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ACQUIRED STIMULUS CONTROL OF DRUG-INDUCED CHANGES IN AGGRESSIVE DISPLAY IN BETTA SPLENDENS[1]

WILLIAM G. BRAUD AND JORGE ERIK WEIBEL

UNIVERSITY OF HOUSTON

Male Siamese fighting fish exhibit stereotyped aggression reactions to their mirror reflections. When distinctive neutral stimuli (flickering colored lights) were repeatedly associated with drug-potentiated aggression (morphine sulfate) and drug-depressed aggression (phenergan), the stimuli came to exert specific stimulus control over aggressive display even after the drugs were discontinued.

Male Siamese fighting fish (*Betta splendens*) exhibit stereotyped aggression reactions when confronted with another male *Betta*, a model of a male *Betta*, or their own mirror reflections. The behavioral components of the aggression reaction include: approach, intense deepening of body and fin coloration, spreading of the median fins, extension of gill covers and branchiostegal membranes, characteristic orientation movements (undulation), tail-beating, biting, and jaw-locking.

Laboratory investigations have demonstrated habituation (Baenninger, 1966), punishment (Adler and Hogan, 1963), classical conditioning (Thompson and Sturm, 1965*a*), and instrumental avoidance conditioning (Otis and Cerf, 1963) of the aggression reaction. Thompson and Sturm (1965*b*) and Hogan (1967) have shown that model- and mirror-elicited aggression could reinforce operant response sequences in *Bettas*. Aggressive display has been shown to be facilitated by the presence of morphine sulfate, sodium salicylate (Walaszek and Abood, 1956), or norepinephrine bitartrate (Marrone, Pray, and Bridges, 1966) in the *Betta*'s water. On the other hand, certain tranquilizing and antihistaminic drugs (Walaszek and Abood, 1956), and epinephrine bitartrate (Marrone *et al.*, 1966) reduce the frequency and/or vigor of aggressive displays.

The present investigators sought to determine whether distinctive neutral stimuli (flickering colored lights) repeatedly associated with drug-potentiated and drug-depressed aggression would acquire specific stimulus control over the aggressive response in non-drugged *Bettas*.

METHOD

Subjects and Maintenance Conditions

Eighteen adult male *Bettas*, each approximately 5.0-cm long, were purchased from a local aquarium supplier. The fish were housed in individual, white, translucent plastic containers (10 by 10 by 8 cm) with perforated tops and bottoms. These containers floated at the surface of an aerated and filtered 10-gal aquarium which was maintained at 24°C, with pH = 7.4. Only six fish were maintained in this environment at a given time. The subjects were fed daily with TetraMin staple food, and were kept on a 9-hr light/15-hr dark cycle.

Apparatus

The subjects were trained and tested in a modified Thompson and Sturm (1965*a*) conditioning apparatus, shown in Fig. 1. This apparatus consisted of two white, wooden enclosures, one containing two 75-w light bulbs and the other containing two 25-w bulbs and six colored (red or green) 7-w bulbs. The enclosures were separated from each other by 12 cm, which allowed six clear plastic containers to fit between them. The containers held the subjects during the experimental sessions. Each container measured 10 by 10 by 8 cm, and had the bottoms and one side covered with opaque white paper. When stacked in rows of three, one above the other, the fish were visually isolated from one another. One of the enclosure's walls adjacent to the six small containers was constructed so that colored light would be transmitted first through a transpar-

[1]Reprints may be obtained from W. G. Braud, Dept. of Psychology, University of Houston, Cullen Blvd., Houston, Texas 77004.

ent plastic sheet (red or green), then through light-diffusing translucent onionskin paper, and into the containers themselves. White light from the other enclosure passed first through onionskin paper, then through a one-way mirror, and into the containers. When the lights behind the colored plastic sheet were illuminated, with those behind the mirror extinguished, the mirror was highly reflective. Reversing the illumination prevented reflection. The two 25-w bulbs and the six 7-w bulbs, which were made to blink on and off irregularly behind the interchangeable red or green plastic sheets, also illuminated the containers whenever the mirror was made reflective. Red or green illumination and mirror presentations were scheduled by automatic timing and switching devices. Two experimenters observed the subjects' aggressive displays during testing, and recorded latencies and durations with individual stop clocks (see below).

Procedure

The subjects were maintained in their translucent containers for one week before the three experimental phases began. The first phase was a pretest procedure, the purpose of which was to determine whether the two colored lights (red or green) exerted any unconditional effects on aggressive display which might bias performance in later phases.

