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Effects of Drugs on the Electric Knife Fish, *Eigenmannia virescens.** (28756)

WILLIAM A. KRIVOY, MONTAGUE LANE AND DONALD C. KROEGER
(Introduced by Edwin L. Smith)
Baylor University College of Medicine and University of Texas Dental Branch, Houston

Although fish have been used for bioassay of drugs(1,2,3), they have not been found generally useful, due in part to problems encountered in quantitating their responses. In previous reports from these laboratories(4,5), it was determined that the electric transparent knife fish, *Eigenmannia virescens,* responded quantitatively to neurotrophic polypeptides and to lysergic acid diethylamide. This paper presents an extension of studies with the electric transparent knife fish to observations on the effects of methylphenidate, pentylenetetrazole, phenobarbital, morphine, and nalorphine.

Methods and materials. Two days before each experiment, the fish was put in an individual 20 l aquarium. The pH of the water in the aquarium was maintained at 8.2 with Longlife Aqua-age buffer (Longlife Fish Food Product, Derville, N. J.) and temperature was kept at 25°C. Since these fish are sensitive to bivalent cations, the water was pre-treated with EDTA, 200 mg per 20 l. On the day of experiment, the aquarium was divided into 2 unequal compartments by means of a plastic grid. The fish was maintained in the smaller compartment. Two silver electrodes (E_1) were placed in opposite corners of the compartment containing the fish. Another pair (E_2) was placed in the larger compartment. Electrodes E_2 were placed approximately 1 cm from each other and as far from the fish as possible without jeopardizing recording because of distance or interference from extraneous signals. Because of their relative positions, electrodes E_1 recorded primarily movement, whereas E_2 tended to treat the fish-generator as a point source, and predominately recorded changes in amplitude. The dependence of E_1 on movement and the failure of movement to modify E_2 were checked when the electrodes were positioned and constituted the major criteria for electrode placement. The sides of the aquarium were made opaque with cardboard and the top was covered with red plastic to allow observation and minimize photic stimulation. The fish was permitted to acclimate to this new environment for a period of 2 hours. Electrical activity was amplified and the information from each electrode pair was recorded on a dual channel tape recorder for one hour. The test drug was then administered and the tape recorder was turned off, except in the case of pentylenetetrazole, in which instance recording was continuous. After an appropriate interval (see Results), recording was resumed. The electrical activity of the fish was also monitored by means of a cathode ray oscilloscope.

E. virescens produce a continuous electrical discharge of a frequency of approximately 300 cps. The frequency is constant for individual fish and was not modified by drugs. However, the amplitude of the signal

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varies spontaneously and also with movement of the fish-generator relative to the electrodes. Amplitude variation is decreased in restrained fish which are mechanically disturbed. The electrical activities of the fish were recorded on tape from each electrode pair. The tape was replayed through an information analysis system to determine amplitude variation (AV) as a function of time. The taped signal was amplified, filtered to remove extraneous electrical noise, rectified, and integrated (the time constant of the integrator was 55 msec). The resultant signal was further amplified, filtered to remove any 60 cycle interference, and converted into a square wave of constant amplitude by means of a square wave amplifier. The number of square waves was counted by a decade counter unit. Whenever 10 pulses were counted, the beam on a cathode ray oscilloscope was deflected. This deflection was photographed on continuously moving film. The average number of deflections of 8 successive 7.5 minute intervals was an index of AV obtained. The problem of quantitative replication of information derived from each fish was achieved by appropriate amplification of the basal recorded potentials so as to produce similar AVs for control periods when fish were compared. This was required to compensate for differences in size of the fish and electrical discharge. The chamber containing the fish was adjusted to a size large enough for the fish to move freely. If the chamber size restricted swimming activity, low AV resulted, similar to the response of the fish to mechanical stimulation or to interruption of its lines of force with a metallic conductor, as previously observed(4,5). Therefore, the size of the chamber and the electrode placement had to be modified for each experiment.

Except where noted in Results, fish were transferred to an aquarium containing fresh buffered water after an experiment, and could be used again after a period of 4 days. Drugs were administered by pipetting them into the aquarium.

