Research

Preexposure to salty and sour taste enhances conditioned taste aversion to novel sucrose

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Conditioned taste aversion (CTA) is an intensively studied single-trial learning paradigm whereby animals are trained to avoid a taste that has been paired with malaise. Many factors influence the strength of aversion learning; prominently studied among these is taste novelty—the fact that preexposure to the taste conditioned stimulus (CS) reduces its associability. The effect of exposure to tastes other than the CS has, in contrast, received little investigation. Here, we exposed rats to sodium chloride (N) and citric acid (C), either before or within a conditioning session involving novel sucrose (S). Presentation of this taste array within the conditioning session weakened the resultant S aversion, as expected. The opposite effect, however, was observed when exposure to the taste array was provided in sessions that preceded conditioning: such experience enhanced the eventual S aversion—a result that was robust to differences in CS delivery method and number of tastes presented in conditioning sessions. This "non-CS preexposure effect" scaled with the number of tastes in the exposure array (experience with more stimuli was more effective than experience with fewer) and with the amount of exposure sessions (three preexposure sessions were more effective than two). Together, our results provide evidence that exposure and experience with the realm of tastes changes an animal's future handling of even novel tastes.

Conditioned taste aversion (CTA) is a reliable and robust form of associative learning wherein a taste (the conditioned stimulus, CS) is paired with malaise (the unconditioned stimulus, US), and thereby rendered aversive (Garcia et al. 1955; Bouton 1994; Bures et al. 1998; Welzl et al. 2001; Reilly and Schachtman 2009). As is true for other forms of simple conditioning, manipulation of any number of factors can alter the potency of taste aversion learning. For example, previous exposure with the conditioning context, or with the CS itself, has been shown to attenuate learning of the conditioned response (CR—typically reduced consumption, Lubow and Moore 1959; Lubow 1973; Tarpy and McIntosh 1977). This CS preexposure effect, which is commonly referred to as latent inhibition, has been characterized as a learned association between a safe outcome and the taste experience (Lubow 1973).

Given that benign taste experiences impact learning, it is reasonable to ask whether even incidental experience with tastes other than the CS might affect the later associability of the CS. This question takes on particular significance given the fact that all human learning with taste occurs on the backdrop of extensive prior taste experiences—experience that a laboratory rat entirely lacks. The handful of studies that have addressed this topic suggest that rats exposed to one taste (either developmentally or as adults) become more accepting of—that is, less neophobic to—the first presentation of a novel taste (although not of novel sucrose; see Miller and Holzman 1981b), compared with rats previously exposed to water alone (Capretta et al. 1975; Tarpy and McIntosh 1977; Miller and Holzman 1981a,b; Franchina and Gilley 1986) and further suggest that exposure to a "strong" novel taste can enhance latent inhibition learning to a "weak" taste presented immediately afterward (Gentle et al. 2006; Merhav and Rosenblum 2008). No effect of prior taste experience on later CTA toward sucrose was noted in these studies, but this apparent null result could simply reflect the general robustness of CTA—that is, floor effects in consumption which could mask between-group differences—or, in the case of the latter study, the competing impact of prior experience on CTA memory (see Discussion).

Exposure to an array of tastes have also been found to facilitate extinction of CTA acquired to novel sucrose (Moran and Katz 2014), a finding consistent with those shown in relation to experience with more complex foods (Gentle et al. 2006). This fact could bespeak either an impact on the initial aversion strength or a change in general sucrose associability (or both). However, no efforts were made in this most recent study to parametrically examine the impact of experience on CTA, and thus no firm conclusions regarding this specific variable could be reached.

Here we have programmatically examined how exposure to a small array of tastes prior to conditioning influences CTA induced to a novel taste. The experimental design was reminiscent of classic latent inhibition protocols, but the CS was explicitly excluded from the array presented prior to conditioning. Our results reveal that preexposure to a taste array indeed alters subsequent CTA to a novel taste, increasing the strength of the aversion. This effect was observed regardless of whether multiple tastes were also delivered during the conditioning session, and regardless of whether the CS was administered via intraoral cannula or lick spout. Further tests confirmed that the effect scaled appropriately with the number of tastes in the exposure array and with the number of exposure sessions.

