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Stimulus Preexposure Reduces Generalization of Conditioned Taste Aversions Between Alcohol and Non-Alcohol Flavors in Infant Rats

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Results of 3 experiments showed that infant rats (age 13–17 days) generalize conditioned taste aversions between alcohol and non-alcohol tastes such as a mixture of sucrose and quinine, apple cider vinegar, or coffee. Nonreinforced preexposure to those tastes reduced generalized aversions between them. Generalization between alcohol and sucrose–quinine was reduced not only after preexposure to both tastes, but also when only the nonconditioned taste was preexposed, whereas with alcohol and vinegar, both tastes had to be preexposed to obtain that effect. In no case was generalization reduced when only the to-be-conditioned taste was preexposed. Previous experience with alcohol alone, as well as with similar gustatory stimuli, may enhance subjects' ability to differentiate them during infantile stages in rats.

Experiences with alcohol are not exclusive to adulthood; exposure to alcohol taste, odor, and/or toxic properties can take place during adolescence, infancy, and even neonatal and fetal stages (Abel, 1984; Fossey, 1993; Mennella, 1999; Noll, Zucker, & Greenberg, 1990). Some of these experiences with the taste and/or odor of alcohol have been shown to modify subjects' response to the drug. Studies with children have shown that previous experience with alcohol's orosensory properties facilitates alcohol identification in preschoolers (Noll et al., 1990) and can also modify their alcohol odor preference (Mennella & Garcia, 2000). Neonates born of mothers who have self-reported frequent alcohol consumption during pregnancy were found to react more to alcohol odor than neonates born of mothers who drank infrequently (Faas, Spontón, Moya, & Molina, 2000).

In studies with animal models, specifically infant rats, conditioned preferences or aversions to alcohol were observed after pairing alcohol odor and/or taste with an appetitive or aversive stimulus, respectively (Molina & Chotro, 1989a, 1989b). Nonreinforced experience with the orosensory characteristics of alcohol during early stages of development has also been demonstrated to induce significant changes in subsequent reactions to alcohol. Fetal exposure to alcohol, for instance, produces a sensitized response to the sensory and toxic properties of this drug (Chotro & Spear, 1997), as well as increased alcohol consumption and alcohol odor preference (Molina, Chotro, & Domínguez, 1995). Moreover, prenatal experience with alcohol has been found to modify postnatal learning about ethanol (Chotro, Córdoba, & Molina, 1991). Exposure to alcohol odor and taste in the nursing environment of infant rats has also been found to modify pups' responsiveness to alcohol taste and odor (Pepino, López, Spear, & Molina, 1999), increasing or reducing consumption of alcohol solutions, but not other flavored solutions (López & Molina, 1999; Molina, Pepino, Johnson, & Spear, 2000). Indirect experience with alcohol, through contact with an intoxicated sibling, has been found to increase alcohol preference in adolescent and infant rats (Hunt, Holloway, Scordalakes, 2001).

In short, a considerable amount of research corroborates the idea that reinforced and nonreinforced experiences with the gustatory and olfactory characteristics of alcohol during early stages of development may induce changes in subsequent responses to alcohol odor or taste, generally enhancing subjects' responsiveness to alcohol and increasing alcohol consumption.

Mere stimulus exposure, without any explicit reinforcement, can also promote learning. For instance, in adult rats, nonreinforced exposure to a pair of stimuli has been found to facilitate subsequent discrimination learning or to reduce generalization between them: That is, a learned aversion to one taste generalizes less to another taste when both have been previously exposed (Honey & Hall, 1989). Recently, this perceptual learning effect has been demonstrated to occur also with infant rats through the use of primary tastes, such as salt or sucrose, or compound tastes with a long enough stimulus preexposure period (Chotro & Alonso, 1999, 2001). In general, infants show poorer discrimination between stimuli and a greater tendency toward stimulus generalization than adults (Gibson, 1969; Spear & Mackinzie, 1994). It has also been acknowledged that stimulus discrimination improves not only with sensory maturation, but also with sensory experience (Campbell & Haroutunian, 1983; Gibson, 1969; Spear & Mackinzie, 1994). It is, therefore, particularly interesting to analyze the benefits of previous stimulus exposure with regard to stimulus discrimination in developing subjects. Furthermore, studying learned behaviors toward alcohol's sensory properties during early developmental stages may help to evaluate the importance of this factor in subsequent responses to this drug and could provide a useful approach to the control of alcohol consumption initiation later in life.

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The aim of this series of experiments was to investigate the extent to which learned experiences with alcohol taste generalize to other tastes and vice versa, and whether preexposure to alcohol flavor and other similar tastes reduces that generalization between stimuli in infant rats, thereby leading to an enhanced differential response to them.

Experiment 1

A first experiment was conducted in order to test the level of generalization of taste aversion learning between alcohol and other non-alcohol tastes. Basically, one of the tastes was aversively conditioned, and the generalization of this aversion to the other taste was tested. Two groups were used in this experiment: one that received a paired treatment (paired group) and another that received an unpaired treatment (unpaired group). A 6% alcohol solution was chosen on the basis of previous studies showing that infant rats seem to perceive the distinct taste of the drug at this concentration (Molina & Chotro, 1989b). The non-alcohol tastes were a sucrose and quinine solution (Experiment 1A), an apple cider vinegar solution (for Experiment 1B), and a decaffeinated coffee solution (Experiment 1C). These solutions and their concentrations were selected on the basis of their degree of relative similarity to the taste of the 6% alcohol solution. For instance, the sucrose and quinine solution has been found to be perceived by adult and infant rats as very similar to the taste of alcohol (Kiefer & Mahadevan, 1993; Molina et al., 1995). Indeed, electrophysiological and behavioral data indicate a stronger generalization between alcohol taste and this sweet–bitter combination than between alcohol and other basic taste compounds in adult rats (DiLorenzo, Kiefer, Rice, & Garcia, 1986). These authors have also reported that adult rats generalize alcohol taste aversions to a sweet–sour mixture, although not as strongly as to the aforementioned sweet–bitter combination. In light of this, apple cider vinegar was selected on the basis of its sweet and sour taste components. Furthermore, this substance has previously been used as an alternative flavor in studies measuring alcohol consumption. In these studies, young rats consumed similar amounts of alcohol and apple cider vinegar solutions (e.g., Bannoura & Spear, 1995). Finally, coffee has also been used as an alternative flavor in several studies with alcohol (e.g., Hunt et al., 2001) and was considered to be more different from alcohol than the first two solutions. Pilot studies were performed to adjust the flavor concentrations in order to achieve uniform baseline intake of the solutions in naive rat pups.

