequency between day and night has been reported in all species observed, with the EOD activity being of higher frequency in the dark. Harder, Schief, and Uhlemann (1964) found mean frequencies of up to 10 Hz in the light and 15 to 20 Hz in the dark for G. petersii. For G. niger Moller (1970) observed 4 to 8 Hz during the day and 6 to 14 Hz at night. While an overlap in the range of frequencies is shown, in each case each individual fish had a higher mean frequency at night.

Gallon, Mandriota, and Thompson (1967) showed the effect of water temperature on the discharge frequency of mormyrids. Over the range of 20 to 30°C they found an increase in mean EOD rate with an increase in temperature with $Q_{30/20}$ (or the ratio of rates at 30° and 20°C) of 2.08 and 2.46 for two G. petersii and 2.81 for a G. mori. They also examined the effect of a 100 msec, 5 to 6 mA DC pulse on the EOD activity of four G. petersii. The ratios of the 2-sec period postshock to the 2-sec period preshock for the fish were 2.43, 1.48, 2.62, and 2.11, an increase of more than 100% in frequency in three of the four cases. The response to shock lasted approximately 10 to 15 sec before a return to baseline frequency occurred.

Mandriota et al. (1965, 1968) successfully modified the EOD activity of several species of mormyrids through conditioning procedures. Using an electric shock as the reinforcement both Pavlovian and operant conditioning were obtained. The conditioned response and the operant response were an increase in instantaneous frequency of EOD activity to at least 2.8 times the individual baseline level.

Movement of the fish as in swimming or in being physically transferred from one tank to another also has effects on the EOD activity. Kramer (1976) presents time interval (TI) histograms of the EOD activity of resting and swimming G. petersii. The resting histogram is trimodal and has a mean frequency of 8.4 Hz. When swimming the EOD activity of the fish increases to 16 Hz and is unimodal. Moller (1970) reported observing "peculiar" discharge patterns in G. niger after transfer to the experimental tank. Experimenters have used various adaptation times in experiments for the fish to become adapted to the new situation. Mandriota et al. (1965, 1968) allowed the fish to adapt overnight. Moller and Bauer (1973) used an adaptation period of a few hours, the time required for the fish to accept and remain within a provided shelter tube. Kramer (1976) in agonistic interaction experiments allowed the resident fish to inhabit the experimental tank for at least three days. In similar experiments Bell, Myers, and Russell (1974) allowed a G. petersii a one hour period to recover from anesthesia before the second fish was also placed into the experimental tank. After the interaction with the first pair had been recorded, the second fish was removed and as many as six different fish were placed, one at a time, in the tank to interact with the first fish. Therefore, in each pairing the first fish had an increased adaptation time to the test tank. Although the transfer is known to affect the EOD activity of mormyrids, none of the experimenters has tried to determine by an examination of the EOD activity when the fish has adapted to the experimental situation. Moller and Bauer (1973) did use a criterion of adaptation, the accepting of a shelter in the experimental tank. Because it is the EOD activity is usually being studied, a criterion based on the discharge behavior would seem to be preferred.

Dewsbury (1966) has suggested that the increases in EOD activity seen in pulse type electric fish may be related to the concept of arousal. This increase in activity is seen in situations that might be expected to increase arousal such as changes in illumination, shock, locomotion and disturbances of the fish. Hagiwara and Morita (1963) have offered the suggestion that an increase in EOD activity increases the information flow of the fish through an increased sampling of its environment. This is not inconsistent with Dewsbury's concept of arousal as in situations that would lead to an increased level of arousal in the fish an increased information flow would usually also be desirable. Support for Hagiwara and Morita was obtained in experiments on electrolocation by Heiligenberg (1975) and Push (Note 2).

In both cases the response used to determine the distance at which the fish, G. niger and G. petersii, detected the object of conductivity different from the water was an increase in the rate of ongoing EOD activity. To distinguish a criterion response from the spontaneously occurring changes in EOD activity Push required the increase in EOD activity to have a duration of at least 2 sec. The data obtained in these two experiments agree with the theoretical model of Heiligenberg (1975) and the experimental results of Belbenoit (1970) which used a conditioned locomotor response to determine the maximal distance for electrolocation.

Bauer (1974) found that G. petersii show individual differences in resting EOD activity. There were differences in the TI histograms of EOD activity both in the number and the location of modes and minima. In several fish these individual resting patterns remained constant over time and Bauer suggested that the fish were showing stationarity in their EOD activity. This is an interesting finding because it indicates that despite the high degree of variability shown in the EOD activity and its sensitivity to disturbance by stimuli of most modalities there are consistencies in the behavior of individual fish.

It is in the social or more particularly agonistic behavior of mormyrids that the extreme variability of the EOD activity has been shown. Russell, Myers, and Bell, (1974) and Bell et al. (1974) studied agonistic activity in <u>G. petersii</u> by placing a second fish, an intruder, into the tank of a resident conspecific. When the second fish was placed into the tank pauses in EOD activity were observed which lasted from 2 to 6 sec. The IPTs observed during attacks were as short as 9 msec (111 Hz). The average EOD frequency of the dominant fish was 21.8 Hz and that of the subordinate fish 13.6 Hz. They also observed a behavior which they called the "echo response" in which the EOD of one fish followed that of the other with an interval of 10 to 14 msec. This extreme range of EOD frequencies, from pauses of up to 6 sec duration to EOD activity of over 100 Hz illustrates the great range and variability of the EOD activity in mormyrids. Kramer and Bauer (1976) and Kramer (1976) studied agonistic behavior in <u>G. petersii</u> and the relationship between EOD activity and motor behavior. The mean frequency of the EOD activity was found to increase in attack behavior to three times that of resting level and two times the level seen in an isolated swimming animal. They also found that the amount of variability and the shape of the TI histogram changed with different behaviors. Attacking behavior is accompanied not only by a large increase in EOD rate but also by increased variability in EOD activity. Two sharp high frequency peaks are seen. A fleeing fish also shows a higher level of variability than a resting or isolated swimming fish with some IPTs being of 1 sec duration. While the range of IPTs resembles most that seen in an attacking fish, the two high frequency modes are not seen. The distribution is unimodal with the peak value being the same as that seen in an isolated swimming fish.

The high variability of EOD activity of mormyrids and the changes in EOD activity correlated with the introduction of stimuli of many modalities make these fish an interesting and challenging subject of study. Bauer's (1974) finding of a stationarity of indivdual resting patterns in the EOD activity of G. petersii suggests that there may be less variability in the EOD activity than is at first apparant. This stationarity may provide a means of determining when a fish has adapted to a new situation. The disturbance of being moved into an experimental tank produces a large increase in high frequency EOD activity, as does most any new stimulus (Scheich & Bullock, 1974). Adaptation times in studies with mormyrids have varied greatly with no standard procedures existing for insuring that a stable baseline of EOD activity exists to measure the experimental manipulations against. If stationarity returns to the EOD activity of a fish it would provide a stable baseline. One part of this series of experiments will be a more extensive analysis of the stationarity of individual resting patterns in mormyrids and an examination of adaptation to a new situation and the usefulness of stationarity as a measure of adaptation to this new situation.

The variability in EOD activity has made the use of naturally occurring responses less valuable for sensory studies with these fish (e.g., Push, Note 2). An extension of the conditioning techniques of Mandriota et al. (1965) to discriminated Pavlovian conditioning would provide a useful means of obtaining a consistency in EOD activity that would enable further electrosensory studies to be done, including instances where no pre-existing EOD response occurs. The effects of several levels of electric shock on the EOD will also be examined to extend the findings of Gallon et al. (1967) to other values and to determine if lower levels of shock will serve as potential US for conditioning.

EXPERIMENT 1

The existence of consistent individual patterning in the EOD behavior of mormyrid fish was first reported by Moller and Bauer (1973). The TI histograms of the control recordings made prior to experiments on social interaction of G. petersii showed consistent individual differences in the spontaneous EOD activity among four fish. Two of the fish had bimodal TI histograms but differed in that the high frequency peak was greatest in one and the low frequency peak in the other. The third fish had a unimodal distribution that was skewed to the left or higher frequencies. The fourth fish had histograms that were much flatter in shape and had either two or three modes. While no systematic exploration was made, as the purpose of this experiment was one of communicative interactions of pairs of fish, the TI histograms presented do seem to show a large degree of consistency in the EOD activity within fish and individual differences between fish. Bauer (1974) specifically addressed this question of consistency in the spontaneous or resting EOD activity of mormyrids. Bauer observed the TI histograms of 12 G. petersii for 3 min periods during the light for 2 to 7 days. Data are presented for five of these fish. For fish H five TI histograms are shown. All the histograms were trimodal with peaks at 18-24 msec, 77-100 msec and 125-133 msec. Bauer described these three modes as the burst activity, first interburst activity, and second interburst activity peaks. The EOD activity was very similar over days with the major change being in the relative heights of the modes. The high frequency mode was the highest on the two recordings made on the first day. In the three subsequent recordings the mode at 77-100 msec was the highest or preferred mode. This similarity among the TI histogram envelopes led Bauer to conclude that there is stationarity of the EOD spike producing process. Fish I, for which five TI histograms were also presented, showed much less similarity in the envelopes. Three of the histograms were unimodal and skewed toward low frequencies; the other two were trimodal. They were somewhat similar in a tendency toward a flat shape without the sharp modes shown by fish H. For five fish, including H and I, a figure showed the locations of modes and minima in the TI histograms. The number of histograms summarized ranged from 8 to 17. Four of the five fish showed trimodal histograms in at least one recording. Bauer reports that stationarity is high in fish H and Q, both of which were trimodal. Fish L which had bimodal TI histograms also showed a high degree of consistency among the ten histograms shown. The other two fish were not as consistent with one or more of the modes not present in every recording. Bauer concluded that the EOD activity has the property of stationarity for an individual G. petersii under resting conditions and was found in 12 individuals followed over two to seven days. While data were not presented for 7 of the 12 fish stationarity did not seem to be present in at least three of the fish for which TI histogram data were reported. The other two fish showed more variability, to the extent that some of the modes were not present in all of the recordings shown, a condition that would argue against a stationarity of the EOD spike generating process in these fish.

While the term stationarity has a specific set of defining properties in the theory of stochastic processes (e.g., Parzen, 1962) it is often used in an approximate manner to mean one in which the measured statistical properties of the process are the same at all times

(Wyman, 1965). Rodieck, Kiang and Gerstein (1962) in developing methods to deal with the spontaneous activity of single neurons (very similar to the spontaneous EOD activity of electric fish in many ways) use the term stationarity when the statistical properties of a short sample of data are independent of the particular choice of sample within a long run of data. This less formal definition of stationarity would appear to be the one implied by Bauer's use of the term.

In the present series of experiments Wyman's (1965) requirement of having the measured statistical properties of the process the same at all times will be used. The aim of the present experiment was to examine the EOD activity of mormyrid fish for the presence of stationarity in a more systematic and extensive manner than has been previously done. Recordings were made throughout the light and dark cycle. This permitted further analysis of the presence of stationarity as a function of time of day, including a determination of the occurence of stationarity during the dark phase. The EOD activity of mormyrids shows differences between day and night. For G. niger, Moller (1970) found that during the day the EOD rate had a frequency of 4 to 8 Hz which increased at dusk to a rate of 8 to 13 Hz at night. G. petersii increases its frequency from 8 to 10 Hz during the light to 15 to 20 Hz at night (Harder, Schief and Uhleman, 1964). Kemmer, Baumann and Altmann (1970) found that G. petersii have trimodal TI histograms during the day with modes at 4.4, 6.5, and 40.0 Hz. At night the shape of the TI histogram changes to a unimodal distribution, skewed toward the lower frequencies. The majority of mormyrid EOD activity is found at frequencies of 36.5 to 4.4 Hz with a mean of 11.0 Hz. Bauer (1974) also reported that most of the fish had trimodal histograms similar to those found in Kemmer et al. (1970). The question arises as to whether mormyrids that show TI histograms of other shapes show the same skewed, unimodal histogram shape in the dark and if there is any relationship between the degree of stationarity shown in the light and in the dark.

The effect of a "typical experimental disturbance" of the fish on the stationarity of its EOD activity was also examined. Transferring a fish from one tank to another causes at least temporary disturbances in the EOD activity. Moller (1970) reported that G. niger when transferred into a new tank changed its usual discharge pattern into a "peculiar" one consisting of groups of double and triple discharges closely following one another. By transferring fish from a community tank to individual tanks after recording them in the community tank and then recording the EOD activity on successive days in isolation information can be obtained on any changes in the EOD activity due to the change from the group situation to isolation, including the effect of the transfer on the discharge activity and the possible emergence of EOD activity that shows stationarity.

Method

Subjects. Eight G. petersii and four Brienomyrus niger obtained from a local supplier were used. Their length was 11 to 15 cm measured from the mouth to the caudal fin. All fish were sexually immature and no attempt was made to sex them. Prior to the start of the experiment the G. petersii were maintained in a 50 gallon community tank containing ten G. petersii and the B. niger were maintained in a 50 gallon tank containing eight B. niger. They had been present in the laboratory for more than one month at the beginning of the experiment.

Apparatus. The experiment was conducted in a small, light proof room 1.5 m by 1.3 m by 2.2 m high. An adjoining room of the same size housed the recording equipment. The individual aquaria were three gallon glass and stainless steel tanks measuring 25 cm by 18 cm with a water depth of 17 cm. The eight tanks were placed on the floor of the experimental room. Cardboard placed between the tanks prevented the fish from having visual contact. Water conductivity was adjusted to 450 µmho at the start of the experiment. Air conditioning in the laboratory maintained the temperature of the tanks within a range of 21 to 23°C. Each tank was provided with an inside filter and an unglazed ceramic tube, 5 cm diameter and 14 cm long, as a shelter for the fish. A stainless steel aquarium hood with a 15 W incandescent bulb on each tank gave approximately 1400 Lux at the water surface directly under the bulb and 700 Lux at the end farthest from the bulb. A timer controlled the lights with onset at 0900 and offset at 2100 giving a 12/12 hour light/dark cycle. The lights of the community tanks were also on this cycle.

The EOD was picked up with stainless steel rod electrodes. The two electrodes were fastened to a piece of acrylic plastic 35.5 cm apart in diagonally opposite corners of the tanks. The electrodes were present in each tank for the duration of the experiment. Shielded cables carried the EOD to the adjoining room where they recorded on two Tandberg tape recorders, models 64X and 6041X, using the 22 Kohm impedance inputs. For the recording of the B. niger in the community tank the pick up electrodes consisted of a 15 cm long section of ABS plastic, 1 cm by 1 cm in cross section, with a single loop of uninsulated wire around each end connected to insulated wire leads. The EOD was preamplified with Grass P-15 battery powered differential preamplifiers having an input impedance of 200 Mohm. The recording of a single fish was assured by monitoring the output of the preamplifier with an oscilloscope.

Procedure. The G. petersii were transferred from the community tank to the individual tanks two weeks prior to the start of the experiment. Two recordings were made of each fish at each of the following times: 0900, 1100, 1400, 1800 and 2030 in the light; 2100, 2300, 0200, 0600, and 0830 in the dark. These times were chosen to sample times both in the light and the dark and the time period around the change in illumination. Each recording was 30 min long. The connection of the tank electrodes to the input of the tape recorder was made a minimum of six hours prior to recording to minimize any effects on the EOD due to impedance changes. These 20 recordings of each fish were distributed over a two-week period with no more than two recordings of each fish made on the same day. A complete schedule of the recording times is included in Appendix A.

The procedure for the B. niger was different. In the light mormyrids remain hidden in shelters in the community tank (Harder, Schief and Uhlemann, 1967). This allowed 60-min recordings to be made of four fish in the community tank immediately after lowering the electrodes into the tank near each of the shelters. Two fish were recorded then were removed and transferred to individual tanks in the experimental room. Each fish was recorded for 60 min after being placed into its individual tank. Sixty-minute recordings were made at 1200 each day for the next 20 days for each fish. The electrodes of each tank remained connected to the inputs of the tape recorders for the three week period.

Tubifex worms were present in each tank at all times. The filters were not changed after the fish were introduced into the tanks to minimize disturbances to the fish. Water loss due to evaporation was made up with demineralized water added at the end of a day's recording period if needed. By the end of the experiment conductivity rose in all tanks to 500 to 550 µmho from the initial value of 450 µmho.

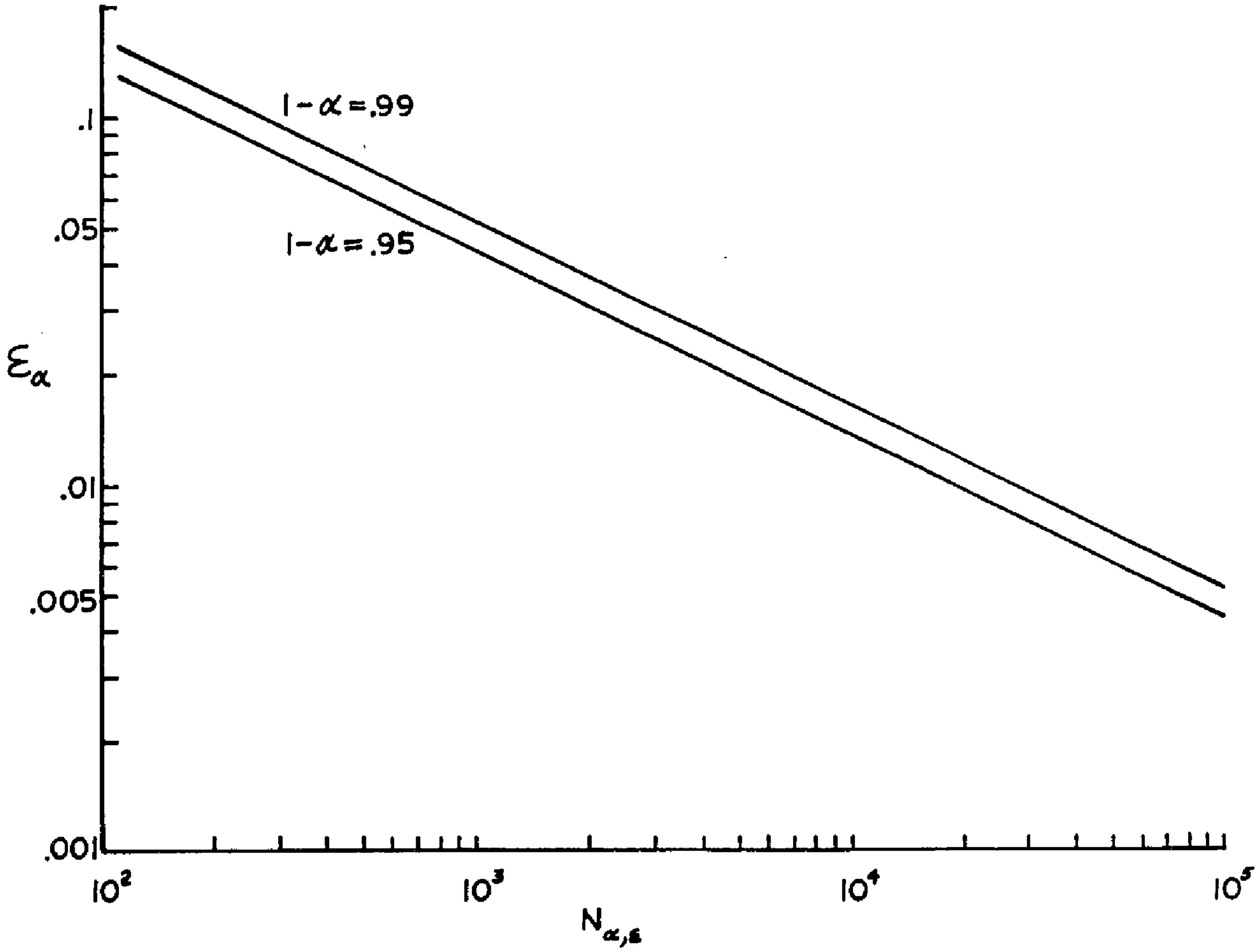
Data Analysis. The data analysis problems of the EOD activity of mormyrids are similar to those encountered in the study of single neuron activity. In both cases the time between events, EODs or spike activity is the only variable. Gerstein and Kiang (1960) discussed the possible types of histogram analysis to be used for data from single neurons. They suggested that for data involving the spontaneous firing of neurons the TI histogram might be the most useful means of analysis as it emphasizes internal timing relationships in the spike train. This method of analysis using TI histograms will be used in this experiment as the behavior of interest is the spontaneous or resting EOD activity of the mormyrids. The degree of consistency of the shape of the TI histograms is a measure of stationarity. This procedure was used by Rodieck et al. (1962) to test the presence of stationarity in the study of single neuron activity.

To assess the presence of stationarity among the TI histograms, Kendall's coefficient of Concordance, $\underline{W}$, will be used (Siegel, 1956). This is a rank order statistic which determines the degree of agreement among several sets of rankings of data. In each of the TI histograms of a given fish and condition, the number of events in each bin of the histogram will be replaced by its appropriate rank, the bin with the greatest number becoming rank 1, etc. The test yields a value of W of 0 to 1 with a value of 1.0 representing perfect agreement among the histograms and a value of 0 total disagreement. In this manner a measure of the actual degree of similarity among the TI histograms, and the presence of stationarity, can be assessed.

The question of the appropriate sample size is a difficult one. If the spike generating process were truly stationary it would not matter as the statistical properties of any sample would be the same as any other. The EOD activity of the fish does in fact show at least one short term behavior that does not have stationarity, burst activity. A burst consists of several short interpulse time (IPT) discharges in sequence. Bauer (1974) has analyzed the EOD activity of G. petersii for this type of sequential dependency by means of "histograms of regulation". In this type of histogram, the total time required for the occurence of the number of EODs in the sample is plotted against the difference in the longest and shortest IPTs in the sample. Sequential dependencies were found when examining EOD discharges in groups of ten. These are due to the bursts in the EOD activity. The analysis of groups of 100 consecutive discharges showed that bursts are composed of groups of less than 100 discharges. The value of variation, a measure of sequential dependencies, also diminishes with groups of 100 discharges and becomes even smaller when groups of 200 discharges are examines. This implies that the long-term effects of any sequential dependencies that may exist are greatly diminished when examining groups of several hundred discharges. This is necessary if stationarity is to exist in the weak sense used in this experiment. The data that Rodieck et al. (1962) analyzed for stationarity possess similar short-term dependencies but they argued that there may still be stationarity in the long run if samples are chosen large enough to reduce these short-term effects. On the other hand if the underlying EOD generating process is changing, samples that are too long may average and obscure these changes in the EOD generating process and lead to a false conclusion of the existence of stationarity. The size of a sample must then be large enough to diminish the short term dependencies that exist, large enough to yield a true picture of the EOD activity of the fish, and not so large as to obscure real changes in the EOD behavior.

There is a method which enables distribution-free confidence bands to be established for cumulative distribution functions, Kolmogorov's statistic (Birnbaum, 1962). By cumulating the TI histograms this statistic may be applied to them. For a given N of events in the distribution, a confidence band of $\pm\varepsilon_\alpha$ with a confidence level $1 - \alpha$ may be determined from Figure 1. For example with an $N = 1000$, the confidence band is approximately ± 4%, with a confidence level of 95%, or ± 5% with a confidence level of 99%. In the present experiment it was decided to try to achieve a confidence band of approximately ± 1% with a confidence level of 95% so that the samples would accurately reflect the EOD activity of each fish. This requires N, the number of EODs in the samples, to be approximately 19000. With the average frequency of a *G. petersii* equal to 10 Hz, 30-min samples should meet this requirement. As the *B. niger* has a lower mean frequency 60-min samples will be recorded. Samples of this size should be long enough that the short-term sequential dependencies will not be a problem. In the case of the *G. petersii* which are to be recorded ten times over the 24 hour light/dark cycle the 30-min recording period is short enough that changes occurring as a function of time of day, if any, should not be obscured.

Figure 1. Distribution-free confidence bands for the cummulative distribution function. For a sample size of $\underline{N}$, the graph shows the size of the confidence band $\pm\varepsilon_\alpha$ for confidence levels of 95% and 99%.



$1-\alpha=.99$
$1-\alpha=.95$
ε_α
.1
.05
.01
.005
.001
10^2
10^3
10^4
10^5
$N_{\alpha,\varepsilon}$

Results

G. petersii. Two of the eight G. petersii died during the recording period and are excluded from the data analysis. Figures 2, 3, and 4 present the TI histograms for the six G. petersii for the ten recordings in the light. In a TI histogram the interpulse times of the fish's EOD are grouped into bins, in this case of 10 msec width. The height of a bin therefore shows the number of responses that occurred with an IPT of that duration. Each bin will be identified by the number of milliseconds at its midpoint. For example, if the 95 msec bin contains 200 responses it means that there were 200 IPTs of between 90 and 100 msec duration. Information about the order of the IPTs is lost in the TI histogram. The TI histogram represents a statistical estimator of the probability density function of the underlying EOD producing process (Sanderson, Kozak, & Calvert, 1973). It is this property which makes them appropriate for a determination of stationarity. The Cynax Histogram Computer (Wright Associates) used to analyze the recordings, has provision for accumulating up to 200 bins of data. With a bin width of 10 msec this allows the determination of IPTs from 0 to 1990 msec with the last bin being all IPTs of greater duration. The TI histograms show 30 bins representing IPTs of 10 to 310 msec duration. This includes greater than 97% of the IPTs in all cases and for most recordings represents greater than 99%. In some recordings almost 2% of the IPTs fall into the last or overflow bin, greater than 1990 msec. Because the fish could not be restrained in this experiment due both to its long term nature and the desire to interfere with the fish as little as possible, it is not possible to determine if these represent actual IPTs of this duration or failures to record the fish because of position changes relative to the electrodes.

Figure 2. TI histograms for GP1 and GP2 for the ten 30-min recordings in the light.

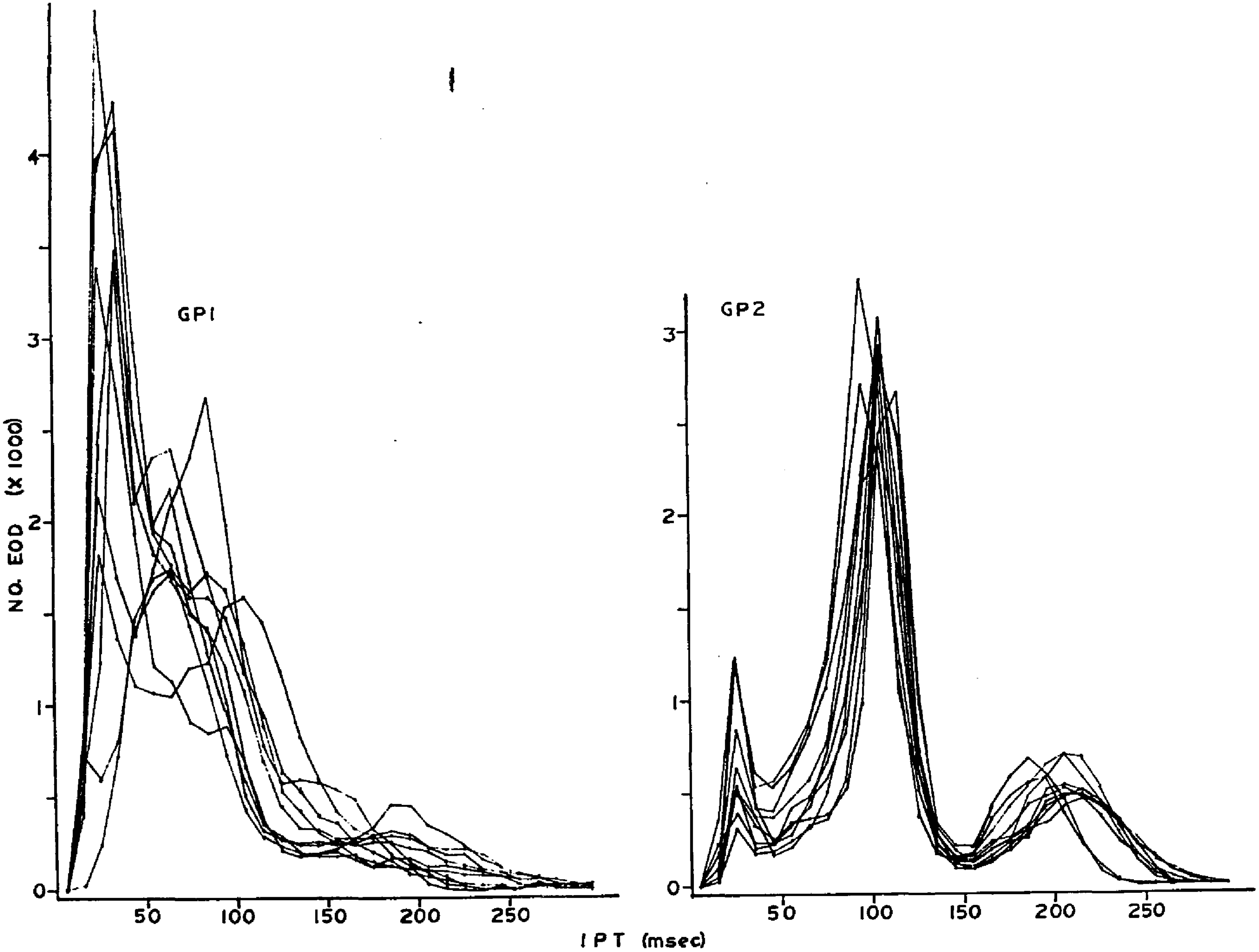

GP1
GP2
NO. EOD (x 1000)
0
1
2
3
4
50
100
150
200
250
IPT (msec)

Figure 3. TI histograms for GP3 and GP4 for the ten 30-min recordings in the light.