On the basis of these experiments, we suggest that experience with tastes, offered without negative outcome, enables an animal to more strongly associate a novel taste with an aversive outcome

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at a later time. This fact has implications for theories of learning, and specifically suggests limitations on the generalizability of experiments performed on animals lacking prior experience with a range of taste stimuli.

Results

Experiment 1: when an array of tastes is delivered during the conditioning session, CTA toward sucrose is attenuated

The experimental protocol spanned 4–5 d total (1 session per day)—2–3 d of exposure to a taste array (non-CS preexposure), 1 d of conditioning and 1 d of testing (Fig. 1). Taste solutions used in the experiments consisted of sodium chloride (N), citric acid (C), distilled water (W), and sucrose (S), which was consistently used as the conditioned stimulus. In all experiments the unconditioned stimulus was a subcutaneous injection of lithium chloride (see Materials and Methods).

As confirmation of the validity of our paradigm, we first tested the expectation that animals should show a relatively weak aversion toward a sucrose CS when the conditioning session includes exposure to N, W, and C in addition to S (compared with control rats that receive S alone in the conditioning session). Tastes were delivered via intraoral cannula (IOC; see Materials and Methods). On conditioning day, rats ($N = 8$) received S via lick spout; to ensure control of exposure (see Materials and Methods), the taste array consisting of N, W, and C was presented via IOC. Control rats ($N = 7$) received the same protocol, but without the delivery of the taste array. We predicted that animals receiving an array of tastes during the conditioning session would, because of overshadowing among those tastes, show a reduced aversion toward S when compared with animals that received S alone (Pavlov 1927; Logue 1979; McLaren and Mackintosh 2002), despite the fact that the S was delivered by a distinct route.

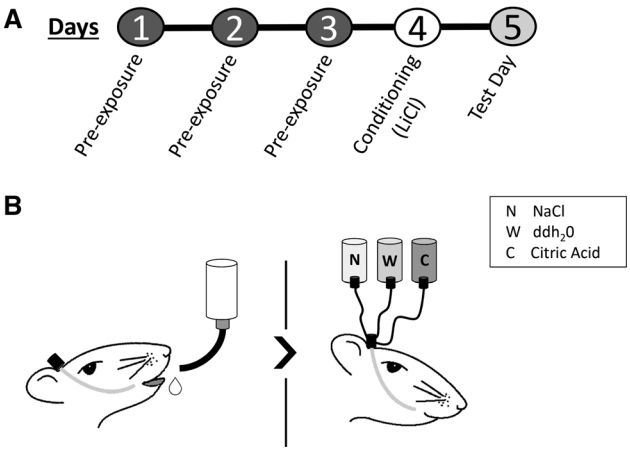

Figure 1. Preexposure paradigm. (*A*) Preexposure paradigm for IOC + Bottle CS delivery experiments. Five-day experimental paradigm where animals receive preexposure to salty and sour tastes or water alone over 2–3 d (dark gray circles days 1–3). Aversions are conditioned on the fourth day across all experiments (white circle day 4). Animals receive bottle and IOC access to sucrose that is immediately paired with LiCl (0.3 M, 0.5% of current body weight) or saline (control). On day 5, aversions are tested by exposure to sucrose via bottle spout for 5 min (light gray circle day 5). Following a 5-min break, animals are then presented with water via lick spout for 5 min. (*B*) During each preexposure session animals are first presented with 5 min of bottle access to a control substance (*left*). Following a 5-min intermission, preexposure session animals are presented with salty (N) and sour (C) tastes in addition to water (W) via IOC (*right*).