Method

Subjects and Apparatus

Subjects were 16- and 17-day-old albino Wistar male and female rats born in the vivarium at the Universidad del País Vasco. A total of 168 pups were used, distributed across three experiments ($n = 56$ for each experiment). Pups were reared with their siblings and progenitors in standard maternity cages lined with pine shavings. The day of subjects' birth was designated Postnatal Day 0 (PD 0). All rats were housed in an acclimatized room and maintained at constant temperature (23 °C) and humidity (50%) levels, with a 12-hr light–dark cycle (lights on at 8 a.m.). Rats had ad-lib access to water and rat chow (maternity formula; Panlab, Barcelona, Spain).

For each experiment, pups were equally distributed, by litter and sex, into two groups: paired ($n = 28$) and unpaired ($n = 28$). For half of the pups in each group, the conditioned stimulus (CS; Stimulus A) was an ethanol solution (ETH, 6% vol/vol), and the alternative stimulus (B) was a solution of sucrose plus quinine (SQ; 2.5% wt/vol and 0.04% wt/vol, respectively) in Experiment 1A, apple cider vinegar (VIN, 0.35% vol/vol) in Experiment 1B, and decaffeinated coffee (COF, 0.625% wt/vol) in Experiment 1C. For the other half, Solution B was ETH, and Solution A was the corresponding alternative taste.

During the experimental sessions, subjects were placed in holding chambers (15 cm long × 8 cm wide × 15 cm high), grouped according to treatment, and maintained at 30 °C by a heating pad placed beneath the chamber. All pups were intraorally cannulated by means of a procedure described in previous studies (e.g., Chotro & Alonso, 2001; Hall & Rosenblatt, 1977). In brief, cannulas are made with 5-cm sections of polyethylene tubing (PE 10, i.d. = 0.28 mm; Clay Adams, Parsippany, NJ). One end of the section is heated in order to form a small flange. A thin wire attached to the nonflanged end of the cannula is placed on the medial internal surface of the pup's cheek. The wire is then pushed through the oral mucosae until the flanged end of the cannula is positioned over the internal surface of the cheek and 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 cannulas were later used to infuse the different solutions during the study. Four hours after cannulation, pups' bladders were voided by gentle brushing of the anogenital area. Then, body weights were registered, and subjects were placed into individual chambers in which they received the intraoral infusion of the corresponding solution. Intraoral infusions were performed with a 10-syringe infusion pump (KD Scientific, Boston, MA) connected to the oral cannula of each pup. The volume infused to each subject was equivalent to 5.5% of its body weight and was administered at a constant rate during 15 min. Pups could either consume or reject the infused solution. At the end of the infusion, pups were immediately weighed and placed into the holding cages. The difference in the pup's pre- and postinfusion weight reflected the amount of fluid consumed. The dependent variable analyzed in all experiments was the percentage of body weight gain (% BWG), calculated by means of the following formula: [(postinfusion weight − preinfusion weight) ÷ preinfusion weight] × 100.

Procedure

Conditioning. On the morning of PD 16, all pups were removed from the home cage, cannulated, grouped according to litter, and placed in heated holding cages (without the dam) for 4 hr during the food-deprivation period prior to the conditioning trial. After the deprivation period, pups were placed in individual chambers, where they received a 15-min administration of Solution A. This was immediately followed by an intraperitoneal injection of lithium chloride (LiCl, 0.5% wt/vol of 0.5 M solution) for subjects in the paired group, whereas subjects in the unpaired group received the same injection 2 hr later. This 2-hr interval has been shown to be enough to avoid an association between the CS and the unconditioned stimulus (US) in infant rats (Hoffmann, Hunt, & Spear, 1991). During the experimental period, pups remained in holding cages grouped according to litter and treatment. Two hours after the injections, subjects were again placed in their maternity cages.

Tests. On PD 17, all pups were again removed from the home cage, placed in heated holding cages, and cannulated. After a 4-hr deprivation period, consumption of Solutions A (conditioning test) and B (generalization test) was tested in two 15-min trials, in counterbalanced order, with a 4-hr interval between trials.

The data obtained during this and subsequent experiments were analyzed with analyses of variance (ANOVAs); when appropriate, post hoc analyses (Tukey's honestly significant difference tests) were also performed. In all these analyses, a rejection criterion of $p < .05$ was adopted.

Results

Mean consumption of solutions (% BWG) for the paired and unpaired groups during conditioning, Test A, and Test B in Experiments 1A, 1B, and 1C are shown in Panels A, B, and C (respectively) of Figure 1. In the interests of clarity, and given that no effect of the counterbalanced solution was observed in any of the three experiments, data were combined across solutions for each experiment.

As can be seen in the three experiments, consumption of both groups was similar on the conditioning day. In Test A, however, a sharper decrease in consumption was observed for paired subjects than for the unpaired controls. This indicates that the paired treatment was effective in producing a taste aversion learning to Solution A. Moreover, in Test B, a sharper decrease in consumption was also observed for the paired group, compared with the unpaired group, in the three experiments, although in Experiment 1C this decrease was not as marked. This seems to indicate that the conditioned aversion observed in the paired group in Test A was strongly generalized between ETH and SQ and between ETH and VIN and, to a lesser degree, between ETH and COF.

The results of each of the three experiments were analyzed with 2 (conditioning) $\times$ 3 (trial) ANOVAs, with consumption (% BWG) as the dependent variable. As mentioned earlier, the main variable, solution, produced no significant effect, nor did it interact with other variables in any of the three experiments. As a consequence, this variable was not included in the following analyses.

