Interpolation of electroconvulsive shock during CS-US interval as an impediment to the conditioning of taste aversion¹

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Rats were allowed to drink sour water for 10 min. Thirty minutes later they were injected with a toxic drug. The taste was subsequently avoided unless ECS had been interpolated during the taste-illness interval. This finding was discussed in relation to a memory-addressing mechanism of unusual properties.

Garcia, Ervin, & Koelling (1966) paired a distinctive taste (CS) with drug-induced illness (US) to establish a taste aversion. Apparently taste-visceral associations can be conditioned after a single pairing despite CS-US intervals of more than 1 h. This finding was difficult to interpret within current learning theory where contiguity between events is considered prerequisite to their association. After ruling out obvious sources of peripheral contiguity (aftertaste, regurgitation), Garcia & Ervin (1968) concluded that a memory or neural stimulus trace of CS bridged the gap to US. This interpretation compliments Hebb's (1949) assertion that associations depend upon the continuance of electrical reverberatory activity (CS stimulus trace) in the nervous system until temporal overlap with the US is achieved. The purpose of this experiment was dual: To test the hypothesis suggesting central mediation of long taste-illness intervals, while simultaneously determining the usefulness of extending Hebb's physiological concept of the mediator to this problem.

ECS is believed to destroy ongoing electrical activity in the brain (Glickman, 1961). If taste-illness intervals are centrally mediated by continuous electrical activity in the brain, then ECS during the CS-US interval should disrupt learning.

SUBJECTS

Forty male rats (Sprague-Dawley, 350-450 g) were housed individually and kept on ad lib chow. Drinking was restricted to a 10 min/day schedule.

APPARATUS

ECS (40 mA for 0.5 sec) was delivered across the ears via alligator-clip electrodes padded with saline-soaked gauze. The CS was the sour taste of standard hydrochloric acid (37%) diluted 0.7 ml/liter tap water. The US was a dose of cyclophosphamide (Cytoxan^R, Mead Johnson Laboratories,

Evansville, Indiana) injected i.p. Cytoxan, a nitrogen mustard derivative, causes symptoms of gastric upset in the rat. Ordinary 100-ml graduated cylinders fitted with drinking tubes were used to measure water intake.

PROCEDURE

On the 8th day of habituation to the drinking schedule, all animals received sour water in their home cages. In addition, all animals were injected with either Cytoxan (66 mg/kg) or isotonic saline 30 min after drinking (CS-US interval). An ECS animal was removed from its home cage, placed on a nearby table where electrodes were attached and the shock delivered. A convulsed rat was returned to the home cage while still unconscious.

Ss were assigned randomly to five groups of eight animals each. One experimental group (early ECS-Cytoxan) was shocked 2 min after CS presentation and another (late ECS-Cytoxan) 25 min after. Both groups received a Cytoxan injection. If the association depends on the continuity of central processes during the CS-US interval, it should make no difference wherein the interval interference occurs. The early ECS-saline control group was also shocked 2 min after CS, but the injectant was saline. This procedure allowed the assessment of ECS as an aversive or amnesia-producing stimulus. The Cytoxan-only control group received only the CS-US pairing in order to show the normal conditioning of a taste aversion. Finally, saline only was a nonconditioning control that received the CS and a saline injection. This group provided a baseline for sour water consumption.

Each animal received one acquisition treatment, then five extinction tests, one every 3rd day. An extinction test involved presentation of CS without subsequent shock or injection. The amount of sour water consumed constituted the dependent measure of conditioning. On alternate days, the animals drank unflavored water as usual.

RESULTS AND DISCUSSION

Mean sour water intake for all groups over all days is plotted in Fig. 1. First extinction day drinking scores were analyzed separately since they most directly reflect differential treatment during acquisition. Analysis of variance indicated that group differences were significant ($F = 15.93$, $df = 4/34$,

$p < .001$), and relevant t test comparisons were calculated.

As is strongly indicated in Fig. 1, the combined ECS-Cytoxan groups were significantly different both from the no-shock Cytoxan-only group ($t = 2.54$, $df = 38$, $p < .02$) and the combined no-conditioning saline groups ($t = 5.97$, $df = 38$, $p < .01$). ECS retarded learning but could not prevent it.

No real difference obtained between early ECS-saline and saline-only groups ($p > .10$). This indicates that shock did not exert an independent effect on sour water consumption. It should be noted that both of these groups evidenced habituation to the novel sour taste by increasing their intake over acquisition day. It was unlikely that ECS resulted in retrograde amnesia for the CS, since habituation to a novel stimulus implies memory for previous encounters. Also nonsignificant were differences between the early and late ECS-Cytoxan groups ($p > .10$). Figure 1 does suggest an increasing divergence over trials that reached significance by the fourth extinction day ($F = 9.09$, $df = 4/33$, $p < .01$; $t = 2.06$, $df = 37$, $p < .05$). It may be that delayed shock allowed for the formation of a somewhat stronger association resulting in increased resistance to extinction. The effect was negligible on the first extinction day, however, justifying the conclusion that point of interpolated interference was not critical.

Garcia & Ervin (1968) argued that taste-visceral associations must be centrally mediated. The present results show that ECS during the taste-illness interval impeded conditioning. Since the affects of

Fig. 1. Mean sour water consumption on acquisition day (A) and extinction days for all groups. Animals injected with cyclophosphamide after drinking sour water (Cytoxan only) reduced their subsequent intakes. Animals convulsed during the taste-illness interval (early and late ECS-Cytoxan) evidenced significantly less avoidance.

