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Effects of age and flavor preexposures on taste aversion performance

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Rats (*Rattus norvegicus*) 19, 30, 90, and 180 days of age received one pairing of 12% sucrose (w/v) and an intraperitoneal (ip) injection (2% body weight) of either .15 M lithium chloride (LiCl) or .9% saline. Testing with a two-bottle choice procedure showed that, relative to saline-injected controls, aversion effects in LiCl groups increased with age at conditioning. In Experiment 2, 19- and 90-day-old rats received zero, one, or three exposures to 12% sucrose prior to pairing sucrose with an injection (ip) of .15 M LiCl or .9% saline. Testing with a two-bottle choice yielded reliably greater aversion effects for adult rats than for pups. However, both age groups showed reliably increasing attenuation of taste aversion with increasing preexposures.

Developmental studies of taste aversion have generally reported reliably greater aversion effects in adult rats than in rat pups (e.g., Baker, Baker, & Kesner, 1977; Campbell & Alberts, 1979; Gregg, Kittrell, Domjan, & Amsel, 1978; Grote & Brown, 1971; Klein, Domato, Hallstead, Stephens, & Mikulka, 1975). Most studies have typically used comparisons between two age groups: adult rats, 65 to 80 days old, and young rats, 29 days of age and younger. To our knowledge (also see G. M. Martin & Timmins, 1980), there has been no report of aversion effects compared over several developmental stages; conclusions based on cross-study comparisons are complicated by differences in procedures and in experimental parameters. Accordingly, Experiment 1 investigated taste aversion in rats 19, 30, 90, or 180 days of age at conditioning. These age levels seem to correspond roughly to preweanling, juvenile, young adult, and older adult stages of development.

GENERAL METHOD

Subjects

Subjects were albino rats (*Rattus norvegicus*) from the same breeder group of 12 female and 4 male Sprague-Dawley descendants from the colony maintained by the Department of Psychology at Virginia Polytechnic Institute and State University. Each litter was culled to eight pups 3 to 5 days after birth (Day 0).

Procedures

The experiment occurred in a room 2.4 x 2.4 x 3.0 m. Illumination level was 80 fc; the light-dark cycle was 14 h/10 h, and light onset was at 0700 h. Room temperature was 78°F ± 2°F.

On Day 1 of the experiment, each rat was housed individually in a single hanging cage, with food and tap water available ad lib for the first 12 h. Water was then discontinued for 12 h to start the deprivation cycle. On Days 2 and 3, each rat received 30 min access to tap water at 0800 h and 1600 h. On Day 4, 30 min water access occurred only at 0800 h.

Day 5 was the aversion training day. Each rat received a 30-min access period to 12% sucrose (w/v) followed, within 15 min, by an injection (ip) of .15 M LiCl or .9% saline (2% body weight). Twenty-four hours after injection, all rats were tested for aversion effects with a two-bottle choice procedure. One bottle contained 12% sucrose; the other contained tap water. The measure of performance was the amount drunk (to the nearest .1 ml), calculated for each bottle as the difference in the bottle's weight from the beginning to the end of the 30-min test period. A sucrose preference index was obtained by comparing sucrose intake to total fluid intake, [sucrose/(sucrose + water)] × 100. Rats received no fluids outside training and testing periods. Food was available continuously except during flavor access. The sucrose solution was mixed with commercial grade sugar and tap water at least 18 h prior to access.

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EXPERIMENT 1

Method

Rats were randomly assigned to cells (n = 10) of a 4 by 2 design: four age groups at conditioning (19, 30, 90, or 180 days old) and two injection conditions (LiCl or saline). Each group contained five male and five female rats. Rats in the 19-day-old group were separated from the dam at 15 days of age and were immediately started in the experiment. Rats in 30-, 90-, and 180-day-old groups were separated from the dam at 21-23 days of age. They were gang caged until Day 1 of the experiment, when they were 26, 86, and 176 days old, respectively.