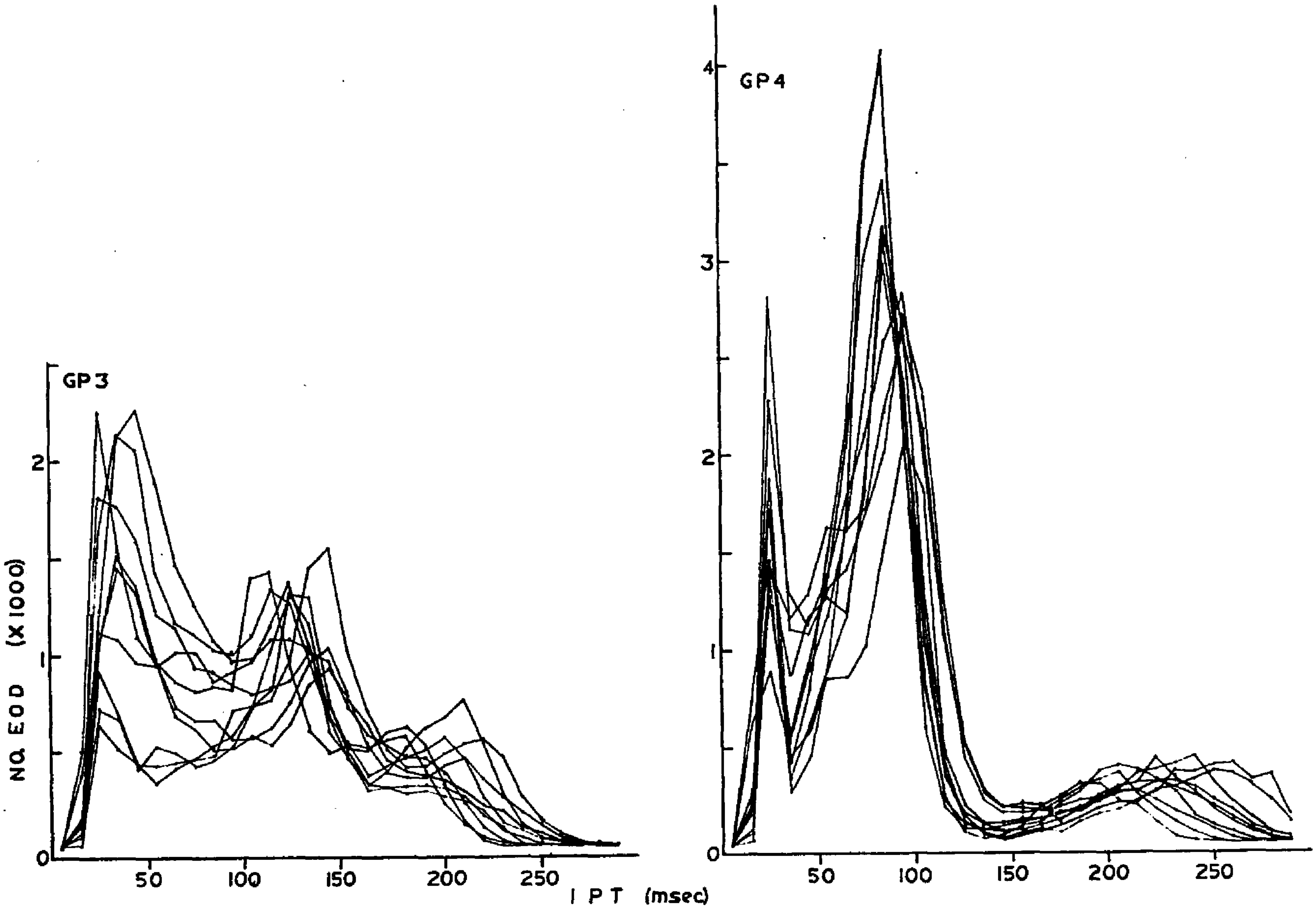
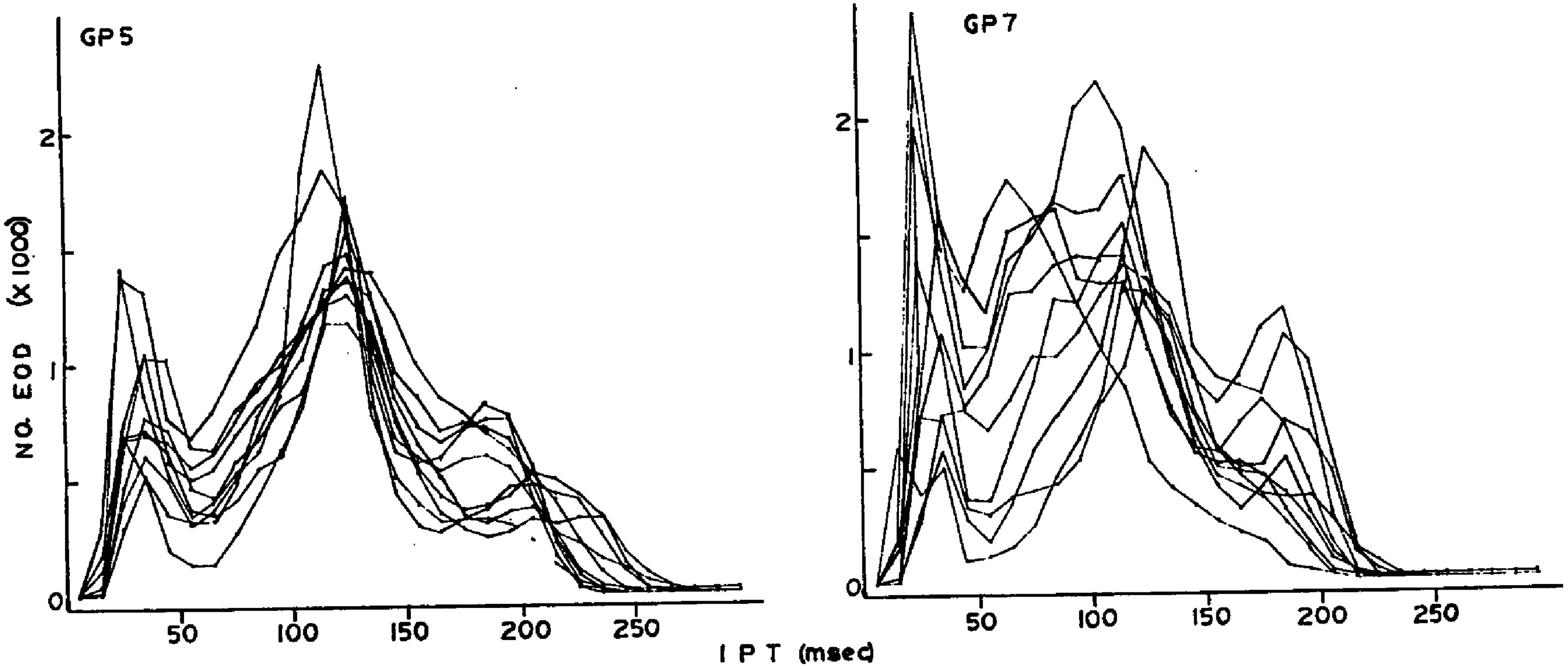

GP3
GP4
NO. EOD (X1000)
I P T (msec)
0
1
2
3
4
50
100
150
200
250

Figure 4. TI histograms for GP5 and GP7 for the ten 30-min recordings in the light.


GP5
GP7
NO. EOD (X 1000)
0
1
2
50
100
150
200
250
I P T (msec)

Moller and Bauer (1973) for G. petersii report that 98% of the IPTs ranged from 10 to 210 msec and the remaining 2% were from 210 to 510 msec. This would seem to indicate that the long duration IPTs found in this experiment resulted from a failure to record the EOD of the fish due to its position relative to the electrodes.

From these histograms it is possible to observe several features of the EOD behavior of the fish. These include modal or preferred frequencies of IPTs, a degree of consistency among the different recordings and individual differences among fish in their EOD activity. A peak in the TI histogram was defined as a mode if it met one of the two following criteria: the bin has 25% more responses in it than either of the adjacent bins; or two or more bins on each side of the bin show a decreasing number of responses. Fish GP2 shows peaks or modes of responding at IPTs of approximately 25 msec, 105 msec, and 205 msec, with the mode at 105 msec more than twice as high as the others. GP1 in contrast shows modes at approximately 30 msec and 65 msec with the main response peak at the higher frequency. GP1 also shows less consistency in its TI histograms recorded at different times. The recordings made in the first week show a single peak at about 30 msec which decreases over days. A second mode developes at approximately 60 to 70 msec and in the last recordings the peak at the high frequencies disappears. This would indicate that even after the two week adaptation period to the experimental tank the EOD activity of some fish is still not consistent. The pattern of GP3 is similar to that of GP1 with modes at 30, 125, and 205 msec. GP3 does not show the one very large dominant modal response as does GP2. While differences are apparent among the different recordings there are great similarities among them. The TI histograms of GP4 are more like that of GP2. The modes are 25, 90, and 225 msec. As was the case with GP1, a significant change over time occurred. The two recordings made first show the highest peaks at 25 msec while in the others the highest peak occurs at approximately 90 msec. Again a high degree of consistency is to be seen among the histograms with the main change being in the heights of the modes. GP5 is also trimodal with modes at 35, 135, and 195 msec. While similar in shape to GP2, GP5 differs in the lower height of the second mode. There is a large degree of consistency among the ten recordings and no consistent changes were noted over days. GP7 is seemingly the least consistent of the fish with large shifts not only in the height of the modes but large shifts in their location. The first peak is at approximately 30 msec and ranges from 15 to 45 msec. The second mode ranges from 75 to 125 msec. A third mode appears in some of the recordings at 185 msec.

Figures 5, 6, and 7 present the TI histograms for the six <u>G. petersii</u> for the ten recordings made in the dark. In comparison with the light recordings it is seen that there is a shift in the distributions toward the shorter IPTs or higher frequencies. Most distributions are either unimodal or have their highest mode at the shorter IPTs. A greater similarity both within and among fish is also apparent in the dark EOD activity compared with the EOD activity in the light. For GP1 six of the ten TI histograms are unimodal with the mode at 25 msec. The other four show an additional mode at about 60 msec. GP2 has a predominance of bimodal histograms with modes at approximately 30 and 80 msec with the mode at 30 msec being slightly higher. GP3 and GP4 show similar patterns with the mode being at approximately 40 msec.

Figure 5. TI histograms for GP1 and GP2 for the ten 30-min recordings in the dark.

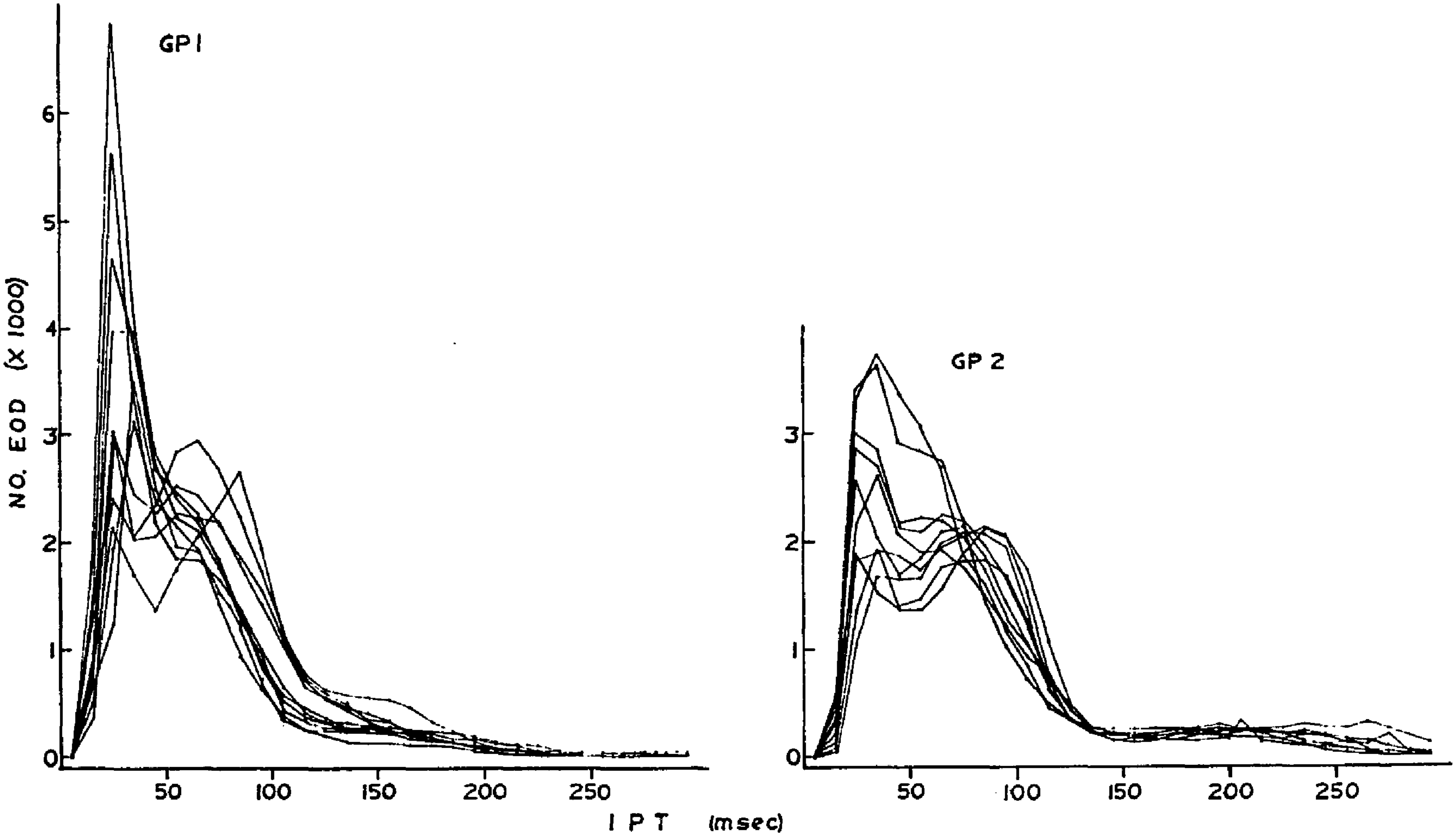

GP1
GP 2
NO. EOD (X 1000)
0
1
2
3
4
5
6
50
100
150
200
250
I P T (msec)

Figure 6. TI histograms for GP3 and GP4 for the ten 30-min recordings in the dark.

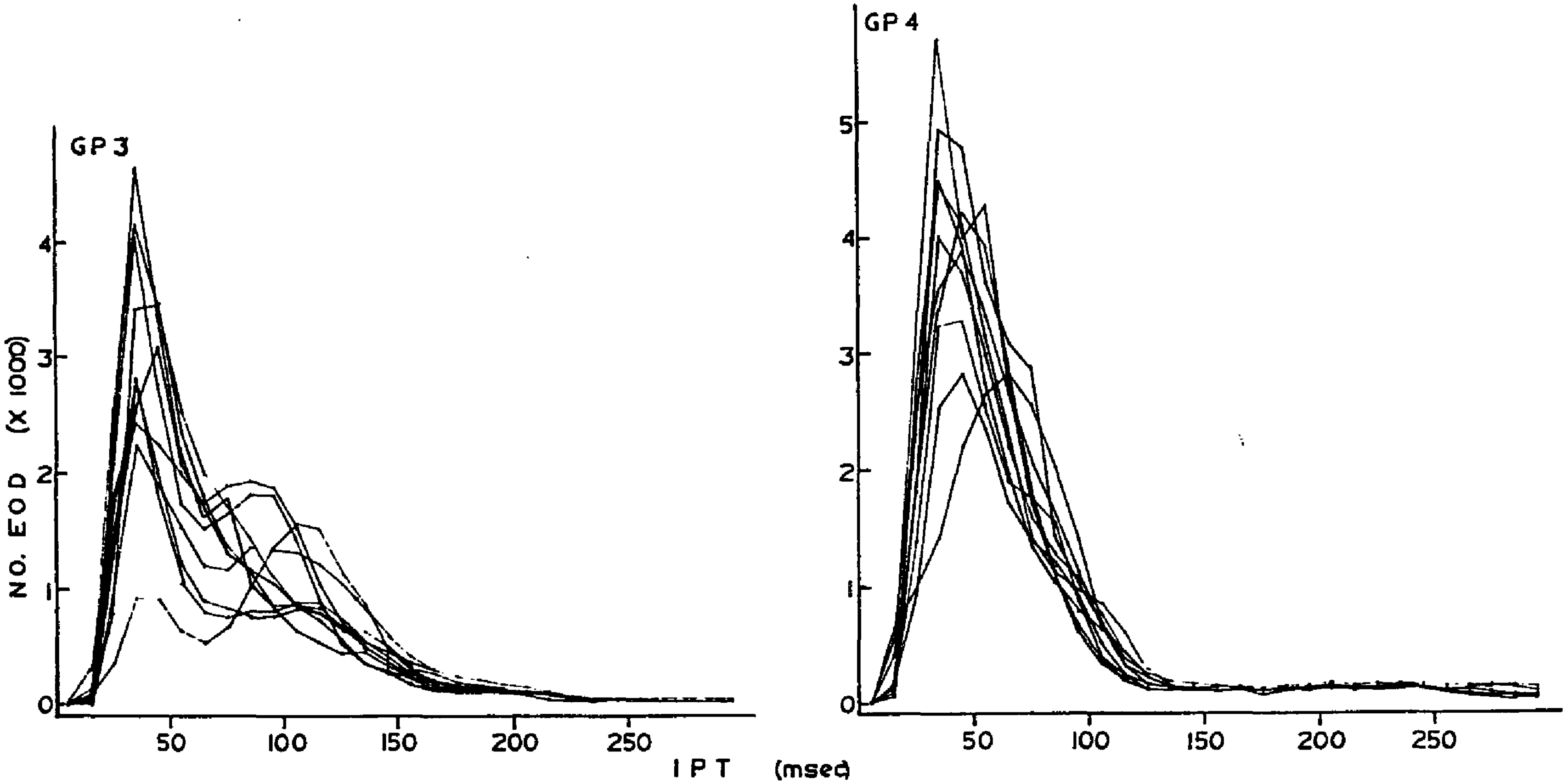
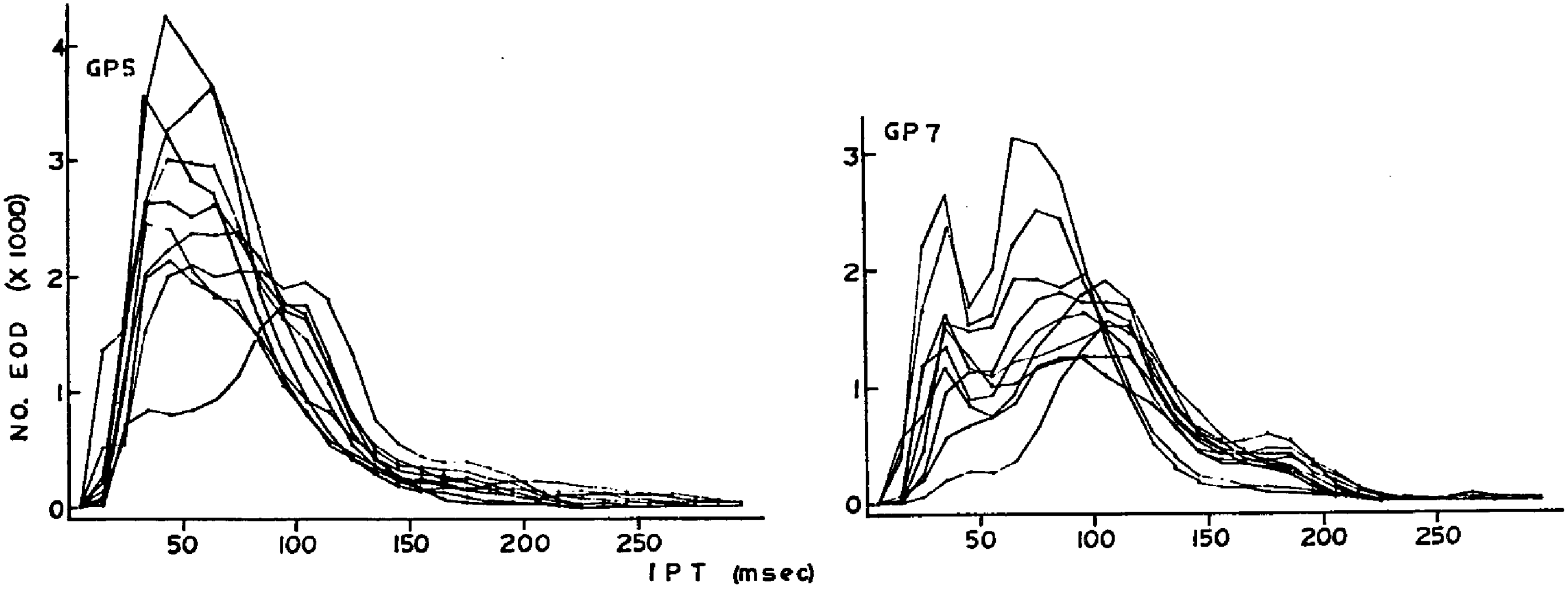

GP 3
GP 4
NO. EOD (X 1000)
0
1
2
3
4
5
50
100
150
200
250
I P T (msec)

Figure 7. TI histograms for GP5 and GP7 for the ten 30-min recordings in the dark.


GP5
4
3
2
1
0
NO. EOD (X 1000)
50
100
150
200
250
IPT (msec)
GP7
3
2
1
0
50
100
150
200
250

The drop in the number of responses at frequencies lower than the mode is sharper in GP4 than GP3. GP3 shows a second mode at longer IPTs in five of the ten recordings. GP5 is unimodal but the modal frequency is lower, approximately 45 msec, and the shape of the distribution is not as sharply peaked. GP7 is different from the others in that not all of the recordings show a peak at the shorter IPTs. In only six of the recordings is a peak found at 35 msec. The other four have a single mode at 95 msec and do not show any high frequency or burst activity mode that is typical of EOD activity in the dark. Some of the histograms have a slight increase also at approximately 175 msec. With the exception of GP7 the recordings in the dark when compared with those made in the light show a change in the shape of the distribution toward that of a single main peak located toward the shorter IPTs of the histograms in the light.

Table 1 presents the mean of the medians ($\overline{Q_2}$) and the interquartile ranges ($\overline{IQR}$) of the TI histograms for the light and the dark for each fish. In each case the $\overline{Q_2}$ is lower for the dark reflecting a higher average frequency of EOD activity. Notice however that there is an overlap of $\overline{Q_2}$ values with GP1 having a lower average IPT in the light than GP2, GP3, GP5, and GP7 do in the dark. GP4 has a lower average IPT in the light than GP7 in the dark. Therefore, while each individual fish shows an increase in average frequency in the dark, average frequency by itself is not enough to separate the EOD activity differences due to light/dark. With the exception of GP2 the $\overline{IQR}$ of all fish is smaller in the dark as a consequence of the tendency toward more sharply peaked, unimodal TI histograms of the EOD activity in the dark. This is not seen in the case of GP2 because of the very high, steeply sloped mode shown in the light TI histograms.

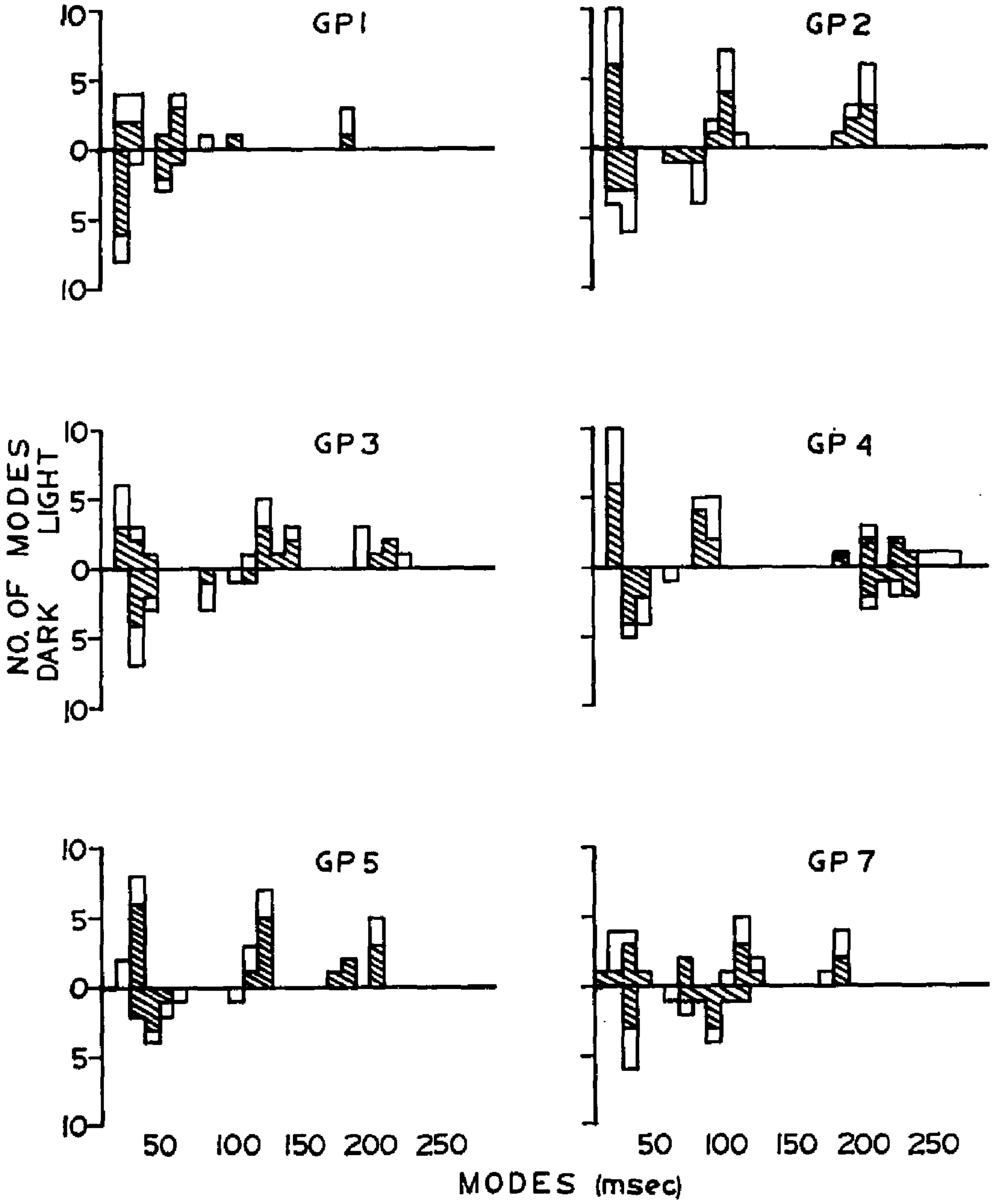
TABLE 1

Mean Values of The Medians and Interquartile Ranges of The Individual TI Histograms.

	Mean Values (msec)			
	Light		Dark	
Fish	Median	IQR	Median	IQR
GP1	66.0	56.8	54.5	47.2
GP2	104.6	49.5	69.4	54.2
GP3	105.0	90.6	68.0	60.1
GP4	84.1	47.3	55.6	40.8
GP5	118.0	69.0	71.6	50.5
GP7	104.5	66.1	91.2	59.1

The modes of the TI histograms from Figures 2 through 7 are shown in Figure 8. Each square represents the occurrence of a mode in a TI histogram. In the light, shown above the horizontal axes, all fish show a trimodal histogram at least once. GP2 and GP4 had trimodal histograms for all ten recordings. The high frequency mode or burst activity is present in 58 of the 60 histograms while the first interburst activity peak is present in 57 or 60 histograms. In both cases it is fish GP1 which does not show both types of activity. All other fish show at least these two peaks on each recording. GP1 has a unimodal distribution five times. While both the burst activity and interburst activity peaks are equally likely to occur, the interburst activity peak is more variable in location both within and between fish. Only 3 of the 58 burst activity peaks occur in other than the 25 msec or 35 msec bins while the interburst activity peak occurs from the 55 to the 155 msec IPT bins. The second interburst activity peak is the most variable of the modes occurring only on 43 of the recordings and in IPT bins from 185 to 275 msec. The two fish, GP3 and GP7, that showed the least consistency in the TI histograms of light EOD activity also show the most variability in the locations of their modes. The first interburst activity peaks of GP7 ranges from 75 to 125 msec and that of GP3 from 115 to 145 msec. The distribution of modes for the TI histograms of the dark recordings, shown under the horizontal axes in Figure 8, reflects the shift toward unimodal distributions in the dark. Forty of sixty distributions are unimodal. Burst activity is present in 54 of 60 histograms. For five of the fish the modal frequency for the burst activity is lower in the dark than in the light. The unshaded squares are the modes from the recordings made around the illumination changes.

Figure 8. The number of modes and their location for each of the ten histograms in the light and the ten in the dark for each fish. The modes for light recordings are shown above the horizontal axes; those for the dark below. Unshaded squares are the recordings which began 30 min before and immediately after the light/dark change.


GP1
GP2
GP3
GP4
GP5
GP7
10
5
0
5
10
NO. OF MODES
LIGHT
DARK
50
100
150
200
250
MODES (msec)

It was expected that these might show a systematic shift toward the pattern of the other light condition, e.g. a trend toward unimodal distributions in the 2030 recordings. No orderly pattern of shifts in location of these modes is apparent in Figure 8.

The coefficients of concordance for each fish for the ten light and ten dark recordings are in Table 2. All values of W are significant at $\alpha < .01$. This does not mean that the recordings for each of the fish are statistically similar, but rather that the samples used were of adequate size to be certain that the degree of concordance or similarity found for each individual fish was reliable. Individual fish have values of W ranging from .72 to .84. The values for the individual fish accord well with the amount of similarity in the TI histograms shown by inspection of Figures 2 through 7. The concordance for all 60 histograms for the six fish is .54 in the light. While this is less than the value for any individual fish it still represents a substantial degree of agreement among the EOD activity of all the G. petersii. This finding is not totally surprising however as the EOD activity of all the fish have many features in common. The burst activity present in most recordings occurs with a small range of IPTs. The interburst activity, while more variable, tends to occur within a limited range.. Also very little EOD activity occurs with IPTs of greater than 250 msec. These features all contribute to the significant degree of similarity among the fish. The concordances for the dark recordings support previously noted features of the EOD behavior in the dark. The W of .84 for all fish reflects the trend toward a unimodal dark TI distribution shown in most fish. Except for GP4 all indivdual fish also increased in similarity of EOD activity in the dark. Fish GP1 reached .99, a

TABLE 2

Coefficients of Concordance, W, for the Individual TI Histograms.

