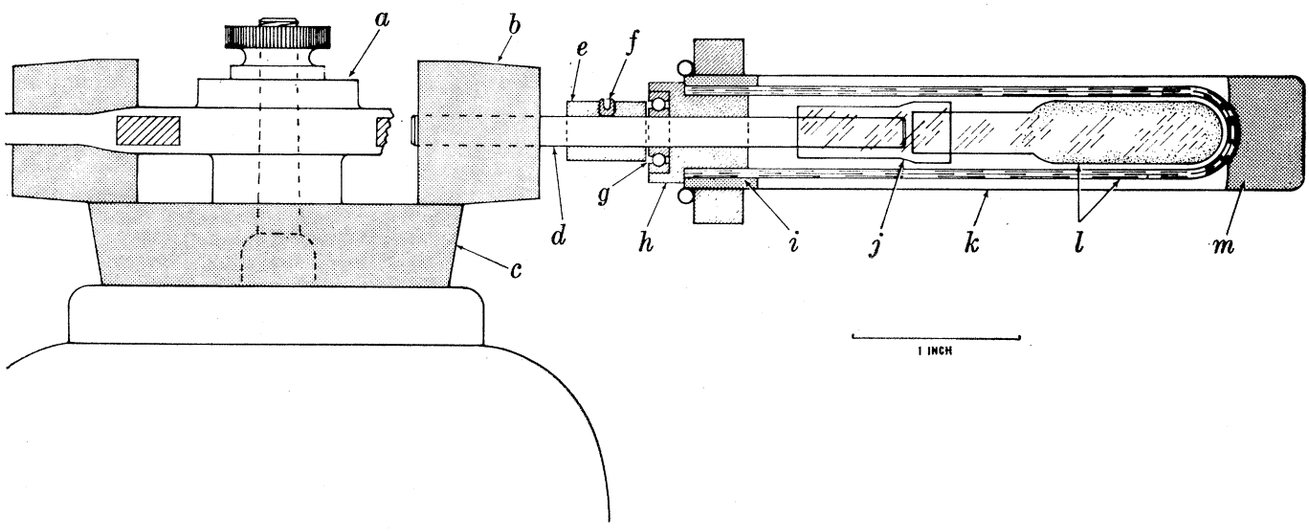

Fig. 1. Homogenizer, longitudinal section. (*a*) Centrifuge head, (*b*) rubber-stopper drive, (*c*) stationary rubber stopper, (*d*) aluminum shaft, (*e*) manganese collar, (*f*) locking screw, (*g*) ball bearing, (*h*) manganese plug, (*i*) rubber gasket, (*j*) plastic-tubing connector, (*k*) metal centrifuge-tube holder, (*l*) ground-glass tube and pestle, (*m*) rubber cushion.

national clinical centrifuge, model 49891H. The rotation of the centrifuge is transferred to rotate the pestle of the grinder, while the centrifugal force of the centrifuge maintains the tissue at the bottom of the tube where grinding is effective. As homogenization proceeds the solubilized material at the bottom of the tube is continually displaced by intact cells or by particles of higher density.

One unit of the device consists of a ground-glass homogenization tube and pestle. The glass shaft of the pestle is attached to an aluminum shaft by a length of plastic tubing. The shaft is centered in the tube by a magnesium plug into which a ball bearing is inserted. The shaft is machined to fit through the bearing with sufficient clearance to allow free adjustment of the shaft for regulation of the depth of the pestle in the tube. A small manganese collar fitted with a set screw, through which the shaft may slide, is adjusted after the pestle is positioned, so that it rests against the inner bearing surface; the collar is then locked to the shaft by the set screw and prevents the pestle from being forced to the bottom of the tube by gravity (in which event abrasion between the two surfaces would soon destroy the homogenizer). The collar also rides on the inner surface of the bearing; when the shaft turns, friction between shaft and bearing is negligible. The homogenizer is mounted on and rotates with the centrifuge, while the shaft of the homogenizer is rotated by friction between the rubber stopper on the end of the shaft and the stationary stopper mounted at the base of the centrifuge. Preliminary tests with slime-mold spores and yeast cells indicate that homogenization is effective and rapid with the homogenizer shaft turning at 3000 to 4000 rev/min.

