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BRIEF COMMUNICATION

Effects of Ethanol and Sodium Phenobarbital on Conflict Behavior of Goldfish *(Carassius Auratus)*[1]

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GELLER, I., D. J. CROY AND R. S. RYBACK. *Effects of ethanol and sodium phenobarbital on conflict behavior of goldfish* (Carassius auratus). PHARMAC. BIOCHEM. BEHAV. 2(4) 545–548, 1974. – Hungry goldfish learned to press a lever for worms obtainable on a 2 min variable-interval schedule of reinforcement. Conflict was induced by rewarding with a worm and punishing with an electric shock any lever responses made in the presence of a flashing light. The resulting suppression of responding was attenuated in fish exposed to sodium phenobarbital. Ethanol solutions were generally without effect.

Rats Ethanol Conflict Sodium phenobarbital

THE POTENTIAL value of conditioned suppression techniques (CER or conflict) for the study of pharmacologic agents has been reported [1,7].

Although conditioned suppression may be established in lower forms of animals such as goldfish [8], most drug behavior studies of this type have been limited to the use of laboratory rats and sub-human primates. In the present investigation a conditioned suppression based on a conflict situation was established in goldfish in order to elucidate the potential anti-anxiety activity of sodium phenobarbital and ethanol. This approach seemed a reasonable one for several reasons. Other researchers have demonstrated that one may obtain drug effects on a conditioned suppression in laboratory fish [22]. They reported an attenuation of a conditioned emotional response in African Mouthbreeder fish injected with reserpine.

In addition, goldfish can easily receive water-soluble drugs [15] such as alcohol, or odorants [20] via the water. Goldfish actually come into equilibrium with the water-alcohol solution in which they swim within 6 hours [15,16]. This is in contradistinction to the rat or mouse where the liver removes alcohol from the body much more rapidly than in man. Consequently, the effect of the rise and fall of blood alcohol due to its absorption and metabolism is avoided and the goldfish can be studied in a steady-state condition. The use of the goldfish in this investigation allowed us to monitor drug effects on behavior until blood concentration of the drugs came into equilibrium with the water drug solution.

METHOD

Six experimentally naive goldfish, 15 to 18 cm long, were obtained from a local dealer. They were maintained individually in 2-1/2 gallon tanks that were kept under constant aeration. Once each week the filters were cleaned and the water was replaced with tap water drawn 24 hr previously. The water was maintained at 72° – 76° F.

A Plexiglas feeder [13] was used to dispense tubifex worms. An experimental chamber contained the lever and

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Plexiglas electrodes. Lever responses were transmitted to counters by a high-gain amplifying integrating system [12]. A 24 V 2 amp a.c. transformer served as the shock source. A detailed account of this apparatus has been reported previously [8]. Programming and recording was done with switching and timing circuits, electrical impulse counters, and a cumulative recorder.

The fish were deprived of food for approximately 2 weeks and were trained to press a lever to obtain the worms. Before introduction of the lever into the experimental situation, the feeder was activated periodically in order to accustom the fish to the feeding location as well as to the sound of the feeder. In cases where fish did not learn to press, a shaping procedure was used [17]. After one session of lever pressing in which every lever response produced a worm, the schedule was changed so that reinforcements were obtainable after variable intervals which averaged 2 minutes (2 min VI).

Experimental sessions of 1 hr duration were conducted at the same time on Monday through Friday of each week. When lever-pressing rates on the 2 min VI schedule of reinforcement became relatively stable, a flashing light of 3 min duration was activated twice during an hour session. The light signalled a change from the 2 min VI to a more desirable continuous reinforcement schedule (CRF). After a number of such stimulus presentations, a punishment contingency was added so that during the flashing light, responses were rewarded with a worm and simultaneously punished with an electric shock. Starting with an initial value of 4 volts, shocks were increased by 0.5 V after every 2 experimental sessions to a maximum value of 8.5 V. This produced moderate suppression in 3 fish and almost complete suppression in the remaining 3.

Drug tests were conducted on Wednesdays or Thursdays of alternate weeks. On these days experimental sessions were increased to 4 hr duration in order that the time course of drug action might be monitored. One hour prior to and throughout an experimental drug session fish were maintained in an 8 liter drug solution which was calculated on a weight/vol. basis and expressed as mg %. Calculated values for sodium phenobarbital represent the total salt. Four hr control sessions were conducted on Wednesdays and Thursdays of the alternate non-drug weeks.

Three of the fish were tested first in alcohol concentrations of 150, 300 or 500 mg %. The remaining 3 fish were tested in phenobarbital concentrations of 62.5, 75 and 100 mg %. Following this phase of the experiment the fish were switched so that the alcohol fish were tested in phenobarbital concentrations of 37.5, 62.5 and 100 mg % and the phenobarbital fish were tested in alcohol concentrations of 150, 300 or 500 mg %. Fish were placed in the drug solution 1 hr prior to the start of an experimental session. A total of 8 conflict trials occurred during each 5 hr session and the total number of shocks taken by a fish under each drug condition was compared with the total number of shocks taken during the 4 hr non-drug control session.

RESULTS

In Fig. 1 are cumulative response records obtained for a fish following a 3 and 5 hr exposure to the drug solutions. The records were obtained during the second and fourth hours of an experimental session. Sodium phenobarbital at 100 mg % produced an extensive attenuation of conflict after three hours of exposure to the drug solution. Shocked responses during the flashing lights increased from 4 during control to 51 under drug. After 5 hr of exposure to the phenobarbital, variable interval responses were reduced considerably. However, during Hour 3, no significant attenuation of conflict was obtained with 500 mg % of ethanol, the dose which also reduced overall responding to a minimum after 5 hr of exposure to the drug solution.