	W*	
Fish	Light	Dark
GP1	.82	.99
GP2	.84	.86
GP3	.73	.95
GP4	.83	.83
GP5	.83	.93
GP7	.72	.88
Comb[a]	.54	.84

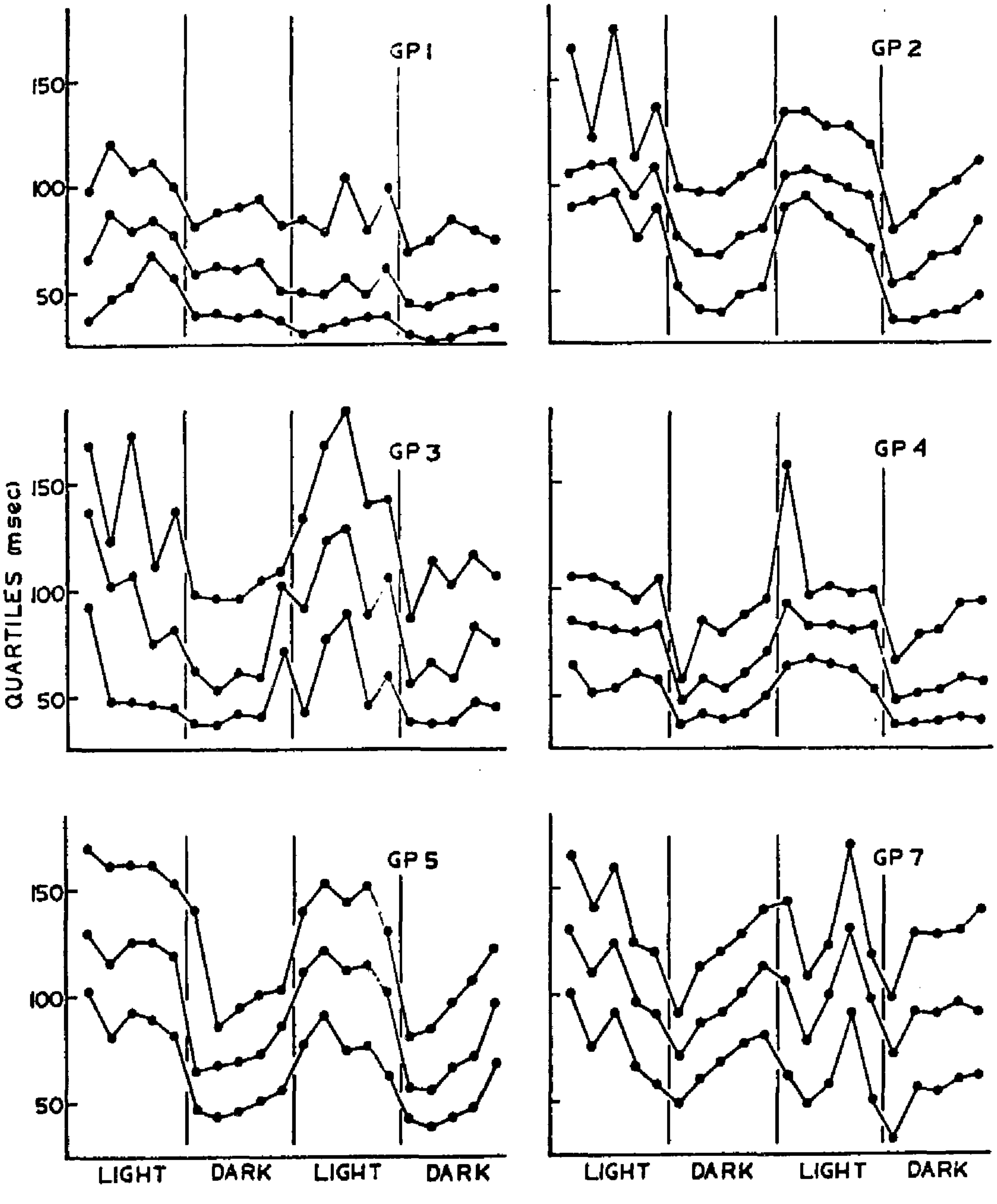
* All values of W are significant, $p < .05$.

[a] The values of concordance for the 60 recordings of the six fish.

close to perfect agreement among the ten dark recordings. These findings do not support the hypothesis of stationarity in the resting or spontaneous EOD activity in these mormyrids as that would require values of W to be close to one in all cases. While a degree of consistency in the EOD behavior is shown, in some cases a very high one, of the definition stationarity was used here (Wyman, 1965) requires that the statistical properties of the samples of data must be independent of the particular choice of sample in time. That is clearly not the case for these fish.

While the modes for the recordings around the illumination changes did not show any systematic variation, there do seem to be some systematic variations in the EOD activity with time of day. Figure 9 presents the quartiles of the TI histograms for each fish. They are in order by time of day of recording. Because the times that the recordings were made over days was randomized, the order on the figure is not the order of the recording. The recordings for the second set of ten recordings replicated the order of the first ten recordings. GP5 and GP7 have increases in the median IPTs throughout both of the dark periods; the average frequency of EOD activity is slowing down. Fish GP2 and GP4 exhibit this same behavior with one exception early in the dark time. GP1 and GP3 also show this frequency decrease over the dark with the exception that an increase in frequency is shown in one 0830 recording for each of them. The pattern with some exceptions then seems to be a gradual increase in average IPT or a decrease in average frequency of EOD activity during the dark period. The quartiles for the light recordings do not have this degree of consistent trend. GP5 and GP7 do show a decline in median IPT for the recordings at 2030, just

Figure 9. Quartiles of the TI histograms of the 30-min recordings for the light and dark.


GP 1
GP 2
GP 3
GP 4
GP 5
GP 7
QUARTILES (msec)
150
100
50
LIGHT
DARK
LIGHT
DARK

before the change to dark. Fish GP3 and GP4 in comparison show a rise in median IPT or decrease in frequency at 2030. The other two fish show one rise and one drop in average IPT. There do not seem to be any orderly progressions due to the increased time in isolation shown by the changes in the quartiles.

B. niger. The effects of change from the community tank to isolation on the EOD activity in B. niger can be seen in Figures 10 through 12. The histograms for the 60 min recordings for each fish are shown for the community tank, the first hour after the move and on the twentieth day in the individual tank. Only for fish BN2 is the EOD activity on day 20 similar to that shown in the community tank. In the other three fish there is a flattening of the TI distribution and a shift toward longer IPTs. No peak of burst activity is seen on day 20. The mode of the burst activity shown by BN2 has shifted from 15 to 35 msec, a change from 66.7 to 28.6 Hz. It would seem possible that the burst activity serves a social function and diminishes or disappears when the fish are in isolation. In the community tank two of the fish had bimodal distributions of EOD activity. BN1 had modes at 25 and 165 msec, with the one at 165 msec being large and sharply peaked. The distribution for BN2 was similar with the modes at 15 and 165 msec. The other two fish had flatter distributions without the clear peaks. The modal value was at 225 msec for BN5 and 235 msec for BN6. After 20 days of isolation the modal values for the three fish with the flat, skewed distributions were: BN1, 215 msec; BN5, 285 msec; and BN6, 305 msec. For BN2 which still retained the bimodal distribution the modes were at 35 and 145 msec. In general the transfer to isolation resulted in a slowing of EOD activity, a reduction of burst activity and a reduction of preferred values of IPTs.

Figure 10. TI histograms for BN1 for the 60-min recordings in the community tank, immediately after being moved to isolation, and the twentieth day in isolation.

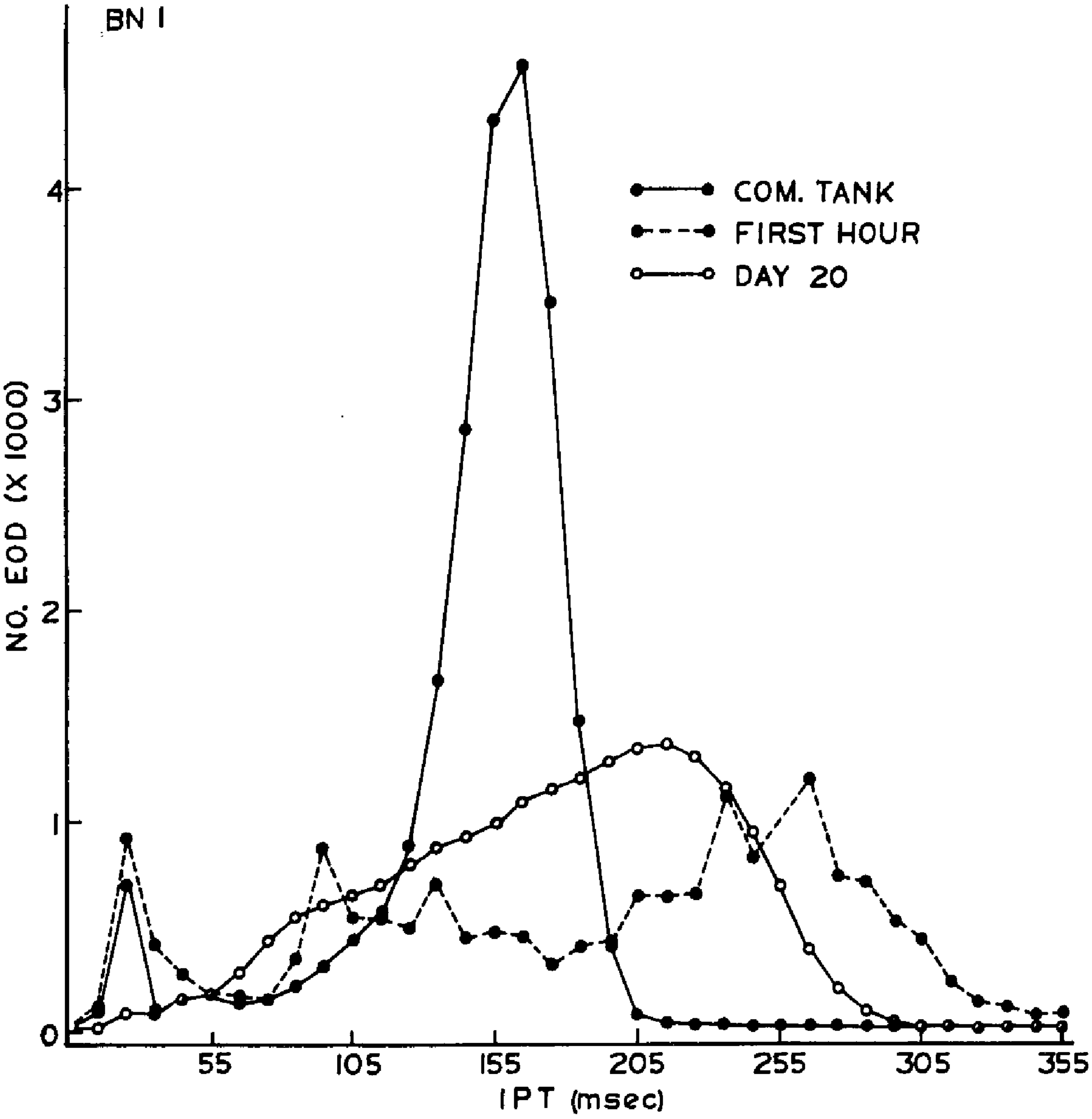

BN I
COM. TANK
FIRST HOUR
DAY 20
NO. EOD (X 1000)
0
1
2
3
4
55
105
155
205
255
305
355
IPT (msec)

Figure 11. TI histograms for BN2 for the 60-min recordings in the community tank, immediately after being moved to isolation, and the twentieth day in isolation.

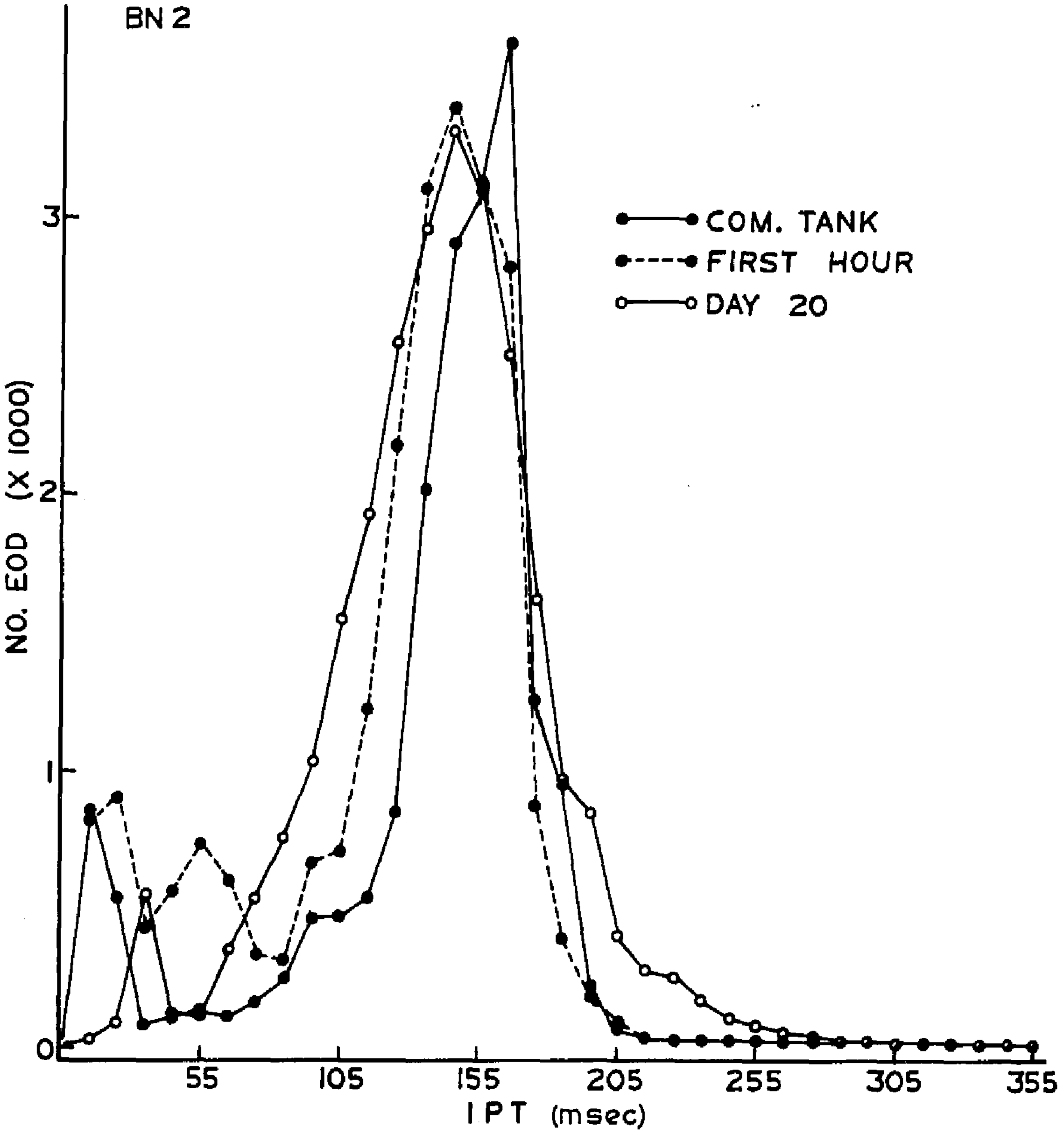
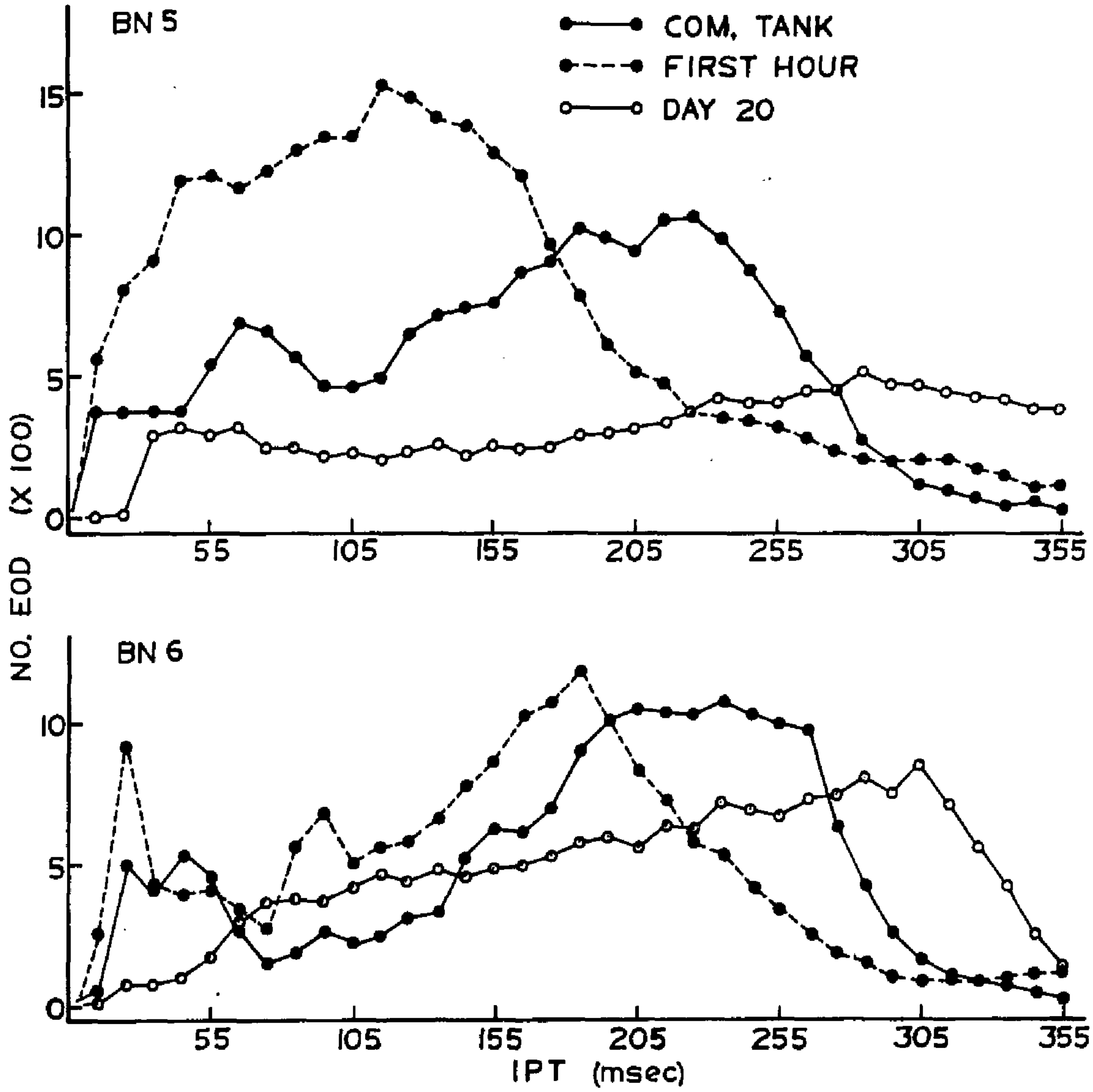

BN 2
COM. TANK
FIRST HOUR
DAY 20
NO. EOD (X 1000)
0
1
2
3
55
105
155
205
255
305
355
I P T (msec)

Figure 12. TI histograms for BN5 and BN6 for the 60-min recordings in the community tank, immediately after being moved to isolation, and the twentieth day in isolation.


BN 5
COM. TANK
FIRST HOUR
DAY 20
15
10
5
0
55
105
155
205
255
305
355
NO. EOD (X 100)
BN 6
10
5
0
55
105
155
205
255
305
355
IPT (msec)

The recovery from the disturbance of being moved and the adaptation to isolation is shown by the quartiles of the TI histograms for each fish for the community tank, the first hour of isolation and the next 20 days is shown in Figures 13 and 14. An increase in the frequency of EOD activity in the first hour after the move is apparent for the fish except for BN1. As was seen in Figure 13, BN1 did show an increase in burst activity but the large increase in low frequency activity caused the median IPT to increase. Each fish shows a slowing of EOD activity on day 1 after 24 hours in isolation. The EOD behavior after this does not show any consistent pattern in any of the fish, although there are trends up or down of four or five days duration. No systematic changes or stabilizations are apparent which could be clearly identified as a product of adaptation to the new situation. Concordances were computed for each fish for the TI histograms by five-day blocks (Table 3). In three of the fish the values of W were quite high for the first five-day block, indicating a high degree of stability of the EOD activity after this short period of adaptation. BN1 is the only fish to show an increase in the values of concordance over the 20-day period, requiring 15 days to reach the level of stability seen in the other fish in the first five days. Increased time in isolation did not lead to a maintained level of stability in all fish. Fish BN6 was less similar in its EOD activity on the second ten days than the first five. BN5 has a large decline to a W of .40 on days 15 to 20, the lowest value obtained by any fish in this experiment. The quartiles of the TI histograms in Figure 14 show that the EOD behavior of BN5 was undergoing a large slowdown over this period. The TI histograms for this period for BN5 are shown in Figure 15. The reduction in burst activity and the shift of the distribution toward longer IPTs accounts for the low value of

Figure 13. Quartiles of the TI histograms for BN1 and BN6 in the community tank (C.T.), immediately after being moved to isolation (F.H.), and the following 20 days in isolation.

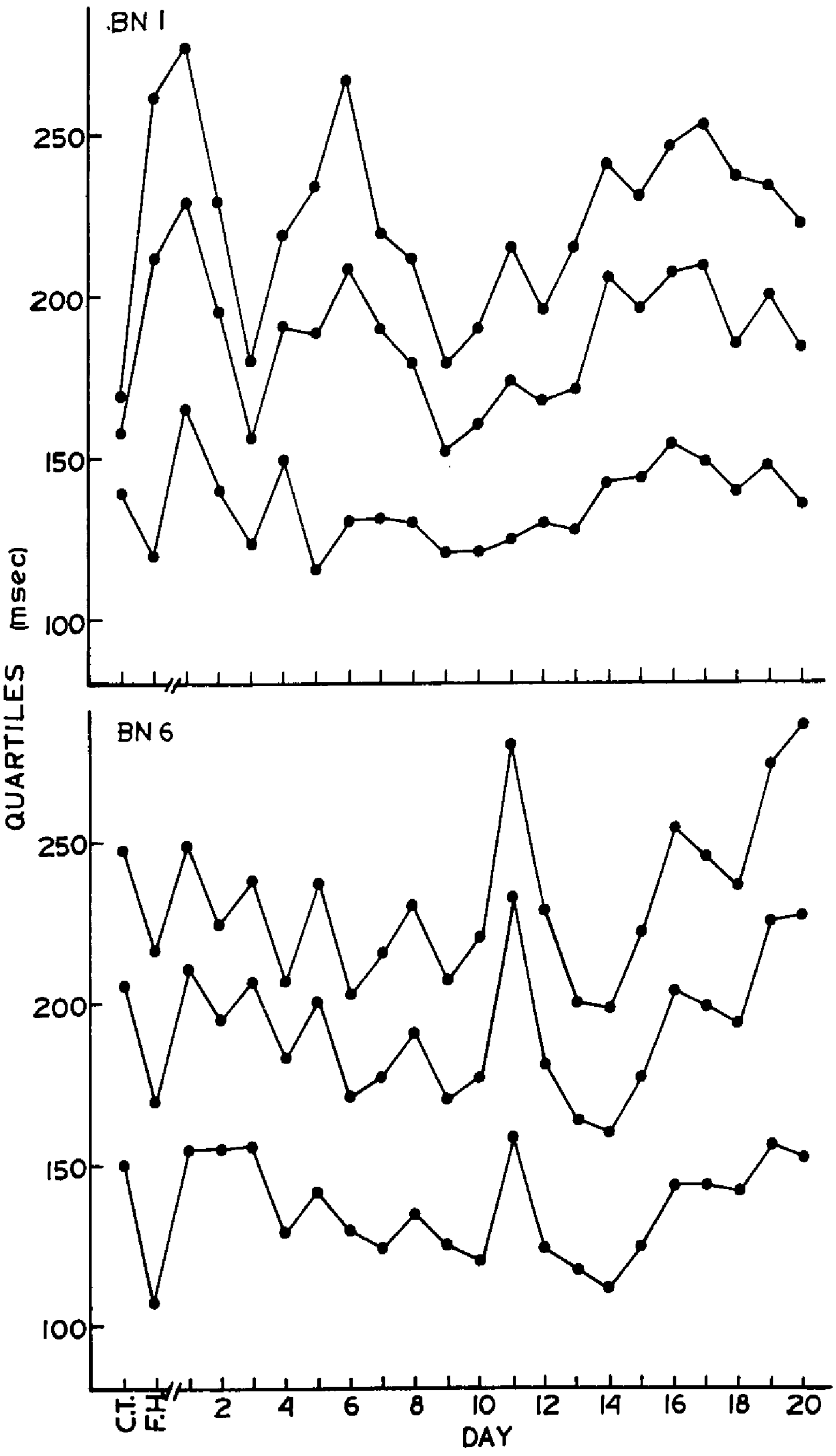

BN 1
BN 6
QUARTILES (msec)
100
150
200
250
C.T.
F.H.
2
4
6
8
10
12
14
16
18
20
DAY

Figure 14. Quartiles of the TI histograms for BN2 and BN5 in the community tank (C.T.), immediately after being moved to isolation (F.H.), and the following 20 days in isolation.

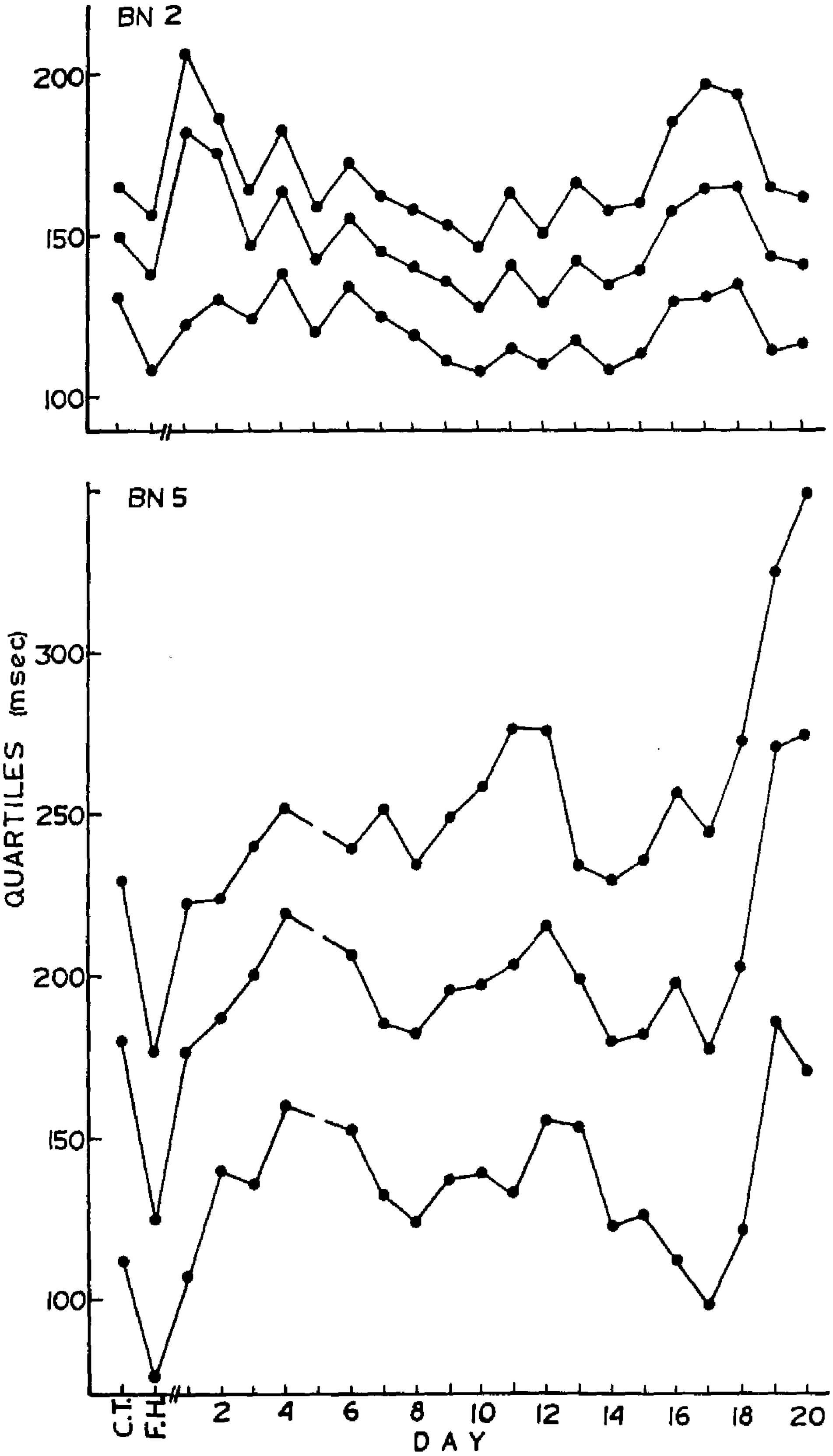

BN 2
200
150
100
BN 5
300
250
200
150
100
QUARTILES (msec)
C.T.
F.H.
2
4
6
8
10
12
14
16
18
20
DAY

TABLE 3

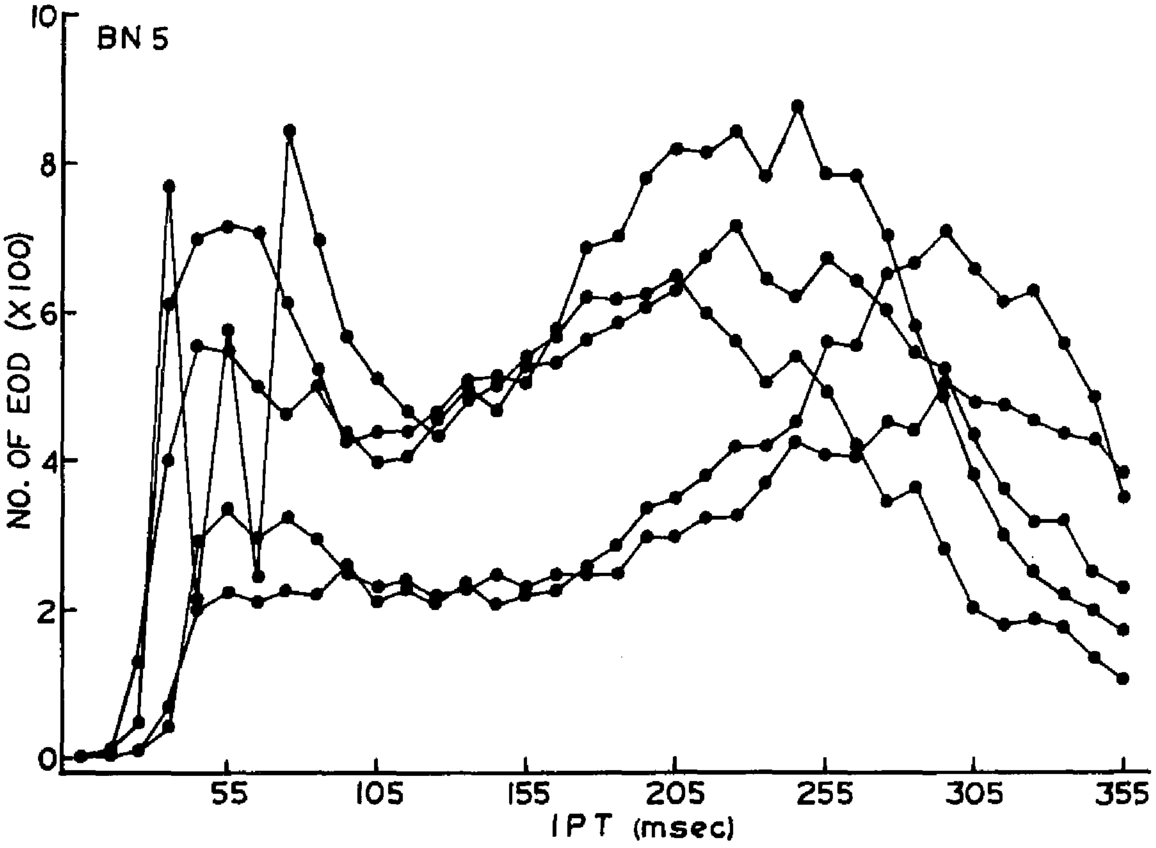
Coefficients of Concordance, W, for the TI histograms of the one-hour recordings for each fish.