Each rat was weighed daily to assess the effects of experimental treatments on development. Mean weights (in grams) on Day 1 were 31.5, 80.3, 300.7, and 345.9 for 19- to 180-day-olds, respectively. Weight differences were not reliable across injection conditions (LiCl vs. saline) on any day of the experiment. On the last day of the experiment, mean body weights (in grams) were 73.2, 136.3, 295.0, and 320.0 for 19- to 180-day-olds, respectively.

Testing for taste aversion began 24 h after injection and continued for 5 consecutive days.

Results

Mean sucrose intakes (in milliliters) on the injection day were 4.0, 5.8, 10.4, and 8.8 for 19-, 30-, 90-, and 180-day-old groups, respectively [$F(3,76) = 3.46$,

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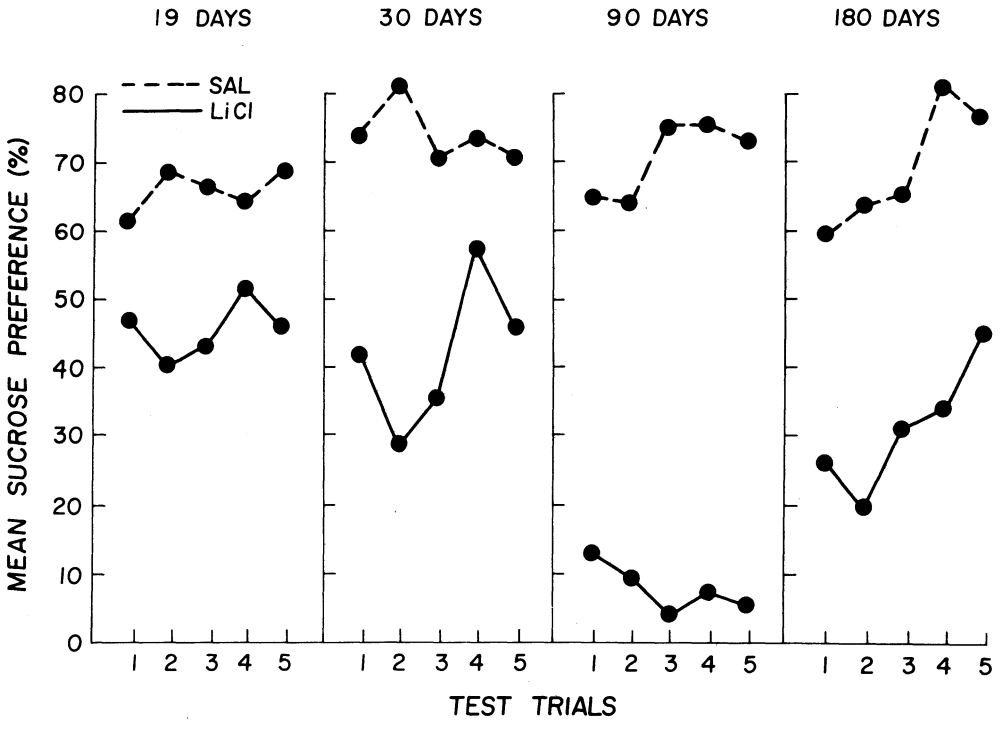

Figure 1. Mean sucrose preference on Test Trials 1-5 for rats injected with .15 M LiCl or .9% saline at 19, 30, 90, or 180 days of age.

$p < .025$]. On Test Trial 1, mean sucrose intakes for 19- to 180-day-olds were 3.1, 4.0, 8.3, and 7.3 ml for saline-injected groups and 1.4, .6, .6, and .8 ml for LiCl groups. Thus, sucrose intake increased with age for saline groups ($p < .03$) but was reliably higher for 19-day-olds than for any other age group in the LiCl condition [$F(3,36) = 3.42, p < .03$].