ECS are believed to occur in the central nervous system, these results should be regarded as evidence supporting the central mediation hypothesis.

Hebb (1949) contended that associations depend upon the continuity of electrical activity in the brain during CS-US interval. The possibility was offered of extending this conception of the associative mechanism to account for mediation of long taste-illness intervals. The sizable aversion shown by ECS-Cytoxan rats argues against such an extension. It is unlikely that ongoing electrical activity could endure ECS.

In concluding, we offer our own conception of the mediator bridging taste-illness intervals: A mechanism could exist that somehow addresses a recent taste memory permitting it to be "called up" upon report of visceral reaction. Electric shock interferes with this mechanism, reducing its efficiency in retrieving the appropriate memory at the appropriate time. The mechanism must be relatively stable judged by its remarkable resistance to electrical interference. Garcia & Ervin (1968) have provided impetus for such speculation by drawing attention to the close anatomical convergence of first-order fibers carrying taste and visceral information upon second-order neurons in the solitary nucleus. It may be that the addressing mechanism takes the form of a functional connectivity or convergence among these second-order cells. By virtue of anatomical proximity, the functional connectivity might be able to maintain integrity, in spite of interference, for unusually long time periods. The rats' eating pattern serves to prevent confounding of the retrieval mechanism, while keeping interference at a low level.

These animals typically abstain from eating for long intervals after sampling a novel tasting meal (Rozin, 1969).

Our speculations are not intended as a theoretical statement, but are offered only because they have guided subsequent research with the interpolated shock paradigm in this laboratory: If isolation from interference preserves the integrity of the addressing mechanism, then by introducing graded amounts of artificial interference into the system (electric shock duration or intensity), the strength of the taste-illness association should be manipulated.

REFERENCES

- GARCIA, J., ERVIN, F. R., & KOELLING, R. A. Learning with prolonged delay of reinforcement. *Psychonomic Science*, 1966, 5, 121-122.
- GARCIA, J., & ERVIN, F. R. Gustatory-visceral and tele-receptor-cutaneous conditioning-adaptation in internal and external milieus. In: *Communications in behavioral biology*, Part A, 1. New York: Academic Press, 1968. Pp. 389-415.
- GLICKMAN, S. E. Perseverative neural processes and consolidation of the memory trace. *Psychological Bulletin*, 1961, 58, 218-233.
- HEBB, D. O. *The organization of behavior*. New York: Wiley, 1949.
- ROZIN, P. Adaptive food sampling patterns in vitamin deficient rats. *Journal of Comparative & Physiological Psychology*, 1969, 69, 126-132.

NOTES

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Theophylline: Induction of self-biting in rabbits¹

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A tentative animal model is presented for studying the self-mutilating behavior seen in children with an abnormality of purine metabolism. Each of four groups of

rabbits was given a quarter-normal diet and daily injections of saline or 46.0, 61.5, or 92.0 mg/kg of anhydrous theophylline (1,3-dimethylxanthine). The major findings were that (1) the greatest number of self-biters were found in the 61.5 mg/kg group, (2) mortality was directly related to drug dosage, and (3) the onset of biting occurred earliest with the highest dosage.

The Lesch-Nyhan syndrome is a familial

disorder of uric acid metabolism and nervous system function (Lesch & Nyhan, 1964). The most striking aspect of the disease is self-destructive biting of the lips and fingers. Clinical symptoms also include cerebral palsy with chorea, athetosis, hypertonia, and mental retardation. The severity of the mental retardation and other behavioral effects of the Lesch-Nyhan syndrome are difficult to assess, not only because of the patient's disability, but also because of the need to keep the patient in restraints. Furthermore, ethical consideration involved with studying human patients requires the exploration of animal models for the examination of relationships between purine metabolism and behavior.

While studying the effects of caffeine toxicity in starved rats, Peters (1966) observed incidental evidence that rats receiving a reduced diet and high doses of the methylpurine caffeine show self-mutilative behavior. In studying the biochemical structure activity relationships in rats receiving another methylpurine, theophylline, McDonald, Morgan, Sage, Sweetman, & Nyhan (in preparation) have also observed self-biting behavior. The purpose of the present experiment was to determine systematically whether or not the administration of theophylline would induce self-mutilation in the rabbit, an animal that, unlike the rat, does not use its teeth in aggressive behavior.

METHODS

Forty-two New Zealand male albino rabbits, weighing 1.6 to 2.9 kg, were housed individually in wire cages and maintained ad lib on water. They were offered a quarter normal daily diet of Purina Rabbit Chow. After receiving the diet for 2 days, four groups of 10 rabbits each were given daily intraperitoneal injections of 0.15N saline or 46.0, 61.5, or 92.0 mg/kg anhydrous theophylline (1,3-dimethylxanthine) in saline for 25 days. Two additional rabbits received 107 mg/kg of theophylline. Dosages were adjusted daily to compensate for weight changes. Theophylline was obtained from Mann Research Laboratories and Nutritional Biochemicals Corporation; it was added to the saline and heated until dissolved, and then cooled to body temperature before injection. Assessments of self-biting behavior were made by two independent Os.

RESULTS

Percentages of rabbits showing self-biting in the saline control and theophylline groups may be seen in Fig. 1. Self-biting was observed in each of the treated groups but not in the control group. The greatest number of self-biters,