The analysis of Experiment 1A revealed a strong and significant effect of conditioning, $F(1, 54) = 475.96$; trial, $F(2, 108) = 78.12$; and the Conditioning $\times$ Trial interaction, $F(2, 108) = 126.17$. Post hoc analyses of this interaction indicated that pups from the paired group consumed significantly less than those from the unpaired group in both Test A and Test B. These post hoc comparisons also revealed that whereas significant differences in consumption were observed in the paired group between the conditioning day and Tests A and B, and between Test A and Test B, no significant differences were observed in the unpaired group between any of the three trials.

A similar profile was observed in Experiment 1B: significant effects of conditioning, $F(1, 54) = 292.30$; trial, $F(2, 108) = 53.94$; and the Conditioning $\times$ Trial interaction, $F(2, 108) = 71.12$. Post hoc analyses of the interaction indicated that both groups differed significantly in Test A and Test B, consumption being lower for the paired group compared with the unpaired group. As in Experiment 1A, significant differences were observed in the paired group between the conditioning trial and Tests A and B, and between Test A and Test B, whereas no significant differences were observed in the unpaired group between any of the three trials.

The analysis of Experiment 1C indicated an effect of the two main variables: conditioning, $F(1, 54) = 142.71$; trial, $F(2, 108) = 24.24$; and the Conditioning $\times$ Trial interaction, $F(2, 108) = 40.47$. Further analyses of the interaction indicated that, as was the case in Experiments 1A and 1B, both groups differed in Test A and Test B, but not in the conditioning trial. These post hoc comparisons also revealed that, whereas no differences among trials were observed in relation to the unpaired group, in the paired group, intake during the conditioning trial was significantly higher than in Tests A and B. Significant differences were also found between Test A and Test B.

The results suggest that infant rats can readily acquire conditioned aversions to the taste of alcohol and other non-alcohol tastes. Furthermore, and of special interest for this study, they also suggest that these aversions can be generalized between flavored solutions, although apparently in different degrees and with a differential response to Tastes A and B. Within-group comparisons revealed that rats respond differentially to Tastes A and B in all

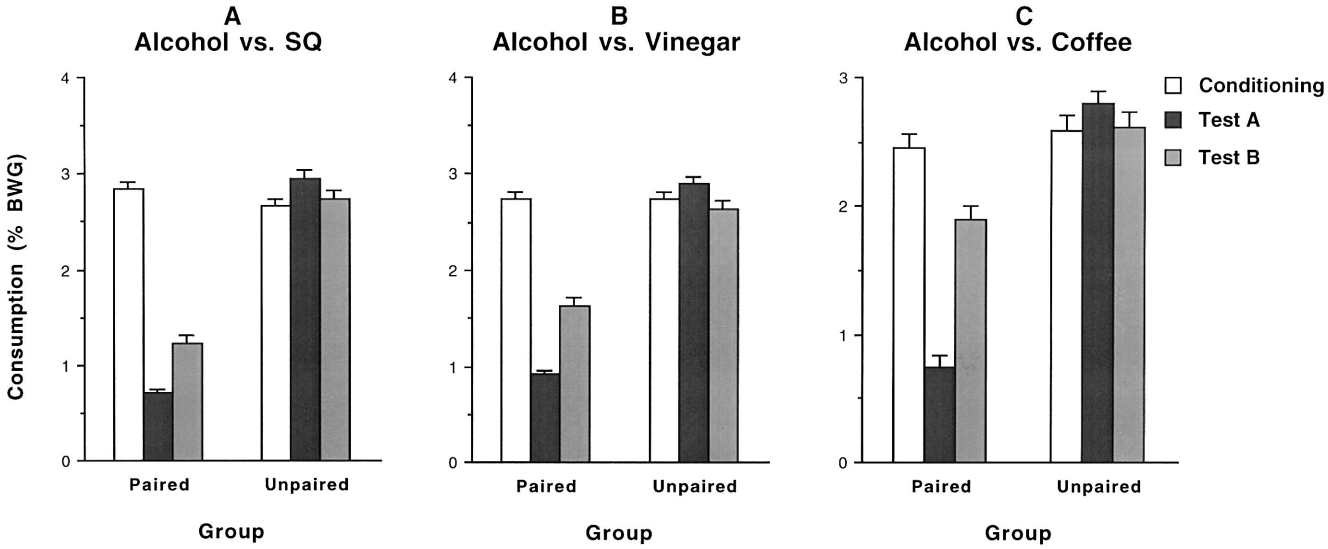

Figure 1. Mean ($\pm$*SEM*) consumption (expressed as percent body weight gain [BWG]) of Solution A during conditioning and Test A and Solution B during Test B, as a function of conditioning treatment (paired or unpaired) and tastes compared in Experiments 1A (alcohol vs. sucrose–quinine [SQ]), 1B (alcohol vs. vinegar), and 1C (alcohol vs. coffee).

three cases, indicating that they discriminate between flavors. As regards the level of generalization, a one-way ANOVA, with consumption of Solution B for the paired groups of the three experiments as the dependent variable, revealed a significant difference, $F(2, 81) = 12.82$. Post hoc analyses indicated that consumption of Solution B for the paired group was lower in Experiment 1A than in Experiments 1B and 1C, the difference in consumption between these last two experiments not being found to be significant. As expected, generalization seems to be stronger between ETH and SQ than between either ETH and VIN or ETH and COF, at least at the concentrations used here.

Furthermore, the results showed once again infants' tendency toward generalization between stimuli. This infantile disposition for generalization was confirmed when alcohol taste was one of the stimuli involved. In light of all the above, it seems worthwhile to ask whether previous experience with these stimuli may help the infant rat to reduce generalization between them or, in other words, to improve discrimination between tastes, as has been the case with other non-alcohol tastes used in previous studies (Chotro & Alonso, 1999, 2001).

Experiment 2

The next step was to analyze the effect of stimulus preexposure on the generalization of the conditioned aversion. Two groups were used in this experiment: one that received alternate preexposure to both the to-be-conditioned stimulus and the to-be-tested one (Group P), and another that did not receive any preexposure (Group NP). Taking into account the results of the generalized aversion and the differential response to Solutions A and B observed in Experiment 1, and in order to ensure enough margin for observing the expected reduction in generalization or enhanced discrimination, the only flavors used in this experiment were ETH and SQ (Experiment 2A) and ETH and VIN (Experiment 2B). Coffee was not included in this experiment because its generalization with respect to alcohol was somewhat lower and no different from that observed with VIN.

