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Effects of Stimulus Preexposure on the Generalization of Conditioned Taste Aversions in Infant Rats

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ABSTRACT: *Generalization of a conditioned taste aversion in infant rats and how this is affected by stimulus preexposure was investigated in a series of experiments. In Experiment 1 generalization of a conditioned aversion between two tastes (sweet and salty) was found, and the effect of tastes preexposure was a reduction in generalization (Experiment 2). However, when these tastes were combined with a common taste (acid) that was less (Experiment 3) or more intense (Experiment 3b), the effect of stimulus preexposure was a stronger generalization of the conditioned aversion. In this case, a reduction on generalization was again observed by increasing the number of preexposure trials to the taste compounds (Experiment 4). In all cases the generalization levels were directly related to the effect of stimulus preexposure on the acquisition rate of conditioning. It can be concluded that, with the appropriate parameters, a reduction of generalization of a conditioned taste aversion can be obtained after taste exposure in preweanling rats.* © *1999 John Wiley & Sons, Inc.* Dev Psychobiol 35: 304–317, 1999

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It has been observed that infants show a great tendency for generalization among stimuli (Spear & Mackinzie, 1994). This has been found in children (Gibson, 1969; Mednick & Lehtinen, 1957) as well as with infant rats, with a broad variety of stimuli (Mellon, Kraemer, & Spear, 1991; Spear & Kucharski, 1984). Nevertheless, a decrease in stimulus generalization is evidenced as the infant organisms develops, a change that seems to respond to the combination of maturational and experiential processes. It is well documented that the ability to discriminate stimuli improves jointly with sensory receptor's maturation (Gibson, 1969). However, infant's poor discrimination capabilities may be also improved by providing them with the appropriate experience (Campbell & Haroutunian, 1983; Spear & Mackinzie, 1994). As Spear and Mackinzie (1994) point out in their review about intersensory integration in the infant rat:

> It should be clear, however, that the failure to discriminate two events does not necessarily imply the lack of a capacity to differentiate those events. Discrimination is presumed to be a consequence of the animal's disposition for organizing events, whereas

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> capacity for differentiation implies limitation on sensory receptors or cognition. (p. 152)

For example, Mellon et al. (1991) observed that preweanling rats show generalized responses to different compounds of visual and auditory stimuli, though discrimination learning was effective when these compounds were differentially reinforced.

Therefore, the absence of sufficient experience with stimuli may be an important determinant of infants' disposition for stimulus generalization. If so, mere exposure to the stimuli might be able to counteract that tendency, helping them with stimulus differentiation which is essential to achieve discrimination between them. Gibson and Walk (1956) showed that permanent and prolonged exposure to two visual stimuli, starting on infant stages of the rat, facilitated subsequent discrimination learning between them during adulthood. However, subsequent studies have shown that preexposure does not need to be prolonged or to start during early infancy to promote this perceptual learning effect (Hall, 1991). In fact, in adult rats a relatively short exposure to a pair of stimuli results in an enhanced discrimination between them. For instance, preexposure to a pair of flavors reduces the generalization of a conditioned taste aversion between them (e.g., Honey & Hall, 1989; Mackintosh, Kaye, & Bennett, 1991; Symonds & Hall, 1995). During the last few years, many researchers have described and analyzed this phenomenon with adult subjects. To our knowledge, however, except for studies on imprinting with chicks and visual cues (e.g., Honey & Bateson, 1996; Honey, Bateson, & Horn, 1994), there are no comparable studies of the perceptual learning capacities in the immature rat or other altricial mammals.

If it is assumed that the lack of experience is one of the factors responsible for infants' disposition for generalization between stimuli, then it would be expected that enough experience with those stimuli would make up for their immature capacity for discrimination, which will result in a reduced generalized response between them (Campbell & Haroutunian, 1983). In other words, their disposition for generalization makes them ideal subjects for studying the beneficial effects of stimulus preexposure on generalization. That was precisely the purpose of the present study: to assess the effects of stimulus exposure on stimulus generalization in infant rats, using gustatory stimuli. Taking into account that with other stimulus modalities (auditory, visual, tactile) infants show broad generalization gradients, the same tendency would be expected with gustatory stimuli. However, considering that this generalization between gustatory stimuli in infant rats has not been reported, it should be first assessed using tastes with which different levels of generalization were observed in adult rats (Mackintosh et al., 1991).

Therefore, a series of experiments investigated (a) if infants' disposition for generalization can be observed between gustatory stimuli, and if so, (b) whether taste preexposure affects the generalization of taste aversion learning in infant rats. This was evaluated with 13- 17-day-old rats using simple or compound tastes and varying the intensity of the common taste and the number of preexposure trials in the last case.

EXPERIMENT 1

The first step was to assess if generalization of a taste aversion conditioning between two primary tastes is observed with rat pups. In Experiment 1, a primary taste (A) was aversively conditioned and the generalization of that aversion to another taste (B) was tested. In addition to the experimental treatment (Group P) in which Taste A, i.e., the conditioned stimulus (CS), was presented paired with the unconditioned stimulus (US), three other control treatments were used to rule out participation of nonassociative processes in the assessment of the generalized response. One consisted of the unpaired presentation of both stimuli (Group UP), and the others in the presentation of only Taste A (Group CS) or the US alone (Group US).

Method

Subjects and Apparatus. Ninety-six 15-day-old rats from 12 litters were used. Subjects were 48 male and 48 female albino Wistar rats born in the vivarium at our university. Pups were reared with their siblings and progenitors in standard maternity cages lined with pine shavings. The day of birth of the subjects was designated postnatal Day 0. All animals were housed in an acclimatized room, maintained at constant temperature (23°C) and humidity (50%), with 12:12 hr light:dark cycle (light onset at 8 a.m.). Rats had ad-lib access to water and rat chow (Panlab, maternity formula).

Pups were distributed, equating litter and sex, into four groups ($N = 24$). For half of the pups in each group, Taste A was a 3% sucrose solution and Taste B was a 1% sodium chloride (salt) solution, and for the other half A was salt and B sucrose.