Many refinements of the apparatus are possible. Tests have been restricted to the cells mentioned. Homogenization of other types of tissues may be improved by mounting a small cutting blade on the pestle shaft; the resultant small fragments of tissue would be further homogenized upon reaching the grinding surface. Specific tasks, such as isolation of intact nuclei from cells, could be accomplished by selection of appropriate ground-glass homogenizers. Sonic vibration or other principles of homogenization might be incorporated in the system. Installation of the device in a refrigerated centrifuge, with homogenizer speed independent of centrifuge speed, should control excessive heating. The method may be of special value for separating soluble from insoluble material during homogenization of tissue.

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Classical Conditioning of Electric Organ Discharge Rate in Mormyrids

Abstract. *Weakly electric fish of the African family Mormyridae emit pulses at variable intervals with a distribution skewed toward longer intervals. Fourteen specimens of the genera* Mormyrops, Gnathonemus, *and* Marcusenius *were classically conditioned to increase briefly their discharge frequency. The unconditioned stimulus was electric shock and the conditioned stimulus was light. These results are novel in that the overt conditioned response involves neither secretion nor movement.*

The rate of discharge of electric organs in freshwater fish of the African family Mormyridae is known to vary spontaneously and to be accelerated (or sometimes decelerated) by a variety of novel or noxious stimuli (*1*). The electrophysiology and morphology of the electric organs in these and other weakly electric fish have been described in detail (*2*). These organs

are presumed to operate as the energy source in an electrosensory system which detects variations in the impedance of the animal's environment (*3*). Much remains to be learned, however, of the behavioral significance of electric organ activity and the variables controlling it.

We are not aware of any systematic attempts to control electric organ activity through behavioral conditioning. Whether new stimulus control of discharge rate can be acquired by classical conditioning, or whether discharge rate can be controlled instrumentally by its consequences, has not been discussed in the literature. Our first experiments employed operant avoidance conditioning, but interpretation of the data required evaluation of classically conditioned effects of the type described in this report (*4*).

It is reasonable to question whether discharge amplitude might also be a response dimension of importance. Although amplitude is often observed to decrease slightly at high rates of discharge, it is usually quite constant at low rates. Moreover, the medullary neurons controlling discharge are electrotonically coupled and behave as a single unit. Thus the discharge appears to be an all-or-none response and frequency is the primary variable open to manipulation (*5*). The variations in amplitude that do occur are a function of frequency and ascribable to refractoriness rather than to some other independent factor.

The subjects of our experiments were fish, 8 to 15 cm long, that belonged to several species of the genera *Mormyrops, Gnathonemus*, and *Marcusenius*. The *Mormyrops* emit diphasic pulses of about 1-msec duration and 0.4-volt amplitude as recorded in aquarium water. The discharges of the other two genera are similar but briefer, lasting about 0.3 msec. The mean discharge rate for specimens we have observed is between 2 and 8 pulses per second, with perturbation to extremes of 1 per minute and over 50 per second. Each fish was confined in a perforated lucite box, which was large enough so that the subject did not necessarily contact the walls, but small enough to prevent its turning around. The box was suspended in a shock-mounted, semidark, aerated aquarium at room temperature. Recording electrodes of silver wire were at the head and tail ends of the box, and stimulating electrodes were placed

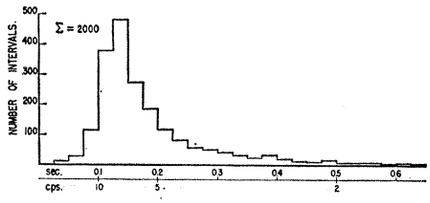

Fig. 1. Histogram of pulse intervals for one specimen of *Mormyrops* sp.

bilaterally. The electric output of the fish was amplified, and each pulse triggered a single oscilloscope sweep. This arrangement allowed generation of a standard pulse which was used for recording and computation; it also permitted continuous monitoring of the discharge and reliability of triggering.