Figure 1 presents mean sucrose preference for each age group on Test Trials 1-5. Comparisons between LiCl and saline groups showed that the magnitude of the aversion effect increased with age up to 90 days and then decreased slightly at 180 days. Analysis of variance (ANOVA) yielded reliable effects for age, injection condition, and test trials (ps $< .03$) and, more important, for the interactions, Age by Injection Condition [$F(3,72) = 3.85$, $p < .01$] and Age by Injection Condition by Test Trials [$F(4,288) = 1.78$, $p < .05$]. A simple-effects ANOVA for LiCl groups revealed reliable effects for age [$F(3,36) = 5.17$, $p < .004$] and test trial [$F(4,144) = 3.27$, $p < .01$]. The ANOVA for saline groups showed no reliable effects ($F < 1$) for any factor (e.g., age, trials, etc.). Paired comparisons with Duncan's multiple-range test for LiCl groups showed that 19- and 30-day-olds differed reliably from 90- and 180-day-olds, but groups within pairs did not differ reliably from each other.

To evaluate whether the taste aversion data reflected lowered fluid intake due to the ingestion-illness contingency for LiCl groups, ANOVA was applied to the data of total fluid intake (sucrose + water) for all groups in testing. Results showed that total fluid intake increased reliably with age ($p < .001$) but was unrelated to injection condition ($F < 1$).

Discussion

Aversion effects were similar for young rats 19 and 30 days old and for adult rats 90 and 180 days old, but the magnitude of aversion was reliably smaller for the younger pair. These data are consistent with reports of age-related deficits in taste aversion learning (e.g., Baker et al., 1977; Klein et al., 1975).

The apparent dichotomy for age effects seems amenable to a neurological explanation. Anatomical, chemical, and physiological development of the central nervous system (CNS) undergoes rapid alteration in the 16- to 25-day-old rat and shows a decline in maturational rate at about 40 days of age (Campbell & Coulter, 1976). If structural or functional CNS immaturity affects the rat's adeptness in encoding stimuli or withholding inappropriate behaviors, then the age effects of this experiment seem to coincide with the developmental range noted for CNS alteration.

On the other hand, the present data may reflect differences in experiential or associative factors for younger animals. For example, Baker et al. (1977), Gregg et al. (1978), Grote and Brown (1972), and Klein et al. (1975) report that reliable aversion effects in rat pups may depend upon relatively particular procedures: brief flavor exposure in training, short temporal intervals between flavor and toxicosis, and use of a two-bottle test procedure. Campbell and Alberts (1979) reported that the strength of conditioning and retention of taste aversion in rat pups depended upon the distinctiveness of the flavor cue in aversion training. Failure to implement exactly the precise procedure for aversion learning in pups might yield inferior performance from them.

Finally, the relatively recent nursing experience for young rats may have attenuated their subsequent acquisition of taste aversion. Galef and Sherry (1973) and L. T. Martin and Alberts

(1979) report that rat pups respond to taste cues transmitted through the dam's milk. Possibly, the sucrose flavor used in training in this experiment and milk carbohydrates obtained from the dam's milk may be similar to each other in taste or nutritional characteristics. The nursing experience may have transmitted taste information to the pups, thereby serving as a relatively recent preexposure treatment for them, compared with being a more distant experience for adult rats. Thus, assuming that flavor preexposures attenuate aversion learning for rat pups as they do for adult rats, the inferior aversion performance of pups may not reflect a deficit in learning capacity but, rather, an impediment to learning following preexposures.

EXPERIMENT 2

While the relative recency of pups' nursing experience may plausibly account for their inferior aversion performance, this explanation presumes that the effects of flavor preexposures in pups are similar to those that have been well documented for adult rats (e.g., Elkins, 1973). To our knowledge, there is only one report (Klein, Mikulka, Domato, & Hallstead, 1976) of flavor preexposure effects on taste aversion in rats of different ages, and that is for only one level of preexposure, four. Klein et al. reported that four preexposures to sucrose reliably reduced sucrose taste aversion in adult rats 65 days old, but not in young rats 23 days old. On the other hand, in a study of 19-day-olds Franchina, Domato, Patsiokas, and Griesemer (1980) reported that zero, one, two, four, or eight sucrose preexposures progressively reduced aversion effects. Since there seems to be little information about the comparability of young and adult rats on preexposure effects and taste aversion, further speculation about the possible role of the nursing experience as a preexposure procedure seems gratuitous. Rather, a more reasonable strategy would be to obtain further information about the parametric effects of preexposure on taste aversion for different age groups. Experiment 2 studied the effects of zero, one, or three sucrose preexposures on sucrose taste aversion in rats 19 or 90 days old at conditioning.