Methylphenidate, pentylenetetrazole, sodium phenobarbital, morphine sulfate, and nalorphine hydrochloride were dissolved in distilled water prior to administration. Concentration of morphine in the aquaria was estimated by a spectrophotofluorometric method(6). Determinations were performed in duplicate and averaged. Standards were prepared from samples of the drugs which were used in the experiment.

Regression lines for methylphenidate and phenobarbital data were calculated by the least squares method for linear data after conversion of the ratio AV post-treatment/AV control (in per cent) to logarithms. Deviation of the calculated lines from linearity was tested by an analysis of variance(7). The differences in mean AVs in the morphine and nalorphine experiments were tested for significance by means of the Student's "t" test(7).

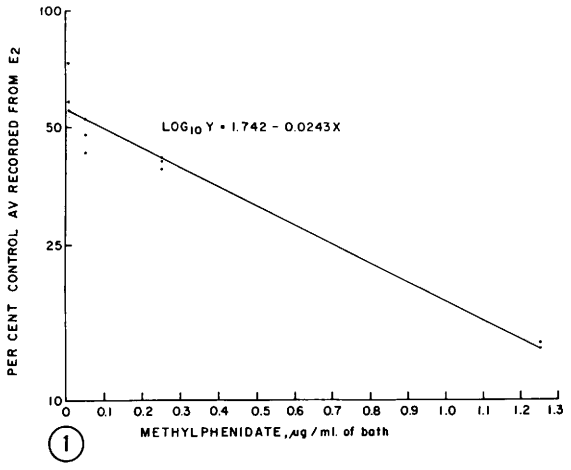
Results. The effects of methylphenidate on the electrical activity of the knife fish are shown in Fig. 1. A linear relationship was found when the logarithm of the AV treated/AV control (in per cent) recorded from E_2 (amplitude dependent electrodes) was graphed against the dose of methylphenidate. The probability that the regression equation deviated significantly from linearity was less than 0.001. Substitution of the value $X = O$ in the regression equation yields the value of $Y = 55.2\%$. However, control activity is 100%. This discrepancy suggested that a large initial fall in AV occurred between 0 to 0.005 μg of methylphenidate per ml of bath. A 50% decrease in AV from control occurred at a concentration of 0.1 μg per ml of bath. No consistent changes in AV were noted in potentials simultaneously recorded from E_1 (movement dependent electrodes).

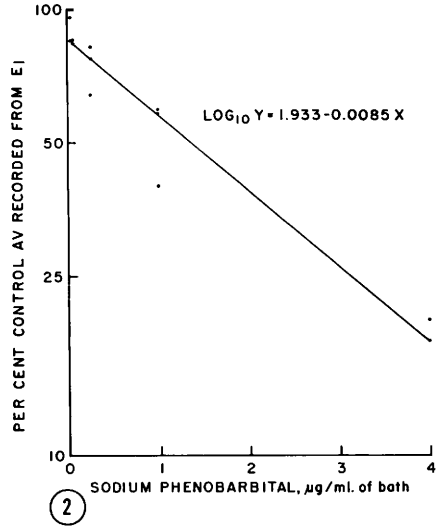
Pentylenetetrazole, in contrast to methylphenidate, did not produce consistent changes in AV recorded from E_1 or E_2 in concentrations of 2.5 to 7.5 μg per ml of bath.

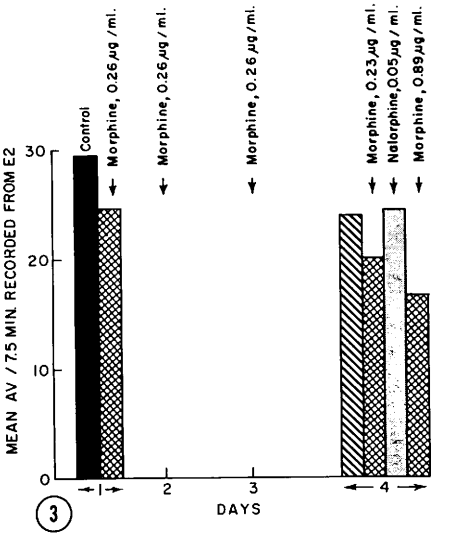
Phenobarbital was investigated in concentrations from 0.025 to 2.0 μg/ml of bath. A linear relationship was found when the logarithm of the AV treated/AV control (in per cent) recorded from E_1 (movement dependent electrodes) was graphed against the dose of phenobarbital (Fig. 2). The probability that the regression equation deviated signifi-

cantly from linearity was less than 0.001. A 50% decrease in AV from control occurred at a concentration of 1.4 μg of sodium phenobarbital per ml of bath. No consistent changes were noted in AV recorded simultaneously from E_2.