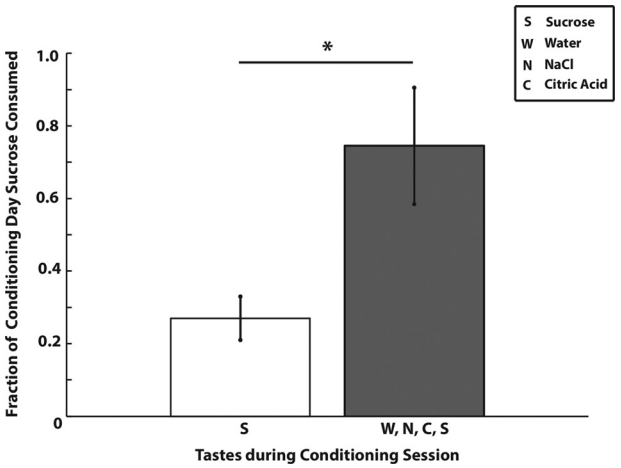

Figure 2. Exposure to a taste array during conditioning attenuates CTA acquired to a novel taste. Rats exposed to a taste array (S, W, N, C) in the conditioning session (gray bar, measured in terms of fraction of sucrose consumption in conditioning sessions) acquire a milder aversion to the sucrose CS, compared with animals who received S alone during conditioning (white bar). *X*-axis represents aversion indices for each animal ($C_{STestConsumed}$ mL/$C_{SConditioningConsumed}$ mL). Error bars represent SEM. (*) $P < 0.05$.

The results of this experiment are shown in Figure 2. An independent samples *t*-test revealed that the fraction of conditioning day consumption (0.74 ± 0.45) on test day was, as predicted, significantly higher ($t_{(13)} = -2.621$, $P < 0.05$; reflecting a weakened aversion) for animals that received a small array of tastes in addition to sucrose during the conditioning session in comparison to animals receiving only sucrose on conditioning day (0.27 ± 0.15). Thus, Experiment 1 confirmed our (and common) expectation of an overshadowing effect (Pavlov 1927; Logue 1979; McLaren and Mackintosh 2002): when multiple tastes are presented within the conditioning session, (even via a delivery method different from that used to deliver the CS) the resultant CTA to sucrose is diminished.

Experiment 2: when an array of tastes is delivered during non-CS preexposure sessions, CTA toward a novel CS is strengthened

We next assessed the strength of CTAs to S when rats were exposed to either an array of tastes (W, N, C) or water only ($N = 17$ and 15, respectively) for three sessions prior to conditioning day (non-CS preexposure; Fig. 3). We tested this using two experimental protocols: one in which, consistent with the previous experiment, we delivered the CS through a lick spout, and a second in which the CS was delivered through the IOC; during the non-CS preexposure sessions, tastes were necessarily IOC administered, in order to ensure equivalent consumption of each across all experiments (see Materials and Methods). We also varied whether the rats received W, N, C in the conditioning session (i.e., we counterbalanced the number of tastes in preexposure sessions and number of tastes in the conditioning session), but as this variable had no statistical impact on the effect described below (i.e., we observed no significant interaction effect in a two-way ANOVA) and was of no theoretical interest, we collapsed across those groups for presentation.

As revealed by one-way ANOVA and shown in Figure 3, non-CS preexposure (W, N, and C) had a significant impact on