During the experimental sessions, subjects were placed in holding chambers ($15 \times 8 \times 15$ cm) grouped by treatment and maintained at 30°C with a heating pad placed beneath the chamber. All pups were in-

traorally cannulated using a procedure extensively described in previous studies (e.g., Chotro & Spear, 1997; Hall & Rosenblatt, 1977). Briefly, cannulae are made with 5-cm sections of polyethylene tubing (Clay Adams, PE 10, i.d. = 0.28 mm). One end of the section is heated to form a small flange. A thin wire attached to the nonflanged end of the cannula is placed on the internal surface of the pup's cheek. The wire is then pushed through the oral muccosae until the flanged end of the cannula is positioned over the internal surface of the cheek while the remainder of the cannula exits from the oral cavity. The entire procedure takes less than 5 s per pup and induces minimal stress. These cannulae were later employed to infuse the different solutions during the study. Four hours after cannulation, pups' bladders were voided by gentle brushing of the anogenital area. Body weights were registered and subjects were placed into individual chambers where they would receive the intraoral infusion of a solution. Intraoral infusions were performed using a 10-syringe infusion pump (KDS) connected to the oral cannula of each pup. The volume administered to each subject was equivalent to 5.5 % of their body weight and was infused at a constant rate for 15 min directly into its mouth, and pups could either consume or reject the infused solution. This forced infusion technique ensures similar exposure to the tastants among subjects, independently from the amount consumed. At the end of the infusion, subjects were weighed and placed into the holding cages. Consumption was determined by the percentage of body weight gain throughout the following formula:

$$[(\text{postinfusion weight} - \text{preinfusion weight})/\text{preinfusion weight}] \times 100.$$

Procedures. The experiment was run in two phases: Conditioning and Generalization Test (see Table 1).

Conditioning. This phase consisted of two trials: On the first trial (postnatal Day 15) all pups were removed from the home cage, placed in heated holding cages, and cannulated. Four hours later, pups received a 15-min administration of either Solution A in Groups P, UP, and CS, or water in Group US. This was immediately followed by an intraperitoneal (ip) injection of lithium chloride (LiCl, 1% v/w of 0.5 M solution) for subjects in Groups P and US, and 2 hr later for subjects in Group UP. This 2-hr interval has been shown to be enough to avoid CS–US association in infant rats (Hoffmann, Hunt, & Spear, 1991). Subjects from Group CS received an ip injection of isotonic

Table 1

Exp.	Groups	Preexposure	Conditioning	Generalization Test
1	P		A-US	
	UP		A/US	B
	CS		A-Saline	
	US		W-US	
2	P-P	A,B, . . . , A,B	A-US	
	P-UP	A,B, . . . , A,B	A/US	B
	NP-P	W, . . . , W	A-US	
	NP-UP	W, . . . , W	A/US	
3	P-P	AX,BX, . . . , AX,BX	AX-US	
	P-UP	AX,BX, . . . , AX,BX	AX/US	BX
	NP-P	W, . . . , W	AX-US	
	NP-UP	W, . . . , W	AX/US	
4	P12-P	AX,BX, . . . , AX,BX	AX-US	
	P12-UP	AX,BX, . . . , AX,BX	AX/US	
	P6-P	AX,BX, . . . , AX,BX	AX-US	BX
	P6-UP	AX,BX, . . . , AX,BX	AX/US	
	NP-P	W, . . . , W	AX-US	
	NP-UP	W, . . . , W	AX/US	

Note. A = 3% sucrose (for half of the subjects; salt for the other half).
B = 1% salt (for half of the subjects; sucrose for the other half).
US = i.p. injection of LiCl (0.15 M, 1% of body weight).
W = water.
Saline = isotonic saline solution (0.9%).
X = hydrochloric acid solution (0.1 M), 0.25% in Experiment 3a and 0.5% in Experiments 3b and 4.

saline solution immediately after the administration of Solution A. Two hours after the injections, subjects were again placed in their maternity cages.

On the second trial (postnatal Day 16), in which the conditioned aversion to A was tested, pups were removed from the maternity cage, placed in the heated holding cage, and cannulated 4 hr before starting the trial. All pups received the administration of Solution A alone.

Generalization Test. That same day (postnatal Day 16) all pups received a generalization test, consisting of the administration of Solution B. On each test, pups received a 15-min oral infusion of the correspondent solution. Both test trials were run separated by a 4-hr interval, and order of tests (consumption of A and B) was counterbalanced within each group.

Results

Results of this experiment are depicted in Figure 1. The left panel of this graph illustrates mean consumption of the conditioned Solution A of the different groups across trials. On the right panel, mean consumption of the nonconditioned Solution B of the different groups during the generalization test is presented.

As can be observed on the left panel, subjects of all groups showed equivalent consumption of Solution A during the first conditioning trial. On the second trial, however, subjects that received the CS paired with the US (Group P) showed less consumption than the remaining three groups. A 4 × 2 (Group × Trial) analysis of variance (ANOVA) of the consumption data from both conditioning trials was done. The rejection level adopted for this and all subsequent analyses was $p < 0.05$. The analysis found significant effects of the two main factors, group, $F(3, 92) = 35.51$ and trial, $F(1, 92) = 11.35$ and their interaction, $F(3, 92) = 46.14$. Post hoc analyses of this interaction revealed that groups differed only on the second conditioning trial; on this trial consumption of Group P was significantly lower than the remaining three groups. At the same time, the effect of trial was only significant for Group P, in which consumption during the second trial was lower than during the first one, i.e., this group showed a conditioned taste aversion.

On the right panel of the figure, results of the generalization test show that those subjects that received the paired treatment during conditioning consumed apparently less Solution B than the other groups. This seems to indicate that the conditioned aversion to A was generalized to B. The analysis of variance of the consumption scores of Solution B confirmed that differences among groups were statistically significant, $F(3, 92) = 21.02$. Further analysis revealed that Group P consumed significantly less than the control groups, while none of the latter differed among them.

All other factors that were counterbalanced in this experiment (litter, sex, order of test, and conditioned solution) were analyzed separately and no significant effects on consumption of Solutions A or B were found.

