Attenuated and enhanced neophobia in the taste-aversion "delay of reinforcement" effect

DENIS MITCHELL, D. WINFIELD SCOTT, and LINDA K. MITCHELL
University of Washington, Seattle, Washington 98195

Rats were made ill immediately or 6 h after drinking either familiar tap water or a novel .1% saccharin solution. Control animals were given sham treatments immediately after water or saccharin consumption. Subsequent two-bottle preference tests between water and saccharin revealed: (1) a "delay of reinforcement" effect across those groups presented with saccharin prior to treatment; (2) a neophobic avoidance of the novel saccharin solution by all groups presented with water prior to treatment; and (3) that the extinction curve of those animals poisoned 6 h after drinking saccharin was similar to the extinction curves of the groups presented with water prior to treatment. The results are discussed in terms of the two-process model of the poison avoidance behavior of the rat proposed by Mitchell, Kirschbaum, and Perry (1975). It was suggested that "conditioned" taste aversions obtained when laboratory rats are poisoned some hours after the consumption of a novel substance consist of two antagonistic nonassociative components, attenuation or habituation of neophobia and the disruption of habituation by the administration of a noxious stimulus during the habituation process.

Poisoned rats will avoid a novel substance even when long time intervals are interposed between consumption of the novel substance and subsequent illness (e.g., Garcia, Ervin, & Koelling, 1966). This seeming violation of a cardinal associative principle has led several theorists to postulate the evolution of a specialized associative mechanism capable of bridging long stimulus-consequence intervals (see Garcia, Hankins, & Rusiniak, 1974; Seligman & Hager, 1971, for reviews).

There are, however, several reasons to question the existence of a *specialized* associative mechanism in the taste-aversion behavior of the rat. First, there is ample evidence that laboratory rats are neophobic, they show a natural avoidance of novel stimuli in a variety of behavioral situations (cf. Mitchell, 1976). Second, the neophobia displayed by laboratory rats is susceptible to enhancement or sensitization by toxicosis (cf. Mitchell, Kirschbaum, & Perry, 1975). Third, the neophobia of laboratory rats can be attenuated by habituation and/or prior experience with novel stimuli (cf. Mitchell, Scott, & Williams, 1973). Fourth, there is no a priori reason to assume that the neophobia of laboratory rats is not routinely enhanced and/or attenuated in the conditioned taste aversion paradigm (cf. Mitchell, Parker, & Johnson, 1976). Finally, the general failure to include non-

contingently poisoned control groups in taste-aversion studies has led most researchers to erroneously assume that any avoidance which follows the use of conditioning procedures is entirely due to associative processes (cf. Mitchell, Kirschbaum, & Perry, 1975).

The present experiment was designed to assess the role of nonassociative factors such as the attenuation and enhancement of neophobia when rats are made ill either immediately or some hours after the consumption of a novel saccharin solution.

Given that a rat avoids a novel solution, how can one know whether the avoidance is due to associative or nonassociative mechanisms?

One of the principal characteristics of conditioned taste aversions is that they are quite persistent and resistant to extinction (Garcia, Hankins, & Rusiniak, 1974). However, neophobic avoidances are characterized as being transitory and easily extinguished, especially when a highly preferred stimulus such as saccharin is used as the novel stimulus (Carroll, Dinc, Levy, & Smith, 1975). Elkins (1973) has suggested that in cases where episodic measurement reveals maximum avoidance across several experimental groups, resistance to extinction is the most suitable measure of aversion strength. Accordingly, the animals in the present experiment were given repeated extinction trials in order to reveal similarities and differences in the strength of their aversion to the novel substance.

METHOD

Subjects

Sixty-six naive male Long-Evans rats, approximately 140 days old and obtained from the colony maintained by the Department

Supported in part by Grant 63-0643 from the University of Washington Alcoholism and Drug Abuse Institute awarded to the first author. We thank J. D. Laycock, W. F. Stephens, and Charlene Haug for their technical assistance, Ron Sigmundi and William Winter for their helpful comments on the manuscript, and Mead Johnson Laboratories for supplying the Cytosan. Requests for reprints should be sent to Denis Mitchell, National Institute of Environmental Health Sciences, P. O. Box 12233, Research Triangle Park, North Carolina 27709.

of Psychology of the University of Washington, were used as subjects.