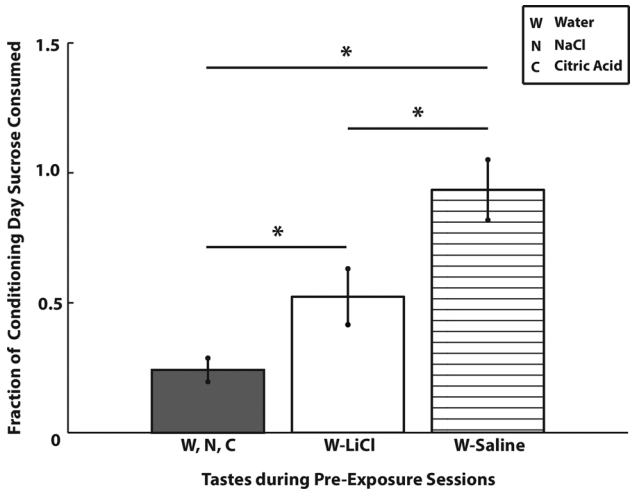

Figure 3. Preexposure to small taste array during preexposure sessions enhances later learning to a novel taste. A one-way ANOVA showed that rats preexposed to W, N, and C across three preexposure sessions (gray bar) significantly enhanced learning (i.e., decreased test day sucrose consumption). Additional analysis demonstrated that both groups (W, N, C) and (W-LiCl) showed significantly enhanced learning than animals given a sham US (W-Saline; stripped bar). *X*-axis represents aversion indices for each animal ($C_{STestConsumed}$ mL/$C_{SConditioningConsumed}$ mL). Error bars represent SEM. (*) $P < 0.05$.

conditioning to novel S ($F_{(1,31)} = 6.35$, $P < 0.05$). CTAs to S were stronger when rats underwent preexposure to an array of tastes (W, N, and C; 0.24 ± 0.18) than when exposed to water alone (W-LiCL; 0.52 ± 0.41) indicating that prior experience with W, N, and C enhanced learning about S. Further analysis additionally demonstrated that both non-pre-exposed rats ($t_{(22)} = -2.47$, $P < 0.05$) and preexposed rats ($t_{(24)} = -6.641$, $P < 0.05$) developed significantly stronger aversions to sucrose in comparison to control rats (0.93 ± 0.34) that received injections of saline instead of lithium chloride (which as expected showed no evidence of aversion learning).

We tested the generalizability of the phenomenon by manipulating variables within the conditioning session. First, we tested whether the impact of innocuous preexposure was robust to CS delivery method: some groups of rats received S via lick spout during the conditioning session—a method that ensured easy-to-measure and strong (under the constraints of the mild US) conditioning, but that required that the S be delivered by a different method than that used to deliver non-CS tastes in preexposure sessions (again, it was vital to deliver N and C via IOC, lest rats refuse to consume the latter, which is aversive); therefore, we also ran groups of rats for which S was delivered solely via the IOC—that is, in an identical manner to the non-CS preexposure array. If the effect that we report is truly a general effect of taste exposure and familiarization, this difference in protocols should have no impact on the basic results.

In fact, non-CS preexposure did strengthen CTA toward novel sucrose regardless of the CS acquisition method. As revealed by a two-way ANOVA with variables experience (preexposed and non-pre-exposed rats) and CS acquisition method (IOC or Bottle and IOC) and displayed in Figure 4A, there was a main effect of preexposure to tastes ($F_{\text{preexposure }(1,29)} = 5.23$, $P < 0.05$). There was no main effect of CS acquisition method ($F_{\text{CS acquisition }(1,29)} = 2.62$, $P = 0.11$), and no significant interaction between the method of CS acquisition and CTA strength ($F_{\text{interaction }(1,29)} = 0.218$, $P = 0.64$). Regardless of whether CS was delivered via IOC and lick spout or via the IOC alone as the non-CS preexposure array, preexposure sessions strengthened learning (Fig. 4B). We therefore did not examine mode of CS acquisition further.

Another variable that can influence the strength of an aversion learned to a specific taste CS is the amount of CS consumption during conditioning—higher initial consumption can lead to stronger learning. Despite our normalization of the aversion index (see Materials and Methods), this fact could conceivably provide an alternative explanation for the above results: non-CS preexposure has been suggested to decrease neophobic responses to certain later-presented novel tastes (although not to sucrose, see Miller and Holzman 1981b); such a reduction in neophobia could have increased CS consumption in the conditioning session, and this potential increase in initial sucrose consumption could conceivably have resulted in stronger conditioning for non-CS preexposed rats.