These results indicate that the conditioning treatment was effective. Presentation of the CS immediately followed by the gastrointestinal illness promoted the formation of an association between them, resulting in taste aversion learning. This conditioned aver-

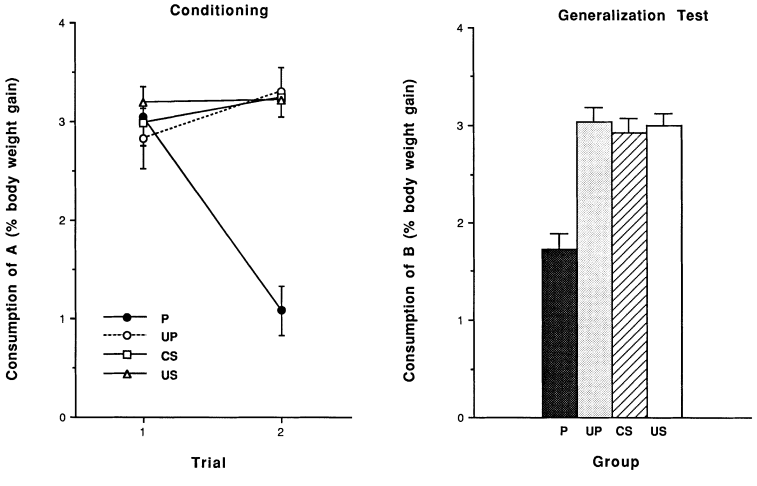

FIGURE 1 Experiment 1. Left panel: Mean (+*SE*) consumption of A for the different groups during conditioning trials. Right panel: Mean (+*SE*) consumption of B for the different groups on the generalization test.

sion to a primary taste seems to generalize easily to another primary taste. The observed aversions may not be attributable to nonassociative processes. On one hand, conditioned and generalized aversion may be discarded as a result of a neophobic reaction because no differences in consumption were detected between control subjects that received novel and familiar tastes on the conditioning test and the generalization test. This is not surprising considering evidence in the literature showing that preweanling rats exhibit none or relatively little neophobic responses to unfamiliar gustatory stimuli (Kalat & Rozin, 1973; Misanin, Blatt, & Hinderliter, 1985; Misanin, Guanowsky, & Riccio, 1983). On the other hand, sensitization derived from the administration of the US also may be discarded as an explanation of the reduced consumption because subjects that received the US alone or unpaired with the CS did not show any aversion.

From these results, it can be concluded that infants' tendency to generalize between stimuli is also evidenced with gustatory stimuli, at least with the parameters used here. Infant rats that learned an aversion to one primary taste easily generalized this conditioned aversion to another primary taste, given that the response to the nonconditioned solution was almost as strong as the one displayed in the presence of the conditioned stimulus.

EXPERIMENT 2

Because a clear generalization of the conditioned aversion was found using two primary tastes, it was considered that there was enough space to observe an effect of preexposure to both stimuli. Hence, the aim of the second experiment was to test if exposure to both stimuli resulted in a reduction of this generalization.

Pups were preexposed to Tastes A and B, then A was paired with the US, and finally generalization of the conditioned response to Taste B was tested. The generalization level of this experimental group was compared to that of pups nonpreexposed to the tastes, pups that received unpaired presentations of A and the US during conditioning, or both.

Method

Subjects and Apparatus. Subjects were 64 Wistar rats 13 to 17 days of age derived from eight litters; half were males and half females. Housing and rearing conditions were similar to those described in Experiment 1 and the apparatus used was the same as in the previous experiment. Subjects were distributed into four groups ($n = 16$ each).

Procedures. This experiment was run in three phases: preexposure, conditioning, and generalization test (see Table 1). General procedures were similar to those described in the previous Experiment.

Preexposure (Postnatal Days 13–14). Pups received two preexposure sessions; each session consisted of three trials with 2-hr intervals between trials. On each trial, subjects received an intraoral infusion (5.5% of body weight) during 15 min. Solutions A and B were administered in alternate trials for half of the pups (Groups P-P and P-NP); the other half received intraoral infusions of water (Groups NP-P and NP-UP). Solutions and order of presentation within and between sessions were counterbalanced.

Conditioning (Postnatal Days 15–17). This phase consisted of three trials performed on consecutive days. On each trial pups received the administration of Solution A. On the first two trials, but not on the last one, an injection of LiCl was given immediately after Solution A (Groups P-P and NP-P) or 2 hr later (Groups P-UP and NP-UP). The remaining details of the procedure were equal to those described on Experiment 1 for Groups P and UP, respectively.

Test (Postnatal Day 17). Consumption of solution B was tested for all pups, as described on Experiment 1.

Results

Results of this experiment are depicted in Figure 2. The left panel of this graph illustrates mean consumption of conditioned Solution A of the different groups along trials. On the right panel, mean consumption of the nonconditioned Solution B for the different groups.

As can be seen on the left panel, consumption of all groups was equivalent on the first conditioning trial. A decrease in consumption of Solution A was observed on the last two trials for subjects that received this solution paired with the US in relation to the unpaired controls. This indicates that paired treatment was effective to produce a taste aversion learning. However, preexposed subjects apparently showed a slower acquisition of that learning. A $2 \times 2 \times 3$ (Preexposure $\times$ Conditioning $\times$ Trial) ANOVA revealed a significant effect of the three main factors: preexposure, $F(1, 60) = 15.40$; conditioning, $F(1, 60) = 132.66$, and trial, $F(2, 120) = 14.88$, as well as significant interactions of Preexposure $\times$ Conditioning, $F(1, 60) = 4.65$, and Conditioning $\times$ Trial, $F(2, 120) = 31.58$. The remaining two interactions were not significant. Post hoc analyses of the Preex-

