Reserpine and conditioned suppression in the fish *Tilapia h. macrocephala*

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African mouthbreeding fish were trained to strike a bar on a VR2 schedule of reinforcement. Over a 10-day period, operant rate of responding was measured on 5 days, randomly interspersed with 5 days on which light (CS) was followed by shock (US) on 10 trials per day in the same environment. The CS was later introduced during operant responding; this resulted in suppression of operant responding. Subsequent injection of reserpine attenuated suppression of operant responding, but saline injection did not. These results probably were a direct function of reserpine administration on rate of recovery of responding following CS onset rather than an indirect function of baseline response rate or "sedation effects."

The literature on conditioned suppression of free operant responding has been recently reviewed by Davis (1968) and Lyon (1968). References are made to several vertebrate species, including two studies on goldfish (Geller, 1963, 1964). The effect of reserpine on this behavior has been studied in several vertebrates, but not in fish. The results of the reserpine experiments are conflicting. Brady (1956), Weiskrantz & Wilson (1956), and Ray (1964) have reported attenuation of conditioned suppression by reserpine in rats and monkeys, whereas Valenstein (1959), Yamahiro, Bell, & Hill (1961), and Kinnard, Aceto, & Buckley (1962) failed to demonstrate this effect in rats and guinea pigs.

Although reserpine has not been shown to affect conditioned suppression in fish, other behavioral effects have been produced in various species of fish by reserpine administration. Keller & Umbreit (1956) have shown that reserpine can eliminate chemically induced behavior effects in several species of fish; Waleszek & Abood (1956) were able to reduce aggression in Siamese fighting fish; and Haralson & Clement (1968) have produced the partial reinforcement effect in the African mouthbreeding fish by administration of reserpine during extinction. In the latter two studies, reserpine produced little or no depression of baseline behavior; this is in marked

contrast to the depression or "behavioral toxicity" observed in most species (Cole, 1960). The work of Blackman (1967) indicates that the supposed reduction of "anxiety" by reserpine in Brady's (1956) experiment may actually have been a secondary result of the reduced baseline rate by reserpine. However, reserpine showed little evidence of depressing the baseline behavioral measure in either the study of Waleszek and Abood or of Haralson and Clement, yet it did affect other kinds of behavior of fish. In view of Blackman's results, this is fortunate, since the aim of the present experiment is to determine if conditioned suppression can be attenuated by reserpine, and lowering of baseline responding by the drug could be explained in Blackman's terms as readily as in terms of attenuation of suppression by the drug. Since VI schedules such as that used by Geller also tend to lower baseline rate of responding, a VR schedule of reinforcement, which tends to elevate the baseline, would be indicated. Geller has suggested another factor that probably also depressed rate of responding in his experiments with goldfish. Referring to his use of the traditional procedure in studies of conditioned suppression, in which the CS-US contingency is superimposed on the free operant response during training until asymptotic performance has been reached, Geller pointed out that "Pre-light and post-light response rates also were reduced considerably, probably owing to a conditioning of some anxiety to the entire experimental environment." Rescorla (1968) has described a procedure that he used to study conditioned suppression in rats that minimizes generalization from training to the test situation. This entails separation in time and space of classical conditioning on CS-US contingencies from training on operant barpressing. Testing for suppression of the operant response by CS is then accomplished by introducing CS into the barpressing situation. In this case at least the first introduction of CS entails no generalization from earlier CS-US pairings to the operant response.

In his experiments, Geller used two electrodes placed vertically to produce US (shock). Kellogg (1958) has studied the effects on the behavior of fish of this arrangement, and has commented as follows: "Animals which were parallel to the electrode plates when originally stimulated therefore required more current to produce movement than would have

been necessary if they had been perpendicular to the electrodes." Geller's Ss were probably forced to assume a position more or less parallel with the electrodes by placing the electrodes closer together than the length of the average fish. Nevertheless, any variation in S length probably tended to increase variability of the measures because of more variability of orientation in small Ss than in large Ss, and probably tended to restrict the freedom of S's locomotor movements both during the period when US was not in effect, but particularly when US was in effect. In the present experiment, this difficulty has been minimized.

The present study was undertaken to determine the effect of reserpine on conditioned suppression of free operant responding in fish. If this can be demonstrated, then the apparent implication of Blackman's results, that baseline behavior rather than drugs may affect conditioned suppression, can be called into question as a general statement pertaining to all conditioned suppression experiments in which drugs are used.

SUBJECTS

Six 2-year-old female African mouthbreeding fish (*Tilapia h. macrocephala*) served as Ss.

