Bait-shyness: A test for toxicity with $N = 2$ ¹

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Bait-shyness in rats induced by conditional pairing of a gustatory stimulus and injections of a test drug is a simple test for toxicosis requiring only a small N . Tests with injections of a nitrogen mustard derivative indicate that the slope of the acquisition curve is related to magnitude of dose and that the maximal response can be obtained most reliably after a large dose in a single trial.

If an animal eats poisoned food and survives, it will subsequently display "bait-shyness," that is, it will reject the specific food which contained the poison. Apparently, these avoidance responses are established by the sequence of olfactory and gustatory discrimination of the poisoned food followed by toxicosis. Bait-shyness, or similar responses, have been reported for a wide variety of mammalian species. Much evidence has been gathered by research workers concerned with practical problems of elimination of rodent infestations (Chitty, 1954). Such responses have been observed in the field and laboratory following the use of a variety of poisons which differ widely in their physiological action (Barnett, 1963). Anecdotes describing the reactions of chimpanzees and monkeys to repeated administration of drugs via food indicate that primates display similar rejection responses. Humans also often report strong aversions for foods associated with nausea and gastric upset. Motion sickness also produces similar gustatory aversions indicating that an exogenous material is not necessary. The "sickness" state is the reinforcer.

An examination of the individual differences reveals a remarkable uniformity of response; hence, it appears that "bait-shyness" might provide an excellent test for toxic illness requiring only small groups of animals. Allowing the animal to ingest the toxin or test material in its food has some obvious disadvantages. Eating is initiated and terminated by the animal, palatability is a problem, and dose is apt to be variable. These problems can be circumvented if the animal is presented with a fluid of known palatability and subsequently injected with the toxin or test material. A previous study has indicated that bait-shyness for saccharin-flavored water could be induced if drinking was followed by injections of apomorphine. The degree of response was cumulative with repeated trials of constant dose (Garcia, Ervin, & Koelling, 1966).

In the present study the injected dose and the number of trials were varied so that the effect upon the magnitude, the acquisition and the extinction of the response could be observed. In addition, Cyclophosphamide (Cytoxan^R, Mead Johnson Laboratories, Evansville, Indiana) a nitrogen mustard derivative used

for treatment of tumors, was used rather than the emetic apomorphine hydrochloride. The chief symptom of the former is a subsequent diarrhea, but it does not produce the violent abdominal spasms in the rat that the latter does. Although the rat does not normally vomit, such spasms could conceivably result in immediate restimulation of the oral receptors by the stomach contents, thus providing temporal contiguity for learning the bait-shy response.

Method

The method is extremely simple, requiring no special equipment, since tests and treatments can be carried out in the animal's home cage. For this study eight young male rats (Sprague-Dawley, 300 gm) were maintained in individual cages with dry Purina Laboratory Chow. Drinking was limited to a 10-min. period at the same time each day, and total fluid intake during this period was the only response measure. After a week of habituation to this schedule, experimental treatment began. On every alternate day (1, 3, 5, etc.) the drinking water was made discriminable by the addition of 1 gm of saccharin per liter of water. On the other days (2, 4, 6, etc.) the water was not so flavored.

Two rats, H_1 and H_2 , received a relatively high dose (66 mg/kg) of Cyclophosphamide on the first saccharin day intraperitoneally injected 5 min. after the bottle containing the discriminable fluid was removed from their cages. These rats received no further injections.

Rats M_1 and M_2 received a medium dose (33 mg/kg) on the first saccharin day via the same route and

Fig. 1. Gustatory discrimination learning in Rat M_1 after two trials in which cyclophosphamide was injected after drinking saccharin flavored water. Decrements in saccharin intake were attended by increased water intake on alternate days. Saline injections had no systematic effect upon intake of either fluid in Rat C_1 .

Fig. 2. The effect of single and fractionated doses of cyclophosphamide upon saccharin-water intake when injections were paired with saccharin in a gustatory discrimination learning paradigm. The magnitude of the decrement and the slope of the acquisition phase bear a closer relationship to dose parameters than does the slope of extinction phase.

time course. This treatment was repeated on the second saccharin day.

Rats L_1 and L_2 were treated in similar fashion with relatively low doses (17 mg/kg) of the drug. This treatment was repeated for a total of five injections. Rats C_1 and C_2 were injected with saline on the same schedule as rats L_1 and L_2 .

Results and Discussion

Animals injected with Cyclophosphamide 5 min. after drinking saccharin-flavored water exhibited a decrement in intake of the flavored fluid when the discriminable fluid was again presented 48 hr. later. Perhaps in compensation, these animals tended to increase their consumption of unflavored water so that a marked differential intake of the two fluids was obvious in any animal receiving the drug injection. Figure 1 illustrates this characteristic pattern, displayed by rat M_1 which received two injections of Cyclophosphamide. In contrast the control animals injected with saline exhibited no systematic differential trend as illustrated by rat C_1 in the same figure.

The scores of all eight animals on the saccharin days are illustrated in Fig. 2. The water intake scores on the alternate days were eliminated for the sake of clarity; since water intake increases as saccharin intake decreases, this information tends to be redundant. Several features of this data are of interest. First, the absolute amount of fluid intake on the second saccharin period for any animal is related to the dose injected after the first saccharin drinking. Second, when the total dose is fractionated into several injections, the effect is attenuated. Note that a single high dose produced the maximal decrement in rats H_1 and H_2 , while approximately the same total dose divided into two injections produced an intermediate effect in rats M_1 and M_2 . The minimal effect was produced in rats L_1 and L_2 which received five injections totaling approximately 125 percent of that received by the other animals. With the light repeated dose, the decrement in saccharin intake appears asymptotic after the first three injections as the two subsequent injections do not increase the decrement. Third, either absolute intake score on the first extinction day or the slope of the acquisition curves appear reliably related to dose parameters; however, the extinction curves do not yield the same orderly array.

All the animals survived the experimental treatment. Diarrhea was evident, particularly in the animals receiving the higher doses. One of two rats given a dose of 130 mg/kg died.

Previous studies demonstrated that gustatory aversions can be induced by various agents which differ in their specific mode of action. Similar responses were produced by ingestion of a toxic salt (lithium chloride), exposure to X-rays (Garcia & Koelling, 1967) as well as by injection of the emetic drug apomorphine (Garcia, Ervin, & Koelling, 1966). These agents probably all produce a general malaise or illness.

On the other hand, gustatory aversions are difficult if not impossible to establish with the peripheral pain induced by injection procedures or by electric shock to the feet (Garcia & Koelling, 1966; Garcia, Ervin, & Koelling, 1966). Hence, the rapid acquisition of a gustatory aversion appears to be specifically diagnostic of internal malaise and especially sensitive to toxoid substances circulating within the animal. For therapeutic agents this method may be used to estimate the maximum dose which can be administered without producing the "side effects" of malaise or illness.

References

- Barnett, S. A. *The rat: A study in behavior*. Chicago: Aldine Press, 1963.
- Chitty, D. (Ed.) *Control of rats and mice*. London: Oxford University Press, 1954.
- Garcia, J., Ervin, F. R., & Koelling, R. A. Learning with prolonged delay of reinforcement. *Psychon. Sci.*, 1966, 5, 121-122.
- Garcia, J., & Koelling, R. A. A comparison of the aversions induced by X-rays, toxins and drugs. *Radiat. Res.*, in press, 1967.
- Garcia, J., & Koelling, R. A. The relation of cue to consequence in avoidance learning. *Psychon. Sci.*, 1966, 4, 123-124.

Notes

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