Fish	W^* (Blocks of 5 days) 1-5	6-10	11-15	16-20
BN1	.68	.73	.90	.93
BN2	.88	.97	.96	.95
BN5	.90	.92	.90	.40
BN6	.88	.95	.73	.85
Comb[a]	.71	.74	.75	.54

[*] All values of W are significant, $p < .05$.

[a] The values of concordance for the 20 recordings of the four fish.

Figure 15. TI histograms for BN5 for five consecutive days illustrating a low value of W = .40.


BN 5
NO. OF EOD (X100)
0
2
4
6
8
10
55
105
155
205
255
305
355
IPT (msec)

concordance. As a comparison, the histograms of BN2 for days 6 to 10, which had a <u>W</u> of .97, are shown in Figure 16. Again, there is a change over days but it is relatively minor. The distributions retain their bimodal shape while the interburst activity mode moves toward shorter IPTs, from 165 to 135 msec (6.1 Hz to 7.4 Hz). As with the <u>G. petersii</u> these values of concordance do not support the hypothesis of stationarity but do show a high, but not constant, amount of similarity in the EOD activity of the fish.

Table 4 gives the medians and interquartile ranges for each fish for the community tank and the mean of the medians and interquartile ranges for the next 20 days by five-day blocks. For all fish the $\overline{Q_2}$ and $\overline{IQR}$ are greater on days 15 to 20 than in the community tank resulting from a slowing down in frequency of the EOD and an increased variability in the TI histograms. Fish BN5 is the only one to show an orderly trend over days with both $\overline{Q_2}$ and $\overline{IQR}$ increasing over the four, five-day blocks.

<u>Discussion</u>

The results of this experiment do not support the hypothesis of stationarity proposed by Bauer (1974). The existance of stationarity would have required that the TI histograms of a fish be identical in shape or nearly so for all the samples made under the same conditions of resting or spontaneous activity. As the TI histograms represent the probability density functions of the EOD activity this identical shape would have meant that the statistical properties of each of the samples was the same and therefore the EOD activity had the property of stationarity. Of the 24 concordances determined in this experiment, only five

Figure 16. TI histograms for BN2 for five consecutive days illustrating a high value of $\underline{W}$ = .97.

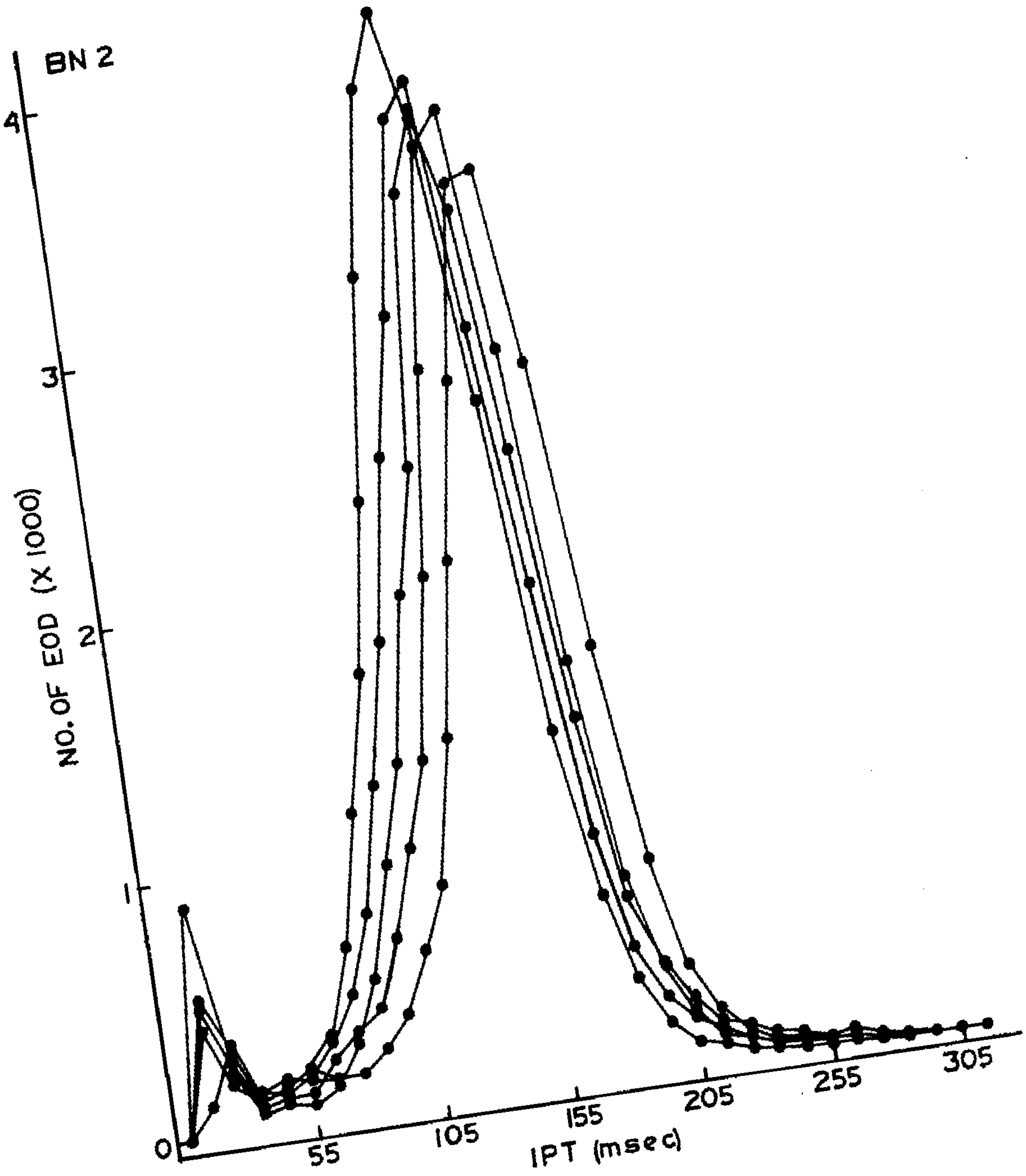

BN 2
NO. OF EOD (X 1000)
0
1
2
3
4
55
105
155
205
255
305
IPT (msec)

TABLE 4

Mean Values of the Median and Interquartile Ranges of the Individual TI Histograms.

Fish	Mean Values (msec)									
			Blocks of Five Days							
	Community Tank		1-5		6-10		11-15		16-20	
	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR
BN1	157.6	30.0	192.0	89.4	177.5	84.6	182.5	86.3	197.6	93.6
BN2	150.7	34.1	162.1	52.4	141.3	38.8	137.8	47.0	154.8	55.1
BN5	179.8	117.1	156.6	78.9	193.4	111.2	195.9	112.3	224.3	151.1
BN6	205.6	98.2	199.8	85.7	177.4	89.6	182.9	99.1	210.1	112.2

are .95 or greater. While true stationarity is not present there is a similarity, often a very substantial one, among the TI histograms of individual fish. This leads to the conclusion that the underlying process of EOD production remains to some degree constant. For the G. petersii in the light, the values of concordance ranged from .72 to .84. As little orderly variation was shown by the EOD activity during the light for individual fish these values of concordance which combine all ten recordings for the light probably do not distort the actual degree of similarity in EOD behavior of a fish. The concordances for the dark were .83 to .99 indicating a greater degree of similarity in EOD activity in the dark even though many of the fish did show consistent changes in average frequency over the dark period. It is also interesting that a greater stability in the EOD-producing process was found in the dark as it is at night that mormyrids are active (Kemmer et al., 1970; Poll, 1959). In general the changes in EOD activity in the dark found in this experiment are similar to that reported by Kemmer et al., (1970). The shift in EOD activity toward a higher frequency unimodal distribution was found in 54 of the 60 recordings. Twenty of the TI histograms also had a second mode which was, however, of lower amplitude than the high frequency peak. The TI histograms for the adaptation experiment with the B. niger revealed only one reliable indicator of disturbance and adaptation, burst activity. All four fish showed an increase in burst activity in the first hour after being moved and then it diminished almost to the point of not being observed at all in three of the fish by day 20. Three of the fish also showed a flattening of the shape of the histograms and a reduction in average frequency over the course of adaptation, while the fourth fish maintained the same EOD activity seen in the community tank. The values of concordance increased over the 20 days for only one fish with the other three showing fluctuations in this statistic. The quartiles of the TI histograms also varied over the 20-day period leading to the conclusion that even when in relatively undisturbed isolation the EOD activity of these fish does not remain totally constant but changes over time. The cause of this variation is not known at present. It is most likely not due to the rise in conductivity from 450 to approximately 500 µmho as DeFazio (1974) found no systematic effects of aquatic conductivity on the EOD activity over a much greater range of conductivities. As there was no increase in the values of concordance shown by the four fish over the 20 days of adaptation, this would not serve as a useful measure of adaptation. It is possible that the adaptation to isolation occurs in a short time, e.g., 24 hours, and the failure of the measures of concordance for the 5-day periods to show an increase is due to adaptation having been completed to its maximum extent in a shorter period of time.

Two of the G. petersii also showed a reduction in burst activity over the course of the experiment even after the 14-day adaptation period, and this lends support to the use of the amount of burst activity as an indicator of the degree of adaptation in mormyrid fish. The reduction, almost to the point of elimination, of burst activity shown by three of the B. niger after being moved to isolation may be related to the lack of conspecific interactions in which high frequency EOD activity plays an important role (Krammer & Bauer, 1976; Moller & Bauer, 1973) or to the general reduction in stimulation due to the smaller and featureless aquaria the fish were moved into in the experiment. While two of the G. petersii showed a reduction in burst activity during the experiment, a burst activity peak was present in 58 of the 60 recordings and even those two recordings without a mode at these frequencies did show a substantial amount of burst activity. It is possible that this represents a species difference in their response to isolation.

Both G. petersii and B. niger showed significant values of concordance when the TI histograms were combined over individuals. This is due to certain features of the EOD activity that all fish have in common as conspecifics and includes a common range of frequencies, the presence of burst activity and similar modal frequencies for the interburst activity peaks. In most cases the individual fish are more consistent than the similarity shown as a group but there is a great variation in the individual values of concordance and, therefore, the EOD activity shown.

As stationarity does not exist for the EOD activity of mormyrids, this reduces the possibility of the fish having consistent individual differences as suggested by Moller and Bauer (1973). The fish do show individual differences. Three of the G. petersii, GP2, GP4, and GP5 can be differentiated from each other in 27 of the 30 recordings solely by location of the first interburst activity peak. The degree of variability shown by the other three G. petersii does not permit them to be identified on the basis of any single sample of their EOD activity. Among the B. niger fish BN2 can easily be identified from the other three fish by the sharply peaked shape of its TI histogram. The changing nature of the EOD activity of these fish seen in the 20 days of recordings for the B niger and in several of the G. petersii raises further difficulties with the possibility of discriminating among them over time. Individual differences do exist, but there is a large overlap in the EOD activity and it changes over time making it impossible to reliably identify each fish from its EOD activity.

EXPERIMENT 2

The aim of this experiment was twofold: to further explore the adaptation effects of mormyrids to new experimental situations; and to study the effects of an electrical unconditioned stimulus (US) on the ongoing EOD activity. As contrasted with the long term, undisturbed adaptation examined in the first experiment, many experiments with mormyrids involve a situation where the fish are placed into the experimental tank for each session with varying amounts of adaptation time allowed. For example Bell et al. (1974) placed a G. petersii into the experimental tank and allowed one hour recovery time from previously given anesthesia. A second fish was then introduced into the experimental tank directly from its home aquarium. After an experimental period of 15 to 45 minutes, the second fish was removed. Additional fish were paired with the first fish, again being moved directly from their home tanks. It is possible that any social interactions found in this experiment are confounded with changes in the EOD activity due only to being moved and to the increasing adaptation time of the first fish relative to the fish with which it was successively paired in the experiment. It would be desirable to establish a baseline of EOD activity to measure experimental changes against. Experiment 1 found a large increase in high frequency activity in the first hour in a new tank and also changes in the EOD activity over days. While the activity never became totally stable over the time course of the experiment, the fish showing continual changes over time, a high degree of concordance was shown by most fish indicating a high level of stability in their EOD activity over time. In this experiment the EOD activity will be examined for stability in fish which are repeatedly placed into and removed from restraint in experimental tanks.

Conditioning techniques have proven valuable in the study of animal psychophysics (Stebbins, 1970). Through the use of a conditioned response, data may often be obtained on sensory systems for which no reliable pre-existing indicator responses are found in the animals behavior. Also in many cases a more precise control over the presentation and specification of the stimuli is possible. Belbenoit (1970) used conditioned locomotion in G. petersii to establish the fish's ability to discriminate plastic and metal rods. The use of a locomotor response makes the precise specification of the stimuli more difficult as the fish's position is not fixed. Through the use of a conditioned EOD response, it would be possible to present stimuli with the fish in a fixed position relative to the stimuli and obtain a more precise determination of the psychophysical data. In the present experiment, the effects of a negative reinforcer, electric shock, on the EOD activity of mormyrids will be systematically explored more fully with the aim of using shock as the reinforcer in the establishment of discriminated Pavlovian conditioning of the EOD activity.

Gallon, et al. (1967) explored the effects of a single, 100 msec, 5 to 6 mA DC pulse on the EOD activity of four G. petersii. With a water conductivity of approximately 220 μmho and an interelectrode distance of 2 cm this is approximately 11 to 14 V/cm. Shocks were delivered every five minutes for one hour, followed by one hour without shock, then another hour of shock delivery on each of two consecutive days. The mean EOD rate was determined for 2-second periods for a 60-second period beginning eight seconds prior to shock delivery. The response to shock was an elevation in EOD rate which lasted approximately 10 to 15 seconds before returning to baseline rate. The ratios of post-shock to baseline mean rates were 2.43, 1.48, 2.62, and 2.10 for the four fish. While the recording methods did not permit the determination of the instantaneous changes to shock they report that EOD activity of over 50 Hz occurs in response to the US. Mandriota et al. (1965, 1967), in their conditioning studies, used a 7.5 mA DC pulse of 100 msec as the US. This corresponds to approximately 20 V/cm. They used a conditioned response or avoidance response of a single occurence of an EOD with a rate of 2.8 times the mean resting rate and report that each US elicited several pulses at a rate greater than this criterion rate. They also report that US levels smaller than the 7.5 mA were not as effective in conditioning. Since an important use of conditioning in mormyrids would be to determine sensory thresholds for the electrosensory systems, the use of US levels of this high magnitude would probably interfere with the receptor systems because the biologically interesting electric signals are of much lower amplitude than this. Moller and Bauer (1973) measured the voltage of six G. petersii out of water, from mouth to tail, and found between .10 to .18 V/cm. Field measurements in water of approximately 500 to 1000 µmho conductivity revealed a value of approximately 1 mV/cm at a distance of 5 cm from the fish. The value of US used by Mandriota et al. is over 1000 times greater than this and it would clearly be advantageous to be able to condition the fish with a lower level of US, preferably one that is within the biologically occurring levels of voltage. Stimulation experiments (e.g., Moller, 1970) have shown that mormyrids do respond to low level electric signals that fall within the naturally occurring range of voltages. Moller (1973) stimulated Marcusenius sp. with recorded discharge patterns of other mormyrids at three different amplitude levels. In all cases, the stimulated fish responded with an increase in EOD activity immediately after the initiation of stimulation even at levels as low as .07 mV/cm. It is possible then that US levels in this biological range of amplitude could serve as a US if this response is maintained over repeated presentations.

The second part of this experiment will, therefore, examine a broad range of US values to determine if lower voltages will serve to produce a reliable response which might then be used in conditioning. US values of .0005 V/cm, .05 V/cm, 1 V/cm, 10 V/cm, and 20 V/cm will be used. The highest of these values is approximately the level used by Mandriota et al. (1965, 1968). The lowest value is approximately ten times the threshold value of .04 mV/cm reported by Moller (1970) for B. niger.

Method

Subjects. Nine G. petersii and nine B. niger obtained from a local supplier were used. Their length was 9 to 13 cm measured from the mouth to the caudal fin. All fish were sexually immature and no attempt was made to sex them. Before being used in the experiment, each species had been maintained in its own 50 gallon community tank. They were present in the laboratory for more than one month at the beginning of the experiment.

Apparatus. During the experiment, the fish were housed in three gallon aquaria in the laboratory. Each tank was provided with a filter, shelter, and light as described in Experiment 1. A timer controlled the tank lights with onset at 0700 and offset at 1900. Some natural light also reached the tanks. Tubifex worms were available at all times in these home tanks.

The two experimental tanks were housed in the room described in Experiment 1. The tanks were made of acrylic plastic and were 38 by 23 by 20 cm with a water depth of 12 cm. The conductivity of the experimental tanks was maintained at 450 µmho by adding to or changing part of the water in the tanks with demineralized water before the experimental sessions each day. Inside filters were in these tanks except during an experimental session when only aeration was provided. Each tank was placed on foam rubber within a styrofoam box, inside dimensions 45 by 31 by 31 cm, to visually isolate the fish and to minimize the effects of vibrations. A restraining device similar to that used by Mandriota et al. (1965) was used in each tank to locate the fish in a relatively constant position with respect to the stimulating electrodes. This box-like device was made of acrylic plastic with inside dimentions of 20 cm long, 4 cm high, and 2 cm wide. Two movable verticle partitions enabled the inside length to be adjusted to accommodate fish of different length. Fastened to these end partitions, at the head and tail of the fish, were silver wire electrodes for recording the EOD. The stimulating electrodes were also silver wire and were fastened to the sides of the restraint with a distance of 2 cm between the electrodes. Holes drilled in the restraints allowed water circulation.

The EOD was amplified by Grass P-15 amplifiers as used in Experiment 1 and then recorded using a Tandberg 6041X. The EOD was recorded on the left channel of the tape. Pulses generated by Tektronix 160 Series modules were recorded on the right channel of the tape to mark the US delivery and the time 30 sec prior to the US for purposes of data analysis. The US was a 100 msec square pulse timed by Scientific

Prototype programming modules. For the low levels of US (.0005 V/cm, .05 V/cm, 1 V/cm) the output of a Hewlett-Packard 350D attenuator set to a minimum of 20 dB attenuation was connected to the stimulating electrodes of each tank. At a setting of 20 dB or greater the attenuator provides a 600 ohm output impedance. The output of a Southwest Technical Company variable voltage DC power supply was switched to the input of the attenuator by a relay to deliver the US. This method provided a constant 600 ohm impedance at the stimulating electrodes to prevent a confounding of impedance changes with the voltage change. For the two high levels of US, 600 ohm resistors were substituted for the attenuators. An attenuator or resistor was connected to the stimulating electrodes continuously during each experimental session and adaptation period. The US levels were calibrated before each session with an oscilloscope by measuring the level of US actually delivered into the tank via the stimulating electrodes.

Illumination in the experimental cubicle was a Westinghouse 1819 pilot light in series with a 125 ohm resistor connected to 26 VDC. The bulb was suspended one meter above and between the tanks and provided an illuminance of 1.4 lux at the surface of the water. Air conditioning in the laboratory maintained the temperature in a range of 23 to 25° C in the experimental and home tanks during the experiment.

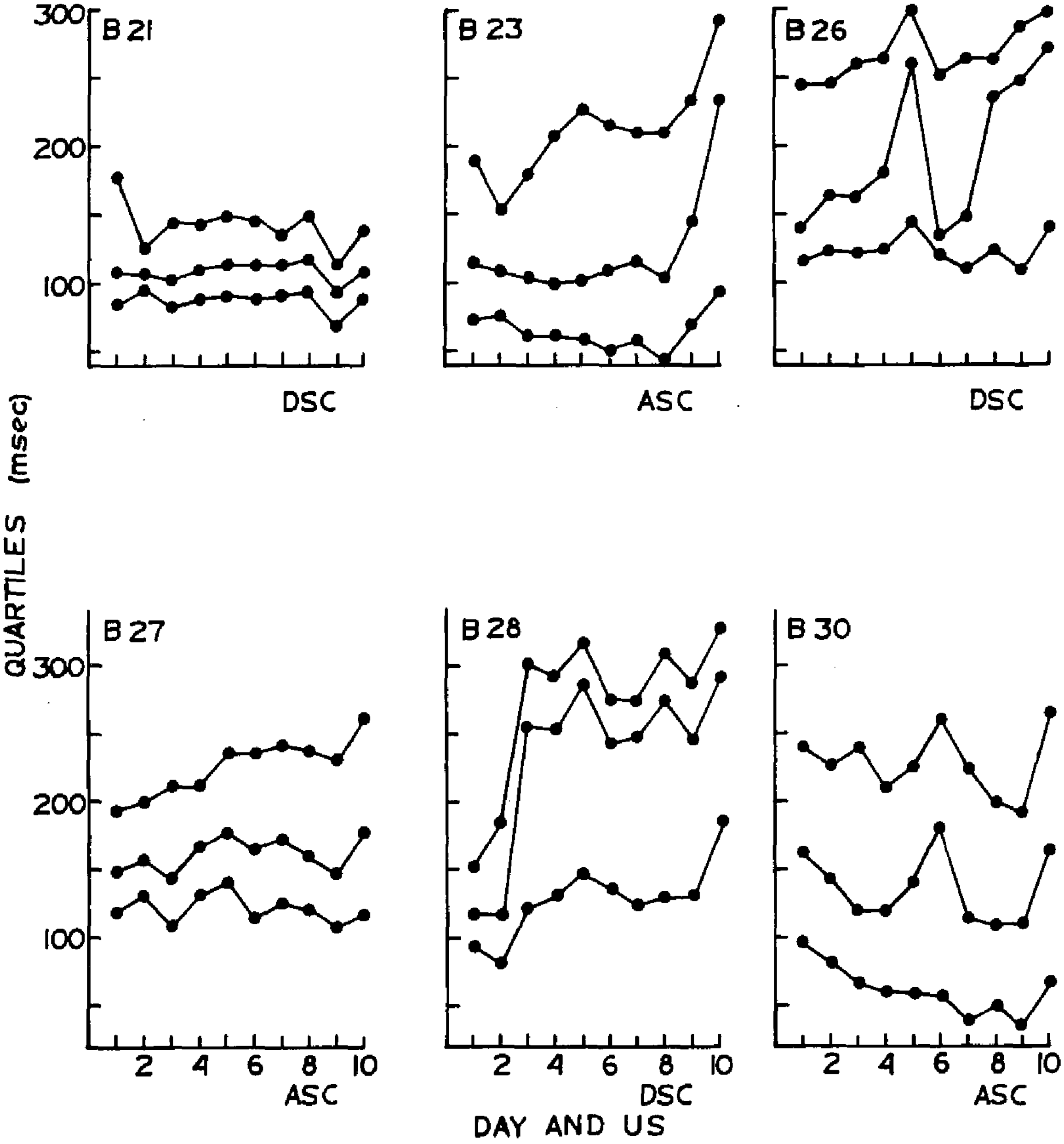
Procedure. All fish were moved from the community tanks to the home tanks two weeks prior to the start of the experiment. Two fish were used in the experiment each day. The first fish was placed into the restraint in the experimental tank at 0900. After 150 minutes adaptation for this fish, the second fish was placed into the other tank and the session was started for the first fish. The 150 minutes required for the 15 US presentations provided the adaptation period for the second fish. The session for the second fish then began at 1400. Each fish was used at the same time of day throughout the experiment.

One level of US was presented in each session 15 times with an interval of ten minutes between each presentation. Each fish received the five levels of US in either an ascending or descending order with a session every third day. Nine G. petersii and three B. niger received two replications of the five levels of US. Six B. niger did not have the US delivered on the first replication. All apparatus was set as if to deliver the appropriate US level except the power supply that provided the DC was not turned on. These fish received the US on the second replication of the five US levels.

Results

Adaptation. The six B. niger that did not receive any US on the first replication of five sessions provide a measure of the effects of repeated change of tank and restraint on the EOD activity. From each session, a 7.5-minute sample of EOD activity was taken consisting of the 30-second period prior to the scheduled time of delivery of each of the 15 USs. The quartiles of the TI histograms of these samples are shown in Figure 17. The first five days shown for each fish, therefore, show the effects on the EOD activity of fish which are placed into an experimental situation where they are restrained, allowed 150 minutes of adaptation to the situation, but are not otherwise distriburbed during the period of recording. No systematic trends between subjects are apparent in the quartiles for the first five days. Fish B21 is very stable

Figure 17. Quartiles of the TI (US - 30) histograms for the six fish that received one replication of the US levels. Each histogram is based on a 7.5-min sample of the 30-sec period prior to the scheduled time of US delivery. On days 6 to 10 each fish received 15 presentations of the US levels in ascending or descending order.


B 21
B 23
B 26
B 27
B 28
B 30
300
200
100
QUARTILES (msec)
DSC
ASC
DSC
ASC
DSC
ASC
2
4
6
8
10
DAY AND US

over the five days, while B26, B27, and B28 show a slowing of EOD activity over the period. B23 and B30 have an increase in high frequency activity as shown by the lower values of Q_1 over days. They also both first increased and then decreased in median EOD frequency. The changes, while showing no consistent patterns among fish, do tend to be gradual from day to day except in the case of B28 which showed a drastic shift in EOD activity on day 3. Even in the case of B28 the other changes are gradual.

The concordances for the TI histograms (Table 5) reflect the impressions gained from the quartiles. Fish B28 has the lowest value of $\underline{W}$, .40. This results from the large shift on day 3. For the other fish, the values range from .78 to .91 in agreement with the gradual changes in the shapes of the TI histograms. Shown in Figure 18 are the five TI histograms for the fish with the highest and lowest values of $\underline{W}$, B27 and B28 respectively. B27 has a high degree of stability over the five days in its trimodal histograms. B28 in contrast shows the changes in EOD activity which were seen in the quartiles. The histogram for day 1 was unimodal in distribution with a high peak from 85 to 125 msec. On day 2 a bimodal histogram with the first peak in the same location as day 1 but lower is seen along with a low frequency peak at approximately 245 msec. The three subsequent days have similar histograms with the main mode at approximately 295 msec and a second, lower mode at approximately 95 msec.