Apparatus

The entire experiment was conducted in an isolated room used only for this experiment. Throughout the experiment, all rats were maintained in individual cages with food (Purina Rat Chow) continuously available. Room-temperature tap water was provided in glass bottles equipped with stainless steel spouts affixed to the front of the cages.

Habituation

For the first 15 days after housing, all rats were maintained with water freely available. The water bottles were placed on alternate sides of the front of the cages each day. On Day 16, and thereafter for the remainder of the experiment, water consumption was restricted by limiting access to a single 30-min period (between 1:30 and 2:00 p.m.) each day. Additionally, all litter trays were emptied and lined with clean brown paper prior to water presentation each day. On Day 20, at the completion of the usual 30-min drinking session, all animals were weighed.

Conditioning

On Day 21, the rats were divided randomly into six equal groups ($n = 11/\text{group}$) and given one of the following treatments: Group P-W-I (poison water immediately) was presented with the usual room-temperature tap water and poisoned immediately upon completion of the 30-min consumption period by administration of a 25-mg/kg IP injection of cyclophosphamide (Cytosan). Group P-W-D (poison water delay) was presented with tap water and poisoned, in the same manner as Group P-W-I, 6 h after the completion of the fluid session. Group P-SAC-I (poison saccharin immediately) was given a 0.1% (w/v) room-temperature sodium saccharin solution and poisoned in the same manner as the preceding groups. Group P-SAC-D (poison saccharin delay) was treated exactly as Group P-SAC-I, except that poisoning was delayed until 6 h after the completion of the fluid session. Group SAL-W-I (saline water immediately) and Group SAL-SAC-I (saline saccharin immediately) were presented with water and saccharin, respectively, and injected with equivalent volumes of saline (0.15 ml) immediately after the consumption period.

Testing

On the day following the conditioning trial (Day 22), and for 9 days thereafter, all subjects were given a two-bottle preference

Figure 1. Mean amount of fluid (water plus .1% saccharin) consumed by the various groups on the 10 preference tests. (Abbreviations: Group P-W-I = poison water immediately; Group P-SAC-I = poison saccharin immediately; Group P-W-D = poison water delay; Group P-SAC-D = poison saccharin delay; Group SAL-W-I = saline water immediately; Group SAL-SAC-I = saline saccharin immediately.)

Figure 2. Mean saccharin preferences of the various groups during each of the 10 preference tests. (See caption of Figure 1 for abbreviations.)

test between the water and saccharin solutions during the usual 30-min fluid sessions. The bottles were positioned off-center in such a way that the spouts were aligned 2 cm apart, 3 cm off the floor, in the center of the front of the cage. During conditioning, the fluid bottles were positioned in the center of the front of the cage. During testing, saccharin bottles were initially placed on the right for half of the rats; for the other half, they were initially placed on the left. Thereafter, the left-right positions were alternated daily. Consumption of water and saccharin solutions (± 1 ml) during each 30-min preference test was recorded for each subject. Testing was conducted under "blind" conditions; the experimenters recording fluid consumption had no knowledge of group assignments. Saccharin preference was calculated as the proportion of total fluid consumption consisting of saccharin (saccharin consumption divided by saccharin plus water consumption).

RESULTS

The mean fluid consumption for all groups on each of the 10 test days is shown in Figure 1. Individual analyses of variance on the total amount of fluid consumed each day indicated no significant differences among the groups on any of the 10 test days (all $F_s < 2.25$, all $p_s > .05$).

Figure 2 shows the mean saccharin preferences for all six experimental groups on each of the 10 test days. An analysis of variance applied to the saccharin preference scores of the six groups on the first test day revealed significant differences among the groups [$F(5,60) = 15.45$, $p < .001$].

Multiple comparisons (Newman-Keuls test) between each group presented with saccharin prior to treatment and its counterpart presented with water indicated that a significant difference existed between each pair (Group P-SAC-I vs. Group P-W-I, $p < .05$; Group P-SAC-D vs. Group P-W-D, $p < .01$;

Group SAL-SAC-I vs. Group SAL-W-I, $p < .01$). Additional comparisons on the first test day saccharin preference scores revealed that the groups receiving saccharin prior to treatment (Groups P-SAC-I, P-SAC-D, and SAL-SAC-I) were significantly different from one another ($p < .01$ in all cases), while the groups receiving water prior to treatment (Groups P-W-I, P-W-D, and SAL-W-I) showed no significant differences ($p > .05$ in all cases).