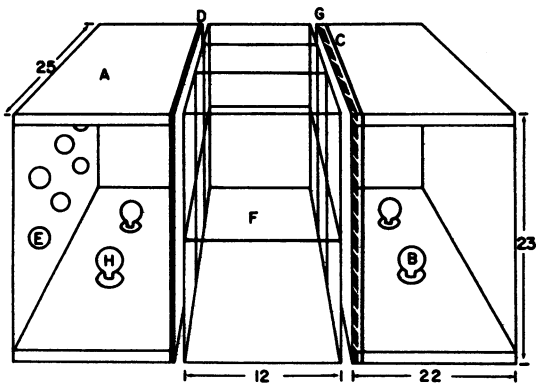

Fig. 1. Diagram of the conditioning apparatus. See text for further details.

One the day before the first training day, all subjects were pretested in undrugged water. The subjects were exposed to 20 randomized (Gellerman, 1933) 120-sec mirror presentations, 10 in the presence of red and 10 in green illumination. Intertrial intervals varied from 60 to 180 sec, with a mean of 120 sec. Two measures of aggressive behavior were recorded: (*a*) latency of the first aggressive reaction, measured from mirror- and colored light-onset, and (b) total time per trial spent aggressing. Gill extension was the indicant aggressive response.

All subjects were tested individually. Two experimenters observed the subject's behavior through the top of the clear plastic container, independently recording latency of the first gill extension and total gill extension per trial with separate stop clocks. Neither experimenter knew a given subject's group membership during test trials; *i.e.*, recording was done in a blind fashion. On none of the 720 latency judgments (20 pretest and 20 posttest trials for each of 18 subjects) did the scores assigned by the two experimenters differ by more than 2 sec. On only two of the 720 duration judgments did the two experimenters differ by more than 2 sec (differences of 4 and 6 sec). In cases of discrepancies, a score based on the mean of the two judgments was assigned.

For each subject, light colors were associated with drug conditions so that any acquired effects would work against the subject's initial response tendencies; *i.e.*, the light in the presence of which the subject responded more rapidly or more frequently during pretesting was associated with the aggression-depressing drug, and *vice versa*, during the training phase.

For the 12 experimental subjects, training consisted of the association of either red or green illumination with pretreatment of a particular drug. The pairing of colors was consistent for each subject, but for half of the subjects red was paired with morphine, and for the other half it was paired with phenergan. During each type of session, the particular illumination (red or green) was paired with the reflecting mirror for 120 sec. Intertrial intervals varied from 60 to 180 sec, with a mean of 120 sec. Fifty trials were given on each of the eight experimental sessions. Morphine and

phenergan sessions alternated, with two days of rest between each session. These rest days assured dissipation of drug effects between treatments.

The aggressive response was potentiated in the following manner. Before a training session, the subjects were placed in individual containers of 500 ml of a morphine sulfate-water solution (40 μg/ml). The subjects remained in this potentiating solution for 20, 24, 28, and 32 min, in that order, on the four successive morphine sessions. The daily increments in exposure time counteracted drug tolerance. The subjects next were placed in individual containers of fresh, undrugged water in the training apparatus, where they remained undisturbed for 15 min before their daily sequence of trials. After training, the subjects were returned to their home containers and fed.

The aggressive response was depressed by immersing the subject in a phenergan-water solution (20 μg/ml). Drug exposure times were 6, 8, 10, and 12 min, in that order, on the four phenergan sessions. The other aspects of the procedure were parallel for both drugs. The subjects were always trained in groups of six animals.

In summary, the 12 experimental subjects experienced differential training in which specific external stimuli (red and green colored lights) were repeatedly and consistently associated, respectively, with morphine-potentiated and phenergan-depressed aggressive behavior. Six additional control subjects experienced the same sequences of events, except that no drugs of any kind were ever administered.

Four days following the last training day, all subjects were tested in undrugged water. This testing sequence was identical in all respects to the original pretesting sequence; the same two response measures were recorded.

A summary of the entire procedure for the experimental subjects is presented in Table 1.

RESULTS

Six of the experimental subjects, although exhibiting aggressive reactions in the pretest phase, showed no evidence of aggression during the posttest. These animals not only failed to display toward their mirror image (all latencies = 120 sec, durations = 0 sec), but failed also to aggress toward live "target" males placed either in an adjacent container or in the subject's own container. Since these animals failed to aggress under any conditions, they were dropped from the experiment and their data were excluded from analyses.