The mean value of $\underline{W}$ for the six fish is .78 which is slightly lower than, but not significantly different from the mean value of .84 for the concordances of the *B. niger* which remained undisturbed in the same

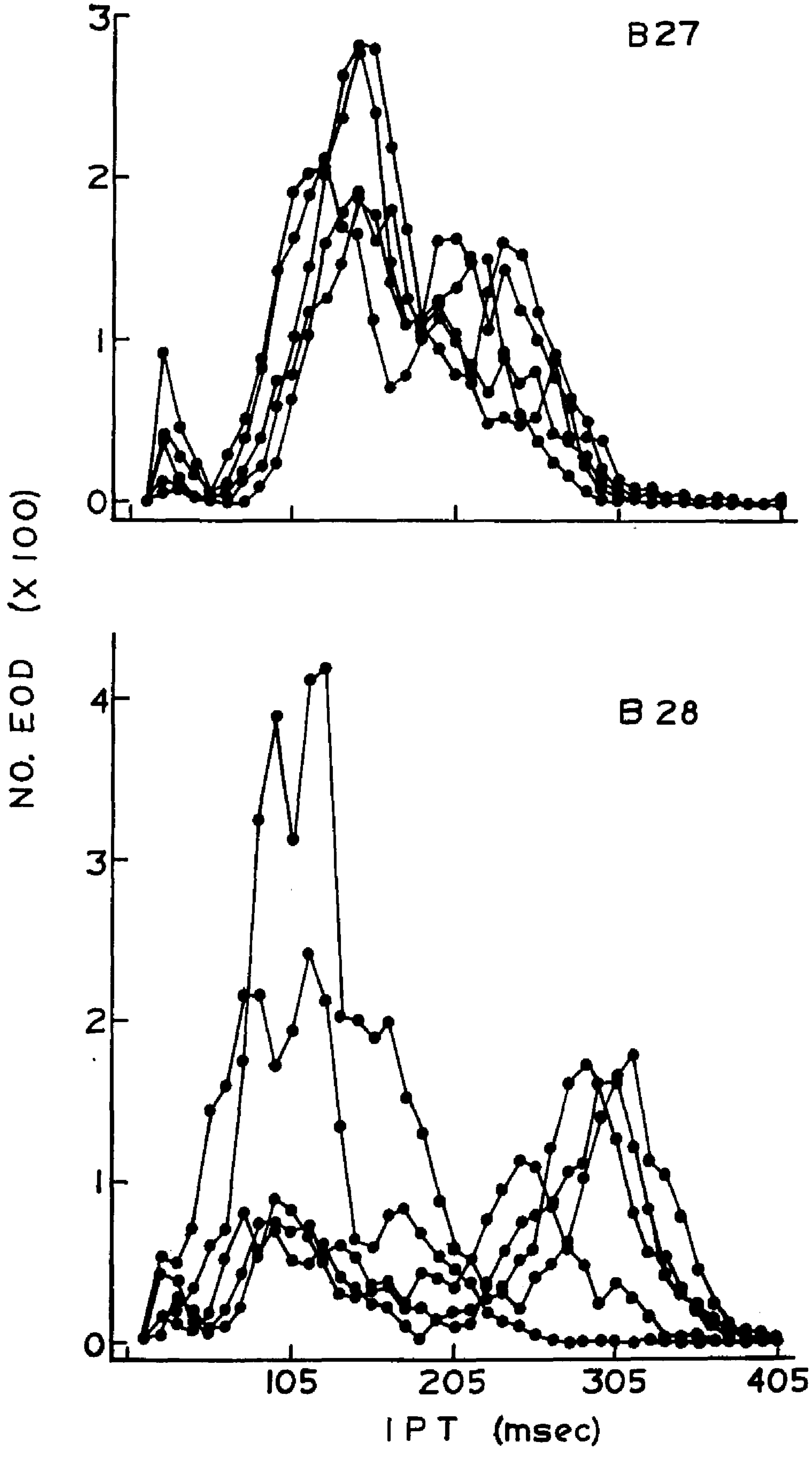
TABLE 5

Coefficients of Concordance, W, for the TI Histograms of the 7.5 minute Pre-US Samples.

	W* for Five Sessions	
Fish	Without US	With US
B21	.88	.87
B23	.89	.60
B26	.82	.80
B27	.91	.86
B28	.40	.65
B30	.78	.71

* All values of W are significant, $p < .05$.

Figure 18. TI (US—30) histograms for the replication without the US delivery for the fish with the highest and lowest values of concordance. For B27, $\underline{W}$ = .91; for B28, $\underline{W}$=.40.


B27
B 28
NO. EOD (X 100)
3
2
1
0
4
105
205
305
405
I P T (msec)

tank for a 20-day period, $\underline{t}(26) = -.89$, $\underline{p} < .05$. B. niger in this situation of repeated placement into a restrained experimental situation, with a limited adaptation time of 150 minutes, show a high degree of stability in their EOD activity.

US Effects. The effects of the introduction of the US on days 6 to 10 on the resting EOD are also shown in the quartiles of Figure 17 and in the values of $\underline{W}$ in Table 5. Five of the fish show a decline in the degree of stability in EOD activity after the introduction of the US. The reduction is not large except for B23 where it changes from .89 to .60. Fish B28 which had the least amount of stability in EOD activity without the US present shows a substantial increase in stability in the five days with US. Shock does not, therefore, always cause an increased variability in EOD activity of the fish. The quartiles of the TI histograms for days 6 to 10 in Figure 17 do not show any consistent changes among fish after the introduction of US. B23 and B26 show a decline in median frequency of responding. Fish B21, B27, and B28 remained relatively stable over the five days of US. The order of presentation of the US levels did not produce consistent changes among the fish.

In the phase of the experiment where the fish received two replications of the US without the preliminary five days of no US, the shock had a much greater effect on the fish and their EOD activity. Of the nine G. petersii and three B. niger which started this experiment only five G. petersii and one B. niger survived for the ten US sessions. The other fish died before receiving one complete replication of the five US levels. G12 and G13 died within three days of the completion of the second replication. While it is not possible to determine for certain the cause of this high mortality rate compared with no deaths in the group that received one replication of US, it seems possible that the five days of restraint without the US helps to prepare the fish for the additional stress of the US. Figure 19 presents the quartiles for the TI histograms for the same 7.5 min period as in Figure 17. The quartiles were not computed when the mean frequency fell below 1 Hz as the sample becomes too small for the reliable computation of the quartiles. Only G10 and B03 show a high degree of stability over days. The EOD activity of G20 shows a large, but orderly, increase in the frequency over days with the exception of a dramatic slow down on day 7. For G12 the last three sessions and for G13 day 5 and the last four sessions had an EOD frequency of less than 1 Hz. G12 declined to .1 Hz on day 10. G13 had a frequency of .2 Hz on day 5 and 0. on days 9 and 10 in this pre-US period. The order of presentation of the US levels may be partly responsible for the effects on the pre-US EOD activity of the fish. The two fish that showed the most stability both received the US levels in an ascending order although G12 which also received an ascending order showed substantial deleterious effects of the US having a mean frequency of .3 Hz for the last three sessions. No effects due to order of US presentation were apparant in the six fish that received only one replication of the US.

In Figures 20 and 21 are poststimulus time (PST) histograms of the responses of individual fish to the five US levels. In a PST histogram the responses are counted in each consecutive time interval from a specific starting point. These PST histograms show consecutive 1-second periods beginning with the point 20 seconds before the US delivery and continuing for 20 seconds post-US. For each fish the response to the

Figure 19. Quartiles of the TI (US - 30) histograms for the six fish that received two replications of the US levels. Each histogram is based on a 7.5-min sample of the 30-sec period prior to the US delivery. The order of the delivery of the US levels is shown for each fish. No quartiles are shown when the mean EOD frequency was below 1 Hz.

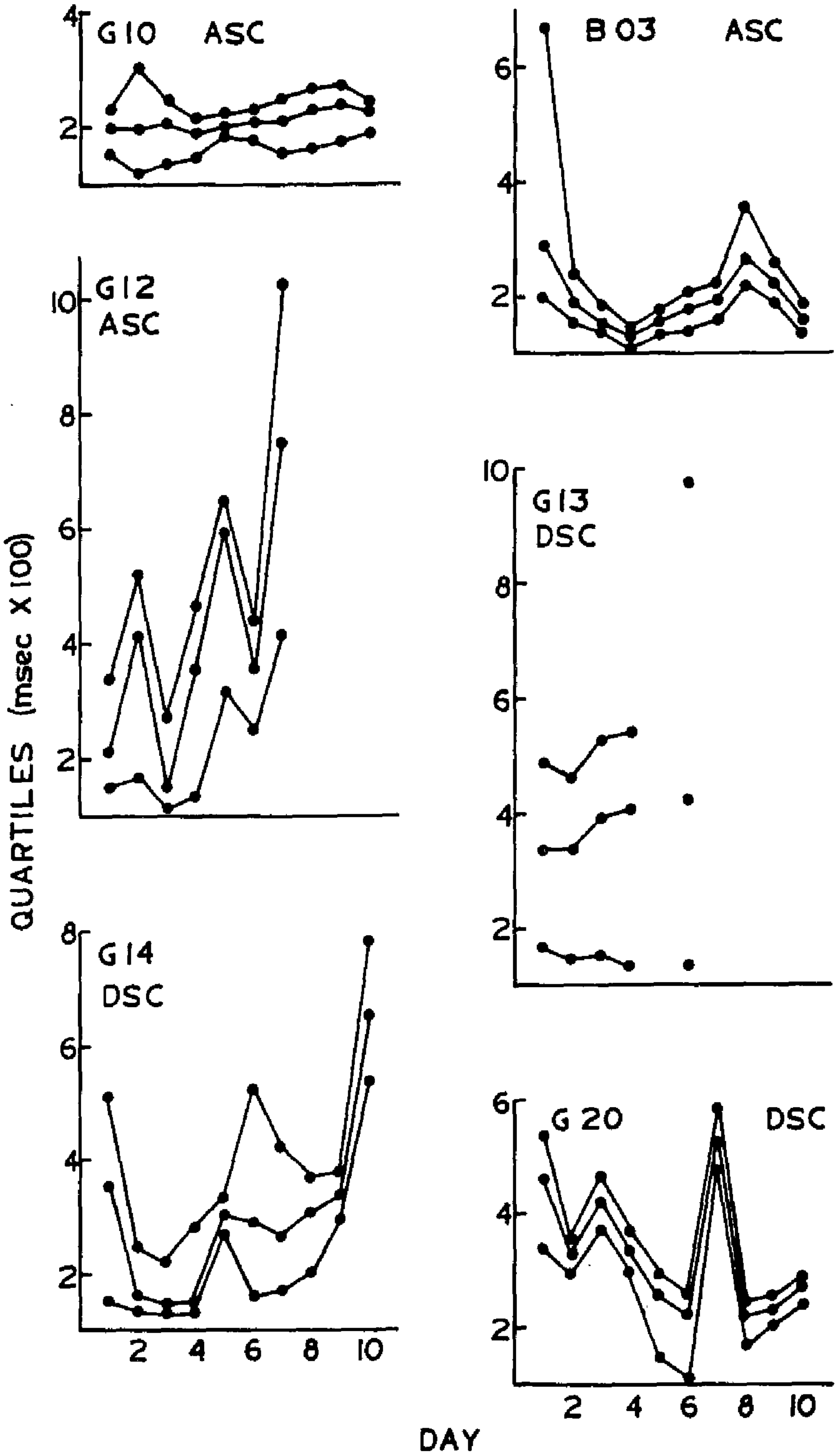

G10 ASC
B03 ASC
G12 ASC
G13 DSC
G14 DSC
G 20 DSC
QUARTILES (msec X 100)
DAY
2
4
6
8
10

Figure 20. PST histograms for the 20 sec before and after US presentation for fish G12, B28, and B30. For each fish five individual trials and the mean values for the first five, last five, and all 15 trails are presented.

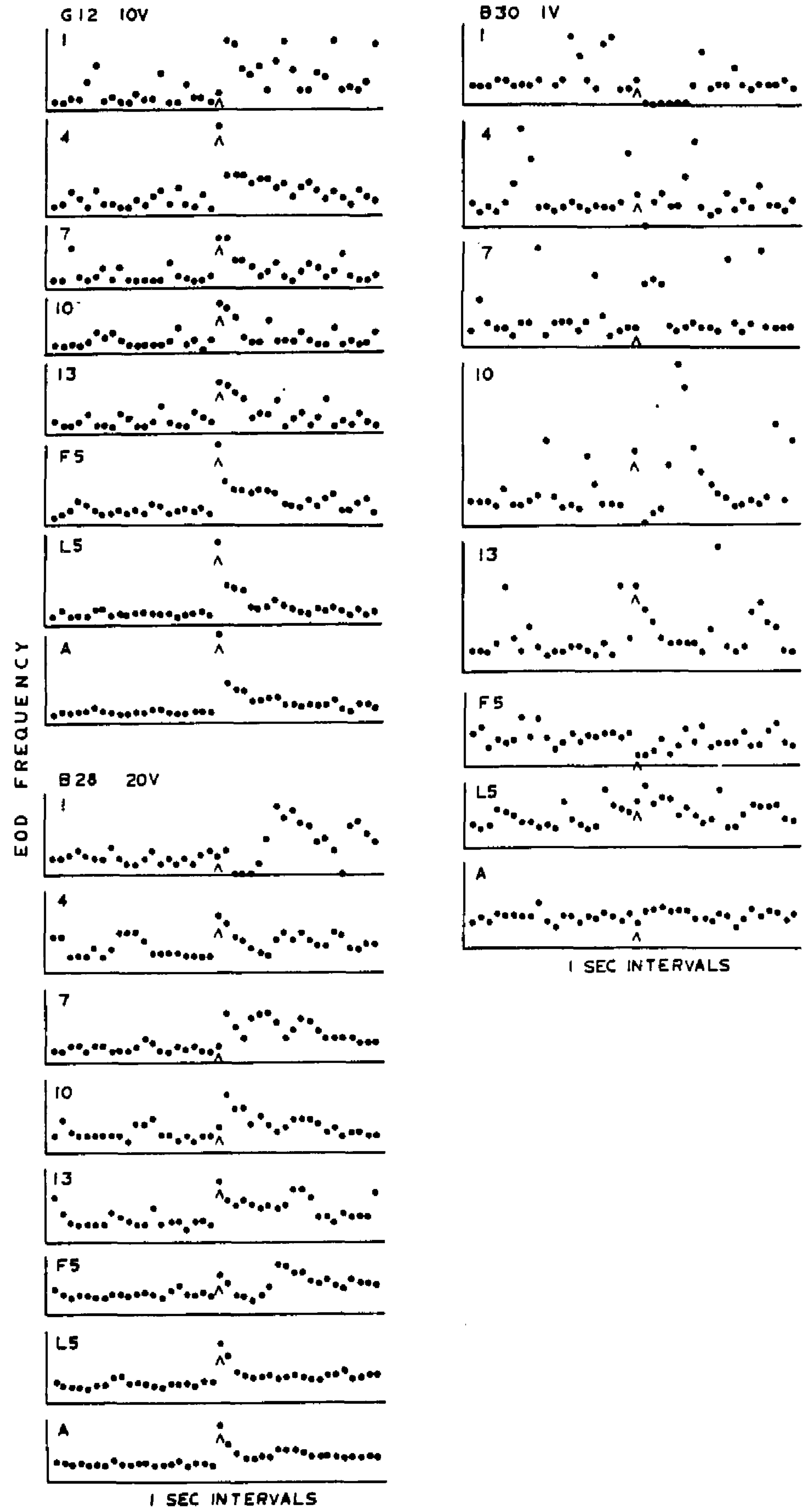
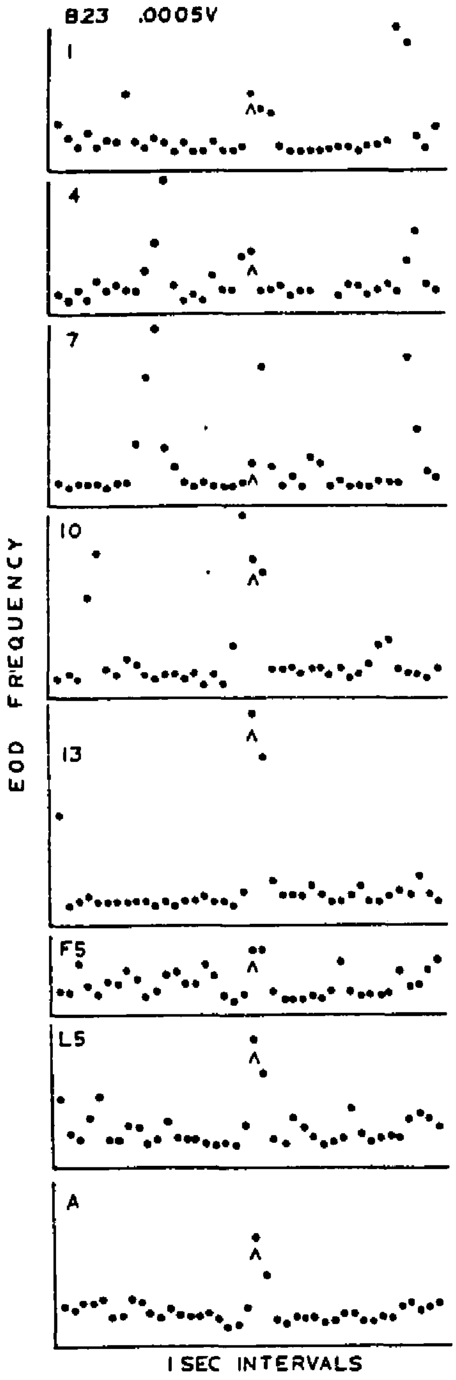
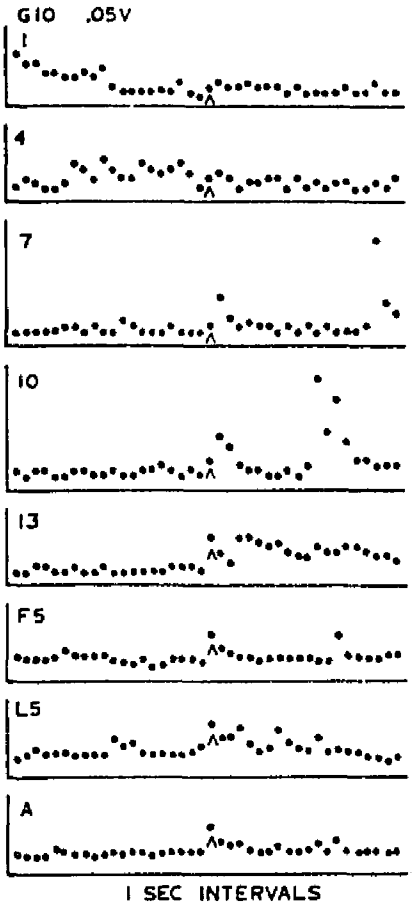

G12 10V
1
4
7
10
13
F5
L5
A
B28 20V
1
4
7
10
13
F5
L5
A
B30 1V
1
4
7
10
13
F5
L5
A
EOD FREQUENCY
1 SEC INTERVALS
1 SEC INTERVALS

Figure 21. PST histograms for the 20 sec before and after US presentation for fish B23 and G10. For each fish five individual trials and the mean values for the first five, last five, and all 15 trials are presented.


B23 .0005V
1
4
7
10
13
F5
L5
A
EOD FREQUENCY
1 SEC INTERVALS


G10 .05V
1
4
7
10
13
F5
L5
A
1 SEC INTERVALS

response to the first, fourth, tenth, and thirteenth individual USs are presented as well as the mean values for the first five, last five, and all 15 US presentations. Two features of the EOD activity are apparent from the histograms; the highly variable nature of the EOD, and the changing nature of the response to the US over presentations. B23 shows pre-US burst activity of higher frequency than the response to the US on both the fourth and seventh trials. This also occurs on the first, fourth, and seventh trials for fish B30. B30 also clearly demonstrated the changing nature of the response to the US. On the first presentation a pause in EOD activity of 6 seconds occurs 1 second after the US. The response becomes more variable over the next several trials and includes a 1-second pause on the fourth and tenth trials. By the thirteenth trial the response becomes the more typical one of a burst which declines to a pre-US level after several seconds. The pause is the dominant feature of the behavior on the first five trials. On the last five trials an increase is seen in the first second after the US, with the maximum frequency reached in the next second. When all fifteen trials are averaged, the response to the US is seen only as a slight decrease in frequency followed by a slight increase for approximately 6 seconds. In contrast, G10 shows an increase in frequency to the US from the first trial but the shape and location of the highest frequency changes. The averaging of the 15 trials smooths out the higher frequency bursts which occur and shows just the response to the US, a sharp rise in frequency which declines to resting or pre-US frequency after approximately 6 seconds. Similar features and changes in the response over trials may be seen in the histograms for the other fish. By considering the mean of the EOD activity over several trials of US presentation the variability in pre- and post-US activity is smoothed out and a clearer picture of the response to the US emerges.

Figures 22 and 23 present the TI histograms for two fish for the 15 30-second periods prior to the USs, TI (US - 30), and the 15 30-second periods beginning at each US, TI (US + 30), for each of the five US levels. For fish B27, shown in Figure 22, the left hand column of histograms is the resting or pre-US activity. Each of the histograms in the right hand column shows the changes in this ongoing EOD activity caused by the US. At .0005 V/cm not much change in the shape of the histogram is apparent. An increase in the high frequency activity was produced by the .05 V/cm US. No increase in burst activity is seen but the overall frequency has increased as the main peak is higher and there is a more rapid decline in the lower frequency responses. At 10 V/cm the shape of the histogram changed greatly for the first time. It became unimodal, with the peak at a higher frequency than the other histograms. Interestingly, there is much less high frequency EOD than in the TI (US + 30) histogram for .05 V/cm. The response to 20 V/cm is similar to that shown to 10 V/cm in showing a shift toward a higher frequency unimodal distribution except that the peak is lower and a trace of the third or low frequency mode is still visible. Figure 23 shows the same histograms for fish G10. G10 received two replications of the US levels and the TI (US - 30) and TI (US + 30) histograms are shown for both replications. More variability is shown in the ten TI (US - 30) histograms than in those of B27. In general the response to the US is similar. A burst of high frequency activity was produced by .05 V/cm in both fish, but not .0005 V/cm or 1 V/cm. The response to 10 and 20 V/cm in G10 is a large increase in high frequency EOD activity with the histograms retaining approximately the same shape as contrasted to the tendency in B27 toward a unimodal shape. There are differences between the first and second replication responses shown at some of the US levels but no consistent changes across levels.

Figure 22. TI histograms for (US - 30) and (US + 30) for B27 for each session of 15 trials for each of the five US levels.

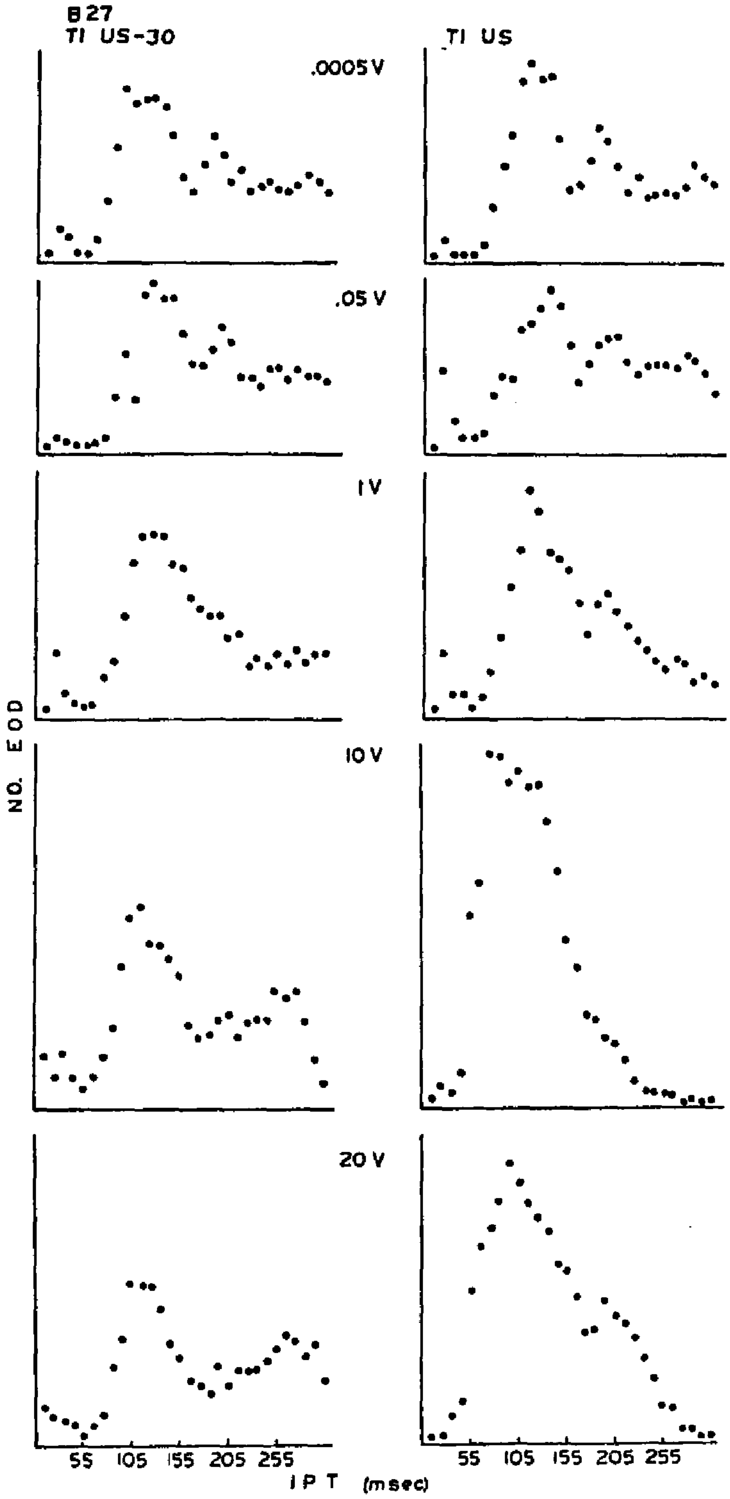
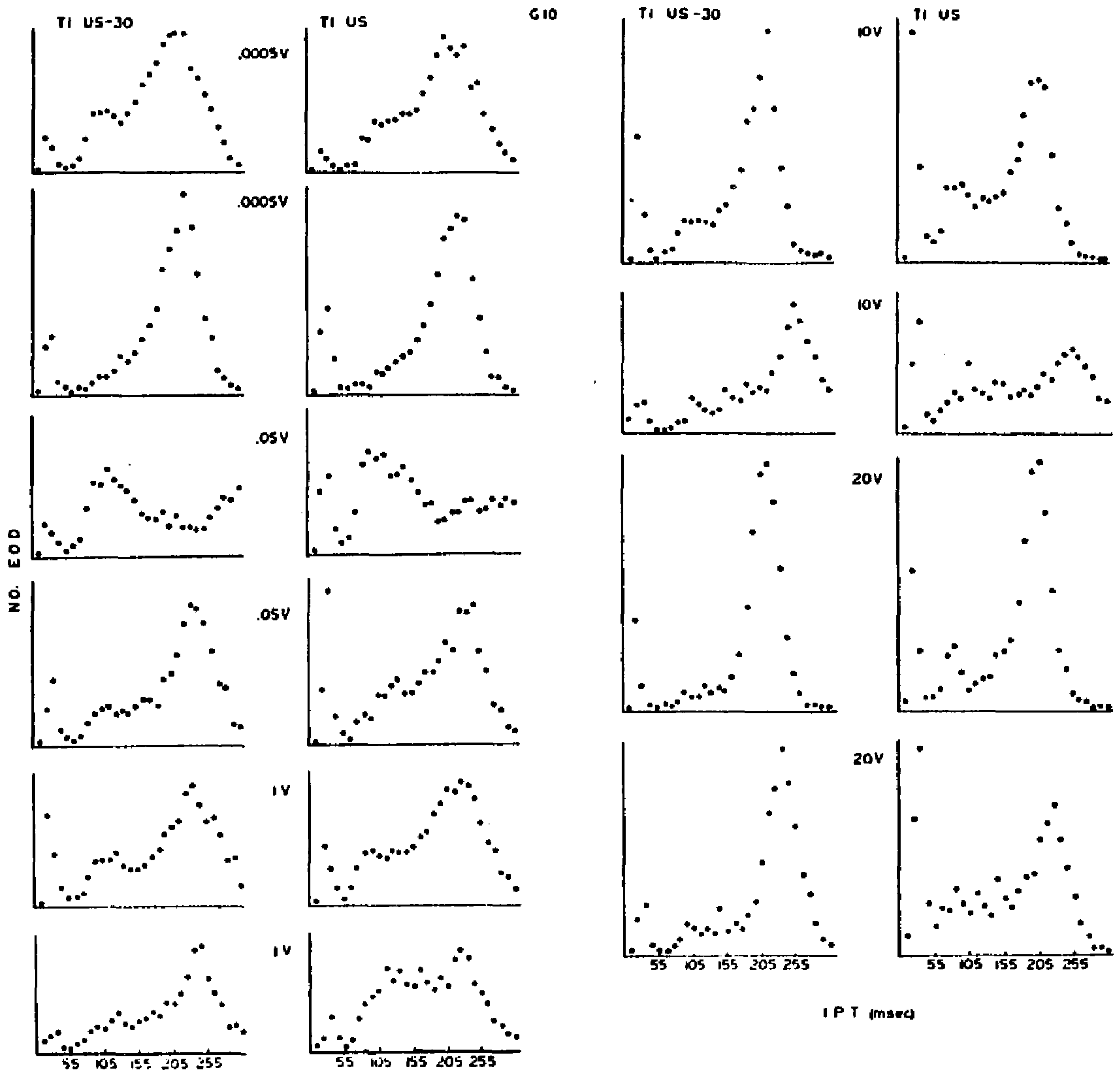

B 27
TI US-30
TI US
.0005 V
.05 V
1 V
10 V
20 V
NO. EOD
55
105
155
205
255
IPT (msec)

Figure 23. TI histograms for (US - 30) and (US + 30) for G10 for each session of 15 trials for each of the five US levels.


TI US-30
TI US
G10
TI US-30
TI US
.0005V
.0005V
.05V
.05V
1V
1V
10V
10V
20V
20V
NO. EOD
55 105 155 205 255
I P T (msec)

The variability in the pre-US EOD activity and the response to the US seen in Figures 20 through 23 require some form of averaging of the EOD activity over trials to produce an orderly measure of the US effect. Table 6 presents four different measures of US response for the fish that received one replication of the US. Each of the measures is based on the mean of the EOD activity for the 15 presentations of each US level in a session. The samples are symetrical time periods before and after the US presentation. For example, the ratio of medians is based on the TI histograms for the 30 seconds preceeding the US, TI (US - 30) and the 30-second period beginning with the US, TI (US + 30). With the medians the smallest effect occurred with the .0005 V/cm US, a ratio of 1.00 or no effect for the mean of six fish. These measures differ because the shape of the response over time is different for the different US levels. In general the four types of response measure show a similar function of US level. Because of large individual differences in the response, only at the two highest levels of US are the measures significantly different from 1.00. A value of 1.00 is expected for any of the measures if no US or other stimulus had occurred. A response measure that is to be used in a conditioning experiment for example must be able to reliably discriminate the response to the US, and CS if any, from the ongoing EOD activity. For this reason the means of the 5- and 1-second samples have an advantage in also being significant at the 1 V/cm level indicating a greater sensitivity to changes in the EOD. Table 7 presents the individual ratios for the six fish based on the 1-second samples. With the exception of B30 the fish show accelerations to all the US levels. B30 slowed its EOD rate to the three lowest levels of US. Even without B30, the data for the two lowest levels would not have been significantly different from 1.00. Five of the fish show a greater value

TABLE 6

Mean Values and Standard Deviations of the Response to the US Levels of the Fish that Received One Replication of the US.