An analysis of variance applied to each of the remaining 9 test days' saccharin preference scores also revealed significant differences among the groups ($p < .001$ in all cases). The major concern of this experiment is the performance of those animals poisoned 6 h after consuming saccharin (Group P-SAC-D) relative to the other five groups. Accordingly, Newman-Keuls multiple range tests were performed comparing Group P-SAC-D with each of the other groups on each of the 10 test days. Group P-SAC-D was found to be significantly different from Group P-SAC-I on all test days ($p < .01$ in all cases), significantly different from Group P-W-D only on the first test day ($p < .01$), and significantly different from Group SAL-SAC-I only on the first 2 test days ($p < .01$ in both cases). However, Group P-SAC-D was not significantly different from either Group P-W-I or Group SAL-W-I on any of the 10 test days ($p > .05$, two-tailed).

The following conclusions can be drawn from these data: (1) Those groups presented with saccharin prior to treatment showed a "delay of reinforcement" effect on the first test day. (2) The animals poisoned 6 h after drinking water (Group P-W-D) showed a greater avoidance of the saccharin solution on the first test day than did those animals poisoned 6 h after drinking saccharin (Group P-SAC-D). (3) There was a transitory (neophobic) avoidance of the novel saccharin solution by all groups presented with water prior to treatment. (4) The extinction curve of those animals poisoned 6 h after drinking saccharin (Group P-SAC-D) was similar to the extinction curves of the three groups presented with water prior to treatment.

DISCUSSION

The present results do not prove that rats poisoned some hours after consuming a novel saccharin solution do not learn a saccharin-illness association. However, they do question the common practice of uncritically attributing any avoidance of a novel solution which follows the use of conditioning procedures entirely to associative processes. We have previously suggested (Mitchell, Hoch, & Fitzsimmons, 1975; Mitchell, Kirschbaum, & Perry, 1975; Mitchell, Parker, & Johnson, 1976) that the conditioning procedures used in the taste-aversion paradigm are

superimposed upon the habituation and dishabituation of neophobia which usually occurs simultaneously. Furthermore, the attenuation and enhancement of neophobia in the taste-aversion paradigm shows the same set of characteristics as the habituation, sensitization, and dishabituation of many other innate avoidance behaviors (cf. Hinde, 1960). For example, Green and Parker (1975) have reported that the attenuation of neophobia to a novel solution is susceptible to "interference" by the presentation of a second novel solution. Though the nomenclature used by Green and Parker (1975) is somewhat different from that used by students of habituation, the "interference" they report is remarkably similar to the "dishabituation" or "disinhibition" of a previously habituated response following the presentation of a novel or noxious stimulus (cf. Galambos, 1956; Hinde, 1970). This characteristic of habituation has been found to occur in a variety of situations (Thorpe, 1963), including studies of the poison-induced avoidance behavior of the rat (Mitchell, Kirschbaum, & Perry, 1975).

The most parsimonious explanation of the present results seems to be that the only group to form an undisputable conditioned avoidance of the novel saccharin solution was Group P-SAC-I, which was poisoned immediately after its initial exposure to saccharin. Group SAL-SAC-I, which was given a forced single-bottle exposure to saccharin without aversion consequences, showed the relatively rapid habituation (attenuation) of neophobia that occurs during single-stimulus training (Domjan & Gillan, 1976). Group P-SAC-D was given the same single-stimulus habituation trial as Group SAL-SAC-I, but the process of habituation was disrupted or dishabituated by the administration of a noxious stimulus 6 h later. Though the evolution of a specialized association mechanism capable of bridging long stimulus-consequence intervals may have considerable heuristic appeal, its existence is not required by this interpretation.

A general failure to appreciate the importance of nonassociative factors in delayed poisoning taste-aversion experiments is evidenced by the near universal omission of the appropriate noncontingently poisoned, or sham-injected, control groups within the various treatment conditions (cf. Garcia, Ervin, & Koelling, 1966; Revusky, 1968; Smith & Roll, 1967). This oversight may have led researchers and theorists alike to prematurely ascribe the "delay of reinforcement" effect solely to associative processes (see review by Garcia, Hankins, & Rusiniak, 1974).