To explicitly examine this possibility, we directly examined whether there was any evidence of learning being a function of

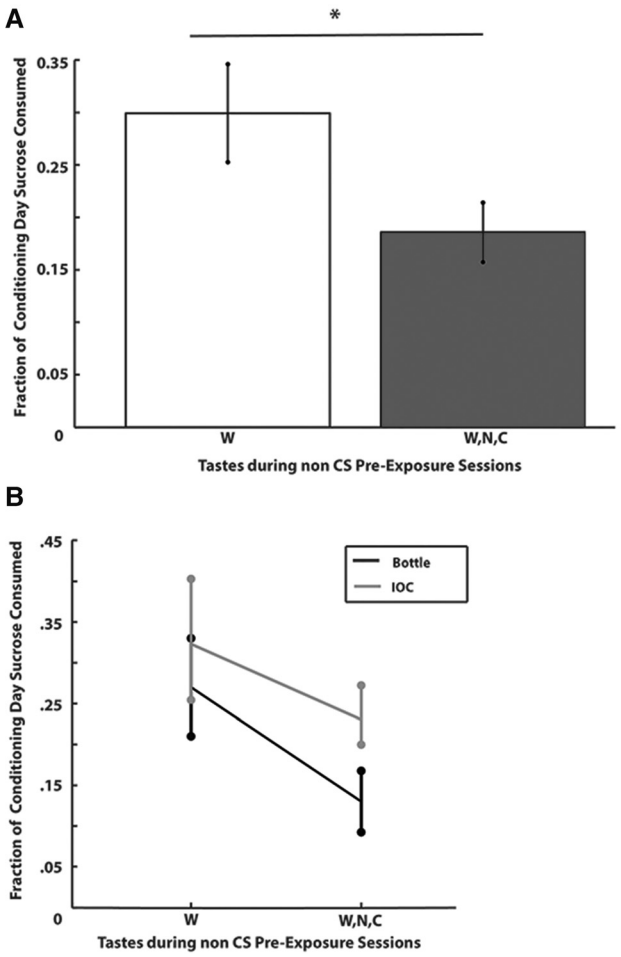

Figure 4. The effect of preexposure to tastes occurs regardless of the CS acquisition method. Animals were preexposed to W or W, N, and C via the IOC during preexposure sessions. Bottle groups received the conditioned stimulus via both IOC and Bottle while the IOC group received the CS via the IOC alone. (*A*) A main effect of preexposure to tastes was found between water (W) and taste array (W, N, C) groups regardless of CS acquisition. (*B*) Aversion strength toward sucrose was stronger in animals who received preexposure to W, N, and C in comparison to animals exposed to water alone regardless of CS acquisition method. *X*-axis represents aversion indices for each animal ($C_{STestConsumed}$ mL/$C_{SConditioningConsumed}$ mL). Error bars represent SEM. (*) $P < 0.05$.

conditioning session consumption, calculating the correlation between the raw S consumption and the aversion index for all rats that had been allowed ad lib access during the conditioning session (i.e., for all rats conditioned by lick spout + IOC). As Figure 5A makes clear, we found no correlation between these variables ($R = -0.002$, $P = 0.98$). Nor was there a trend toward more conditioning day consumption for rats receiving more preexposure tastes, or even significant differences across animals receiving W, W, and N or W, N, and C during preexposure sessions ($F_{(43,4)} = 0.897$, $P = 0.63$; Fig. 5B). Thus, it does not appear that a reliable impact of non-CS preexposure on CS consumption during the training session consumption underlies the strengthening effect that the preexposure has on aversion learning (see Discussion).

Experiment 3: strength of aversion with non-CS preexposure scales with the number of tastes in exposure sessions

If our interpretation of the above results is correct—if prior experience with an array of tastes enhances later learning regarding a novel taste—then several implications follow. It stands to reason, most generally, that this enhancement, if truly linked to prior experience with tastes, should scale with the amount of preconditioning taste experience—operationalized either in terms of: (1) number of tastes; or (2) number of sessions.