Measure[a]	US Level (V/cm)				
	.0005	.05	1	10	20
Median (US + 30) / Median (US - 30)[b]	1.00	.99	1.12	1.39*	1.68*
S.D.	.14	.05	.15	.34	.50
Mean (US + 30) / Mean (US - 30)[c]	.97	.93	1.04	1.32*	1.61*
S.D.	.12	.10	.06	.20	.26
Mean (US + 5) / Mean (US - 5)[c]	1.27	1.21	1.54*	2.13*	2.60*
S.D.	.40	.62	.31	.65	.80
Mean (US + 1) / Mean (US - 1)[c]	1.82	1.42	2.26*	3.38*	3.93*
S.D.	.95	1.04	1.00	1.41	1.62

* Significantly different from 1.00, t (6), $p < .05$.

[a] All measures are ratios of response measures taken for equal periods pre- and post-US.

[b] The medians are from the TI histograms.

[c] Mean frequencies for the specified time periods.

TABLE 7

Measure of the Response[a] to the US for the Fish that Received One Replication of the US Levels.

	US Level (V/cm)				
Fish	.0005	.05	1	10	20
B21	1.5	1.4	2.1	2.5	2.2
B23	3.6	1.2	2.7	5.1	6.5
B26	1.7	3.4	3.4	4.2	5.2
B27	1.5	1.2	2.1	3.4	2.9
B28	1.9	1.0	2.6	4.0	3.7
B30	.8	.3	.7	1.1	3.0

[a] Each measure is the ratio of the mean frequencies for the periods one second pre- and post-US.

to .0005 V/cm than to .05 V/cm. Three of the fish also respond with a smaller increase in EOD activity to the 20 V/cm than to the 10 V/cm US. The values for the fish that received the two replications of US are in Table 8. For the first replication of the five US levels, all of the fish except G12 show a monotonically increasing function of frequency to the increasing US levels. For the four highest levels of US, the mean values of response for all six fish is significantly different from 1.00, $\underline{t}(6)$, $\underline{p} < .05$. A value of 1.00 for this ratio measure would indicate no response to the US. The data for the second replication of the US levels was less orderly. Two of the fish, G12 and G13, had no EOD activity on one session each, and seven sessions where the EOD activity fell below 1 Hz making the computation of the response measure invalid. This lack of normal EOD activity in two fish and the less orderly nature of the responding by the other fish may be due to the deliterious nature of the high levels of US on the fish.

A different method of defining a response to a US was used by Mandriota et al. (1965). The mean frequency of a sample of resting EOD activity was determined and a criterion response of a frequency 2.8 times this mean resting frequency was established for each fish. To assess classical conditioning on a trial, a response was considered to have occurred if a criterion response occurred during the CS and no criterion response occurred during an equal time period in the intertrial interval. Table 9 tabulates the number of these criterion responses to the US for each of the fish that received one replication of the US. The criterion frequency was set at 2.8 times the mean for the 15 30-sec periods preceeding the US for each fish. The number of times this frequency was exceeded in both the 30-sec prior- and post-US period was determined for

TABLE 8

Measure of the Response[a] to the US for the Fish that Received Two Replications of the US Levels.

	US Level (V/cm)				
Fish	.0005	.05	1	10	20
G10	1.0 1.6	2.1 4.7	2.7 3.0	2.9 4.4	3.6 5.2
G12	.9 1.1	3.8 7.2	2.5 b	7.6 b	13.3 b
G13	b b	2.6 b	3.7 b	7.1 b	7.8 20.4
G14	1.3 1.1	1.8 4.0	2.1 2.5	2.3 4.2	5.9 4.7
G20	.8 1.2	2.0 1.4	2.5 1.7	4.0 2.2	7.2 3.4
B03	2.0 1.3	2.1 1.2	2.2 4.9	2.6 3.6	3.3 3.1

[a] Each measure is the ratio of the mean frequencies for the periods one second pre- and post-US.

[b] The EOD frequency for the period prior to the US was less than 1 Hz for these sessions. For G12, 20 V/cm; and G13, .05 V/cm no EOD activity occurred.

TABLE 9

Number Criterion Responses[a] in the TI Histograms of the 30-second Periods Pre- and Post-US.

	US Level (V/cm)									
	.0005		.05		1		10		20	
Fish	Pre-US	Post-US	Pre-US	Post-US	Pre-US	Post-US	Pre-US	Post-US	Pre-US	Post-US
B21	59	55	279	155	116	44	36	108	80	203
B23	717	1073	677	506	908	861	347	701	322	1452
B26	114	210	281	368	85	271	144	413	137	621
B27	58	23	22	137	109	116	266	523	82	413
B28	324	321	266	92	298	225	246	723	126	472
B30	674	419	896	617	857	911	989	872	499	462

[a] A criterion response is defined as at least one occurrence of an EOD frequency 2.8 times that of the baseline frequency.

each US level. While the criterion response is determined in the same way for each fish, there are large differences in the number of responses among fish. For example at 20 V/cm, B21 has 203 while B23 has 1452 criterion responses. This is reflected in the different shapes of the TI histograms shown by the fish not only in their resting patterns but also in their response to the various US levels. Only on the two highest levels of US do a majority of the fish show more criterion responses post-US than pre-US.

Discussion

The results of the first part of this experiment show that a high degree of stability can exist in the EOD activity of mormyrids in situations other than undisturbed isolation. Over five days of being moved from their home tank to the experimental tank where they were placed in restraint, the B. niger had concordances only slightly lower than was seen in the group with 20 days of undisturbed isolation. This finding is important for the conduct of certain types of experiments with mormyrids in which it is necessary to move fish into an experimental tank shortly prior to the experiment. Under these conditions with short adaptation times, one can expect a relatively stable baseline of EOD behavior. For the B. niger the introduction of the US, including very high levels of shock, did not result in large reductions in this stability of EOD activity over days. For the group of fish that received two replications of US, instead of five days of restraint without shock, only two of the six fish showed stability in their EOD activity over the ten sessions. One of the two was a B. niger. The other four G. petersii showed large daily variations in pre-US EOD activity apparently due to the deleterious effects of the US presentations. Four of the nine G. petersii and two of the three B. higer that began this part of the experiment did not survive one complete replication of the five US levels. Two of the G. petersii that survived all ten sessions ceased or slowed greatly in their EOD activity. While the reasons for the more severe effects in the two replication phases are not proven it would seem that the five sessions without the US, but with restraint, provided an additional adaptation to the experimental situation that served to reduce the stress of the total experimental situation sufficiently to both reduce mortality and help maintain normal EOD activity.

All of the US levels were effective in modifying the ongoing EOD activity of the fish. For the lower levels of US the effect is small and variable and can be seen primarily in changes in the shapes of the TI histograms. As the lower levels are in the range of naturally occurring electric events for the fish, this variability might relate to pre-existing responses in individual fish. As an aim of this experiment was to develop an adequate US for conditioning, a more easily detected and quantified measure than the TI histogram is needed. The response measures of the ratios of mean EOD activity for equal 1- or 5-second periods around the US would seem to best serve this purpose. For the B. niger this measure allows the detection of the response to the US levels of 1, 10, and 20 V/cm to be made. For the G. petersii the .05 V/cm US also produced changes in the EOD that are reliably detected. As each of these levels of US produces a response that is reliable and easily measurable, they may serve as USs for conditioning.

The 10 V/cm level in the present experiment was most similar to that used in Gallon et al. (1967). It produced a current of approximately 4.5 mA as compared to 5 to 6 mA. With G. petersii they found ratios of

1.48 to 2.62 for the period including 2 seconds after the US. The present experiment found ratios of 2.5 to 5.1 for M. species and 2.3 to 7.6 for G. petersii. It is expected that these would be higher as they are based on a 1-second period around the US as compared to the 2-sec periods used by Gallon et al. Using the criterion response method of Mandriota et al. (1965) a large variation was found in the occurrence of responses to the US levels among fish. Only at 10 and 20 V/cm did a majority of the fish show an increase in the number of criterion responses to the US. The reported lack of success in conditioning with US levels of less than 7.5 ma may be due to an insensitivity of this measure to the changes in the EOD activity produced by lower levels of US.

EXPERIMENT 3

The purpose of this experiment was to extend the findings of Mandriota et al. (1965) of the classical conditioning of the EOD activity in mormyrids to discriminated classical conditioning and to determine if conditioning could be obtained with a lower value of US by the use of a different response measure. Mandriota et al. successfully established classical conditioning in several species of mormyrids using an increase in illumination as the CS and an electric shock as the US. The duration of the CS was 5.1 sec and a 7.5 mA DC shock was delivered during the final .1 sec of the CS. A CR was the occurrence of an EOD with an instantaneous rate at least 2.8 times the mean resting rate at the beginning of the experiment. Because the fish will sometimes show rate increases greater than this criterion value spontaneously, the data were examined in blocks of 20 trials and the number of criterion responses that occurred in these conditioning trials was reduced by the number of criterion responses that occurred in 20 blank trials of equal duration spaced throughout the 20 conditioning trials. Control groups were also used. Within 100 conditioning trials the level of adjusted criterion responses rose from 20% to over 60% and reached a maximum of 70% in the next block of trials, demonstrating classical conditioning of the EOD. It was also reported that conditioning was not as effective with shock levels lower than 7.5 mA, a level high enough to cause a visible jerk in the fish.

A procedure to establish discriminated classical conditioning in mormyrids using the EOD activity as the response would be a useful technique for sensory and psychophysical studies. Most studies on electrolocation ability in electric fish have been concerned with the conductivity of objects and little is known about the ability of the fish to detect differences in size, shape, separation, or distance (Scheich and Bullock, 1974). By using a discriminated classical conditioning procedure, it would be possible, for example, to study the effect of distance on the fish's electrolocating ability by using identical objects placed at different distances as the CS + and CS −. Similarly objects differing only in shape could be used as the CSs. The establishment of discriminated responses to the two stimuli would then be evidence for the fish's ability to discriminate the objects. Using a discriminated classical conditioning procedure, Woodard and Bitterman (1971) established significant differential responding to red and green stimuli with goldfish. The response in this experiment was shuttling, a component of the general increase in activity induced in goldfish by shock presentation. The use of some aspect of EOD activity as the response measure would allow a more constant positioning of the fish with respect to the stimuli than a motor response does. The continuous nature of the EOD activity in mormyrids poses similar problems in the definition of the conditioned response to those encountered in cardiac conditioning where the response must also be measured against a baseline frequency (Hall, 1976).

Because of possible damaging effects of the shock US on the electrosensory system of mormyrids, it would be desirable to obtain conditioning with the lowest values of shock possible. The results of Experiment 2 found that the criterion response used by Mandriota et al. (1965) is not as sensitive to effects of lower values of US. A response measure based on total number of EODs for an equal period pre and post US showed reliable responding to US levels as low as .05 V/cm in *G. petersii*.

In this experiment, the four levels of shock that produced reliable responses in Experiment 2 will be used as the US in a discriminated classical conditioning procedure. The EOD activity will be recorded permitting analysis in several different ways, including the criterion response of Mandriota et al. (1965). The recordings will also enable an examination of the time course of the conditioned response, when conditioning occurs, and a comparison of the response to the CS + and the CS -.

Method

Subjects. Eight G. petersii obtained from a local supplier were used. Their length was 9 to 12 cm measured from the mouth to the caudal fin. All fish were sexually immature and no attempt was made to sex them. Before being used in the experiment, they were maintained in a 50 gallon community tank. They were present in the laboratory for more than one month at the beginning of the experiment.

Apparatus. During the experiment the fish were individually housed as in Experiment 2. The experimental tanks, fish restraints, and apparatus were the same as in Experiment 2 with the addition of the lights used to provide the CS + and CS - and the additional programming equipment necessary to control the discriminated Pavlovian conditioning. In addition to the pilot light as described in Experiment 2, three incandescent light bulbs, two 15 W and one 7.5 W, were used to provide the increase (+ △ light) and decrease (– △ light) in illumination that served as the stimuli. The intertrial illumination was provided by the pilot light and the 7.5 W bulb giving 16 lx. For the + △ light the two 15 W bulbs were turned on for a total illumination of 52 lx. For the – △ light the 7.5 W bulb was turned off, leaving only the pilot light for an illumination of .7 lx. A Gerbrands tape programmer timed the intertrial intervals. The EOD was amplified and recorded as in the previous experiment except that the CS + trials were recorded on the left channel and the CS - trials on the right channel to facilate data analysis. Pulses generated by Tektronix 160 series modules were recorded on the other channel to indicate 5-sec pre-CS, the CS, and the US delivery.

Procedure. All fish were moved from the community tank to the home tanks two weeks prior to the start of the experiment. One fish was used in the experiment each day. A session began at 0830 with one hour of adaptation. The experimental session began with 40 trials of CS only presentation, 20 each of CS + and CS -, without US presentation. This was followed by 200 discriminated conditioning trials with the US being one of the four voltage levels of Experiment 2; .05 V/cm, 1 V/cm, 10V/cm, 20 V/cm. This is followed by 200 trials with reversal of the CS + and CS - with the same US level. Next, the fish received 100 trials with the stimuli again reversed and with a US level of 20 V/cm for all fish. The CS duration was 5.1 sec with the US delivery during the last .1 sec. The intertrial interval was variable, with a mean of 1 min. CS + and CS - trials and the CS only trials were presented in a pseudorandom order with at most two trials of one type in a row. The trial order and the components of the variable intertrial interval are presented in Appendix B. Table 10 gives the US level and initial CS + for each of the eight fish.

Results

The analysis of the results of the conditioning procedures using the criterion response of Mandriota et al. (1965) is presented in

Table 10

US Level and Initial CS + for Each of the Fish.

	Initial CS +	
US Level (V/cm)	+ Δ Light	- Δ Light
20	G54	G60
10	G62	G55
1	G61	G59
.05	G58	G57

Figure 24. In the CS only phase, the - △ light elicited a greater mean number of responses than the increase in light for all fish, 8.25 vs. 6.62. On the second block of 20 trials the response to both stimuli decreased producing a mean of 5.75 to the light decrease and 5.25 to the light increase.

Taking as a criterion for discriminated responding a greater number of responses to the CS + than to the CS - on the last three blocks of 20 trials, none of the eight fish shows conditioning in both the conditioning and reversal phases. Five of the eight fish show discriminated responding once, in either the conditioning or reversal phase. In every case this was when the decrease in light served as the CS +. When the stimuli were again reversed and the US was 20 V/cm for all fish, four instances of discriminated responding occurred. Three of these were with the light decrease as CS +, and one (G62) with the increase in light as the CS +. With the exception of fish G61 there is an increase in the number of criterion responses shown to the stimuli when the responses to the positive and negative stimuli are combined for the conditioning and reversal phases. Fish G61 produced less than half as many responses as any other fish and less than one third as many criterion responses as the other fish receiving a US of 1 V/cm.

Figures 25 and 26 present the results for the individual fish with the EOD activity analyzed differently. The mean frequency of the EOD for each of the blocks of 20 trials is shown for the 5-sec pre-CS and during the 5-sec CS. As with the analysis by criterion response, the - △ light stimulus produced a greater change in EOD activity than did

Figure 24. Number of criterion responses for each individual fish for the conditioning experiment shown by blocks of 20 trials.

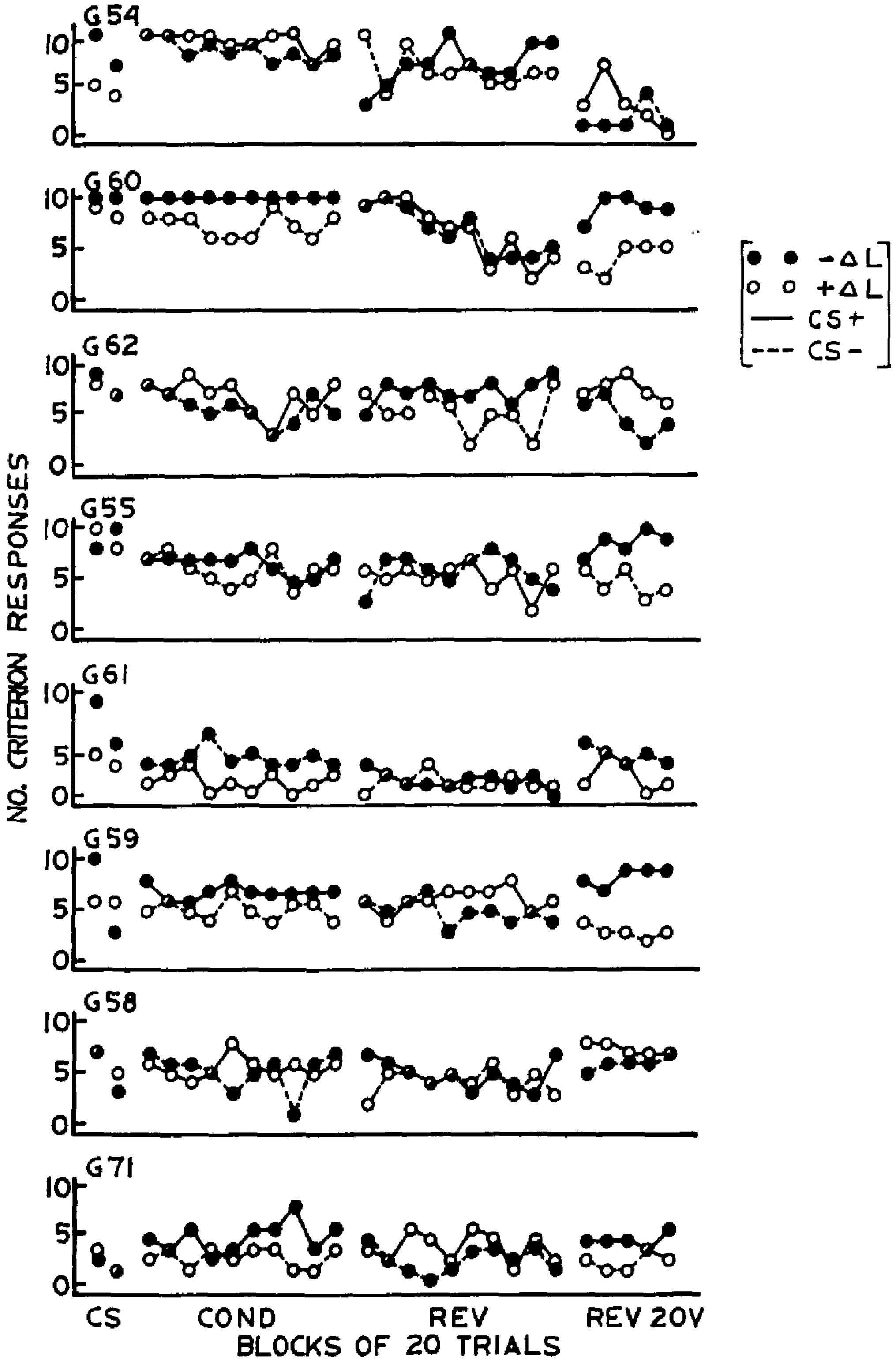

G54
G60
G62
G55
G61
G59
G58
G71
10
5
0
NO. CRITERION RESPONSES
CS
COND
REV
REV 20V
BLOCKS OF 20 TRIALS
● ● −ΔL
○ ○ +ΔL
— CS+
--- CS−

Figure 25. Mean EOD frequency for blocks of 20 trials during the conditioning experiment for G54, G60, G62, and G55.

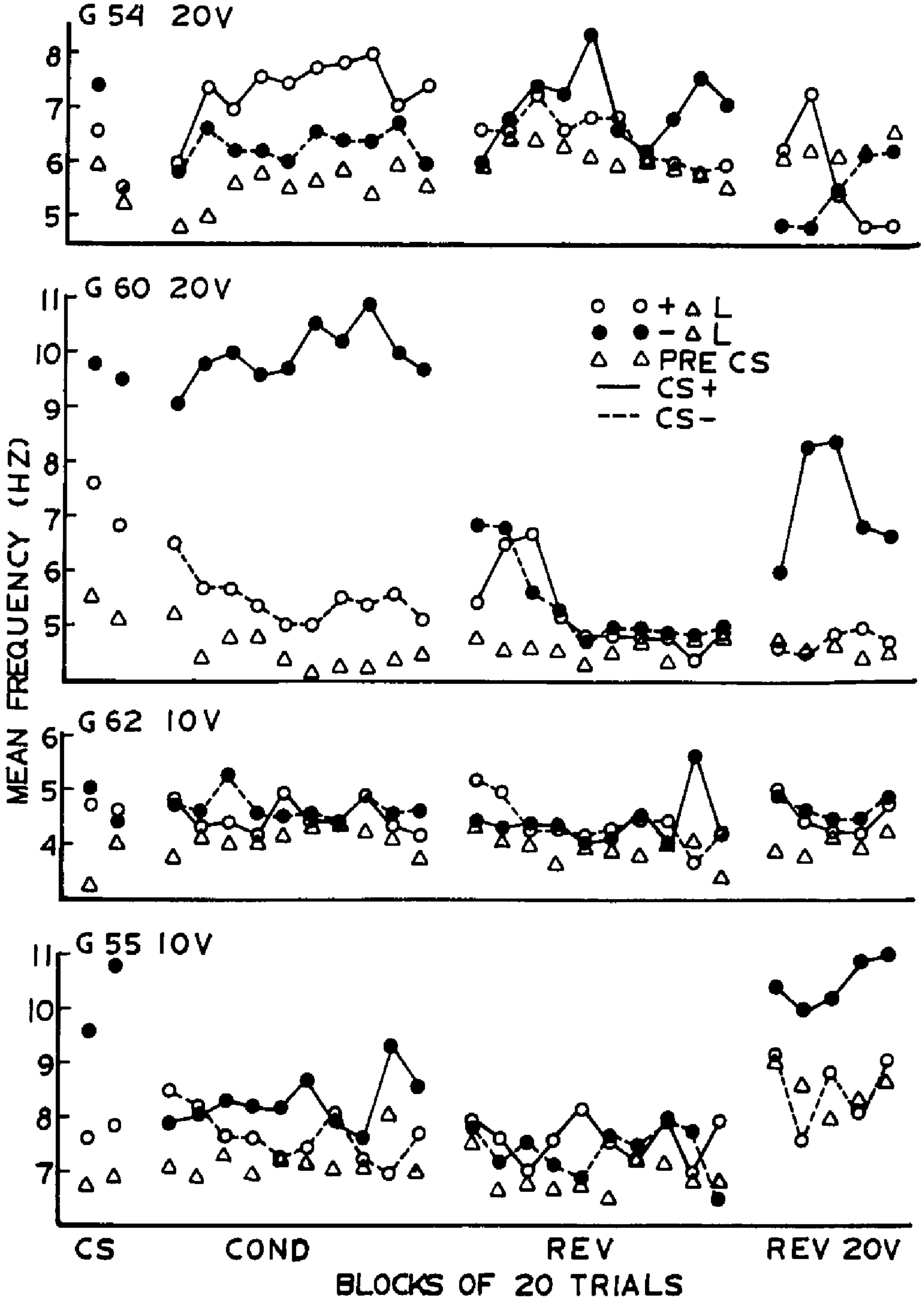
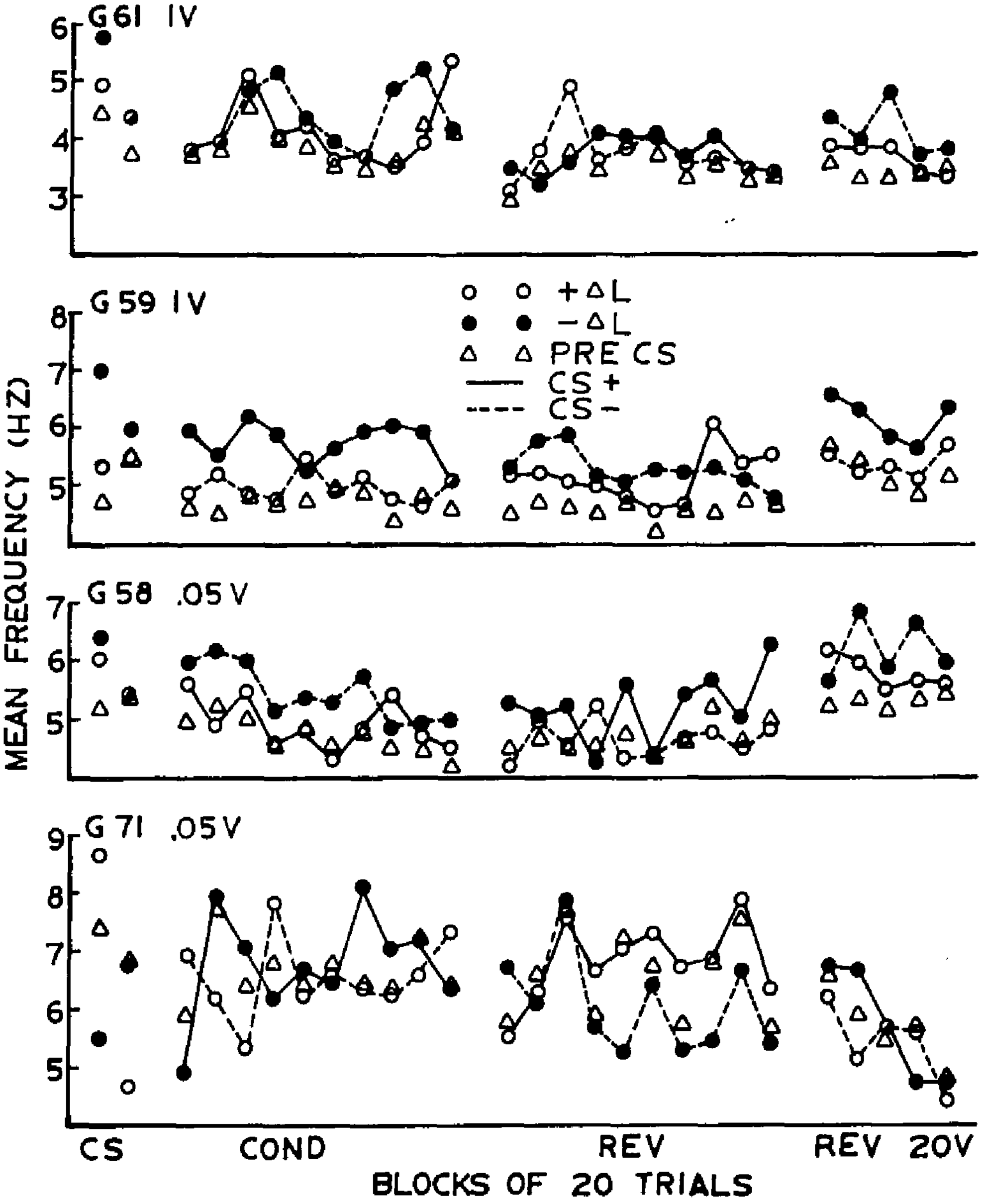

G 54 20V
8
7
6
5
G 60 20V
11
10
9
8
7
6
5
○ ○ + Δ L
● ● − Δ L
Δ Δ PRE CS
—— CS +
- - - CS −
MEAN FREQUENCY (HZ)
G 62 10V
6
5
4
G 55 10V
11
10
9
8
7
CS
COND
REV
REV 20V
BLOCKS OF 20 TRIALS

Figure 26. Mean EOD frequency for blocks of 20 trials during the conditioning experiment for G61, G59, G58, and G71.


G61 1V
G59 1V
G58 .05V
G71 .05V
○ ○ +ΔL
● ● −ΔL
Δ Δ PRE CS
—— CS +
---- CS −
MEAN FREQUENCY (HZ)
3
4
5
6
7
8
9
CS
COND
REV
REV 20V
BLOCKS OF 20 TRIALS

the + △ light. The mean frequency for the eight fish was 7.05 and 6.60 Hz for the first and second blocks of 20 trials for the light decrease and 6.44 and 5.60 for the light increase. With the exception of G71, all fish showed an increase in EOD activity to both CS stimuli. Eight of the sixteen conditioning and reversal phases show a difference in the expected direction on the last three blocks of trials, five for the - △ light CS + and three when the + △ light was the CS +. This response measure would seem to be more sensitive than the criterion response measure as more instances of discriminated behavior were found and that three of these, as compared with zero using the criterion response, occurred to the light increase as the CS +. Two fish, G54 and G59, show a discriminated response in both the conditioning and reversal phases with US levels of 20V/cm and 1 V/cm respectively. With the reversal with the US of 20 V/cm only three of the fish showed differential behavior to the two stimuli, and these two fish had previously shown the same discrimination. Because of the possibility of changes in the EOD activity over the 5-sec CS period, an analysis was made of mean EOD frequency for the first and second halves of the CS periods. The second half mean frequencies showed discriminated behavior for six of the conditioning and reversal phases, not detecting the differences in the reversal phases for G58 and G59 that the analysis of the whole 5-sec period had. The results for the 20 V/cm reversal phase were the same. The analysis of the first half of the CS showed that little of the discriminated responding to the CS + and CS - occurred at the beginning of the CS periods. A difference in responding using this measure was found on only two of the conditioning and two of the 20 V/cm reversal phases and all of these were also found by the other measures.