Method

Subjects and Apparatus

A total of 112 pups were used, distributed across two experiments ($n = 56$ for each experiment). Subjects were 13–17-day-old male and female rats. Housing and rearing conditions were the same as those described for the previous experiment. Apparatus and solutions were the same as described before, except that coffee was not used. Thus, solutions for Experiment 2A were ETH and SQ; and for Experiment 2B, ETH and VIN. The solution concentrations were the same as in the previous experiments.

Procedure

Preexposure. On PD 13 and PD 14, pups received two preexposure sessions (one per day); each session consisted of three trials with a 2-hr interval between trials. In each trial, subjects received a 15-min intraoral infusion of the corresponding solution. In Experiment 2A, Group P received Solutions A and B (ETH and SQ) in alternate trials, and Group NP received water. In Experiment 2B, pups from Group P received the same treatment as in Experiment 2A, but with ETH and VIN, and pups from Group NP received water. Solutions and order of presentation during sessions were counterbalanced in both experiments.

Conditioning. All pups received two conditioning trials, one on PD 15 and the other on PD 16, in which Solution A was paired with the US.

Tests. Test A and Test B were similar to those described for Experiment 1. All other procedures used in this experiment were similar to those described for Experiment 1.

Results

The results of Experiment 2A are shown in Figure 2. The left-hand panel of the figure illustrates the mean consumption of Solution A for both groups during both conditioning trials and Test A. The right-hand panel shows the mean consumption of Solution B for both groups during Test B. Solutions A and B were ETH and SQ, counterbalanced.

As shown in the left-hand panel, consumption for both groups (Group P and Group NP) was similar during the first conditioning trial. Nevertheless, in the second conditioning trial and in Test A, a marked decrease in consumption of the conditioned solution was observed for all subjects. However, Group P showed a higher consumption than Group NP in the second conditioning trial, indicating a retardation in conditioning as a consequence of stimulus preexposure.

This descriptive analysis was confirmed by a 2 (preexposure) $\times$ 3 (trial) ANOVA, with consumption of Solution A as the dependent variable. Because no effects of solution were found in either of these experiments (as was the case in Experiment 1), this variable is not included in either this or subsequent analyses. The ANOVA indicated a significant effect of preexposure, $F(1, 54) = 7.18$; trial, $F(2, 108) = 285.71$; and the Preexposure $\times$ Trial interaction, $F(2, 108) = 6.55$. Post hoc analyses of the interaction revealed that Group P consumed significantly more Solution A than Group NP only during the second conditioning trial.

The results given in the right-hand panel of Figure 2 show that subjects preexposed to both tastes (Group P) consumed more Solution B in Test B than pups from Group NP. A one-way ANOVA, with consumption of Solution B as the dependent variable, confirmed that Group P consumed significantly more than Group NP, $F(1, 54) = 66.40$.

Furthermore, within-group comparisons revealed that differential consumption of the conditioned solution (A) and the nonconditioned solution (B) was found in Group P, but not in Group NP. This indicates that the generalization of conditioned aversion between alcohol and SQ was so strong that it led to an undifferentiated response to the CS and the tested stimulus. However, a differential response appeared after stimulus preexposure.

The results of Experiment 2B are shown in Figure 3, which illustrates the mean consumption of Solution A during conditioning and test trials (left-hand panel) and the intake of Solution B during Test B (right-hand panel). Solutions A and B were ETH and VIN, counterbalanced.

As was the case in Experiment 2A, both, preexposed and nonpreexposed subjects reduced their intake of the CS across conditioning trials, with preexposed pups showing a slight retardation of conditioning in Trial 2. The 2 (preexposure) $\times$ 3 (trial) ANOVA, with consumption of Solution A as the dependent variable, indicated no significant effects of preexposure, $F(1, 54) = 2.60$; a significant effect of trial, $F(2, 108) = 193.67$; and a Preexposure $\times$ Trial interaction, $F(2, 108) = 5.64$. Post hoc analyses revealed that