Method

Subjects and Design. Sixty rat pups and 60 adult rats from the same sources as in Experiment 1 were randomly assigned to cells (n = 10) of a 3 by 2 by 2 design: three preexposure levels (zero, one, or three), two ages (19 or 90 days), and two injection conditions (LiCl or .9% saline).

Procedure. All treatments and test and measurement procedures were as those described for Experiment 1, except on Days 3 and 4. On those days, rats received preexposure procedures: on Day 3, at 0830 h and 2030 h and on Day 4, at 0830 h. Group 3PRE received 30 min access to 12% sucrose at each time; Group 1PRE received 30 min access to tap water on Day 3 and one preexposure to sucrose on Day 4 (at 0830 h). Group 0PRE received access to tap water in each period. On Day 5, aversion training procedures were those from Experiment 1.

Results

Mean sucrose intakes (in milliliters) on the injection day following zero, one, or three preexposures were 3.85, 3.60, and 3.62 for rat pups ($F < 1$) and 7.68, 9.92, and 10.45 for adult rats [$F(2,54) = 5.35$, $p < .01$]. Preexposure to sucrose reliably facilitated sucrose intake in adult rats, but not in pups.

Table 1 presents mean sucrose intakes (in milliliters) on Test Trial 1. Sucrose intake was reliably lower for LiCl groups than for saline groups ($p < .001$). Preexposure reliably increased sucrose intake for LiCl groups of both ages [$F(2,54) = 9.37$, $p < .001$], but for saline groups, preexposure affected intake reliably only for adults ($p < .01$). Sucrose intake was generally lower for adult rats than for pups in LiCl groups, but this difference was not reliable ($p = .05$-$.10$). The data of the injection day and Test Trial 1 suggest that the similarity/difference between young and adult rats in showing preexposure effects depended upon the incidence of aversion training. Pups and adults were similar

Table 1
Mean Sucrose Intake (in Milliliters) on Test Trial 1 For Each Age Group In LiCl and Saline Conditions Following Zero, One, or Three Preexposures

	Preexposure					
	19-Day-Old Group			90-Day-Old Group		
	0	1	3	0	1	3
LiCl	.90	1.15	3.10	.80	.55	2.40
Saline	3.00	3.15	3.75	6.35	7.35	8.30

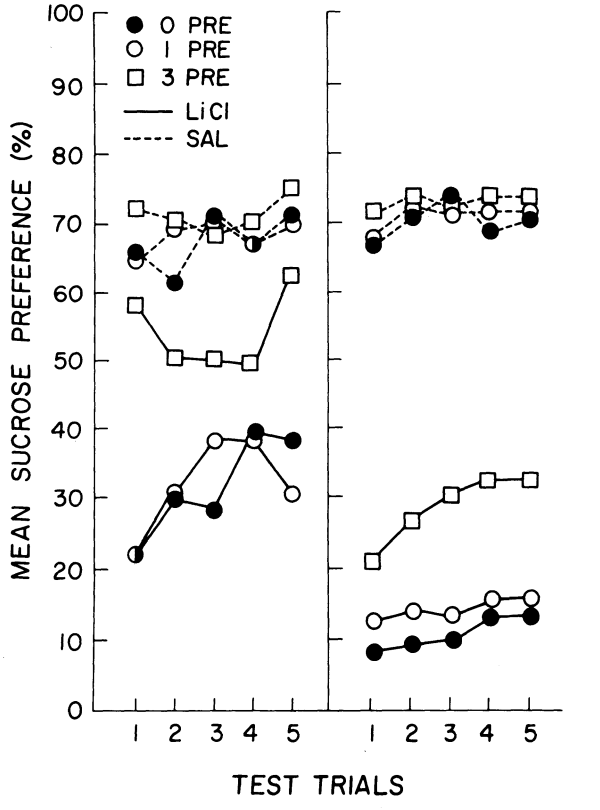

Figure 2. Mean sucrose preference on Test Trials 1-5 for 19- (left side) and 90- (right side) day-old rats that received zero, one, or three sucrose preexposures prior to pairing sucrose with LiCl or saline injections.

in preexposure effects following LiCl injection. However, on Day 5 and following saline injection, preexposure promoted ingestion reliably in adult rats but negligibly in rat pups.