Figures 27 to 34 are PST histograms for each individual fish for the last block of 20 trials in each phase of the experiment. The mean frequency for consecutive .5-sec intervals is shown for the 5-sec pre-CS, the CS, and the 5 sec after the termination of the CS. For all US levels except .05 V/cm an unconditioned response is seen to the US which occurred on CS + trials. This indicates that the three highest levels of US were still effective even after 200 presentations, a much greater number of US presentations than was used in Experiment 2. The failure to obtain conditioning then is not due to a diminuition of the effectiveness of the US, at least for the three higher levels of US. The individual fish had a large variation both in the magnitude and nature of the response to the stimuli used as the CS + and CS - in the CS only phase. Fish G60 showed a very extreme response to the -△ light reaching a frequency of almost 16 Hz approximately halfway through the 5-sec CS period. G62 and G58 showed very little response to the stimulus change in either amplitude or in the degree of variability, with the remaining fish showing an increase in frequency of EOD activity and an increased variability to the stimuli. The phases marked with an asterik are the last blocks of 20 trials from phases that showed conditioning. There does not seem to be any consistency in the nature of the responses to the CS + or CS - stimuli among the individual fish that showed a differentiated response. There was a consistency within the responding to the different phases of fish that did show conditioning. For example, G54 responded with an initial increase, a drop in frequency, and then an increase to a higher level. On the reversal with 20 V/cm, the response is similar in nature but does not show the recovery to the higher level and as a result did not have a higher mean frequency of EOD to the CS + than to the CS - and did not

Figure 27. Post stimulus time histograms for G54 for the Last block of 20 trials in each phase of the experiment. An asterisk marks those phases where discriminated conditioning occurred.

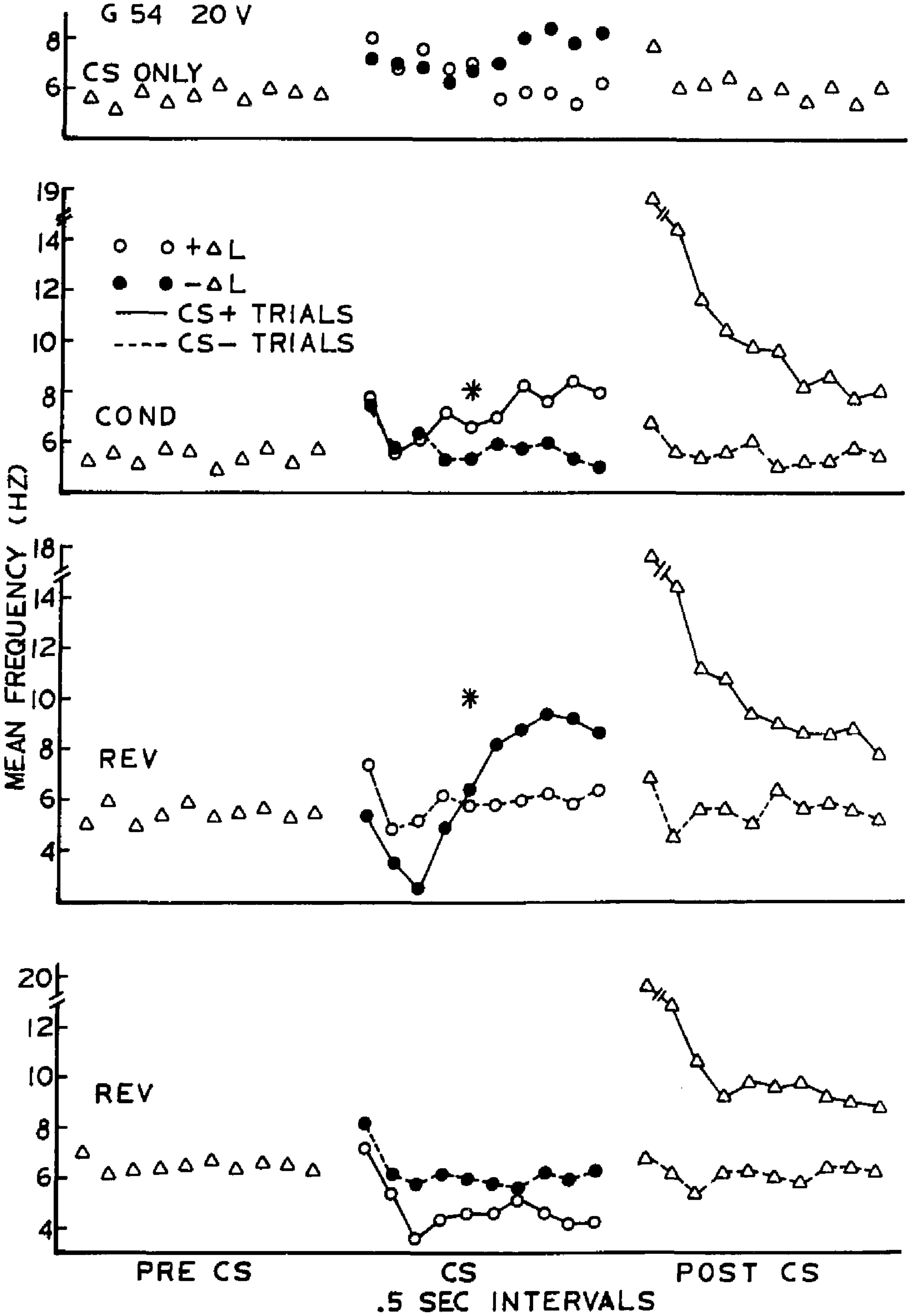

G 54 20 V
CS ONLY
○ +ΔL
● −ΔL
—— CS+ TRIALS
---- CS− TRIALS
COND
REV
REV
MEAN FREQUENCY (HZ)
8
6
19
14
12
10
8
6
18
14
12
10
8
6
4
20
12
10
8
6
4
PRE CS
CS
POST CS
.5 SEC INTERVALS

Figure 2. Post stimulus time histograms for G60 for the last block of 20 trials in each phase of the experiment. An asterisk marks those phases where discriminated conditioning occurred.

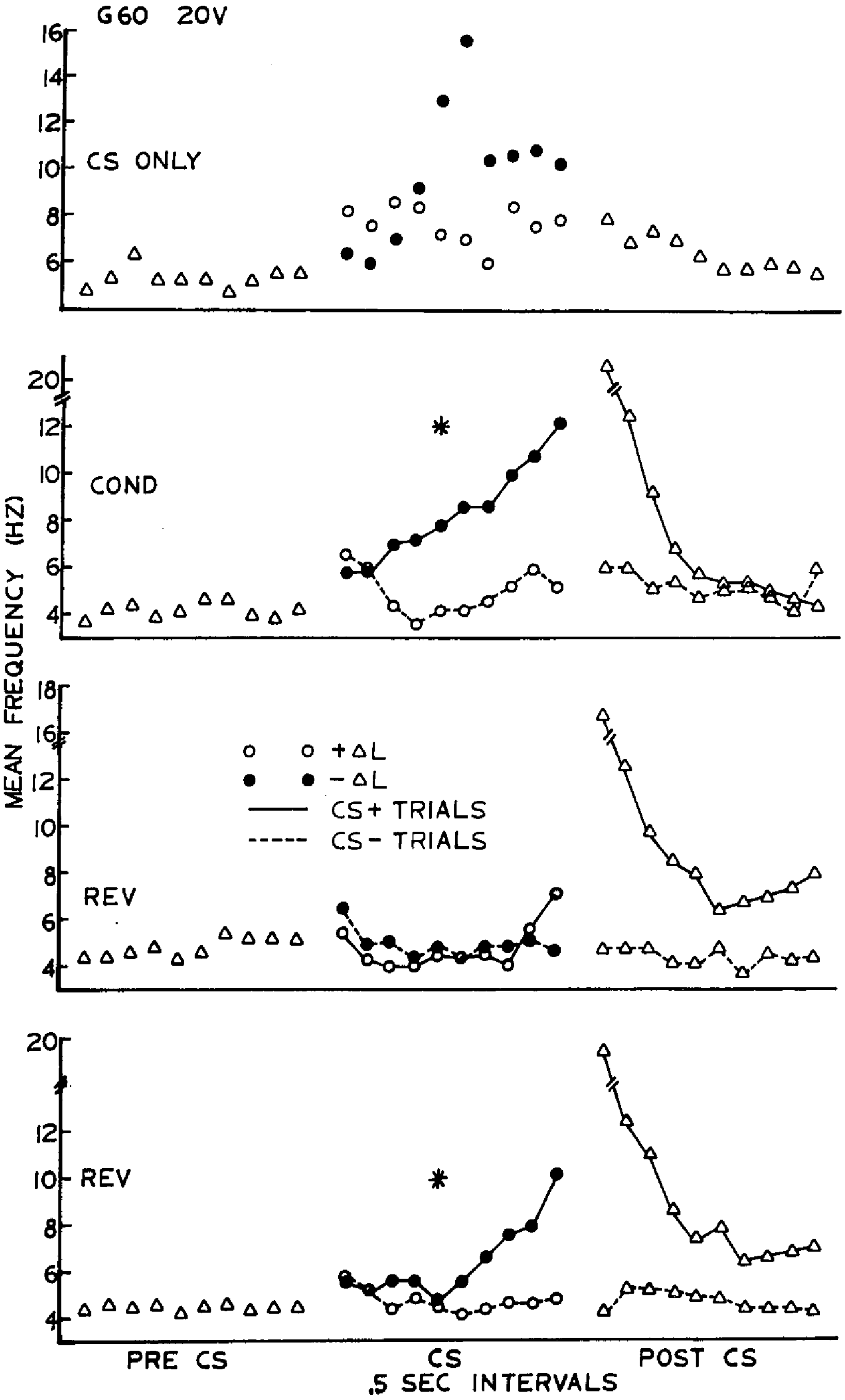

G60 20V
CS ONLY
COND
REV
REV
MEAN FREQUENCY (HZ)
16
14
12
10
8
6
20
12
10
8
6
4
18
16
12
10
8
6
4
20
12
10
8
6
4
*
*
○ ○ +ΔL
● ● −ΔL
CS+ TRIALS
CS− TRIALS
PRE CS
CS
POST CS
.5 SEC INTERVALS

Figure 29. Post stimulus time histograms for G62 for the last block of 20 trials in each phase of the experiment.

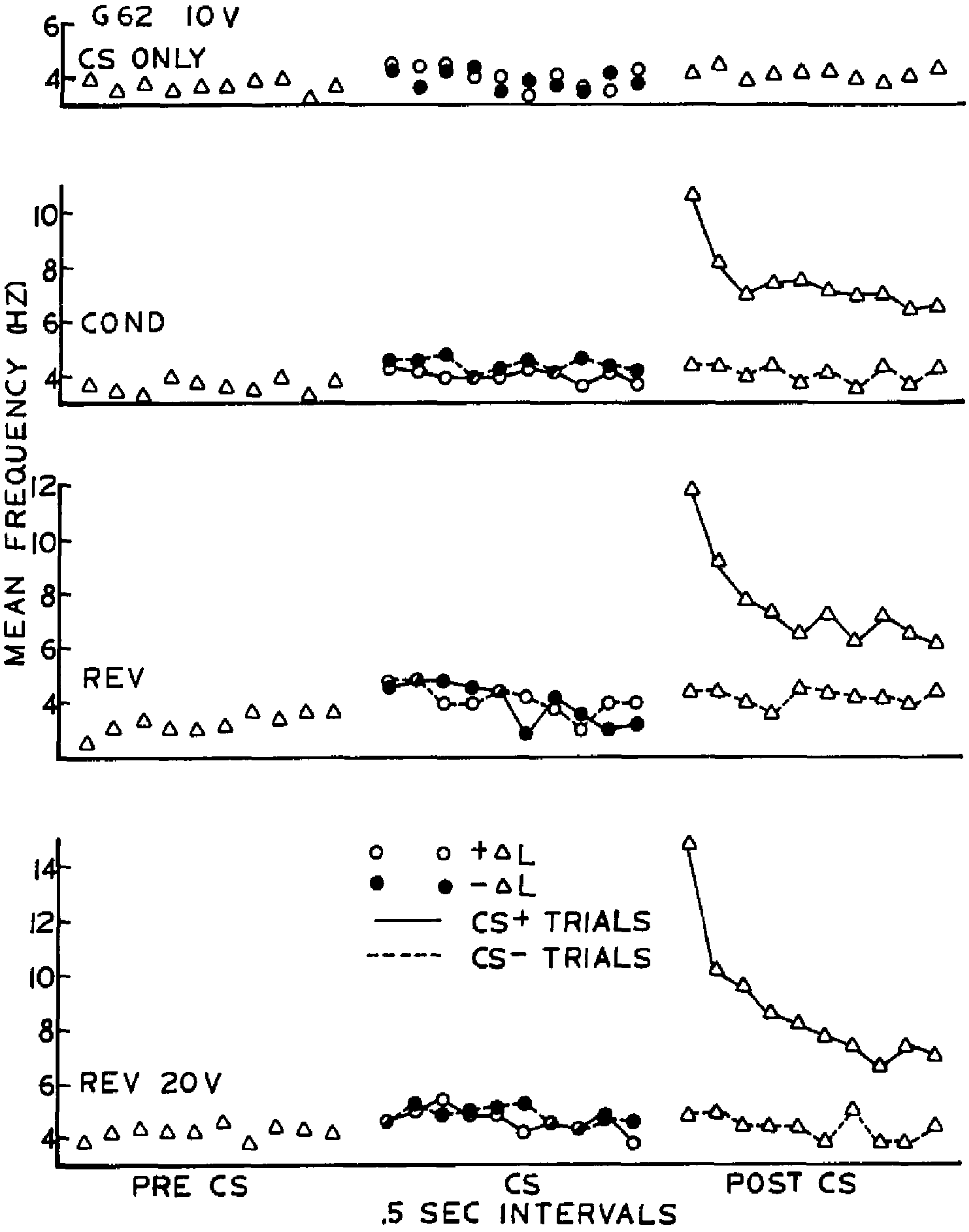

G62 10V
CS ONLY
COND
REV
REV 20V
MEAN FREQUENCY (HZ)
6
4
10
8
6
4
12
10
8
6
4
14
12
10
8
6
4
+ΔL
−ΔL
CS+ TRIALS
CS− TRIALS
PRE CS
CS
POST CS
.5 SEC INTERVALS

Figure 30. Post stimulus time histograms for G55 for the last block of 20 trials in each phase of the experiment. An asterisk marks those phases where discriminated conditioning occurred.

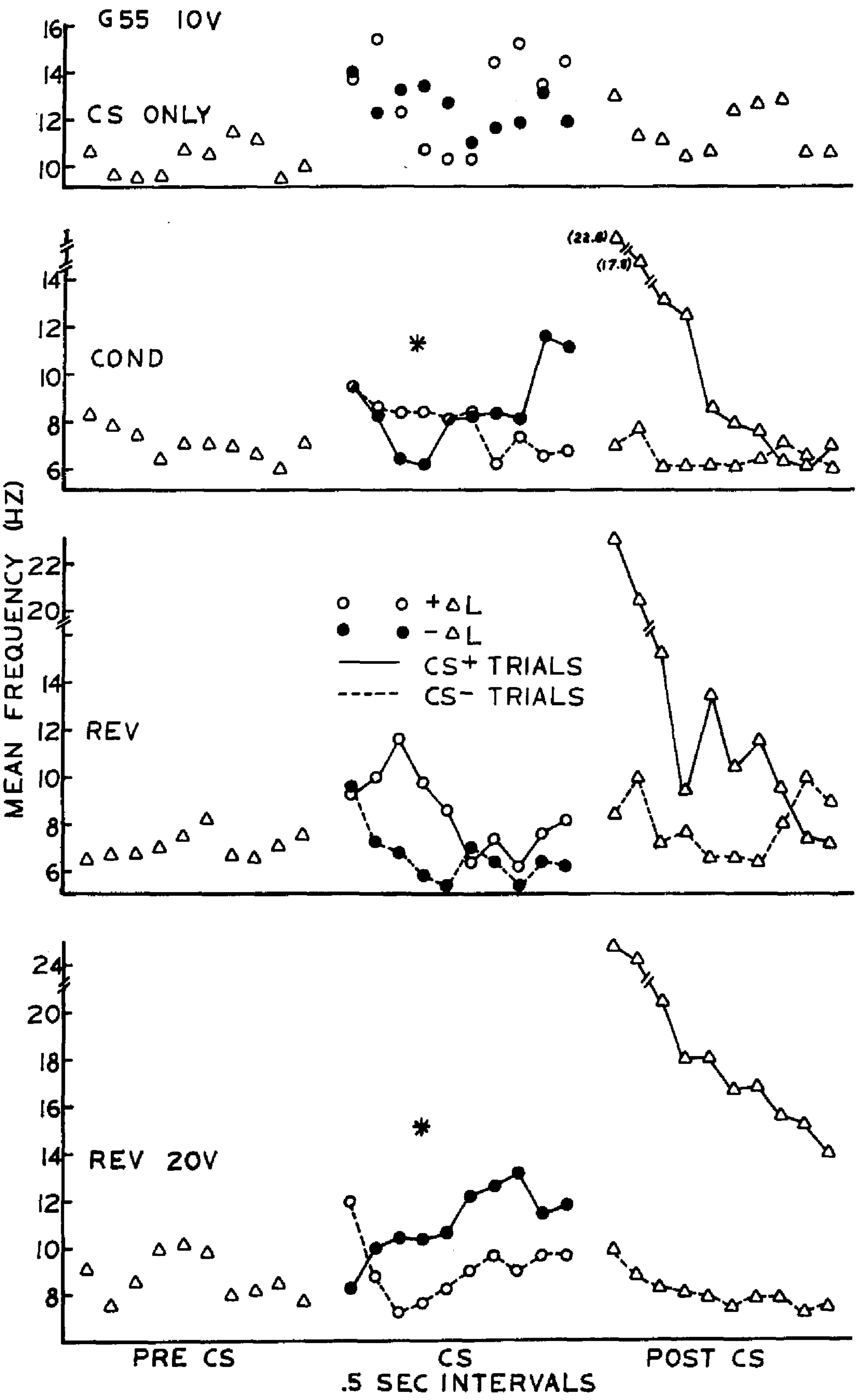

G55 10V
CS ONLY
COND
REV
REV 20V
MEAN FREQUENCY (HZ)
16
14
12
10
8
6
22
20
24
18
(22.8)
(17.8)
*
○ ○ +ΔL
● ● −ΔL
CS+ TRIALS
CS− TRIALS
PRE CS
CS
POST CS
.5 SEC INTERVALS

Figure 31. Post stimulus time histograms for G61 for the last block of 20 trials in each phase of the experiment.

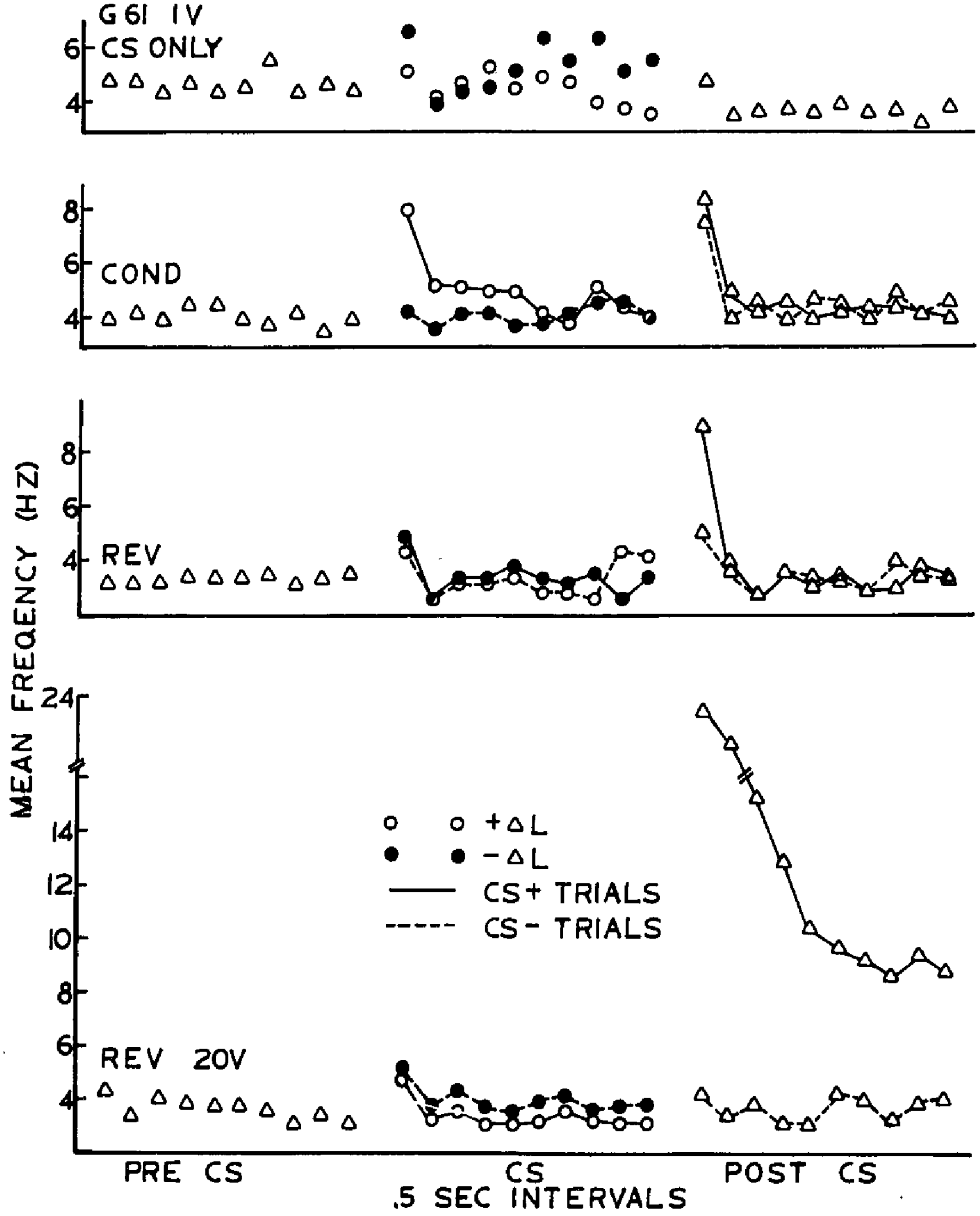

G61 1V
CS ONLY
6
4
8
6
COND
4
8
6
REV
4
24
14
12
10
8
6
REV 20V
4
MEAN FREQENCY (HZ)
○ ○ +ΔL
● ● −ΔL
CS+ TRIALS
CS− TRIALS
PRE CS
CS
POST CS
.5 SEC INTERVALS

Figure 32. Post stimulus time histograms for G59 for the last block of 20 trials in each phase of the experiment. An asterisk marks those phases where discriminated conditioning occurred.

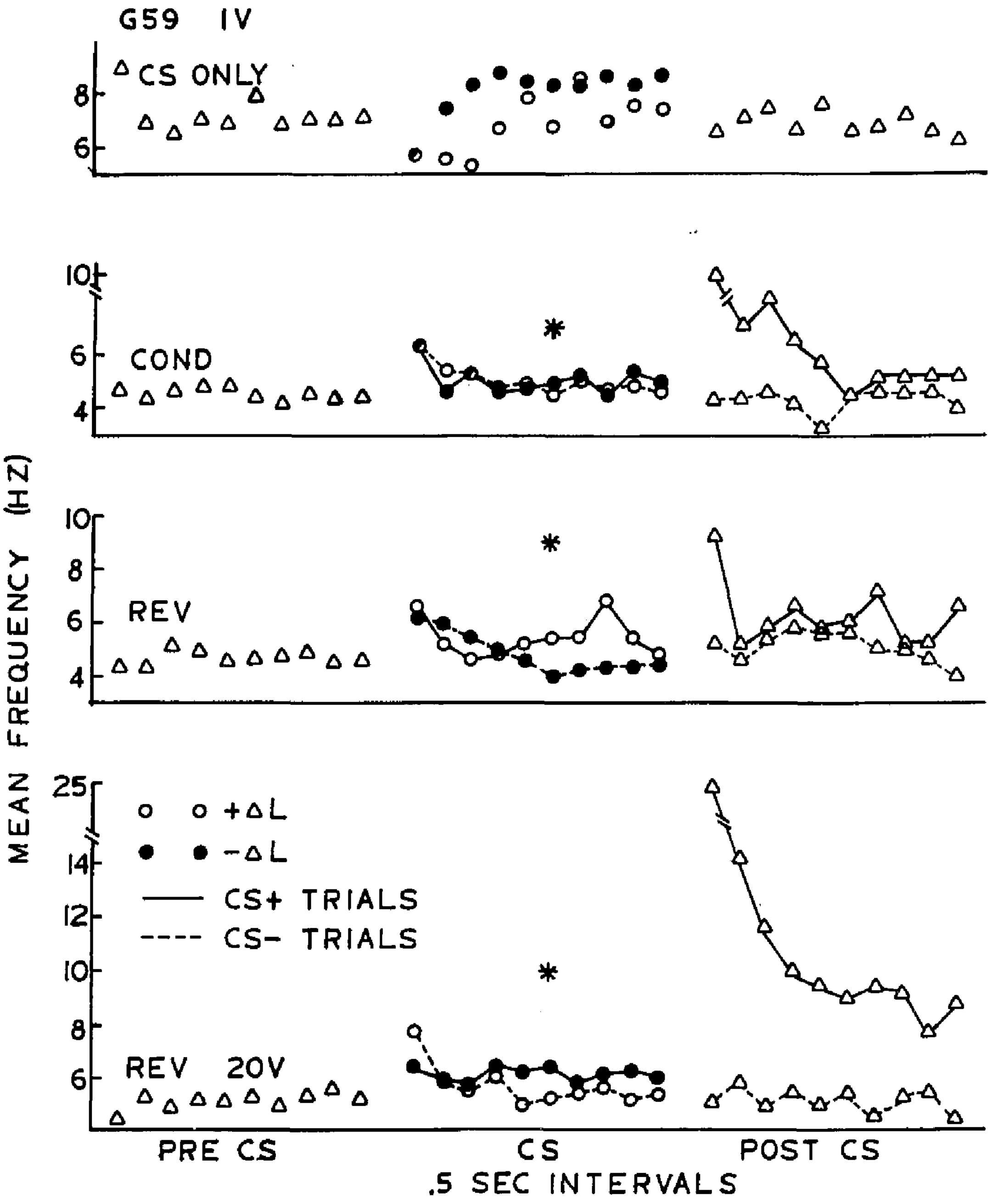

G59 IV
CS ONLY
COND
REV
REV 20V
MEAN FREQUENCY (HZ)
6
8
4
10
12
14
25
*
○ ○ +ΔL
● ● −ΔL
—— CS+ TRIALS
---- CS− TRIALS
PRE CS
CS
POST CS
.5 SEC INTERVALS

Figure 33. Post stimulus time histograms for G58 for the last block of 20 trials in each phase of the experiment. An asterisk marks those phases where discriminated conditioning occurred.