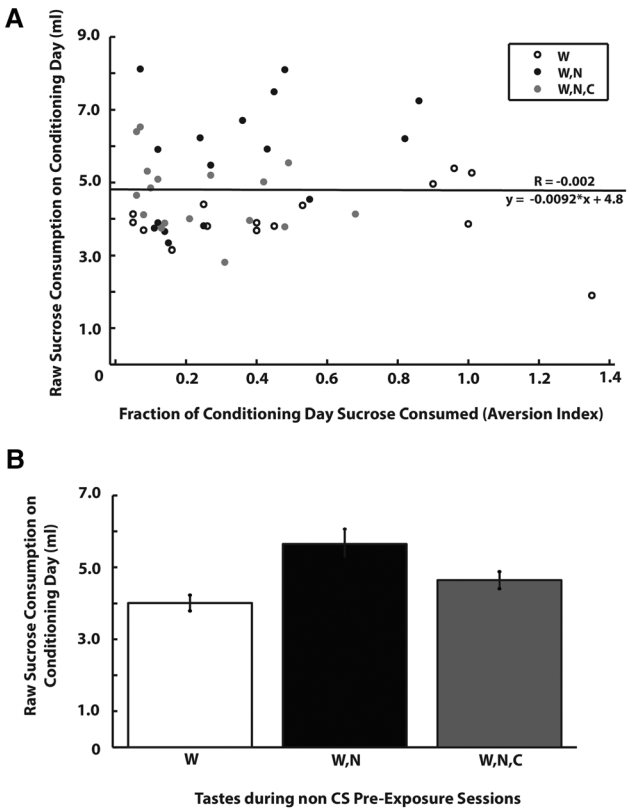

Figure 5. Conditioning day consumption and aversion strength. (*A*) Aversion indices were not correlated with the amount of raw CS consumed on conditioning day across all taste preexposure groups (see legend). *Y*-axis represents raw sucrose consumption (mL) on conditioning day. *X*-axis represents aversion indices for each animal ($C_{STestConsumed}$ mL/$C_{SConditioningConsumed}$ mL). (*B*) Raw consumption of novel sucrose did not scale with number of tastes delivered during preexposure (one-way ANOVA, $P > 0.05$). Error bars represent SEM.

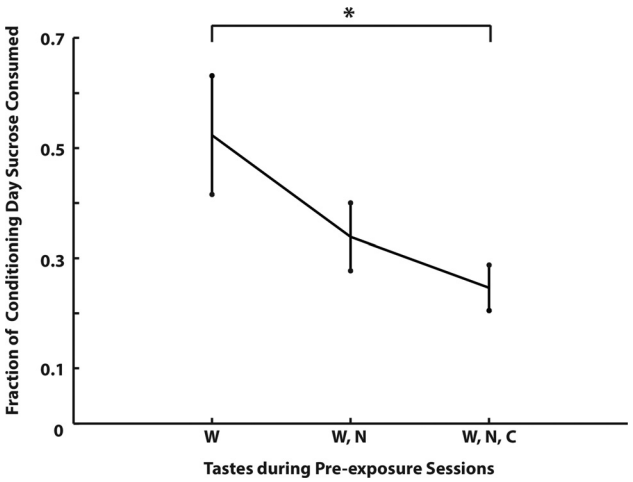

Figure 6. The preexposure effect scales with number of tastes in the preexposure session. Animals were preexposed to W, W, and N, or W, N, and C during three preexposure sessions. Strength of conditioning increased with the size of the preexposure array. *X*-axis represents aversion indices for each animal ($C_{STestConsumed}$ mL/$C_{SConditioningConsumed}$ mL). Error bars represent SEM. (*) $P < 0.05$.

We tested the first version of this prediction with Experiment 3, in which we compared the magnitude of CTA formed to S for rats who underwent preexposure to either to W only ($N = 15$), W, and N (one taste plus water, $N = 16$), or W, N, and C (two tastes plus water, $N = 17$). We specifically predicted that aversions learned to novel S would be stronger the more tastes were experienced in non-CS preexposure sessions (once again we performed this test on rats that either received an array of tastes during the conditioning session or not, but once again, a two-way ANOVA demonstrated that this conditioning-session factor did not interact significantly with the preexposure effect).