The histogram of pulse intervals in a 370-second sample of spontaneous activity of a fish adapted to the restraining box is seen in Fig. 1. Instantaneous frequency varied from 25 per second to 0.76 per second. The distribution is skewed toward lower frequencies with a mean of 5.40 per second and a mode at 7.27 per second. This particular specimen had a higher mean discharge rate than most of the fish from which we have recorded, but the variability of discharge frequency was typical.

In the experiments proper the conditioned stimulus was illumination for 5.1 seconds by two 6-volt lamps (GE No. 47) mounted outside the aquarium 8 cm from the fish's eyes on a line through the eyes. During conditioning trials an electrical stimulus was delivered for the final 0.1 second of the conditioned stimulus and the two stimuli were terminated simultaneously. The shock was a rectangular pulse of approximately 7.5 ma, but, because the fish was shunted by the water, the current actually passing through it was considerably smaller. Shock intensity was the maximum available from the apparatus and caused a visible jerk; smaller values were not as effective in conditioning.

To arrive at a criterion for conditioned acceleration of discharge rate we took into account the range and probability of spontaneous changes in rate and the magnitude of unconditioned rate changes elicited by shock. We chose as a response criterion the occurrence of an instantaneous rate at least 2.8 times the mean resting rate at the beginning of the experiment. With this value every shock elicited several pulses at a rate greater than criterion rate, but spontaneous accelerations to criterion were not too numerous to obscure the conditioning process. Criterion rates for fish used in these experiments ranged from 6.9 to 15.0 pulses per second.

Each pulse interval was automatically compared to the criterion interval. Pulses occurring at intervals shorter than the criterion were recorded on a multichannel inkwriter at a paper speed of 25 mm/min. We wanted a continuous record of pre-experimental and intertrial spontaneous accelerations to criterion, but we did not record the time of each pulse.

The main findings are summarized with reference to three groups of fish: a conditioning group (C) of five fish, a pseudoconditioning or sensitization control group (S) of five fish, and a control group (U) of four fish which received the unconditioned stimulus alone. In the final data analysis we used a measure of the occurrence of conditioned responses which was adjusted for intertrial activity. This measure was defined for each block of 20 trials as the percentage of presentations of the conditioned stimulus in which one or more responses occurred in the 5 seconds before presentation of shock, minus the percentage of an equal number of 5-second samples (blank trials or base-line probes), one from each intertrial interval, in which accelerations to criterion rate appeared without stimulation. The blank sampling periods were programmed in varying positions within each intertrial interval. Conditioned-response measurements for control group U were identical to those for conditioning group C except that during the conditioning phase the stimulus lamps were disconnected. For the control group S, the lamps were off during the 5 seconds preceding shock, and they were on during the same time intervals as the blank trials for group C. The periods preceding shock were used as blank trials in computing the adjusted percentage of conditioned responses.

The procedure began with 2 to 16 hours of adaptation to confinement in the apparatus. During this period measurements were taken of the spontaneous discharge rate in the absence of light and shock, and the criterion for conditioned and unconditioned responses was established. All groups then received 100 presentations of light alone for adaptation and evaluation of the frequency of accelerations

to the criterion rate during light-on and light-off periods. In the conditioning phase, group C received 200 pairings of light and shock; group U, 200 presentations of shock only; and group S, 100 presentations of light and 100 presentations of shock never directly paired as noted above (*6*). The extinction phase consisted of 100 presentations of light alone. All phases followed each other in immediate succession. Intertrial intervals for all treatments were 40, 60, or 80 seconds and averaged 60 seconds.

The results appear in Fig. 2. In adaptation to light alone no statistically meaningful difference between groups was seen so the data for the 14 fish were pooled. A *t*-test comparison of the mean adjusted percentages of responses on the first and fifth block of trials was significant at the .05 level. It is not yet clear whether responding to light alone adapts completely or remains at a low level. The trend of both the adaptation and extinction data suggests that, if unconditioned responding to light alone were a persistent effect, it would be a small one.