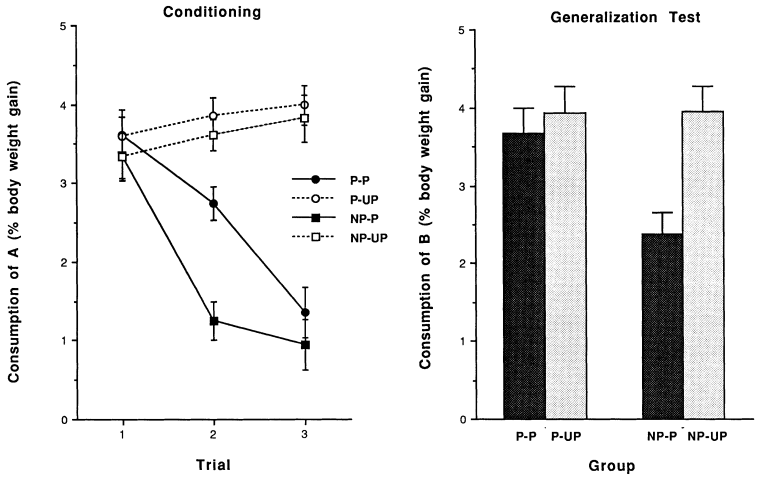

FIGURE 2 Experiment 2. Left panel: Mean (+*SE*) consumption of A for the different groups during conditioning trials. Right panel: Mean consumption of B on the generalization test as a function of conditioning (P or UP) and preexposure treatments (P or NP).

posure × Conditioning interaction indicated that Group P-P differed significantly from Group NP-P, whereas Groups P-UP and NP-UP did not differ between them. The analysis of the second interaction indicated that the effect of conditioning was significant only on Trials 2 and 3. During those trials consumption of Groups P-P and NP-P was significantly lower than Groups P-UP and NP-UP. A separate analyses of the results obtained on Trial 2 confirmed the significant main effect of preexposure, $F(1, 60) = 10.46$, of conditioning, $F(1, 60) = 41.87$, and the interaction between them, $F(1, 60) = 5.36$. Post hoc analyses confirmed that paired groups consumed less than unpaired after one conditioning trial. Of more importance here is the significantly higher consumption of Group P-P than Group NP-P. A similar analysis of Trial 3 revealed only a main effect of conditioning, $F(1, 60) = 153.95$, showing that at this point equivalent levels of conditioning were shown by Groups P-P and NP-P.

On the right panel of the figure, results of the generalization test show that those subjects that received the paired conditioning treatment consumed apparently less that the unpaired groups; however, this difference was remarkably reduced in the preexposure condition. This seems to indicate that conditioning to Taste A was generalized to Taste B, and that this generalization was reduced by taste preexposure. The 2 × 2 (Preexposure × Conditioning) ANOVA revealed significant effects of preexposure, $F(1, 60) = 6.90$, and conditioning, $F(1, 60) = 14.02$, as well as a significant interaction between both factors, $F(1, 60) = 7.10$. Post hoc analyses of the interaction confirmed the initial interpretations showing that preexposure affected the consumption of subjects that received paired treatment during conditioning. Group P-P significantly differed from Group NP-P whereas Groups P-UP and NP-UP did not differ from one another. The significant difference between Groups NP-P and NP-UP, attributable to generalization of the conditioned aversion, was not observed between Groups P-P and P-UP.

These results indicate that in infant rats a clear generalization of a conditioned taste aversion can be obtained with simple primary tastes and that it can be reduced when subjects have been previously exposed to the stimuli. Oppositely, results of studies with adult rats seem to indicate that generalized aversions between simple primary tastes are not easily found (Formaker, & Hill, 1990; Mackintosh et al., 1991). In general, with adult subjects, in order to obtain a generalized conditioned aversion between two tastes and to observe the beneficial effect of stimulus exposure on reducing that generalization, the similarity between stimuli has to be increased by the addition of a third common taste (Mackintosh et al., 1991; Symonds & Hall, 1995). For adults, therefore, it seems clear that when stimuli are more similar there would be more opportunities for obtaining the beneficial effects of stimulus preexposure on its differentiation. Considering this differential result between infants and adults, it would be interesting to study whether this pattern of results is still found with infant rats when augmenting stimulus similarity. Differences in the effect of increasing stimulus similarity could indicate that mechanisms underlying the effect of reduction of generalization after stimulus preexposure are different or work in different ways in infants than in adults subjects.

EXPERIMENT 3

The aim of Experiment 3 was to determine the effect of stimulus exposure on generalization, when increasing the similarity between stimuli. To test this, each of the tastes employed earlier (A or B) was presented in compound with a third common taste (X), varying in intensity from relatively low in Experiment 3a to relatively high in Experiment 3b.

EXPERIMENT 3A

Method

Subjects and Apparatus. Sixty-four Wistar rats 13 to 17 days of age derived from eight litters were used; 32 males and 32 females. Housing and rearing conditions were equal to those described for previous experiments. The apparatus was the same as described for the previous experiment except that the solutions were compounds of two tastes—AX and BX, salt with hydrochloric acid and sucrose with hydrochloric acid, respectively. Concentrations of sucrose and salt were the same as in previous experiments; the concentration of hydrochloric acid was 0.25 % of a 0.1 M solution.

Procedures. Stimulus preexposure, conditioning, and testing procedures were similar to those described in Experiment 2 except that compound tastes were used as stimuli, as described earlier (see Table 1).

Results

The results are shown in Figure 3. The left panel of this figure illustrates mean consumption of the conditioned Solution AX for the different groups along trials. On the right panel, mean consumption of the nonconditioned solution BX of the different groups on the generalization test is shown.

As can be observed on the left panel, consumption of Solution AX was equivalent among the four groups on the first conditioning trial. On Trials 2 and 3, a decrease in consumption was observed for those subjects that received paired presentations of the CS and US during conditioning in relation to those that received unpaired presentations. Yet, contrary to what has been observed in the previous experiment, those subjects that received preexposure seem to reduce their intake even more, suggesting that a faster conditioning occurred. However, a $2 \times 2 \times 3$ (Preexposure $\times$ Conditioning $\times$ Trial) ANOVA indicated no effect of preexposure but significant effects of conditioning, $F(1, 60) = 243.89$, of Trial, $F(2, 120) = 39.96$, as well as a significant Conditioning $\times$ Trial interaction, $F(2, 120) = 78.51$. Further analysis of this interaction indicated that the effect of conditioning was significant on Trials 2 and 3. During those trials, consumption of Groups P-P and NP-P were significantly lower than that of Groups P-UP and NP-UP. These results did not confirm the first impression of the figure about the preexposure effect upon the rate of conditioning.