Figure 2 presents mean sucrose preference on Test Trials 1-5. Comparison of LiCl and saline groups provided reliable evidence of taste aversion effects [injection condition, $F(1,108) = 226.44$, $p < .001$]. Adult rats showed reliably greater aversion effects than did rat pups [Age by Injection Condition, $F(1,108) = 16.44$, $p < .001$]. The effect of age appeared reliably in LiCl groups ($p < .001$), but not in saline groups ($F < 1$).

Sucrose preexposure reliably reduced taste aversion effects for both age groups, the effect being nondifferential for young and adult rats [preexposure, $F(2,108) = 11.16$, $p < .001$, and Age by Preexposure, $F < 1$]. Three preexposures yielded reliably greater reduction of aversion than did one or no preexposure ($p = .001$). Sucrose preexposure reliably altered preference performance in LiCl groups but not in saline groups [Preexposure by Injection Condition, $F(2,108) = 3.45$, $p < .02$].

To evaluate whether LiCl-saline group differences reflected suppressed fluid intake following the ingestion-illness contingency for LiCl groups, ANOVA was applied to the data of total fluid intake (sucrose + water) in testing for all groups. There were no reliable differences involving injection condition for any component of the ANOVA.

Discussion

Sucrose preexposure reliably reduced the magnitude of sucrose taste aversion for 19- and 90-day-old rats. The failure of the ANOVA to yield a reliable Age by Preexposure interaction for test-trial performance prevents the conclusion that preexposure yielded differential aversion effects between age groups. Apparently, rat pups, like adult rats, retained taste information from prior exposure periods and, on the basis of stimulus generalization between preexposed and training flavors, showed reduced aversion effects. These results are consistent with expectations from the data of Capretta and Rawls (1974), Franchina et al. (1980), and Galef and Sherry (1973) and are discrepant from those of Klein et al. (1976). The discrepancy from the Klein et al. data may likely reflect procedural differences, which have been discussed in an earlier report (see Franchina et al., 1980).

However, sucrose preexposure yielded similar effects across age groups in testing only for the LiCl-injected condition. Preexposure effects differentiated between age groups on the injection day and following saline injection on Test Trial 1. Specifically, sucrose preexposure reliably facilitated sucrose intake on the injection day and on Test Trial 1 for adult rats but negligibly affected intakes for rat pups at comparable points.

Since sucrose access on the injection day constituted the initial presentation of that flavor for Group 0PRE, comparison of Groups 0PRE, 1PRE, and 3PRE provided an indication of flavor neophobia for 0PRE. Facilitation of intake for adult rats indicated that neophobia occurred and subsequently dissipated following preexposure. The lack of a reliable difference among Groups 0PRE, 1PRE, and 3PRE for rat pups suggested that neophobia did not occur or that preexposure procedures failed to attenuate neophobia. These data are consistent with Baker et al. (1977) and inconsistent with Gemberling, Domjan, and Amsel (1980).

Lack of evidence for flavor neophobia in rat pups may indicate a taste similarity between the dam's milk and sucrose. Thus, the recent nursing experience of pups may have constituted preexposure procedures that obliterated differences among Groups 0PRE, 1PRE, and 3PRE. On the other hand, because of the relative recency of birth, pups may be unfamiliar with a large number of objects and events, and unfamiliarity may not provide a sufficient basis for differentiating among stimuli. Thus, the unfamiliarity of taste cues may not deter ingestion for rat pups as it does for adult rats, and, consequently, neophobia may be precluded from appearing in pups.

The results of Experiment 2 suggest that young and adult rats may be differentiable on the basis of preexposure effects in neophobia and taste aversion learning. Pups and adult rats seem to be similar to each other in showing preexposure effects on aversion learning but may differ on preexposure effects in neophobia. These results suggest that conclusions about age-related differences in performance based on taste cues may depend upon the demand characteristics of the measurement task.

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