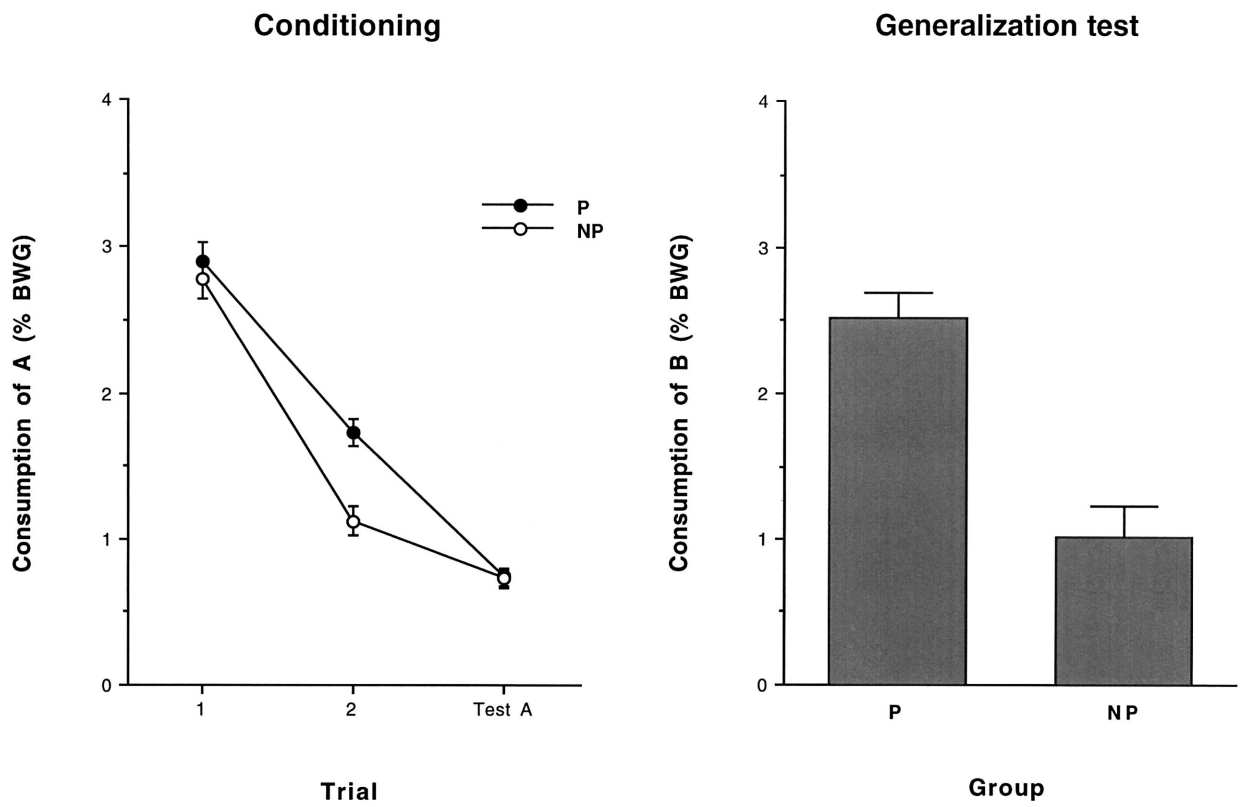

Figure 2. Mean (±*SEM*) consumption (expressed as percent body weight gain [BWG]) of Taste A during both conditioning trials and Test A (left) and of Taste B during Test B (right), as a function of preexposure in Experiment 2A. Tastes A and B were alcohol and sucrose–quinine, respectively, counterbalanced. P = preexposed; NP = non-preexposed.

in the second conditioning trial, Group P consumed significantly more than Group NP.

As can be seen in the right-hand panel of Figure 3, subjects in Group P showed a higher consumption of the nonconditioned solution than subjects in Group NP. A one-way ANOVA confirmed that Group P consumed significantly more than Group NP, $F(1, 54) = 22.31$.

In addition, within-group comparisons between consumption in Tests A and B revealed significant differences for both groups, P and NP. These results, together with the previous ones, suggest that the differential behavior toward ETH and VIN was intensified by stimulus preexposure.

This experiment confirms the previous results showing a generalization of the conditioned aversion between ETH and SQ and between ETH and VIN, the former generalization being stronger than the latter. Moreover, all these results suggest that although infants tend to generalize conditioned aversions between the taste of alcohol and other non-alcohol flavors, previous nonreinforced experiences with both stimuli may help to reduce that generalization and enhance discrimination between them.

Previous results with infant rats suggest that a reduction in generalization is observed whenever an effect of latent inhibition is detected during conditioning (Chotro & Alonso, 1999, 2001). That is, if stimulus preexposure produces a retardation in conditioning, then generalization may appear to be reduced. Although no difference in the final conditioned response was observed during Test A of this experiment, a difference could be masked by a floor effect in consumption. The conditioned strength of Stimulus A could indeed be lower in preexposed subjects than in non-preexposed subjects, given that conditioning was slowed down and, therefore, generalization would be lower as well. So, it is not clear whether stimulus preexposure enhances discrimination between stimuli or just reduces the level of conditioning and, hence, of generalization. In other words, from these results it cannot be inferred whether the reduced generalization stems directly from a stimulus differentiation learned during the preexposure period (perceptual learning effect) or simply from a retardation of conditioning (latent inhibition effect) that affects the subsequent generalization of the conditioned taste aversion. This relationship between latent inhibition and reduction of generalization appears to exist in the present experiments with regard to the tastes of ETH, SQ, and VIN. However, the question remains as to whether preexposure to just the to-be-conditioned stimulus or the alternative taste produces similar reductions in generalized aversion. This was addressed in the following experiment.

Experiment 3

In a previous study with infant rats, it was observed that whenever stimulus preexposure resulted in a retardation of conditioning,