On the right panel, it can be observed that subjects that received the paired treatment consumed less than

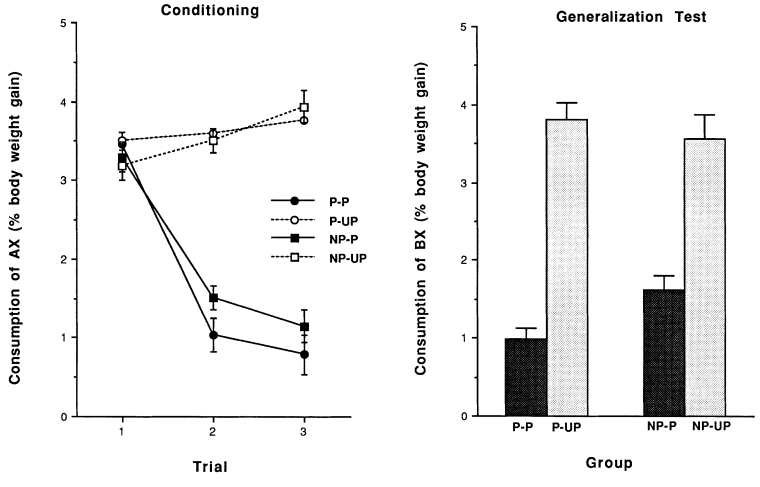

FIGURE 3 Experiment 3a. Left panel: Mean (+*SE*) consumption of AX for the different groups during conditioning trials. Right panel: Mean consumption of BX on the generalization test as a function of conditioning (P or UP) and preexposure treatments (P or NP).

the unpaired groups, yet, this difference seems to be more marked between the preexposed subjects. The 2 × 2 (Preexposure × Conditioning) ANOVA revealed only a significant effect of conditioning, $F(1, 60) = 118.11$. Although Group P-P seemed to show a stronger generalized aversion when compared to Group NP-P, the Preexposure × Conditioning interaction reached only borderline significance, $F(1, 60) = 3.98$, $p = 0.08$.

These results suggest that increasing the similarity of the stimuli enhances the generalization between them, as was expected. Surprisingly, preexposure did not reduce this generalization, as was the case with simple tastes. Moreover, there was a trend for preexposure to increase generalization, although the statistical support for this last interpretation is marginal.

EXPERIMENT 3B

An increase of similarity between stimuli, by the addition of a third common taste, seems to be the factor responsible for this strong generalization observed in preexposed subjects of Experiment 3a as compared to Experiment 2. Thus, with the aim to replicate and even potentiate this result, it was thought that by increasing this similarity even more by augmenting the concentration of the common taste, the effect of stimulus preexposure glimmered in Experiment 3a should be easily obtained. Therefore, in this experiment pups were subjected to similar preexposure and conditioning procedures to those of Experiment 3a with the sole difference that the concentration of the common taste was raised.

Method

Subjects and Apparatus. Subjects were 64 Wistar rats 13 to 17 days of age derived from eight litters; half were males and half females. Housing and rearing conditions as well as the apparatus used during the experiment were the same as those described in previous experiments. Subjects were distributed within four groups ($n = 16$).

Procedures. Stimulus preexposure, conditioning, and testing procedures were the same as in Experiment 3a, with the sole difference that the concentration of hydrochloric acid (X) compounded with sucrose and salt (A and B) was doubled: 0.5 % (of a 0.1 M solution).

Results

Results are depicted in Figure 4. Mean consumption scores of the conditioned taste AX of the different groups along trials are shown on the left panel.. On the right panel, mean consumption of the nonconditioned taste BX of the different groups during the generalization test is represented.

As can be observed on the left panel, as was the case with Experiment 3a, consumption of AX was equivalent on the first conditioning trial for all groups, whereas on the following two trials a decrease in consumption was shown by subjects that received the paired conditioning treatment when compared to consumption levels of the unpaired controls. On Trial 2, subjects that received paired treatment on conditioning and preexposure seemed to consume even less of the conditioned solution than those that received the same

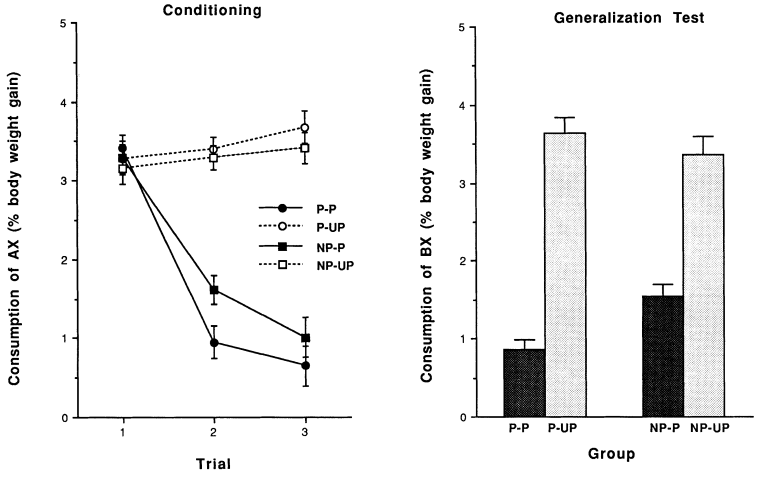

FIGURE 4 Experiment 3b. Left panel: Mean (+*SE*) consumption of AX for the different groups during conditioning trials. Right panel: Mean consumption of BX on the generalization test as a function of conditioning (P or UP) and preexposure treatments (P or NP).

conditioning treatment but no preexposure. These impressions were confirmed by the results of the 2 × 2 × 3 (Preexposure × Conditioning × Trial) ANOVA, which found significant main effects of conditioning, $F(1, 60) = 225.99$, and trial, $F(2, 120) = 52.12$, as well as two significant interactions: Conditioning × Preexposure, $F(1, 60) = 5.23$, and Conditioning × Trial, $F(2, 120) = 81.43$. Further analysis of the first interaction indicated that Group P-P showed significantly lower consumption than Group NP-P whereas Groups P-UP and NP-UP did not differ between them. The analysis of the second interaction revealed that the effect of conditioning was significant on Trials 2 and 3. During those trials consumption of Groups P-P and NP-P was significantly lower than Groups P-UP and NP-UP. Separate analysis of the results on Trial 2 found a significant Preexposure × Conditioning interaction, $F(1, 60) = 5.70$, indicating that the strongest aversion shown by Group P-P occurred in this trial. A similar analysis of Trial 3 revealed only a main effect of conditioning, $F(1, 60) = 275.72$, showing that at this point equivalent levels of conditioning were shown by Groups P-P and NP-P.