In Table 1 are shown the total number of conflict shocks for 5 fish under each drug condition. The sixth fish died prior to initiation of the drug phase of the experiment. The control values are based on at least six 4 hr experimental sessions.

Sodium phenobarbital attenuated conflict in all fish. Maximum attenuation was obtained for 20 and 23, those fish least suppressed during control sessions. A smaller, but significant effect was obtained for 17 and 19, those fish most suppressed during control periods. Conflict attenuation was obtained with ethanol in one instance, the 150 mg % dose for Fish 20. For all other dose levels in all fish ethanol was without effect on conflict. Similar negative findings have been obtained in preliminary experiments with fish exposed to ethanol concentrations as low as 50 mg %.

DISCUSSION

A number of investigators have attempted to demonstrate ethanol's action on aversively controlled behavior of laboratory animals. Our essentially negative findings with ethanol agree with the findings of several of these studies [3, 6, 11, 21] but are not in accord with others [2, 4, 5, 14, 18]. However, it is almost impossible to cite any parallels or differences to account for similarities or dissimilarities in findings because of the many diverse experimental procedures that have been employed.

It is of interest to note that the degree of sodium phenobarbital effects on experimentally induced conflict of goldfish varied in part as a function of the baseline controls. The most extensive effect was obtained for 20 and 23, the fish least suppressed under control conditions while a smaller, but significant attenuation of conflict was obtained for 17 and 19, the fish with the greatest suppression of responses during control periods. Conflict attenuation in goldfish under phenobarbital is in agreement with previous research in which the same experimental methods were used with rats [9,10].

The conflict technique has been shown to be extremely sensitive to the effects of drugs which have clinical utility as anti-anxiety agents [7]. Sodium phenobarbital, a drug used previously as an anti-anxiety agent [19] and shown to attenuate conflict in rats, effectively attenuated conflict in goldfish. If one allows that conflict attenuation is a measure of anxiolytic activity, then our data do not support an anti-anxiety action for alcohol.

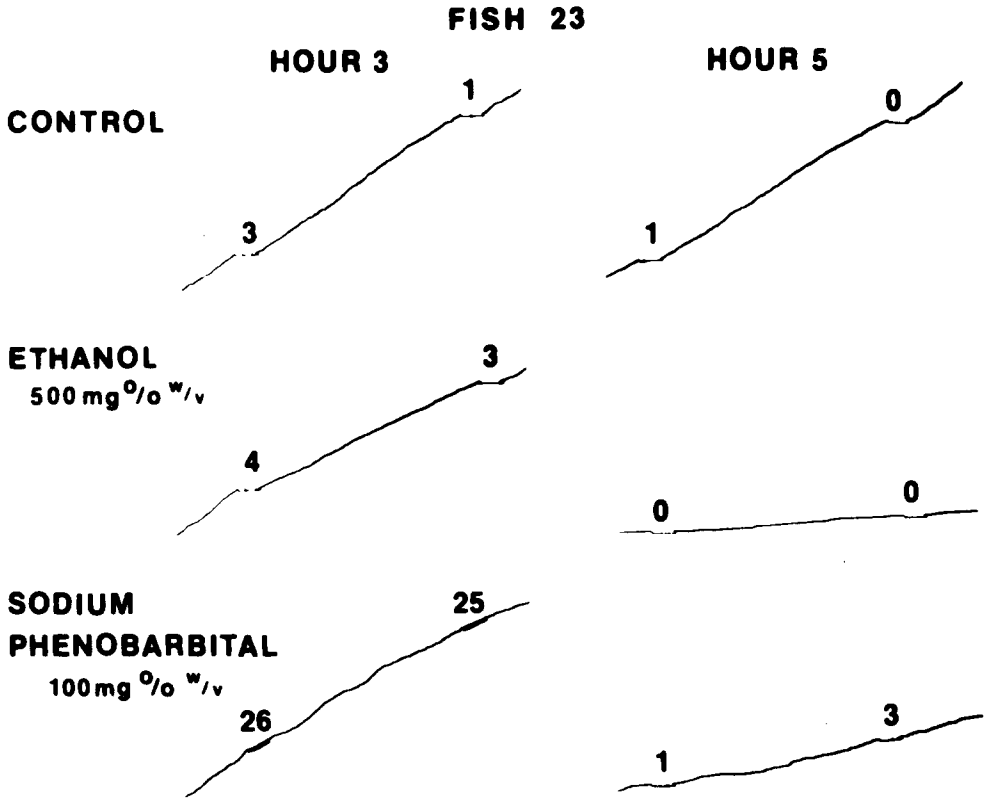

FIG. 1. Effects of ethanol and sodium phenobarbital on conflict in the goldfish. Pen offsets indicate tone periods. Upward pips of the pen and numbers represent responses that were rewarded with a worm and punished with shock simultaneously.

TABLE 1

EFFECTS OF SODIUM PHENOBARBITAL AND ETHANOL ON CONFLICT BEHAVIOR OF GOLDFISH

Fish	Control	Number of Shocks Taken: Ethanol mg/100 ml			Na Phenobarbital mg/100 ml			
	$\bar{X}$ ± S.E.	150	300	500	37.5	62.5	75	100
20	21.0 ± 7.10	38	9	12	140	113		78
22	8.0 ± 0.78	10	5	8	18	26		24
23	25.0 ± 4.40	20	18	27	57	84		103
17	5.0 ± 0.74	2	7	2		13	10	12
19	4.5 ± 0.23	6	6	4		4	10	12

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