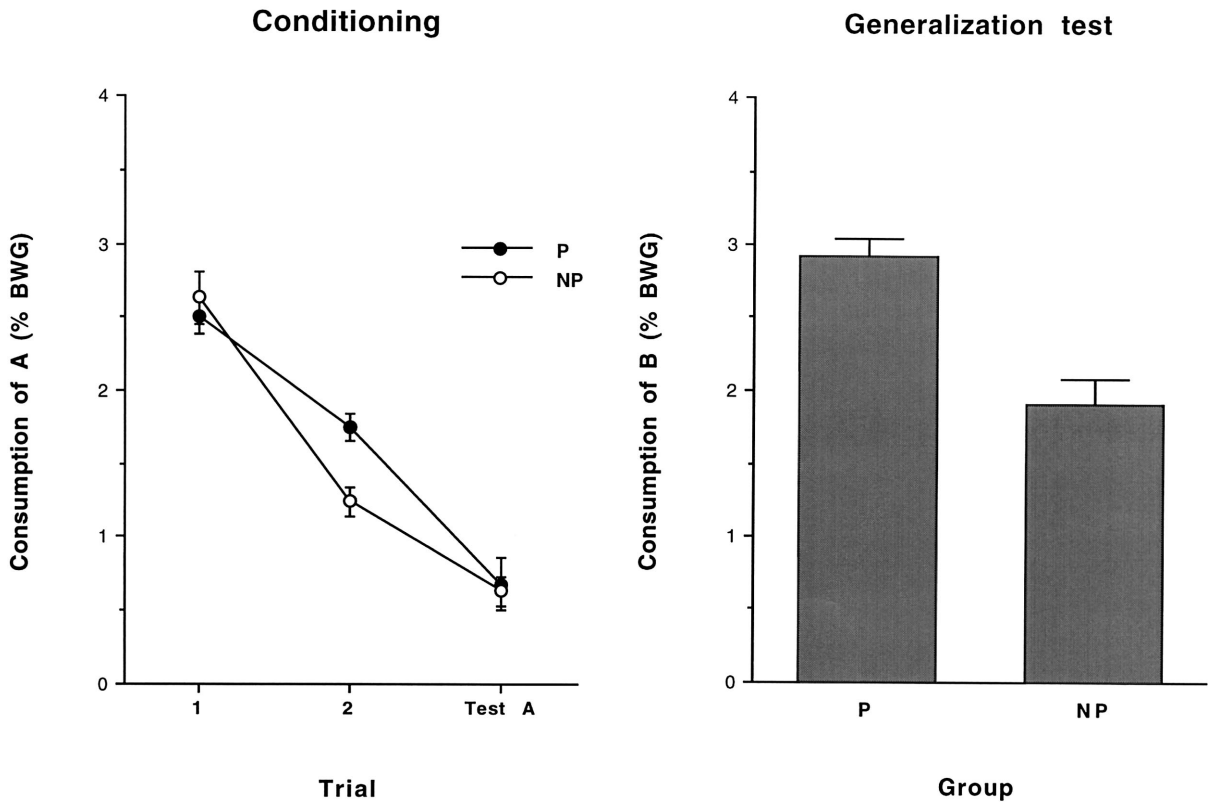

Figure 3. Mean (±*SEM*) consumption (expressed as percent body weight gain [BWG]) of Taste A during both conditioning trials and Test A (left) and of Taste B during Test B (right), as a function of preexposure in Experiment 2B. Tastes A and B were alcohol and vinegar, respectively, counterbalanced. P = preexposed; NP = non-preexposed.

a reduced generalization was obtained, whereas an increased generalized aversion was observed when stimulus preexposure produced a facilitation of conditioning (Chotro & Alonso, 1999, 2001). Those results suggested that reductions in generalization were directly related to the occurrence of latent inhibition during conditioning. If so, preexposure to the CS (A) alone should result in retarded conditioning, and therefore a reduction in generalization would be observed. On the other hand, preexposure to the alternative nonconditioned taste (B) would not produce a latent inhibition effect (it would, at the most, be a generalized latent inhibition effect); consequently, less reduction in generalization should be expected in this case. Conversely, if stimulus preexposure facilitates stimulus discrimination, experiences with Taste A or with Taste B would equally result in a reduced generalization. In any case, preexposure to both stimuli would be expected to produce better knowledge of stimuli and, therefore, better discrimination between them. These hypotheses were tested in this experiment, which analyzed the effect of preexposing either both stimuli or just one of them on the generalization of a conditioned aversion.

Method

Subjects and Apparatus

A total of 112 pups were used, distributed across two experiments (n = 56 for each experiment). Subjects were 13–17-day-old male and female rats distributed into four groups for each experiment, on the basis of stimulus preexposure: Group AB, Group A, Group B, and Group NP. The rats' housing and rearing conditions were the same as those described for the previous experiment. The apparatus was the same as described for Experiment 1. Solutions for Experiment 3A were ETH and SQ; and for Experiment 3B, ETH and VIN. The solution concentrations were the same as in the previous experiments.

Procedure

Preexposure. On PD 13 and PD 14, pups received two preexposure sessions (one per day); each session consisted of three trials, with a 2-hr interval between trials. During each trial, subjects received a 15-min intraoral infusion of the corresponding solution. In Experiment 3A, Group AB received Solutions A and B (ETH and SQ) in alternate trials, Group A received only Solution A (ETH or SQ) alternated with water, Group B received equivalent trials of Solution B (SQ or ETH) and water, and Group NP received only water. In Experiment 3B, Group AB received both solutions (ETH and VIN), Group A received only Solution A (ETH or VIN) and water, Group B received equivalent trials of Solution B (VIN or ETH) and water, and Group NP received water. Solutions A and B and order of presentation during sessions were counterbalanced in both experiments.

Conditioning. All pups received two conditioning trials, one on PD 15 and the other on PD 16, in which Solution A was paired with the US for all subjects as in Experiment 2.

Tests. Tests A and B were similar to those described for the previous two experiments. All other procedures were similar to those described for the previous two experiments.

Results

The results of Experiment 3A are shown in Figure 4. The left-hand panel illustrates the mean consumption of Solution A for all four groups during both conditioning trials and Test A. The right-hand panel shows the mean consumption of Solution B for all groups during Test B. Solutions A and B were ETH and SQ, counterbalanced.

As shown in the left-hand panel, consumption for Groups AB, A, B, and NP was similar during the first conditioning trial. All groups showed a decrease in consumption across trials. Nevertheless, in the second conditioning trial, Group AB and Group A showed a higher consumption of Solution A than Group B or Group NP, indicating a retardation in conditioning as a consequence of preexposure to the CS. However, this difference seems to disappear in Test A. A 4 (preexposure) × 3 (trial) ANOVA revealed a main effect of preexposure, $F(3, 52) = 6.73$, and of trial, $F(2, 104) = 824.53$, with no interaction between the two variables. Post hoc analyses of these effects indicated that all groups significantly reduced their consumption across conditioning trials and that Groups AB and A consumed significantly more Solution A than Groups B and NP.

The right-hand panel reveals that the consumption of Solution B was higher in Groups AB and B than in the other two groups. A one-way ANOVA confirmed that differences between groups were significant, $F(3, 52) = 23.80$. Post hoc analyses revealed that Groups AB and B showed a higher consumption than Groups A and NP.

Within-group comparisons revealed significant differences between the consumption of Solutions A and B for all groups except Group NP. So, apparently, the undifferentiated response to A and B showed by the non-preexposed subjects was affected by preexposure, particularly by preexposure to the two stimuli or to only the tested stimulus (B).

Results suggest that with these stimuli, ETH and SQ, preexposure to both tastes (A, B) or only to the non-conditioned taste (B) reduced the magnitude of the generalized aversion, whereas preexposure to just the CS (A) did not affect the level of generalization of the conditioned aversion.