Studies with morphine and with nalorphine are shown in Fig. 3 and 4. In these experiments the bath was not changed. The mean AV per 7.5 min recorded from E_2 during the second hour after administration of each drug is shown in the figures. Fig. 3 illustrates that 0.26 μg of morphine per ml of bath produced a decrease in AV to 84% of control ($p < 0.2$). Morphine sulfate, 0.26 μg/ml of bath, was added on days 2 and 3.







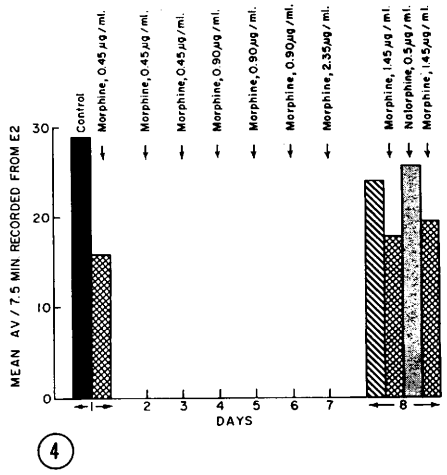


FIG. 1. Effects of methylphenidate on electrical activity of knife fish recorded from E_2. Fish were placed in fresh bath water after each determination. The 95 per cent confidence limits of the slope are −0.0169 to −0.0317.

FIG. 2. Effects of phenobarbital on electrical activity of knife fish recorded from E_1. Fish were placed in fresh bath water after each determination. The 95 per cent confidence limits of the slope are −0.0058 to −0.0112.

FIG. 3. Effects of morphine and nalorphine on electrical activity of knife fish recorded from E_2. Fish was maintained in the same bath water throughout experiment.

FIG. 4. Effects of morphine and nalorphine on electrical activity of knife fish recorded from E_2. Fish were maintained in the same bath water throughout experiment. The chemically determined concentration of morphine in bath following final addition of morphine was 9.2 μg/ml (morphine added to bath, 9.3 μg/ml).

The mean AV on day 4 did not differ from the activity recorded after the first dose of morphine. Addition of 0.23 μg of morphine per ml of bath on day 4 reduced AV to 83.4% of the immediately preceding control ($p<0.2$). Nalorphine, 0.05 μg/ml of bath, restored AV to the activity preceding the last addition of morphine ($p<0.2$). A larger dose of morphine, 0.89 μg/ml of bath, then depressed AV to 68.3% of the activity after nalorphine ($p<0.01$).

Fig. 4 demonstrates that 0.45 μg of morphine per ml of bath depressed AV to 55% of control ($p<0.001$). Similar additions of morphine were made on each of the next 2 days, then 0.90 μg per ml for 3 days, and finally 2.35 μg/ml on the 7th day. On the 8th day the mean AV had increased to 83% of the control on day 1. Following 1.45 μg/ml, the AV decreased to 75% of activity recorded one hour before drug addition ($p<0.001$). Nalorphine, 0.5 μg/ml, increased AV to 144% of the activity after the last morphine addition ($p<0.001$). Morphine, 1.45 μg/ml, then reduced activity to 75% of that after nalorphine ($p<0.001$). In both experiments, changes in AV recorded from E_1 paralleled those from E_2, but they were less pronounced.