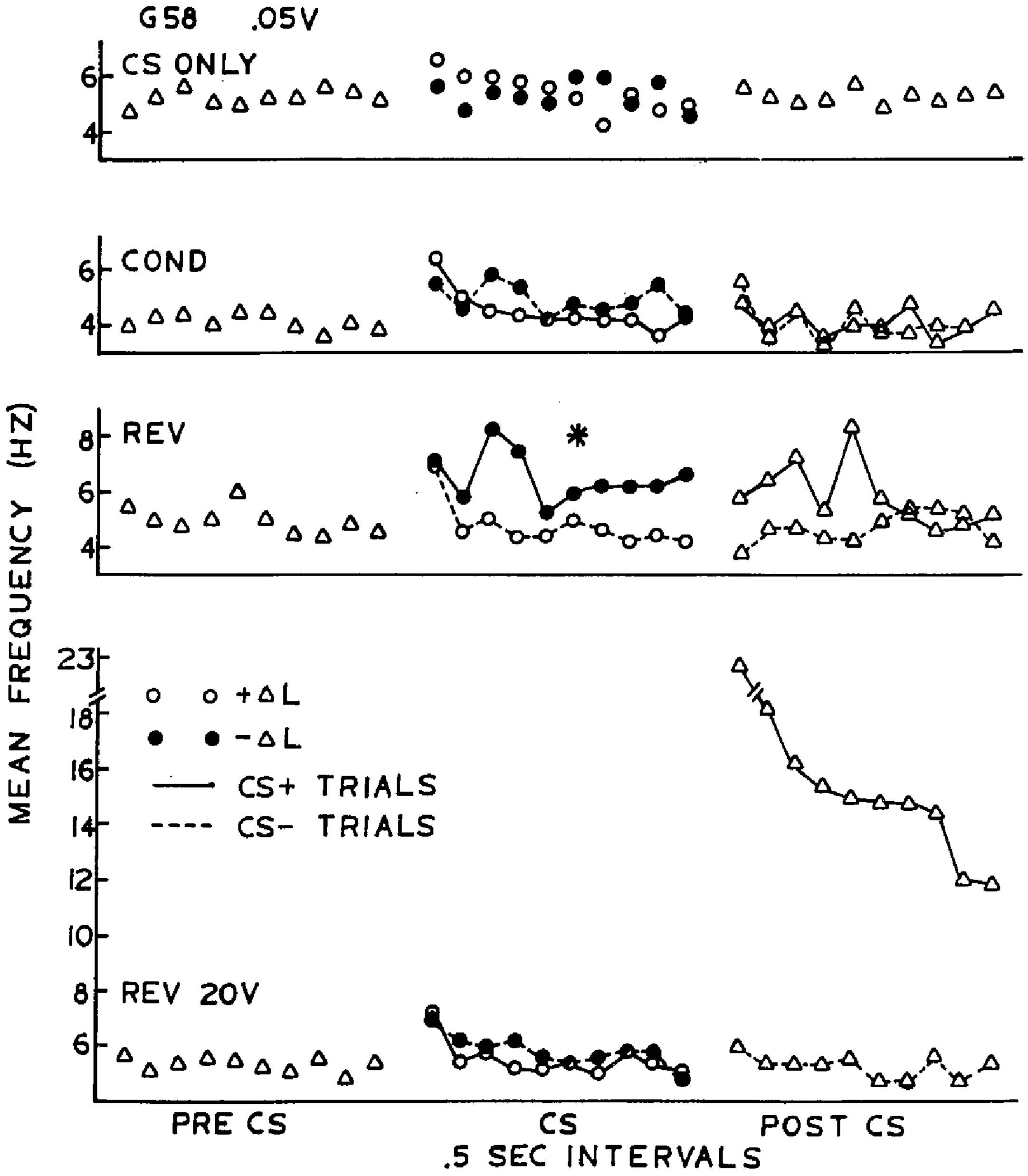
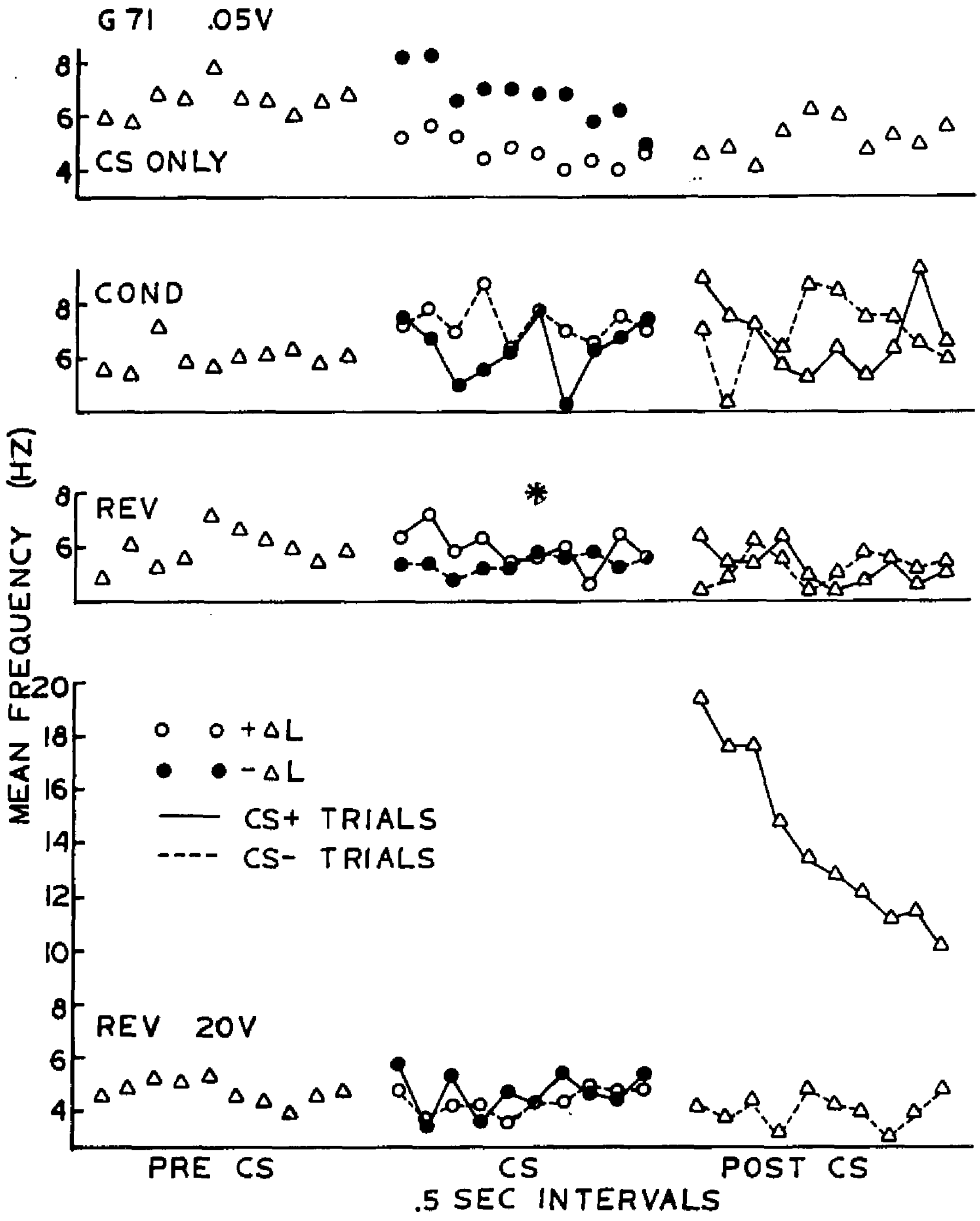

G58 .05V
CS ONLY
COND
REV
REV 20V
MEAN FREQUENCY (HZ)
6
4
8
23
18
16
14
12
10
*
○ ○ +ΔL
● ● −ΔL
CS+ TRIALS
CS− TRIALS
PRE CS
CS
POST CS
.5 SEC INTERVALS

Figure 34. Post stimulus time histograms for G71 for the last block of 20 trials in each phase of the experiment. An asterisk marks those phases where discriminated conditioning occurred.


G 71 .05V
CS ONLY
COND
REV
REV 20V
MEAN FREQUENCY (HZ)
8
6
4
20
18
16
14
12
10
○ ○ +ΔL
● ● -ΔL
CS+ TRIALS
CS- TRIALS
*
PRE CS
CS
POST CS
.5 SEC INTERVALS

show conditioning. This drop and then rise in frequency is also shown by G55 to the - △ light on the initial conditioning phase but not on the reversal with the 20 V/cm US where conditioning also occurred. G60 showed a fairly steady increase over the 5-sec CS period. The remaining cases where conditioning occurred (indicated by the asterisk) did not show any consistent pattern. In the cases where conditioning did not occur the response was similar to both stimuli and in most cases did not differ much from the ongoing EOD activity shown prior to the CS onset. In a few cases, CS onset caused a very brief increase in EOD activity to both the CS + and CS -.

Discussion

This attempt to extend the classical conditioning techniques of Mandriota et al. (1965) to discriminated conditioning was not sufficiently successful to establish a procedure for the determination of sensory thresholds. While discriminated conditioning was established in some cases, only in two of the fish, G54 and G59,were conditioning and reversal both successful, indicating that the obtained results were not just related to the increased EOD activity seen to the decrease in illumination. Of these two fish, G54 received a US of 20 V/cm and G59 a US of 1 V/cm. This result with G59 indicates that with some fish it may be possible to establish discriminated conditioning with lower levels of US as desired. Using the criterion response measure all of the instances of discriminated behavior occurred when a decrease in illumination was the CS +. When the mean frequency was considered as the response, more instances of discriminated behavior occurred but eight of eleven were again to the decrease in light as CS +. In view of the finding that with both of these response measures, greater EOD activity occurred to the light decrease in the CS only phase, an interaction would appear to have occurred between the light decrease as CS and the US. It was this type of interaction that this procedure was intended to overcome, but did not. This may relate to the concept of preparedness (Seligman, 1970) which states that all stimuli are not neutral and not equally able to be conditioned. Welker and Wheatly (1977) found a similar result in experiments on conditioned suppression with rats. When the CS + was an increment in luminance level and the CS - a decrease in luminance level discriminated behavior was quickly established with a high suppression level to the CS + and little change in behavior to the CS -. The reverse conditions failed to produce differentiated suppression behavior. They attribute these findings to the association of bright situations with danger in the rat and relate this to Seligman's preparedness concept. It is possible that the results found in this experiment are also related to this concept. In mormyrids both general activity and the EOD activity increase in the dark and it is to the light decrease stimulus that most of the conditioning occurred.

The parameters used in this experiment were those successfully used for classical conditioning by Mandriota et al. (1965). As many of the parameters of the conditioning situation such as intertrial interval, CS duration, and so forth have major effects on the conditioning process (cf. Kimble, 1961) it is possible that a change in one or more of these parameters may lead to successful discriminated conditioning in mormyrids.

GENERAL DISCUSSION

Stability and Individual Resting Patterns

While the finding of stationarity was not supported, a high degree of stability in the distribution of inter-pulse times (IPTs) of electric organ discharge (EOD) activity was found both within and between fish. The most stable feature was the burst activity peak which showed a similar and quite stable location in all of the fish, with the exception of the later recordings of three of the B. niger in isolation. All of the B. niger also showed an increase in burst activity immediately upon transfer to isolation which declined in the fish as the time in isolation increased. These findings along with previous results on the increase in burst activity induced by stimuli of almost any modality (Scheich & Bullock, 1974) and the arousal hypothesis of Dewsbury (1966) would support an interpretation that the increase in activity is related both to arousal, and to an increased information flow (Hagiwara & Morita, (1963). There was a decrease shown by three of the B. niger over days in burst activity to the point where the TI histograms no longer had a mode in the frequency range for burst activity. This may have occurred due to the almost stable and unchanging conditions in isolation, and may represent a species difference as it did not occur in any of the G. petersii. The similarity in frequency of burst activity shown by all of the fish may indicate an optimal rate of discharge activity for information processing by the electrosensory system, a question that would be best answered by an electrophysiological study of the system of the electroreceptors and areas of the brain of the fish involved in electroreception. It is evident from agonistic studies (e.g. Bell et al., 1974; Krammer & Bauer, 1976) that the fish are capable of sustained periods of higher frequency discharge activity in social situations where these higher frequencies may be serving a signaling function rather than a sensory function of increased information flow.

The interburst activity peaks or peaks of lower EOD frequency seen in the fish are very labile in comparison to the burst activity peak. They show differences in number, location, and sharpness of the mode between and within fish. Fish had varying degrees of stability in the locations of these peaks. The fish that changed the most in shape of their distributions of IPTs were the three _B. niger_ which also showed the greatest reduction in burst activity. In the other fish the degree of stability, especially in the location of the burst activity and main mode was quite large. The third, or lowest frequency mode was the most variable, both in location and its presence or absence. One possible hypothesis that would account for these findings is that these modes, other than the burst activity one, serve a social function of individual recognition. While the existence of dominance hierarchies has not been shown, dominance relationships can exist among pairs of fish (e.g. Krammer, 1974, 1976). Those modal or preferred frequencies could serve to identify individual fish, or at least a dominant fish, and thereby provide the individual recognition necessary for the establishment of a dominance order. The stability found in the TI histograms in these experiments would be a necessary feature of the use of specific frequencies for individual recognition. It is not proposed that mormyrids can process all the information that exists in a TI histogram of the EOD activity. Several of the fish showed distributions that contained very high, narrow peaks. A distribution of IPTs of this nature could possibly be relatively easy for another fish to identify and could, therefore serve as an individual recognition signal. The single _B. niger_ that maintained the same distributional shape after moving to isolation had such a sharply peaked distribution. The loss of modal frequencies and the flattening of the distributions of the other three B. niger supports an individual recognition role for these modes; in isolation there is no longer any need to maintain specific distributions. It should be remembered that even at these lower frequencies of EOD activity the electroreceptive function is still active, even if at a reduced level. Further research will be necessary to determine the role of preferred frequencies in the life cycle of the fish. An important starting point would be to establish a dominance situation in a community tank, beginning with a single pair of fish, and making recordings similar to those done in Experiment 2 of this study. The shape of the TI histograms could then be examined in relationship to the dominance position of the fish. If the hypothesis of sharply defined modes serving an identification function is correct, it would be expected that the dominant fish should have such TI histograms. The distributions would also be expected to be very stable over days. Subordinate fish might be expected to show less sharply peaked, less stable histograms. By adding and removing fish, and by placing fish for which the dominance position and histogram shape is known into isolation, further tests of these hypotheses could be made and they would provide further insight into the nature and significance of the variability, or lack of it, in the EOD activity of mormyrids.

Adaptation

The examination of the adaptation of individual mormyrids to a new situation was examined in Experiments 1 and 2 with the aim of providing a way of establishing when a fish had adapted to the new aquarium through an examination of its EOD activity. One important finding was that both the G. petersii and B. niger show changes in the EOD activity over days even in undisturbed isolation. The B. niger had a high degree of stability in the TI histograms for the first five days of isolation after being moved from the community tank and the degree of stability did not increase for the group with each successive five days of adaptation showing increases and decreases for individual fish. For some of the fish, the shape of the TI histogram continued to slowly change over days, but in most cases they still retained a high degree of stability for the four five-day blocks. The amount of burst activity was the only feature of EOD activity that did show a large change with the move and subsequent isolation. There was a large increase in this high frequency activity within the first hour after being moved and a large drop in the next 24 hours. Three of the four B. niger showed a further drop in burst activity over the 20 days of the experiment to a level below that seen in the community tank. The fish in Experiment 2 which received a 2.5 hour adaptation period also had a high degree of stability for the samples of EOD activity over the five days without the US presentations. This would indicate that a stable baseline of EOD activity against which experimental manipulations could be measured can be obtained even when the fish does not remain in the same tank for long periods of time. Adaptation to a new situation does occur in these fish as evidenced by the reduction in burst activity and the high degree of stability shown over days but the fish never show a total stability. There are changes occurring in the distribution of EOD activity even in an isolated, undisturbed fish. A procedure that would be useful in many experimental situations would be to place the fish into the experimental situation and record samples over several days to obtain samples of the EOD activity. With these samples, the degree of stability of the fish's EOD could be evaluated and a better estimate of the range and trend of EOD activity could be made to provide a baseline sample against which the effects of the experimental manipulation could be evaluated. As with other features of the EOD activity of mormyrids examined in this series of experiments, large individual differences in the EOD were observed in the degree of stability in isolation and in the amount of change in the TI histograms both from those recorded in the community tank and over the time in isolation.

US Effects

The response of the fish to the electric shock US levels depended both on the measure used and the individual fish. In examining the mean values of four different response measures (Table 6), the most sensitive measure appeared to be one based on the mean frequencies for one-second periods pre- and post-US. For the mean values of the responses for *B. niger* this measure produced the largest values for all five US levels. For this reason and because of the ease in measuring it, this was the response measure chosen for the conditioning in Experiment 3. Using this measure, a response occurred to the three highest levels for the *B. niger* and four highest for the *G. petersii*.

The differences in the responses of individual fish to the US levels were shown in Tables 7 and 8. *B. niger* B30 responded with an initial slowing of EOD frequency to the three lowest levels of US, while the other five fish showed an increase or no response to these US levels. Two of the *G. petersii*, G12 and G20, responded with a slowing of EOD activity to the lowest level, .0005 V/cm. With the highest levels of the US all fish responded with an increase in EOD frequency, the increase varying among fish. The lower levels of US are in the range of biologically occurring electrical events. It is possible that fish that slowed their EOD activity were responding to these pulses as if they represented the EOD activity of other fish. In agonistic interactions a slowing of the EOD activity has been found to represent a submissive response (Krammer, 1976). B. niger B23 responded to the .0005 V/cm US with an average frequency increase of 3.6 times. This was a greater increase than to the other levels except the 10 and 20 V/cm ones. It is possible that this fish was a dominant one and was responding with a threat to this US as if it were another fish. Given the differences in EOD activity shown it would seem that the responses to the more "natural" US levels are possibly determined by the prior history and dominance status of the fish. This could also be further explored by establishing dominance relationships among the fish prior to the experiment with the various US levels.

Conditioning

The attempted extension of the conditioning obtained by Mandriota et al. (1965) to discriminated Pavlovian conditioning was not totally successful. The conditioned discrimination obtained under some conditions indicates that discriminated procedures might be successful if some parameters were changed. In particular the successful conditioning of G59 with a US of 1 V/cm indicates that the procedure will work with at least some individual fish. The question remains as to whether a change of parameters would lead to success with more of the fish, or whether the fish are showing individual differences in their conditioning behavior similar to the individual differences they displayed in Experiments 1 and 2.

One factor contributing to these individual differences may be that the US potency for these fish may not vary unidimsionally. This was seen in Experiment 2 where the response to the US levels differed in direction as well as magnitude among the fish. Fish B30, for example, responded to the three lower levels of US by slowing its EOD rate. These are the levels of US most similar to the biologically occurring levels, such as EOD activity of conspecifics. This suggests the possible use of a combination of temporal patterning and signal strength to establish USs that may be most salient to the receiving fish. One possibility would be the use of agonistic patterns of artificial fish EOD activity as a US. The individual nature of the responses to this type of pattern would however necessitate the use of individually determined conditioned responses for each of the fish. The type of response elicited by the US would be determined by the history of the fish.

In many respects the problems involved in conditioning EOD activity are similar to those reported in cardiac conditioning. Hall (1976) discusses the results of many of these experiments and concludes that several aspects of the cardiac response system cause difficulties in obtaining a classically conditioned response. Several of the difficulties Hall describes are methodological ones. First is the problem of defining the response, since the heart is continuously active and the response must be described as a change of rate. Secondly, the intertrial period must be long enough for the heart to return to a baseline rate of response. Thirdly, the orienting response, the original response to the CS, may include changes in the organism's heart rate. Another factor that may influence conditioning is the fact that a variety of events including some that are under voluntary control of the subject have an influence on heart rate. In cardiac conditioning there is some controversy over whether the conditioned response is one of acceleration or deceleration. All of these factors that produce difficulties in cardiac conditioning would seem to have direct parallels in attempts to condition the EOD activity of mormyrids. The EOD is typically continuous and more variable in frequency than heart rate. The changes produced by the stimuli last for several seconds and it is necessary to allow the rate to return to a baseline level between trials. It is certainly true that a variety of factors can influence the EOD activity of mormyrids. These include the introduction of almost any stimulus change, the EOD activity of a conspecific, locomotion, and the "spontaneous" variation seen in the resting, undisturbed fish even when in isolation.as seen in the data from Experiments 1 and 2.

In Experiment 3, the orienting response is seen in the change in frequency shown by the fish to both stimuli in the CS only phase of the experiment (Figures 27-34). As Hall argues the response seen in conditioning to the CS-US compound is produced by an interaction of the responses to these two stimuli. Some part of the individual differences seen in the results of the conditioning may be due to the differences in the orienting response and also the different responses shown to the US levels, especially the lower ones by the individual fish. A further complicating factor in this experiment arises due to the fact that the trials were started independently of the fish's EOD activity. As this EOD activity is continuously changing and reflecting some measure of the state of the fish, the stimuli were being imposed on a variable baseline of responding and perhaps different internal states of the fish. One additional feature which probably contributed to the failure to obtain discriminated conditioning was the differential EOD responses obtained to the two stimuli used as CS + and CS —. Generally the decrease in light gave rise to a large increase in EOD activity in the fish. Most of the successful conditioning phases in Experiment 3 occurred when the light decrease was the CS +.

There are several possible changes in procedure that might lead to successful discriminated Pavlovian conditioning. One possibility would be to change the length of the CS. The nature of the response in several of the fish that conditioned showed an initial increase followed by a decrease and then a gradual increase in EOD frequency. It is possible that a longer CS duration would permit the full development of the conditioned response. The spacing of trials is another variable that should be examined. While the variable intertrial interval with a mean of one minute used in this experiment allowed a return of EOD activity to baseline frequency, the possibility exists that a longer interval would be more effective. CS stimuli that caused a smaller response or no response in the fish might allow discriminated conditioning to be obtained because of less interference from the CS stimuli themselves. While this would be useful for a study of conditioning in this unusual response system, it would not be useful in psychophysical studies of mormyrids as many of the stimuli of interest, e.g., frequency difference thresholds, do produce responses in the fish prior to conditioning. One possibility would be to attempt the use of EOD threat or agonistic patterns as the US. This would have the advantage of being a US of a much lower amplitude than the US caused by Mandritoa et al. (1965) and would not cause interference or damage to the electrosensory system. Using these patterns as a US may make it necessary to use different conditioned responses for individual fish as the response to agonistic EOD patterns may by different depending on the prior history of each fish.

There are few data in the conditioning literature on individual differences in conditioning; most experiments typically report only group data with no mention of the variability within the groups. In a review of cardiac conditioning McCanne and Sandman (1976) suggest that more attention should be paid to individual differences in cardiac conditioning because of the large individual differences shown by the cardiac response in situations other than conditioning. This suggestion would seem to apply equally well to attempts to condition the EOD activity of mormyrids.

Since EOD activity in mormyrids presumably serves both sensory and communicative roles the analysis of either function is made more difficult. If the ideas presented in this discussion are supported by further research it will help clarify some of the individual differences shown in every aspect of the EOD activity examined in the present series of experiments. It will be necessary in future research to take the individual fish's history into account as it appears to be influential even in unexpected stiuations such as the response of a fish to electric shock. The recommendation of McCabbe and Sandman (1976) that more importance be placed on response differences among individuals would seem to be well taken in the study of the EOD activity of mormyrids.

APPENDIX A

TABLE A

Schedule of the Recordings of Experiment 1 for Each Group[a].

First Sequence

	Date							
Time	6/28	6/29	6/30	7/1	7/2	7/3	7/4	7/5
0200				1			1	
0600				1			2	
0830					1			2
0900					1			2
1100	1	2						
1400	1	2						
1800			1			2		
2030	2	1						
2100	2	1						
2300			1			2		

Second Sequence

	Date							
Time	7/7	7/8	7/10	7/11	7/12	7/13	7/14	7/15
0200		1				2		
0600		1				2		
0830							1	2
0900							1	2
1100			2	1				
1400			2	1				
1800	1				2			
2030			1	2				
2100			1	2				
2300	1				2			

[a] Group 1--fish GP1, GP3, GP7
Group 2--fish GP2, GP4, GP5

APPENDIX B

TABLE B

Sequence of CS + and CS - Trials for The Pavlovian Conditioning

Trial No.	CS
1	+
2	-
3	+
4	-
5	+
6	+
7	-
8	-
9	+
10	+
11	-
12	-
13	+
14	+
15	-
16	+
17	-
18	-
19	+
20	-

TABLE C

Components of the One-Second Variable Interval

(all times in Seconds)

40
80
80
40
60
60
40
40
60
80
60
80
40
80
40
60
80

Bauer, R. Electric organ discharge activity of resting and stimulated Gnathonemus petersii (Mormyridae). Behaviour, 1974, 50, 306-323.

Belbenoit, P. Conditionnement instrumental de l'électroreception des objects chez Gnathonemus petersii (Mormyridae, Teleostei, Pisces). Zeitschrift für Vergleichend Physiologie, 1970, 67, 192-204.

Bell, C. C., Bradbury, J., & Russell, C. J. The electric organ of a mormyrid as a current and voltage source. Journal of Comparative Physiology, 1976, 110, 65-88.

Bell, C. C., Myers, J. P., & Russell, C. J. Electric organ discharge patterns during dominance related behavior displays in Gnathonemus petersii (Mormyridae). Journal of Comparative Physiology, 1974, 92 201-228.

Bennett, M. V. L. Electric organs. In W. S. Hoar & D. J. Randall (Eds.), Fish physiology (Vol. 5). New York: Academic Press, 1971. (a)

Bennett, M. V. L. Electroreception. In W. S. Hoar & D. J. Randall (Eds.), Fish physiology (Vol. 5). New York: Academic Press, 1971. (b)

Bennett, M. V. L., & Grundfest, H. Studies on morphology and electrophysiology of electric organs. III. Electrophysiology of electric organs in Mormyrids. In C. Chagas & A. P. de Carvalho (Eds.), Bioelectrogenesis. Amsterdam: Elsevier, 1961.

Birnbaum, Z. W. Introduction to probability and mathematical statistics. New York: Harper & Bros., 1962.

Bullock, T. H. Seeing the world through a new sense: Electroreception in fish. American Scientist, 1973, 61, 316-325.

Bullock, T. H. General introduction. In A. E. Fessard (Ed.), Handbook of sensory physiology (Vol. III/3). New York: Springer-Verlag, 1974.

DeFazio, A. The effect of acquatic conductivity on the electric organ discharge of Gnathonemus petersii. Unpublished master's thesis. Hunter College of the City University of New York, 1974.

Dewsbury, D. A. Stimulus-produced changes in the discharge rate of an electric fish and their relation to arousal. The Psychological Record, 1966, 16, 495-504.

Fessard, A. E. & Szabo, T. Physiology of electroreceptors. In A. E. Fessard (Ed.), Handbook of sensory physiology (Vol. III/3). New York: Springer-Verlag, 1974.

Frey, A. H. & Eichert, E. S. The nature of electrosensing in the fish. Biophysical Journal, 1972, 12, 1326-1358.

Gallon, R. L., Mandriota, F. J., & Thompson, R. L. Effects of temperature and shock on electric organ discharge rate in *Gnathonemus*. *Proceedings of the 75th Annual Convention of the American Psychological Association*, 1967, *2*, 131-132.

Gerstein, G. L., & Kiang, N. S. An approach to the qualitative analysis of electrophysiological data from single neurons. *Biophysics Journal*, 1960, *1*, 15-28.

Hagiwara, S., & Morita, H. Coding mechanisms of electroreceptor fibers in some electric fish. *Journal of Neurophysiology*, 1963, *26*, 551-567.

Hall, J. F. *Classical conditioning and instrumental learning: A contemporary approach*. Philadelphia: J. B. Lippincott Company, 1976.

Harder, W., Schief, A., & Uhleman, H. Zur Funktion des elektrischen Organs von *Gnathonemus petersii* (Gthr., 1862) (Mormyriformes, Teleostei). *Zeitschrift für vergleichend Psysiologie*, 1964, *48*, 302-331.

Harder, W., Schief, A., & Uhleman, H. Zur Empfindlichkeit des schwachelektrischen Fisches *Gnathonemus petersii*, (Gthr., 1862) (Mormyriformes, Teleostei) gegenüber elektrischen Feldern. *Zeitschrift für Physiologie*, 54, 89-108.

Heiligenberg, W. Theoretical and experimental approaches to spatial aspects of electrolocation. *Journal of Comparative Physiology*, 1975, *103*, 247-272.

Kalmijn, A. J. The detection of fields from inanimate and animate sources other than electric organs. In A. E. Fessard (Ed.), *Handbook of sensory physiology* (Vol. III/3). New York: Springer-Verlag, 1974.

Kemmer, W., Baumann, B., & Altmann, G. Impulsratenanlyse der Entladungen des schwach elektrischen Fisches *Gnathonemus petersii* zur Feststellung seiner Lang- und Kurzzeitperiodik unter dem Einfluss aüsserer Zeitgeber. *Verhandlungsbericht der Deutschen Zoologischen Gesellschaft*, 1970, *64*, 287-292

Kimble, G. A. *Hilgard and Marquis' conditioning and learning* (2nd ed.). New York: Appleton-Century-Crofts, 1961.

Kundsen, E. I. Spatial aspect of the electric field generated by weakly electric fish. *Journal of Comparative Physiology*, 1975, *99*, 103-118.

Kramer, B. Electric organ discharge interaction during interspecific agonistic behaviour in freely swimming Mormyrid Fish. *Journal of Comparative Physiology*, 1974, *93*, 203-235.

Kramer, B. Flight associated discharge patterns in a weakly electric fish, *Gnathonemus petersii* (Mormyridae, Teleostei). *Behaviour*, 1976, *59*, 88-95.

Kramer, B., & Bauer, R. Agonistic behavior and electric signaling in a mormyrid fish, *Gnathonemus petersii*. *Behavioral Ecology and Sociobiology*, 1976, *1*, 45-61.

Lissmann, H. W., & Machin, K. E. The mechanism of object location in *Gymnarchus niloticus* and similar fish. *Journal of Experimental Biology*, 1958, *35*, 451-486

Mandriota, F. J., Thompson, R. L., & Bennett, M. V. L. Classical conditioning of electric organ discharge rate in mormyrids. *Science*, 1965, *150*, 1740-1742.

Mandriota, F. J., Thompson, R. L., & Bennett, M. V. L. Avoidance conditioning of the rate of electric organ discharge in mormyrid fish. *Animal Behaviour*, 1968, *16*, 448-455.

McCanne, T. R., & Sandman, C. A. Human operant heart rate conditioning: The importance of individual differences. *Psychological Bulletin*, 1976, *83*, 587-601.

Moller, P. 'Communication' in weakly electric fish *Gnathonemus niger* (Mormyridae) I. variation of electric organ discharge (EOD) frequency elicited by controlled electrical stimulation. *Animal Behaviour*, 1970, *18*, 768-786.

Moller, P. Elektrische Signale bei Fischen. *Umschau*, 1973, *11*, 342-343.

Moller, P., & Bauer, R. 'Communication' in weakly electric fish, *Gnathonemus petersii* (Mormyridae) II. interaction of electric organ discharge activities of two fish. *Animal Behaviour*, 1973, *21*, 501-512

Parzen, E. *Stochastic processes*. San Francisco: Holden-Day, 1962.

Poll, M. Récherches sur la faune icthyologique de la région du Stanley Pool. *Annales du Museum du Congo*, 1959, *71*, (ser. in-8°, Zoologie).

Rodieck, R. W., Kiang, N. Y.-S., & Gerstein, G. L. Some quantitative methods for the study of spontaneous activity of single neurons. *Biophysics Journal*, 1962, *2*, 351-368.

Russell, C. J., Myers, J. P., & Bell, C. C. The echo response in *Gnathonemus petersii* (Mormyridae). *Journal of Comparative Physiology*, 1974, *92*, 181-200.

Sanderson, A. C., Kozak, W. M., & Calvert, T. W. Distribution coding in the visual pathway. *Biophysical Journal*, 1973, *13*, 218-244.

Scheich, H., & Bullock, T. H. The detection of electric fields from electric organs. In A. E. Fessard (Ed.), *Handbook of sensory physiology* (Vol. III/3). New York: Springer-Verlag, 1974.

Seligman, M. On the generality of the laws of learning. *Psychological Review*, 1970, *77*, 406-418.

Siegel, S. *Nonparametric statistics for the behavioral sciences*. New York: McGraw-Hill, 1956.

Stebbins, W. C. Principles of animal psychophysics. In W. C. Stebbins (Ed.), *Animal psychophysics: The design and conduct of sensory experiments*. New York: Appleton-Century-Crofts, 1970.

Suga, N. Coding in tuberous and ampullary organs of a gymnotid electric fish. Journal of Comparative Neurology, 1967, 131, 437-451.

Szabo, T. Les organes électrique des Mormyrides. In C. Chagas & A. P. de Carvalho (Eds.), Bioelectrogenesis. Amsterdam: Elsevier, 1961.

Welker, R. L., & Wheatley, K. L. Differential acquisition of conditioned suppression in rats with increased and decreased luminance levels. Learning and Motivation, 1977, 8, 247-262.

Woodard, W. T., & Bitterman, M. E. Classical conditioning of goldfish in the shuttlebox. Behavior Research Methods and Instrumentation, 1971, 3, 193-194.

Wyman, R. Probablistic characterization of simultaneous nerve impulse sequences controlling dipteran flight. Biophysics Journal, 1965, 5, 447-471.

REFERENCE NOTES

1. Moller, P. Personal communication, June 29, 1977.

2. Push, S. Electric organ discharge response and spatial aspects of electrolocation in the pulse-type fish, *Gnathonemus petersii* (Mormyridae, Teleostei). Unpublished Honors Thesis, City College of New York, 1977.