The results of Experiment 3B are shown in Figure 5. In this experiment, using ETH and VIN as gustatory stimuli, a similar profile was observed with regard to conditioning (left-hand panel). The 4 (preexposure) × 3 (trial) ANOVA, with consumption of Solution A as the dependent variable, revealed significant effects of both main factors: preexposure, $F(3, 52) = 3.31$; trial, $F(2, 104) = 453.21$; and the Preexposure × Trial interaction, $F(6, 104) = 3.36$. Post hoc comparisons indicated that Group NP

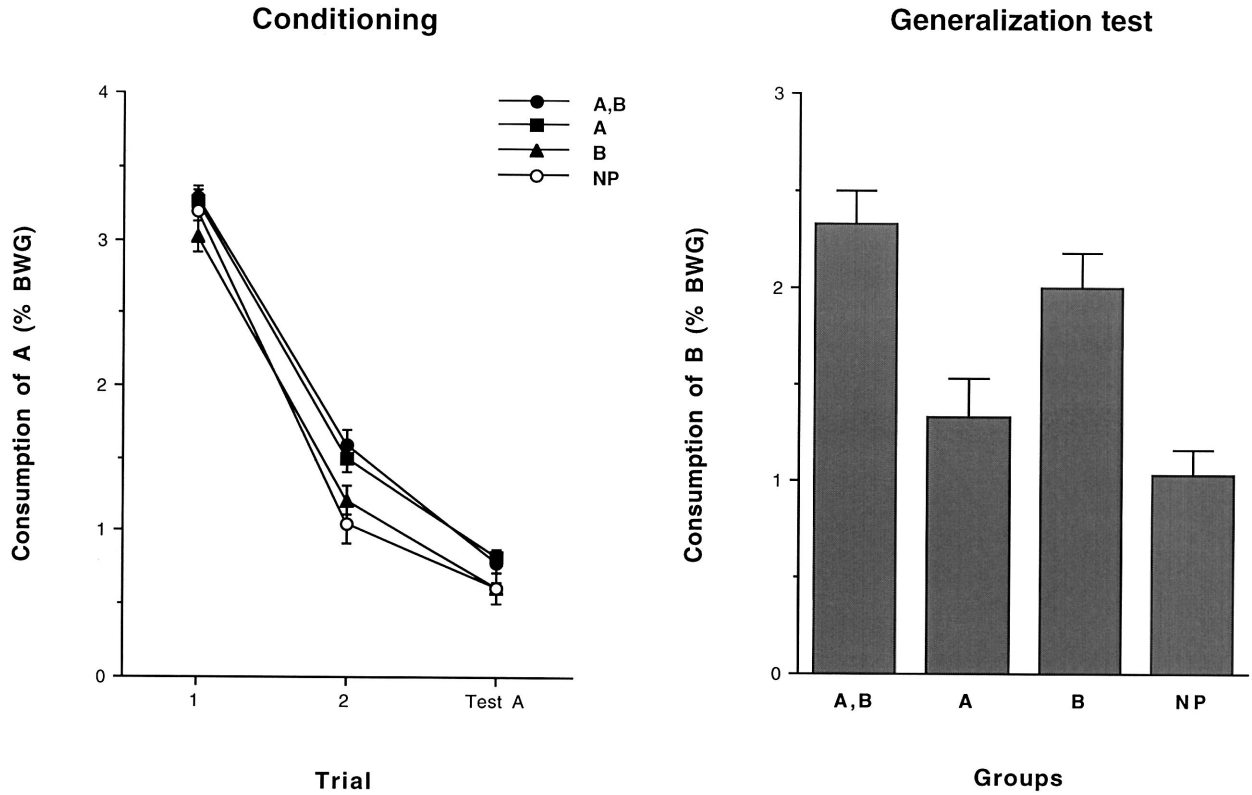

Figure 4. Mean (±*SEM*) consumption (expressed as percent body weight gain [BWG]) of Taste A during both conditioning trials and Test A (left) and of Taste B during Test B (right), as a function of preexposure in Experiment 3A. Tastes A and B were alcohol and sucrose–quinine, respectively, counterbalanced. NP = non-preexposed.

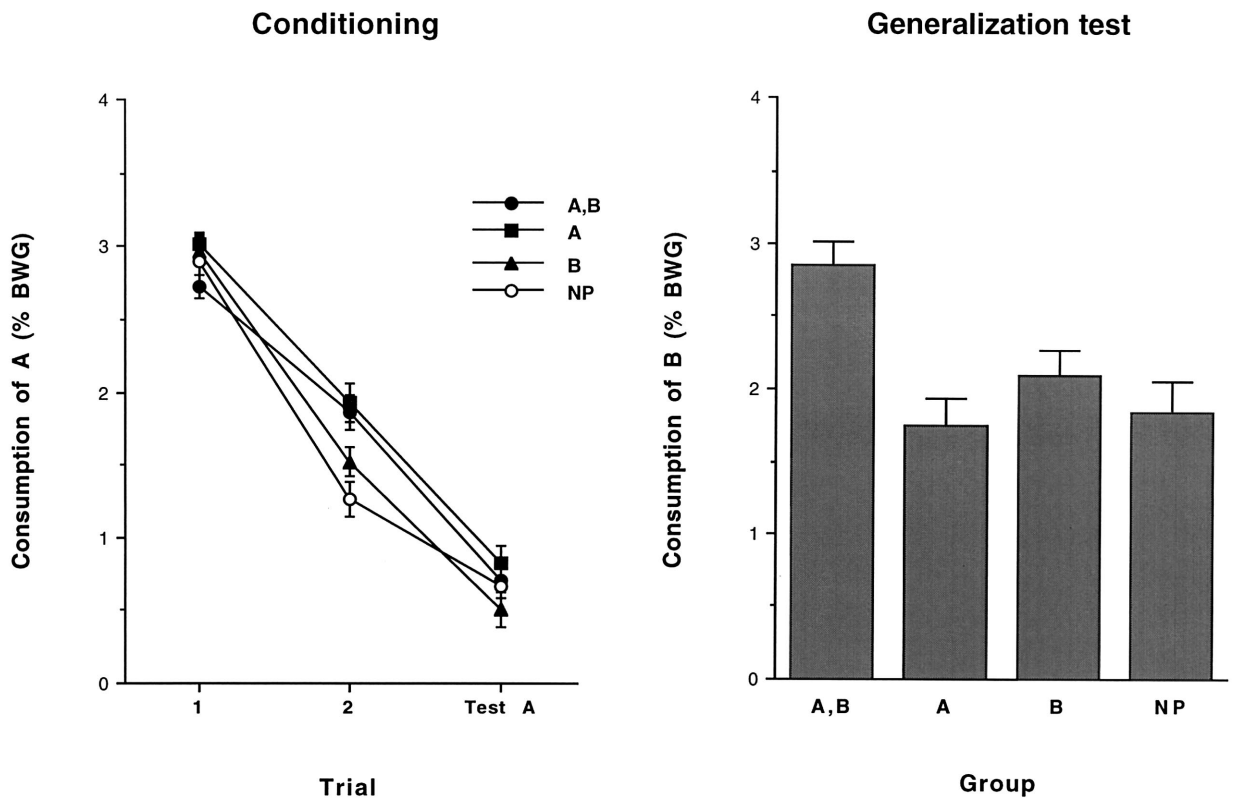

Figure 5. Mean (±*SEM*) consumption (expressed as percent body weight gain [BWG]) of Taste A during both conditioning trials and Test A (left) and of Taste B during Test B (right), as a function of preexposure in Experiment 3B. Tastes A and B were alcohol and vinegar, respectively, counterbalanced. NP = non-preexposed.

consumed less than Groups AB and A during the second conditioning trial.