As revealed by one-way ANOVA and shown in Figure 6, our prediction was borne out: the more diverse the array of tastes available during exposure sessions, the stronger a CTA was learned to novel S ($F_{(2,47)} = 3.75$, $P < 0.05$); post hoc Scheffe tests revealed that the aversion index was significantly lower following preexposure to W, N, and C (0.24 ± 0.11, $P = 0.03$) than it was following preexposure to W alone (0.52 ± 0.41), with the aversion following preexposure to W and N falling approximately half way between the two extremes (0.33 ± 0.24). While the intermediate level of this variable did not differ significantly from either extreme (a common result for adjacent values on a curve), ancillary analysis of the entire data set revealed a significant negative linear correlation between number of tastes and testing-session consumption ($R = -0.353$, $P < 0.05$). These results confirm and extend the results of Experiment 2, showing that the more diverse a rat's prior tasting experience, the stronger the later aversion learned to a novel taste.

Experiment 4: strength of aversion with non-CS preexposure scales with 2 and 3 preexposure sessions

With Experiment 4 we further tested the most primary implication of our hypothesis. We reasoned that, to the extent that the magnitude of a CTA to novel S is increased by prior taste experience, CTA magnitude should scale not only with the number of tastes in the non-CS preexposure array (as shown in Experiment 3) but with the number of exposure sessions themselves, as well. Specifically, we investigated how the impact of W, N, and C preexposure on later aversions to novel sucrose differed depending on

whether the array was presented for 2 ($N = 9$) or 3 ($N = 9$) preexposure sessions.

We also ran an additional group of rats ($N = 9$) that received only a single session of W, N, and C preexposure. This group was added to explicitly test whether novelty of the tastes is a determining factor in the impact of innocuous preexposure: as noted in the Introduction, stimulus novelty is often cited as being a powerful parameter in a learning paradigm (Miller and Holzman 1981a; Lubow 2009); in particular, it appears that only novel stimuli can cause "behavioral tagging," a theoretical process whereby presentation of a stimulus 1 h before (or 2.5 h after) can potentiate subthreshold learning—a phenomenon showing a certain similarity to that described here (but see Discussion; Ballarini et al. 2009).

We reasoned that if novelty alone determines the impact of preexposure on later learning, we would see decreased enhancement of learning (i.e., higher testing session consumption) with increasing numbers of preexposure sessions. If, on the other hand, experience alone determines the phenomenon, we would see the opposite result.

The surprising result of this experiment, which accords with neither of the simple hypotheses above, is shown in Figure 7. A one-way ANOVA revealed a significant effect of session ($F_{(3,49)} = 4.06$, $P < 0.05$), and post hoc tests (Tukey–Kramer) revealed to reflect a "U-shaped curve" that is, two sessions of preexposure (0.41 ± 0.35) enhanced learning less than either one single session (0.15 ± 0.04, $P = 0.04$) or three sessions (0.13 ± 0.11, $P = 0.02$) and was not significantly different then animals exposed to water alone (0.25 ± 0.16, $P = 0.15$; Fig. 7). Both amount and novelty of innocuous experience appear to exert powerful impacts on later learning; our basic hypothesis is confirmed, but Experiment 4 also reveals what appears to be an entirely separate but countervailing influence of novelty (see Discussion).

Discussion

Here, we investigated how experience with a small array of tastes, delivered before conditioning and without negative outcome, affects a later aversion learned toward a novel taste. We found, as expected, that presentation of a taste array during the conditioning session weakens the learned aversion to the novel CS—an effect that is likely due to overshadowing among the proffered tastes (Pavlov 1927; Logue 1979; McLaren and Mackintosh 2002). Perhaps more surprising was the finding that preexposure to the same taste array (i.e., non-CS preexposure) strengthens the later aversion to the novel CS, an effect that scaled appropriately with the number of tastes presented within the array and with the number of days for which the array was delivered. Together, these results allow us to suggest that experience with tastes—experience that does not include exposure to the eventual CS—nonetheless exerts an influence on how this CS is later handled in a learning situation.