The latencies and durations of the aggressive reactions of the remaining 12 subjects are presented in Fig. 2 and 3, respectively. Each entry represents the median response score of 10 trials. During neither pretesting nor posttesting did the subjects evidence any consistent differential responsiveness to stimulus color *per se*, in terms of either latency or duration of display. Nor did the subjects respond differentially, during pretesting, to those stimuli later to be associated with the different drug states. After training, however, all six experimental subjects evidenced enhanced aggressive behavior in the presence of the stimulus previously associated with the aggression-potentiating drug, and decreased aggressive behavior in the presence of the stimulus previously associated with the aggression-depressing drug. This effect occurred whether the stimulus light was red or green. These stimulus-controlled changes in incidence of aggressive reactivity were significant for both latency and duration measures ($p = 0.016$, binomial test).

The behavior of the six control subjects did not vary with changes in the light stimulus, nor did it differ from its pretest values.

DISCUSSION

Before their association with the drug effects, the red and green illuminations did not dif-

Table 1

Summary of the Experimental Procedure

Pretest	*Training*	*Posttest*
Color 1 + mirror and Color 2 + mirror (no drug)	Color 1 + mirror + morphine Color 2 + mirror + phenergan or Color 1 + mirror + phenergan Color 2 + mirror + morphine (drugs on alternate training days)	Color 1 + mirror and Color 2 + mirror (no drug)

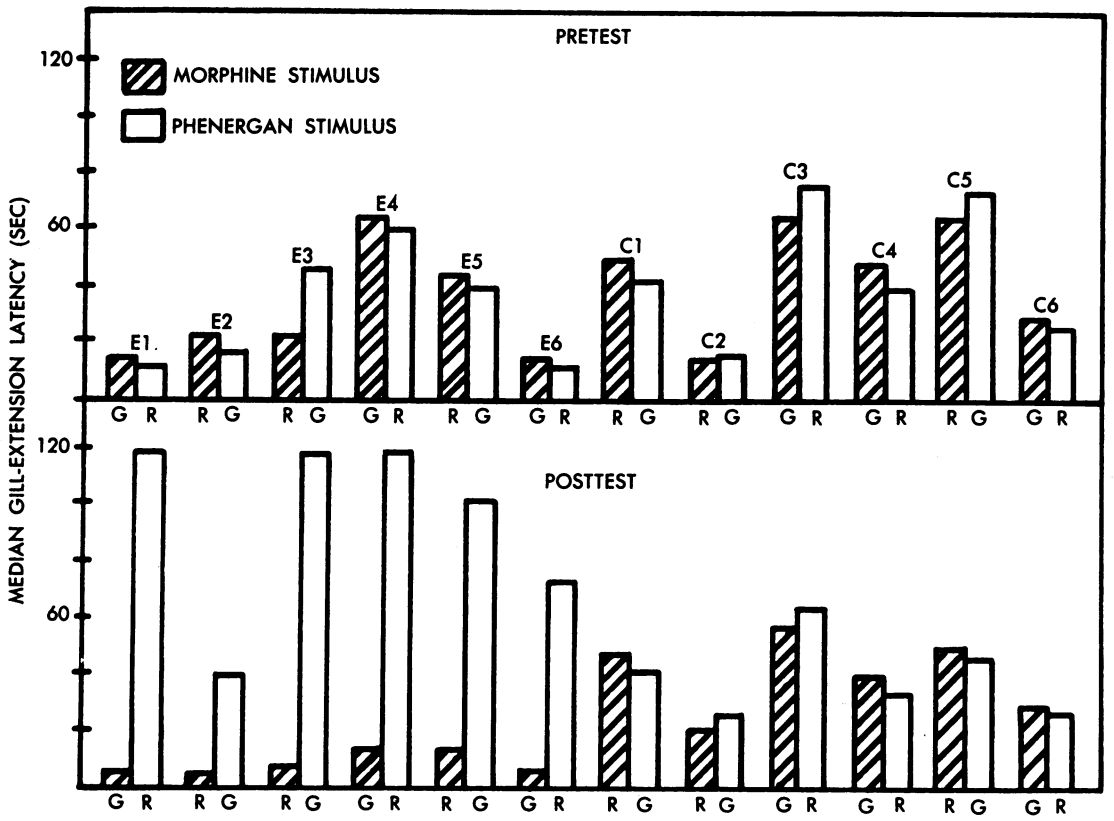

Fig. 2. Median latency in seconds of gill extension for 120-sec test trials. Letters R and G indicate red and green stimuli.

ferentially affect display. After training, however, the experimental subjects aggressed quicker and longer in the presence of the stimulus associated with morphine, and slower and shorter to the stimulus associated with phenergan. The control subjects, which experienced the same light presentations as did the experimental subjects, but without drugs, exhibited no changed behavior. Note that the results provide a consistent and impressive within-subjects demonstration of both an increasing and a decreasing effect of stimuli paired with different drugs, with counterbalancing of visual stimuli.