During Test B (see Figure 5, right-hand panel), Group AB consumed more Solution B than the other three groups. This was confirmed by a one-way ANOVA indicating that differences between groups were significant, $F(3, 52) = 7.49$. Post hoc analyses revealed that Group AB consumed significantly more than any other group.

However, within-group comparisons revealed significant differences between the consumption of Solutions A and B in all conditions. So, the differential response to A and B was observed in every case and was apparently only affected by preexposure to A and B.

On the whole, these results showed that, similar to what was found in Experiment 2, preexposure to both stimuli reduced generalization of the conditioned aversion between ETH and SQ and between ETH and VIN. However, contrary to Experiment 2, there was no clear correspondence between conditioning and generalization. Preexposure to A and B reduced generalization in both Experiments 3A and 3B, but only in the latter was it preceded by a latent inhibition effect. Furthermore, preexposure to the CS alone also produced a retardation of conditioning in Experiment 3B, although, contrary to what was conjectured, no reduction in generalization was observed in Group A, with ETH, SQ, or VIN as alternative tastes. Moreover, when the stimuli were SQ and ETH, preexposure to only the nonconditioned taste (Group B) resulted in a reduced generalized aversion that was not preceded by a retardation of conditioning. Nevertheless, this effect was not observed when tastes were VIN and ETH. Perhaps the strong level of generalization observed with SQ and alcohol favored the observation of different stimulus preexposure effects.

General Discussion

The results of the three experiments show that infant rats generalize conditioned taste aversions between alcohol and other non-alcohol tastes, such as a mixture of sucrose and quinine, apple cider vinegar, or coffee, as a function of similarity between stimuli (Experiment 1). Data also show that previous nonreinforced experience with alcohol and one of the other mentioned tastes (sucrose–quinine, apple cider vinegar) results in a reduced generalization of the conditioned taste aversion between them (Experiments 2A and 2B). Finally, when alcohol and sucrose–quinine were used as stimuli, preexposure to both stimuli as well as to the nonconditioned one alone reduced the generalized aversion (Experiment 3A), whereas when alcohol and vinegar were used, both tastes had to be preexposed in order to obtain that effect in rat pups (Experiment 3B). In no case was reduced generalization found when only the to-be-conditioned taste was preexposed.

Generalization of conditioned taste aversions between alcohol and other tastes have been reported before, but only with adult rats (DiLorenzo et al., 1986; Kiefer & Mahadevan, 1993), and this is

the first time that these results have been clearly observed with infant rats. The high level of generalization between stimuli, however, is not surprising, because of the well-known infantile disposition for stimulus generalization (Spear & Riccio, 1994). This characteristic makes rat pups ideal subjects for studying the beneficial effects of prior experience on stimulus discrimination.

In this study, previous experience with the taste of alcohol and other non-alcohol flavors was effective in reducing generalization between those two stimuli. However, considering that in most cases preexposure produced a retardation of conditioning in preexposed groups, the question arose as to whether the reduced generalization was due to a better stimulus discrimination between Stimuli A and B or to a weaker conditioning of A. This was further analyzed by preexposing subjects to the CS (A) alone or to the alternative taste (B). Contrary to what was expected, the effect of preexposure to just one of the stimuli on generalization was not dependent on the occurrence of a retardation in the acquisition of conditioning. In some cases, exposure to the to-be-conditioned stimulus alone produced an effect of latent inhibition on conditioning but did not produce a significant reduction in the generalization of that conditioning. On the other hand, exposure to the alternative taste reduced generalization without a latent inhibition effect being observed in any case. This challenges our previous conclusions from studies with non-alcohol tastes in infant rats (Chotro & Alonso, 1999, 2001) and seems to indicate that familiarity with only the to-be-differentiated stimulus may be enough to produce a reduction in generalization.

With adult rats, different effects on generalization have been found after exposure to only one stimulus. In some studies, a reduction in generalization between a pair of stimuli (A and B) was reported after preexposing only Stimulus A (e.g., Honey, 1990). In such investigations, the possibility that the reduced generalization was the result of the decreased associative strength suffered by A after preexposure cannot be discarded. Various other studies reported no differences between preexposing only B or only A (e.g., Symonds & Hall, 1995). Another set of studies reported a stronger reduction in generalization after preexposing B than after preexposing only A, or even after preexposing both stimuli (Sanjuán, Alonso, & Nelson, 2002). Finally, some studies using context conditioning have shown that a short preexposure to Context A may facilitate its conditioning and, at the same time, reduce generalization to Context B (e.g., Kiernan & Westbrook, 1993).

The results of this study suggest that enhanced discrimination between a pair of stimuli is observed when subjects have previous experience with the nonconditioned stimulus, although a contrasted experience with both stimuli appears to be much more effective, at least in infant rats.

Focusing on alcohol, these results suggest that infants may learn to discriminate between the flavor of alcohol and other similar tastes by simple exposure to them and that previous experience with alcohol seems to lead to a better recognition of this drug. Studies with human neonates and infants have shown that identification of alcohol is enhanced in those subjects with previous experience with the drug (Faas et al., 2000; Mennella & Garcia, 2000; Noll et al., 1990). It can be speculated, then, that the mechanisms underlying this improved recognition of alcohol's sensory characteristics reported in human infants may be related to those mechanisms that control the effect of enhanced alcohol discrimination observed in this study with infant rats.

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