Discussion. The results indicate that the electric knife fish, *E. virescens,* responded in a quantitative and characteristic manner to methylphenidate, phenobarbital, morphine, and nalorphine. Methylphenidate produced a 45% depression of AV recorded from E_2 at a concentration of 0.005 μg/ml of bath and an 87% depression with 1.25 μg/ml. The dose-log response over this concentration range was linear. The fact that the regression line does not go through 100% activity when projected to zero dose suggests that methylphenidate might have more than one mechanism of action, or that the drug acts with different degrees of rapidity on more than one body function or organ system. Concentrations below 0.005 μg/ml of bath were not investigated. The bioassay system employed here is admittedly crude when one considers precise site or mechanism of action. The changes in electrical activity need not be due to a direct action on the nervous system. A similar lack of specificity exists in the majority of bioassay techniques, especially those utilizing intact higher organisms. While there can be some question as to the precise nature of the potentials being measured and the mechanisms of action of the drugs on the fish, it is apparent that changes take place which permit quantitation of drug activity. This system also differentiates between various drugs. Thus, pentylenetetrazole, another central nervous system stimulant, produced no consistent changes in AV recorded from E_1 or E_2. Since in this instance recording was continued immediately upon addition of drug to the bath, a drug action of short duration would not explain the lack of apparent effect. Phenobarbital produced a depression of AV from E_1, but no consistent changes in AV from E_2. Morphine also produced depression primarily of AV from E_2 and the dose response was observed. However, morphine produced similar but less pronounced effects in AV from E_1. Thus, qualitative differences exist between the responses of the fish to the different types of central nervous system depressants. The significance of the facts that methylphenidate induced changes primarily in the system recording discharge of the generator, that phenobarbital affected the electrode system primarily influenced by movement of the fish, and that morphine affected both, is uncertain.

The morphine experiments clearly demonstrated the diminished response of the fish to high concentrations after repeated additions of drug to the bath. This may represent tolerance to morphine. The presence of morphine in the bath in concentrations consonant with the quantity of morphine added to the bath was verified chemically. Nalorphine apparently reversed the depressant effects of the preceding morphine dose, but did not prevent the fish from responding to morphine administered in high concentrations subsequently. The data on morphine are incomplete. The fish died when morphine was withdrawn. This may represent physical dependence. Since the fish are difficult to ob-

tain, we elected to perform a minimum number of experiments with morphine in order to utilize the available specimens for other purposes. However, the data presented here do indicate that this preparation may be useful for measurement of addiction liability, since nalorphine was able to antagonize the action of morphine.

Our studies with *E. virescens* were initiated because we needed a rapid bioassay for drugs which modify behavior and conditions did not permit us to undertake the more conventional bioassay methods. Our information is insufficient for correlation of emotional response of the fish with the observed results. Despite the shortcomings of this system which have been cited, two cardinal points are in its favor: (1) it can be used for precise and rapid bioassay of a variety of drugs, and (2) the fish respond to concentrations approximately the same as those which have effect in man. These properties have been shown to apply to neurotrophic polypeptides, lysergic acid diethylamide, methylphenidate, phenobarbital, morphine and nalorphine.

Summary. Studies with the electric transparent knife fish, *E. virescens,* have been extended to include observations on methylphenidate, pentylenetetrazole, phenobarbital, morphine, and nalorphine. Characteristic qualitative and quantitative responses were obtained with methylphenidate, phenobarbital, and morphine. Pentylenetetrazole produced no consistent effects. Nalorphine reversed the depressant effects of a preceding dose of morphine. The data suggested that the fish developed tolerance to morphine as well as physical dependence on this drug. The system studied appeared to be suitable as a bioassay technique. The observed drug effects were obtained with concentrations approximately the same as those which have effects in man.

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Effect of Environmental Changes upon Antiviral Action of Interferon.* (28757)

GEORGE E. GIFFORD†
National Institute for Medical Research, London, England

Changes in the environment of cells have been reported to modify the antiviral action of interferon. Reduction of oxygen tension (1) and low pH(2) have been reported to increase the antiviral action of interferon. We have investigated the effect of changes in oxygen tension, pH, and temperature upon the action of interferon on vaccinia virus infection of chick cells. The effects of these changes upon the plating efficiency of vaccinia virus have also been studied.

Materials and methods. Interferon production. Chick interferon was prepared in chick fibroblast monolayers infected with large doses of Chikungunya virus as described by Ruiz-Gomez and Isaacs(3). Interferon-containing preparations were dialyzed against 0.1

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† Present address: Dept. of Microbiology, Univ. of Florida College of Med., Gainesville.