As previously stated, several authors have suggested that neophobia can be enhanced or sensitized by poisoning (cf. Carroll, Dinc, Levy, & Smith, 1975; Mitchell, Hoch, & Fitzsimmons,

1975). For example, Domjan (1975) has reported that noncontingent lithium chloride injections result in a small, though sometimes not reliable, increased avoidance of novel casein and vinegar solutions. In the present experiment, one would expect that those rats poisoned prior to saccharin presentation (Groups P-W-I and P-W-D) would show a significantly greater avoidance of the saccharin than those rats which were not poisoned prior to their first exposure to saccharin (Group SAL-W-I). Failure to demonstrate a significant sensitization effect in the present experiment may be due to the formation of a weak conditioned avoidance of water (Group P-W-I), to a "floor effect" imposed by the use of a two-bottle test, or to fortuitous group differences in initial neophobia or saccharin preferences.

REFERENCES

- CARROLL, M. E., DINC, H. I., LEVY, C. J., & SMITH, J. C. Demonstrations of neophobia and enhanced neophobia in the albino rat. *Journal of Comparative and Physiological Psychology*, 1975, **89**, 457-467.
- DOMJAN, M. Poison-induced neophobia in rats: Role of stimulus generalization of conditioned taste aversions. *Animal Learning & Behavior*, 1975, **3**, 205-211.
- DOMJAN, M., & GILLAN, D. Role of novelty in the aversion for increasingly concentrated saccharin solutions. *Physiology and Behavior*, 1976, **16**, 537-542.
- ELKINS, R. L. Individual differences in bait shyness: Effects of drug dose and measurement technique. *Psychological Record*, 1973, **23**, 349-358.
- GALAMBOS, R. Suppression of auditory nerve activity by stimulation of efferent fibers to cochlea. *Journal of Neurophysiology*, 1956, **19**, 424-437.
- GARCIA, J., ERVIN, F. R., & KOELLING, R. A. Learning with prolonged delay of reinforcement. *Psychonomic Science*, 1966, **5**, 121-122.
- GARCIA, J., HANKINS, W. G., & RUSINIAK, W. Behavioral regulation of the milieu interne in man and rat. *Science*, 1974, **185**, 824-831.
- GREEN, K. F., & PARKER, L. A. Gustatory memory: Incubation and interference. *Behavioral Biology*, 1975, **13**, 359-367.
- HINDE, R. A. Factors governing the changes in strength of a partially inborn response, as shown by the mobbing behaviour of the chaffinch (*Fringilla coelebs*). III. The interaction of short-term and long-term incremental and decremental effects. *Proceedings of the Royal Society of London, Series B*, 1960, **153**, 398-420.
- HINDE, R. A. Behavioural habituation. In G. Horn & R. A. Hinde (Eds.), *Short-term changes in neural activity and behaviour*. New York: Cambridge University Press, 1970.
- MITCHELL, D. Experiments on neophobia in wild and laboratory rats: A reevaluation. *Journal of Comparative and Physiological Psychology*, 1976, **90**, 190-197.
- MITCHELL, D., HOCH, N. E., & FITZSIMMONS, M. Effects of neophobia sensitization on the rat's preference for earned food. *Behavioral Biology*, 1975, **13**, 519-525.
- MITCHELL, D., KIRSCHBAUM, E. H., & PERRY, R. L. Effects of neophobia and habituation on the poison-induced avoidance of interoceptive stimuli in the rat. *Journal of Experimental Psychology: Animal Behavior Processes*, 1975, **104**, 47-55.
- MITCHELL, D., PARKER, L. F., & JOHNSON, R. Absence of a generalization decrement in the poison-induced avoidance of interoceptive stimuli in the rat. *Physiological Psychology*, 1976, **4**, 121-123.
- MITCHELL, D., SCOTT, D. W., & WILLIAMS, K. D. Container neophobia and the rat's preference for earned food. *Behavioral Biology*, 1973, **9**, 613-624.
- REVUSKY, S. H. Aversion to sucrose produced by contingent x-irradiation. *Journal of Comparative and Physiological Psychology*, 1968, **65**, 17-22.
- SELIGMAN, M. E. P., & HAGER, J. L. (Eds.). *Biological boundaries of learning*. New York: Appleton-Century-Crofts, 1971.
- SMITH, J. C., & ROLL, D. L. Trace conditioning with x-rays as aversive stimulus. *Psychonomic Science*, 1967, **9**, 11-12.
- THORPE, W. H. *Learning and instinct in animals*. London: Methuen, 1963.

(Received for publication August 30, 1976;
revision accepted November 26, 1976.)