On the generalization test (right panel), it can be observed that pups that received paired presentations of the CS and US showed lower consumption levels of the nonconditioned solution compared to their unpaired controls. It also can be seen that the preexposure treatment seems to have increased this aversion. The 2 × 2 (Preexposure × Conditioning) ANOVA found no effect of preexposure but a significant effect of conditioning, $F(1, 60) = 136.51$, and a significant interaction of both factors, $F(1, 60) = 5.73$. Post hoc analyses of this interaction revealed that the effect of preexposure was significant in the paired condition. While Groups P-UP and NP-UP showed high and equivalent levels of consumption of Taste BX, Group P-P showed significantly lower consumption than Group NP-P.

To explain the results of Experiment 3, consider that when Taste A is presented in compound with another taste (X) followed by the US, Stimuli A, X, and the configuration of both might gain associative strength. The level of generalization of conditioned aversion from Compound AX to Compound BX may not be attributed to the associative strength acquired by the distinctive taste of the compound (A), but to the one gained by the common taste (X). The strong generalization obtained in this experiment may be explained if Taste X was more salient than A. If so, Taste X might have overshadowed the conditioning of Taste A. As a consequence of that overshadowing, Taste X would gain greater associative strength than Taste A, resulting in a greater aversion to Compound BX in the generalization test. The same conclusion may be reached when considering that presentation of a compound of flavors might produce a configuration or a third combined solution if it is assumed that this configuration is mainly characterized by Taste X on both compounds.

Assuming that the high generalization between Compounds AX and BX was due to a stronger conditioning of the more salient, common Taste X, it would be expected that preexposure to both compounds should have resulted in a weakening of this generalization and not the opposite effect. Twice the exposure to X than to A and B would result in Taste X suffering more latent inhibition, overcoming the beneficial effect of its salience on conditioning level and hence on the effects described earlier. However, the possibility remains that the well-known effect of latent inhibition operates in a different way in infants. Hoffmann and Spear (1989) have shown that, using parameters with which a retardation of conditioning would be observed in adult rats, a facilitatory effect may be found with infants; and that in order to observe latent inhibition infants need more experience with the stimuli, i.e., more preexposure trials (Hoffmann & Spear, 1989). This was assessed in the following experiment.

EXPERIMENT 4

The aim of Experiment 4 was to confirm the results found in Experiment 3b and to test the effect of increasing the number of stimulus preexposure trials on the generalization of the conditioned taste aversion with compound and similar tastes. More episodes of stimulus exposure would be expected to retard conditioning of the common elements of the stimuli and hence might result in a reduction of generalization. This hypothesis was evaluated by increasing the total number of preexposure trials from 6 to 12 (6 for AX and 6 for BX).

Method

Subjects and Apparatus. Subjects employed in this experiment were 60 Wistar rat pups 13 to 17 days of age (30 females and 30 males) derived from seven litters. Housing and rearing conditions as well as the apparatus were the same as those described in previous experiments. Subjects were distributed within 6 groups (– = 10). The solutions were the same as those used in Experiment 3b, i.e., with the higher concentration of hydrochloric acid: 0.5 % (of a 0.1 M solution).

Procedures. Stimulus preexposure, conditioning, and test procedures were exactly the same as those described for the previous experiment (see Table 1). Besides the four groups employed in Experiment 3b, two new groups were added here: Groups P12-P and P12-UP. These two groups were treated the same as Groups P-P and P-UP of that experiment (here named P6-P and P6-UP, respectively) except for the number of trials during preexposure phase. They received 6 alternate trials to Tastes AX and BX on each of the two sessions (a total of 12 preexposure trials) with 1-hr intervals between trials.

Results

Results are depicted in Figure 5. The left panel shows the mean consumption of conditioned Solution AX of the different groups along trials. The right panel shows mean consumption of the nonconditioned Solution BX of the different groups during the generalization test.

It can be seen that no differences in consumption were observed for the first conditioning trial. However, on the following two trials all subjects that received the paired treatment during conditioning consumed less than the correspondent unpaired controls, which show a stable and high consumption across trials. This seems to indicate that beyond the different preexposure conditions, the paired treatment was effective to produce a taste aversion learning. However, consumption on the second conditioning trial appears to depend on the number of preexposure trials. Short preexposure seems to have facilitated conditioning, while the long preexposure produced a retardation of it, when compared to the non preexposure condition.

The results of the 3 × 2 × 3 (Preexposure × Conditioning × Trial) ANOVA revealed significant effects of the three main factors: preexposure, $F(2, 54) = 14.23$, conditioning, $F(1, 54) = 264.02$, and trial, $F(2, 108) = 41.40$. Significant interactions were observed between preexposure and conditioning, $F(2, 54) = 3.89$, and conditioning and trial, $F(2, 108) = 70.35$. Post hoc analyses of the Preexposure × Conditioning interactions indicated that preexposure did affect only the consumption of paired groups, i.e., consumption of Group P12-P was higher than Group NP-P and Group P6-P, whereas the consumption of Group P6-P was significantly lower than Group NP-P. Analyses of the Conditioning × Trial interaction revealed that the effect of conditioning was significant on Trials 2 and 3; during those trials consumption of paired groups was significantly lower than the unpaired.