When light and shock were paired in the next phase, the mean adjusted incidence of conditioned responses in group C rose from 20 percent to a maximum of 70 percent. The unadjusted incidence of responses reached 80 percent or more in all fish of this group. In none of the fish of group U did it exceed 20 percent, and in only one fish of the S group did it reach 80 percent, and this was during the first block of unpaired presentations of light and shock. The Mann-Whitney U-test was used to compare pairs of groups on the total adjusted conditioned responses during the first 100 conditioning trials, adjusted conditioned responses during the fifth block of conditioning trials, and adjusted conditioned responses during the first 60 extinction trials. Over the first 100 conditioning trials, C > U ($p = .008$), C > S ($p = .016$), and S > U ($p = .016$). In the fifth block, C > U ($p = .016$) and C > S ($p = .028$), but group S and group U did not differ ($p = .21$). In the first 60 extinction trials C > S ($p = .028$), but for C vs. U, $p = .095$, and for S vs. U, $p = .452$. (All p values are for a one-tailed test.) As conditioning progressed, an increase in the duration of the conditioned accelerations appeared. The trend in duration was reversed in extinction.

When the data from group S during adaptation and during conditioning

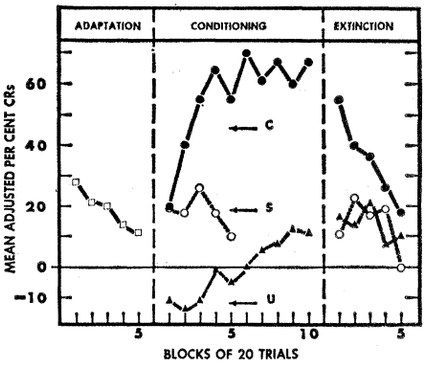

Fig. 2. Grouped results in blocks of 20 trials for conditioning (C) and control groups (U and S). The ordinate is a measure of conditioned response (CR's) adjusted for intertrial activity as explained in the text. Data for all groups are pooled in the first phase, adaptation to light alone.

were compared, no evidence was found for a sensitization effect. The incidence of responses remained low or decreased in contrast to the growth of responses in the C group. The upward trend of the data from group U suggested a low level of temporal conditioning. However, the trend was contributed to by negativity of the adjusted percentages of conditioned responses during the first 100 presentations of shock. This effect resulted from persistence into the blank trial period of the unconditioned accelerations. The existence of temporal conditioning is therefore somewhat doubtful.

As further evidence of the amenability of electric organ activity to classical conditioning, all control subjects were later conditioned successfully. This brought the total number of fish conditioned by the present procedure to 14. Reconditioning of fish in group C was accomplished readily with positive savings scores. Modified procedures on additional control and experimental fish replicated the general results.

The accelerations elicited by light were a result of association of light and shock by classical delayed conditioning. The results are not attributable to unconditioned responding to light alone (adaptation trials), to generalized elevation of discharge rate by shock alone (blank trials), to temporal conditioning (group U), or to sensitization (group S).

There may be some question as to whether we have not in fact conditioned body movements which secondarily evoke acceleration of the discharge. To be sure, conditioned movements were often noted, and movement and discharge rates may be correlated. However, we have seen numerous instances during pre-experimental adaptation runs where movements and increases in discharge rate occur independently. Vigorous swimming can also take place while the electric organ is silent, and large rate increases occur while no movement is visible. Finally, the acceleration during light stimulation can begin before obvious movement starts.

The electric discharge may be regarded as an investigatory, observing, or perceptual response. It is distinctive among responses that have been conditioned in that neither movement nor secretion is involved. Electric organs are derived embryologically from skeletal muscle, which suggests that they be classed with systems that can be controlled by operant techniques. Work in progress demonstrates that frequency changes are amenable to operant conditioning (*7*). In many Gymnotids, acceleration of discharge rate can be readily evoked, and preliminary experiments indicate that in these forms also both operant and respondent conditioning are possible.

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6. The number of shock trials in group S was reduced to 100 on the basis of our experience with group U. Although all U controls were subsequently conditioned, some of them did not survive the entire procedure. Reducing the shock trials to 100 in group S enabled us to save each specimen.
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