APPARATUS

They were housed in separate tanks, 30 x 15.2 x 25.4 cm high, for the duration of the study. Two black electrodes, made of acrylic plastic covered on one side by copper-covered screen and completely coated with a resin-graphite mixture, formed the floor and roof of S's tank.³ With the screened sides facing inward in the tank, the top electrode was suspended 12.7 mm below the waterline. This horizontal placement of the electrodes was used in place of Geller's vertical placement in order to prevent the changes in voltage gradient along S's long axis described by Kellogg (1958) as a function of lateral turns during swimming. The largest gradient in the vertical arrangement occurs when S's long axis is 90 deg to the electrode surface, and the lowest gradient occurs when S's axis is parallel with the electrodes. By using a horizontal placement, extreme differences in overt behavior and, presumably, the felt intensity of the shock that depends on S's orientation during shock were averted. The upper electrode had a 2.5-cm square hole cut into its body midway along its length for the delivery of reinforcement by means of an automatic reinforcing apparatus described by Haralson & Ralph (1966). An identical hole, 13 cm distant, was cut on the opposite side of the same electrode, and a manipulandum similar to that described by Haralson, Gillman, & Ralph

Fig. 1. Cumulative response curves for six Ss: A—the last trial of CRF training; B—the last trial of VR2 training; C—the last trial of VR2 operant training (interspersed with suppression training); D—the suppression test trials; and E—test of the effect of reserpine on suppression. Dashed lines define the length of the CS period.

(1965) was inserted through it at the beginning of experimental sessions. Responses were recorded on a Model A Moduprint printout counter.

PROCEDURE

Stage A

Fish were trained to an operant bar-striking response for 10 consecutive daily trials on a CRF schedule for 60 reinforcements per trial. A 40-W incandescent bulb (later CS), placed 12.7 mm from the outside of the tank near the bar, was turned on for 1-min intervals during this period of operant training; no reaction to it was observed.

Stage B

Six additional training sessions were run on a VR2 schedule for 30 reinforcements per session.

Stage C

On 10 subsequent daily training sessions, five operant VR2 sessions were randomly interspersed with five sessions in which S received 10 trials of light (CS) and a 15-V ac shock. CS was on for 30 sec, during the last 5 sec of which US was applied. The interval between trials varied from 2 min to 1 h over a 5-h period. The voltage level used during the experiment was determined for each S by varying shock intensity in the ascending and descending order until S's dorsal fin twitched noticeably when the shock circuit was closed without producing convulsive movements or swimming on the side, which is characteristic when high shock levels are used. This voltage was then used as the US for S. Voltages varied from 12 to 17 for the six Ss. The bar was not present during these shock sessions. Only operant

responding or shock sessions occurred on any given day.

Stage D

The first suppression test occurred on Day 11. The bar was lowered into the apparatus, and operant responding continued for a period of 2 min. CS was then presented for 30 sec. S remained in the apparatus, and was allowed to continue responding until the usual complement of 30 reinforcements had been delivered. US was not applied in this stage. On Day 13, Ss received 10 additional CS-US pairings flanked by free operant sessions on Days 12 and 14.

Stage E

On Day 15, all Ss were run as on Day 11, except that 4 mg/kg of body weight of reserpine⁴ was injected i.m. in four Ss and an equal volume of normal saline was injected in two Ss 1 h before the beginning of the test session. This injection procedure and dosage was similar to that described by Haralson & Clement (1968).

RESULTS

In Fig. 1, the results of five stages of experimentation are shown in the form of cumulative curves in which responses are plotted at 15-sec intervals through most of the record, but at 10-sec intervals from 20 sec before CS onset to 10 sec after the interval in which the first post-CS response was made. The VR2 schedule of reinforcement produced a higher rate of responding than did the CRF schedule (A and B), and this was unaffected by interspersing VR2 with suppression training (C). Suppression was produced by CS in all Ss (D), and was reduced in a subsequent suppression test (E) in reserpinized Ss, but was increased in the

saline controls. Using the *t* test, this difference in mean suppression was significant at $p < .001$. No change in rate of responding occurred from Stage D to Stage E in reserpinized Ss except during the period of suppression.