In order to check if the number of preexposure trials affected the rate of conditioning, separated analyses of the results of Trial C2 were done. Results of the analysis showed a significant effect of preexposure, $F(2, 54) = 9.45$, and conditioning, $F(1, 54) = 98.25$, and the interaction between them, $F(2, 54) = 4.46$. The analysis of the interaction confirmed the results previously described and, in addition, showed that Group P6-P consumed significantly less than Groups NP-P and P12-P and that Group P12-P consumed more than Group NP-P. A similar analysis of Trial 3 showed only a significant effect of conditioning, $F(1, 54) = 316.40$, indicating that after two conditioning trials all paired groups reached similar levels of conditioning independently of the exposure treatment.

The right panel of the figure shows that all pups

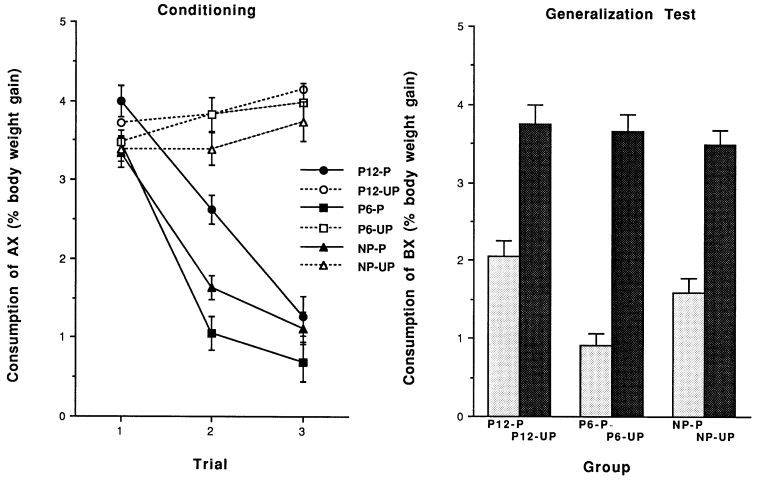

FIGURE 5 Experiment 4. Left panel: Mean (+*SE*) consumption of AX for the different groups during conditioning trials. Right panel: Mean consumption of BX on the generalization test as a function of conditioning (P or UP) and preexposure treatments (P12, P6, or NP).

that received the CS paired to the US during conditioning on the generaliztion test had lower consumption than the correspondent unpaired controls. Consumption of the former subjects seems to differ among them according to their preexposure treatment. Thus, subjects that received more preexposure showed higher consumption whereas subjects that received less preexposure showed lower consumption, both in comparison to the nonpreexposed subjects. These descriptive results were confirmed by the results of the ANOVA, which revealed significant effects of both main factors, preexposure: $F(2, 54) = 8.73$ and conditioning: $F(1, 54) = 231.74$, and the interaction between them, $F(2, 54) = 3.40$. Further analyses of this interaction showed that the effect of preexposure was significant among paired groups. Intake was significantly higher in Group P12-P and significantly lower in Group P6-P when compared to Group NP-P.

The results of this experiment confirmed and extended those of Experiment 3b. Three exposure trials to Compound AX and three to BX facilitated subsequent conditioning of Compound AX and increased generalization to Compound BX. Six preexposure trials to each of the compounds, however, had a reversal effect on conditioning and generalization, i.e., retarded conditioning of AX and reduced generalization to BX. Therefore, the increase of the number of preexposure trials to the compound tastes resulted in the expected effect on the generalization of aversion learning, the same effect found with simple tastes and less preexposure trials. This reduction on generalization was preceded by a retardation on the rate of conditioning. These results appear to point to a direct relation between the degree of generalization and the speed of conditioning promoted by stimulus exposure. In summary, at this age, exposure to compound and very similar tastes seem to result in a facilitation or a retardation of the conditioned aversion together with an increase or reduction on its generalization, respectively, depending on the number of preexposure trials.

GENERAL DISCUSSION

The results of the present study indicate that stimulus preexposure affects the generalization of a conditioned taste aversion in infant rats. With simple primary tastes infants show a clear generalization of the conditioned taste aversion, which is reduced by stimulus preexposure. When using compound and more similar tastes, a strong generalization was also observed and preexposure could either increase or reduce generalization. Relatively few preexposure trials to the compound tastes resulted in strong generalization between them, and with more preexposure trials generalization was reduced.

A closer analysis of these results denotes a strong parallelism between the effects of stimulus preexposure on the acquisition rate of conditioning and on the degree of generalization. That is, the reduction in generalization was observed whenever stimulus preexposure induced a retardation on the rate of conditioning, whereas a stronger generalized aversion was evidenced when preexposure facilitated conditioning, though in all cases after two conditioning trials levels of conditioning were equivalent in all paired groups.

The nonmonotonic effect of preexposure on conditioning observed with the compound tastes—retardation or facilitation of conditioning—seems to depend upon the amount of stimulus preexposure. A similar effect of stimulus preexposure on conditioning was reported by Hoffmann and Spear (1989). They found a facilitation of conditioning with stimulus preexposure in young infant rats with parameters with which a retardation was obtained with older subjects, and a latent inhibition effect was observed with the younger pups only after increasing the number of preexposure trials (Hoffmann & Spear, 1989). Under certain special conditions, stimulus preexposure has been also found to produce a biphasic effect on conditioning in adult rats. For example, 5 min of contextual preexposure for 3 days facilitated context–shock conditioning in adult rats when compared to subjects that received none or 1 day of preexposure, whereas 7 days of preexposure produced latent inhibition (Takigasaki, 1993). Using a conditioned taste aversion paradigm, with a complex taste and a single conditioning trial, it also has been found that preexposure enhanced conditioning whereas this same procedure resulted in a latent inhibition effect after increasing the time of stimulus exposure or with less time but using more simple tastes (Bennett, Tremain, & Mackintosh, 1996). These last findings are interpreted within the frame of the process of "unitization" conceptualized by McLaren, Kaye, and Mackintosh (1989). These authors suggest that stimuli should be regarded as sets of elements and that each time a stimulus is presented only a subset of these elements is sampled. They postulate as well that every time a set of elements is sampled together, they become associated together. Thus, a brief presentation of a complex stimulus during a single conditioning trial will result in the conditioning of only the sampled subset of elements and a full conditioned response will be displayed only by those subjects that fortuitously sampled the same elements during conditioning and test. Therefore, conditioning may be facilitated if the CS is preexposed, allowing the subject to sample a wider range of CS elements and

to associate them with one another. Thus, even if the elements sampled on the test are not the same as those sampled during conditioning, they will be able to retrieve a complete representation of the remaining conditioned elements and to elicit the conditioned response (McLaren et al., 1989).