The present results lend additional support to the findings of other investigators that the aggressive reactions of *Betta splendens* can be controlled by a classical conditioning-like process. Of greater interest, however, is the finding that supporting stimulus for such a conditioning-like process may be a pharmacological agent. Such a finding is consistent with early reports of salivary conditioning to drug stimuli (Crisler, 1930), and with more recent evidence (Levitt, 1964). A most interesting implication of the present findings is that organismic states appropriate to specific drugs (states conducive to increased or decreased aggression) may occur in the absence of those drugs. Notice that the present paradigm differs from a typical classical conditioning one in that, in the present case, the drug is effective throughout the training session; this differs from discrete unconditioned stimulus presentations in the classical paradigm.

A possible, although speculative, explanation of the failure of six of the animals to exhibit aggression in any form following drug treatment is that the aggression-depressing drug, phenergan, may have exerted some relatively permanent effect in these particular animals. This argument is given some substance by the experimenters' qualitative observations that the six nonresponsive animals exhibited considerable blanching of body and fins which persisted throughout testing. The

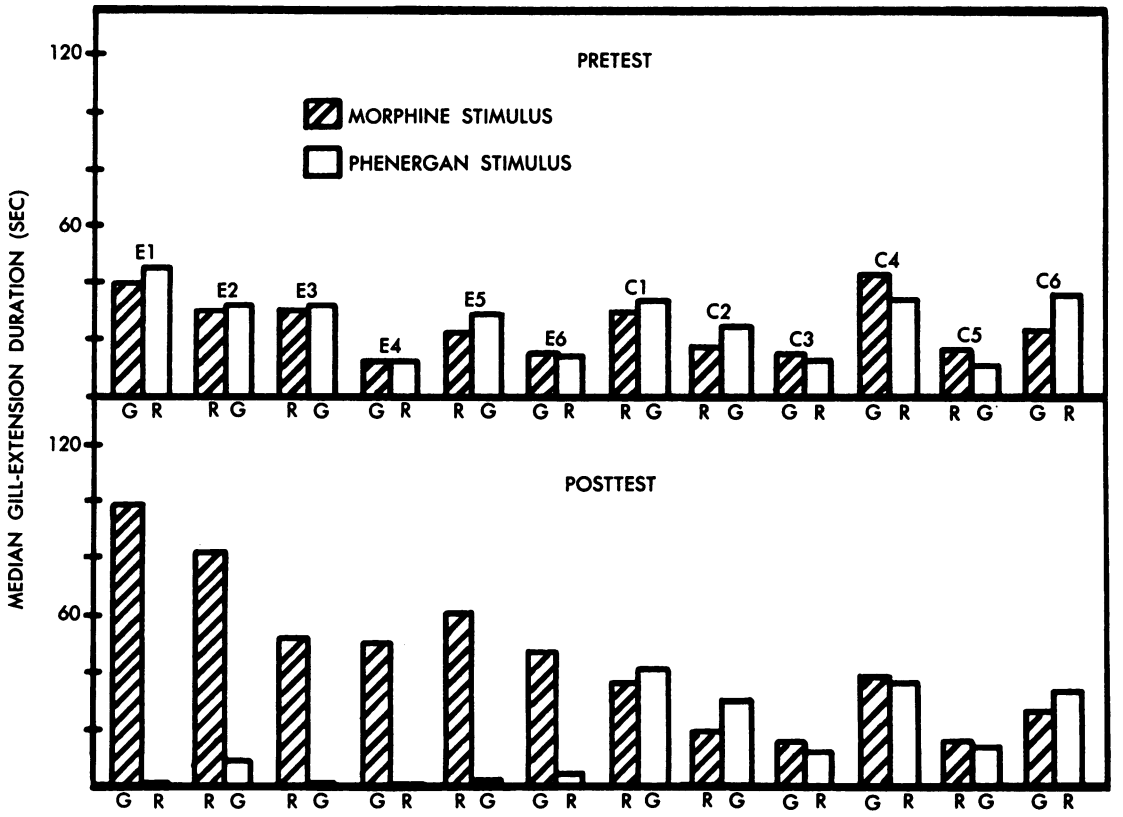

Fig. 3. Median duration in seconds of gill extension for 120-sec test trials. Letters R and G indicate red and green stimuli.

experimenters also noticed that these same animals responded very sluggishly during training. It is interesting that Baenninger (1968) reported some dramatic individual differences in reactivity to epinephrine and norepinephrine in the same species. The possible differential susceptibility of these fish to drug effects suggests another useful paradigm for the investigation of individual differences.

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