Observations of the behavior were made during the period in which the cumulative measures were being made. Prior to Stage A, when the light came on Ss showed no overt reaction to it. Similarly, no reaction was noticed on the first two trials of Stage C when US was contingent on CS. Onset of US produced jerky swimming movements and swimming on the side during these early trials.⁵ On Trials 3-5, however, all Ss began to orient toward the light immediately after its onset and to swim toward the bottom of the tank, where they remained until it was terminated. This behavior occurred more quickly as suppression training progressed. The behavior that occurred following CS onset on suppression tests in Stages D and E differed radically from that following CS onset in Stage C or during operant and suppression Retraining Trials 12-14, which occurred between Test Stages D and E. During these test trials, all skeletal locomotor activity and operant responding ceased immediately following presentation of CS. The eyeballs turned downward, the hard fins came erect, the body color darkened, and, starting at 1-2 sec after CS onset, S sank to the bottom of the tank, slowly at first but accelerating with time until the pelvic fins sharply struck the bottom (this behavior was probably a function of contraction of the swim bladder as in so-called light shock). No difference was apparent between control and reserpinized Ss with respect to this behavior. These observations are in agreement with the quantitative results shown in Fig. 1, D and E, i.e., abrupt cessation of responding following presentation of CS, and immediate resumption of pre-CS rate of responding after the next response had been made.

DISCUSSION

These results indicate that reserpine attenuates suppression of operant bar-striking in fish and that this effect is not a function of baseline rate of responding. The characteristic reduction by reserpine of baseline response rate found in

rats, monkeys, and guinea pigs was not observed in this fish. This agrees with the results of Waleszek & Abood (1956), in which reserpine reduced aggressive behavior in fighting fish, and also with the results of Haralson & Clement (1968), in which reserpine produced the PRE in the African mouthbreeder, in both instances without noticeable sedation.

Quantitative results and visual observation of the behavior of Ss indicate that the CS produced suppression at its onset in both control and experimental Ss, as it did in Rescorla's (1968) rats. The effect of reserpine on conditioned suppression in this study, therefore, is on the rate of recovery from the suppressive effect of CS.

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NOTES

1. Now at the University of Washington.

2. Now at State University of New York at Stonybrook.

3. This horizontal placement was suggested to us by Robert A. Stewart.

4. Serpasil used in this experiment was donated by Ciba Pharmaceutical Co.

5. S probably felt less pain as a function of the lowered voltage gradient produced by this attitude. However, the horizontal position of the electrodes probably produced fewer extraneous effects on the data than the vertical placement used by Geller. Both effects can be eliminated by using two pairs of vertical electrodes mounted at right angles to each other and alternately activated by a commutator at ½-sec intervals. Substitution of a grid scrambler or a bi-stable vibrator should prove superior to the commutator.

Peripheral shock, implanted electrodes and artifactual interactions: A renewed warning¹

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Peripheral shock delivered to animals with intracranial electrodes may induce flows of current through the electrodes that effectively stimulate the brain. This cross talk relates to the adequacy with which shock and electrode circuits are "isolated" from ground. Capacitive coupling to ground, which is less easy to measure, may be as important as resistive connections. Such artifactual brain stimulation may have motivational properties that attenuate aversive properties of the shock. It may also give rise to afterdischarges that are not evident behaviorally. This problem of cross talk cannot be ignored in research with peripheral shock of animals with intracranial electrodes.

This note is prompted by a recent paper (Brunner, Rossi, Stutz, & Roth, 1970) that deals with the retrograde effects of brain stimulation on shock-motivated behavior. The study is not atypical procedurally of other work that has appeared in the literature, and our comments are not directed exclusively to it. These studies contain a potential artifact, which, in the absence of additional information, makes it virtually impossible to evaluate their findings. The artifact in question can have major behavioral consequences. We would like to reemphasize a warning that we offered several years ago (Schwartzbaum & Donovick, 1965) and present some additional observations on the

consequences of the artifactual interaction to which we referred.

In experiments involving peripheral shock of animals with implanted electrodes, cross talk may occur between the shock and electrode circuit, whether used for stimulation or recording, unless certain precautions are observed. This interaction results essentially from the use of shock circuits that are resistively or capacitively referred to ground and from the availability of ground connections through the electrode circuit. Although the problem can be stated simply, it is not always recognized in practice since isolation from ground is a relative matter, and one typically does not assess capacitive coupling to ground. If a shock source is inadequately "isolated" from ground and if there exists resistive or capacitive coupling to ground in the electrode circuit, then application of shock to the animal's body may induce a flow of current through the electrode circuit. Elements in the electrode circuit may basically offer a ground connection that parallels that of the shock circuit. The flow of current will depend upon shock parameters and the ground impedance of the two circuits.

Capacitive coupling to ground represents a subtle problem. It may arise, e.g., through capacitance between a cable and grounded shield, as often used for electrode leads. The reactance offered by such a coupling relates to its capacitance and the frequency parameters of the imposed voltage. With 60-cycle ac shock or even pulsed dc shock, assuming inadequate isolation from ground, electrophysiologically significant flow of current may be generated through