The facilitation of conditioning after stimulus exposure observed here could be interpreted in a similar way, considering that the facilitatory effect was observed after one conditioning trial and that the compounded tastes employed may be perceived as a complex stimulus by infant rats. Perhaps, without preexposure, the compound CS presented during the first conditioning trial was not perceived completely by the infant rat and, therefore, conditioning was not as strong as with stimuli that were more easy to sample. Indeed, when comparing the level of conditioning after the first conditioning trial of nonpreexposed subjects in the present set of experiments, with compounded tastes conditioning does not occur as readily as with simple tastes; however, after a short stimulus preexposure conditioning reached comparable levels.

In relation to this there are studies, using the orienting response (OR) as an index of attention and information processing, which demonstrate that duration of attention to a stimulus is longer the younger the organism, the more complex the stimulus are, or both (Richardson, Hayne, & Campbell, 1992). One explanation they consider is that younger subjects require more time to process novel stimulus information than do older ones and that processing time is also increased with complex stimuli. In consequence, brief stimulus exposure before conditioning can help processing complex stimuli facilitating it. The need of longer time for stimulus processing in younger organisms may indicate a poorer ability for filtering out what is familiar or important and what is not, apparently due to their insufficient sensory experience. Thus, as the stimulus becomes more familiar with more exposure, with less attention on the irrelevant aspects of it and more focus on the essential ones, make information processing more efficient, therefore allowing good discrimination between stimuli. In summary, adding a third common taste to make stimuli more similar and therefore harder to discriminate brought about another consequence—stimuli were made more complex and apparently more difficult to be processed for the immature rat.

The stronger generalization observed in those cases when preexposure facilitated conditioning could also be explained within the theory of stimulus representation of McLaren et al. (1989). They consider that when two stimuli (AX, BX) containing unique (A, B) and common elements to both (X) are preexposed, excitatory connections between the various elements of both stimuli will be initially established. These excitatory connections between common and unique elements of the two stimuli will conduct them to treat stimuli as equivalent, responding in a similar way to both. This effect could be interpreted as an acquired equivalence between exposed stimuli, and has been observed in infant rats (e.g., Spear, Kraemer, Molina, & Smoller, 1988). Within this theory they also have proposed an explanation for the perceptual learning effects that may be considered in order to explain the reduced generalization observed here after stimulus preexposure.

Generalization of a conditioned aversion from one stimulus, AX, to a second stimulus, BX, has two sources. One is the associative strength that the common element of the stimuli (X) gained during conditioning and the other is the result of the evocation of the unique element (A), via the X–A excitatory link, by the presence of the common element (X) in the generalization test (BX). Those two sources of generalization can be attenuated by stimulus exposure. When AX and BX are presented, their common elements are preexposed twice the unique elements, suffering more latent inhibition. Consequently, the common elements will acquire less associative strength during conditioning, resulting in less generalization. The other source of generalization, which might be first facilitated during preexposure by the formation of excitatory links between unique and common elements (X–A and X–B) mentioned earlier, might be later counteracted by the formation of mutual inhibitory links between unique stimulus elements (A and B). That is, the evocation of the unique element of the CS (A), by the excitatory link X–A, would be canceled by the inhibitory link formed between A and B when BX is presented on the test, resulting in a reduced generalized response, i.e., an enhanced discrimination between stimuli (McLaren et al., 1989).

From the present results, it cannot be deduced which is the contribution of each of the stimulus elements to the retarded conditioned response to the CS or to the reduced generalized aversion to the alternative stimulus. Therefore, these mechanisms cannot be either discarded or corroborated as responsible for the reduction on generalization observed after stimulus preexposure in Experiments 2 and 4. However, the close relationship between the effects of preexposure on conditioning and generalization observed in infants could be pointing to different processes underlying similar results in infant and adults. In most studies about perceptual learning with adult rats, stimulus preexposure generally produces a latent inhibition effect; however, preexposed groups do not always show a reduced generalization. In other words, the parallelism between conditioning and generalization is not al-

ways observed. Moreover, Symonds and Hall (1995) reported that some ways of preexposing the stimuli promoted latent inhibition effects followed by no reduction on generalization while some others promoted the opposite, i.e., no latent inhibition effect and a reduction of generalization (Experiments 2 and 3).

In summary, the present results provide a clear indication that infants' disposition to generalize among stimuli can be observed with gustatory stimuli and represent, to our knowledge, the first evidence that generalization can be reduced with the appropriate stimulus exposure in infant rats. These results also indicate that preexposure does not seem to reduce generalization when stimuli are not completely processed. In this case preexposure seems first to enhance stimulus processing, which results in facilitation of conditioning and a strong generalization of the aversion to the nonconditioned stimulus. But, once processing is complete and a strong conditioning occurs, extra stimulus exposure appears to retard conditioning and to reduce generalization. Whether this reduced generalization responds either to a general lower level of conditioning (latent inhibition effect) or to a differential latent inhibition of the common and unique elements coupled with the establishment of inhibitory connections between the unique features of the stimuli (perceptual learning effect) cannot be concluded from the present results. Further research will address the mechanisms and developmental processes underlying this phenomenon in order to understand whether the reduction in generalization observed after stimulus exposure in infant rats indeed reflects an enhanced discrimination between stimuli, as is assumed to occur with adults. If so, these experiments could represent a reliable first approach to analyze how stimulus processing and discrimination change with development by means of sensory experience as well as the participation of perceptual, attentional, and memory-related processes.

NOTES

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