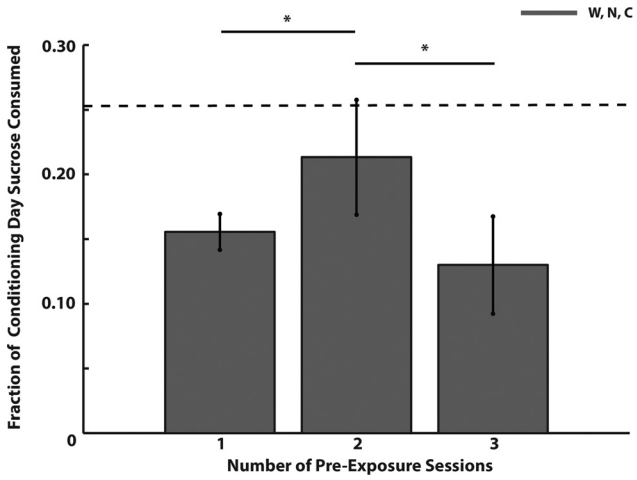

Figure 7. Two distinct preexposure effects revealed in animals who received exposure to W, N, and C for different numbers of sessions. A one-way ANOVA demonstrated a main effect of preexposure sessions ($P < 0.05$). Tukey–Kramer post hoc tests revealed that animals exposed to W, N, and C for 2 d developed a significantly weaker aversion than animals preexposed for one or three sessions ($P < 0.05$). Preexposure for two sessions did not produce aversions different from that of animals exposed to water alone (horizontal line of reference). *X*-axis represents aversion indices for each animal ($C_{STestConsumed}$ mL/$C_{SConditioningConsumed}$ mL). Error bars represent SEM. (*) $P < 0.05$.

Previous studies (Braveman and Jarvis 1978; Miller and Holzman 1981a,b) have reported that exposure to an array of tastes attenuates neophobia (the tendency to avoid novel tastes) to a different taste (although no such effect was observed for sucrose, the taste CS used in our study) but reported no interference with or augmentation of CTA. The differences between our results and previous studies may reflect floor effects in the latter—the learning induced by the typically used US (Smith 1968) is strong enough that further strengthening may not be observable. Here, we used a much milder US, a modification that resulted in a milder aversion (see also: Stone et al. 2005), which in turn allowed us to observe relatively subtle differences in magnitudes of learning.

The fact that preexposure to a set of tastes has been suggested to minimize neophobia of course introduces the possible confound that the increased aversions observed here might be a direct function, not of the exposure itself, but of the increased consumption of novel sucrose in conditioning sessions (i.e., the attenuation of neophobia) that followed such exposure. We observed no such relationship—there was no trend toward stronger conditioning following higher S consumption in conditioning sessions. This is perhaps not surprising, as neophobia is a phenomenon that appears only to certain tastes at certain concentrations (Monk et al. 2014) and is seldom observed in response to sucrose (Miller and Holzman 1981a,b; Franchina and Gilley 1986). We conclude that the increased associability of novel sucrose following familiarization to a small array of tastes is caused, not by obvious confound, but by the direct effect of experience.

As to how the specific effect of general experience with tastes might be more precisely conceptualized, we can currently only speculate. Previous experience with tastes other than the CS might enhance attention to the newly experienced taste by increasing its novelty (Kutlu and Schmajuk 2012). This suggestion jibes well with latent inhibition studies, wherein exposure to the CS causes a reduction in the conditioning effect (Lubow and Moore 1959; Lubow 1973; McLaren and Mackintosh 2002), a reduction that has been suggested to reflect the loss of novelty (Roman and Reilly 2007; Reilly and Schachtman 2009). It is possible that the learned association of a "safe outcome" (or "no outcome") with the diverse taste array reduces the associability of those specific tastes, thereby increasing attention to the novel CS as the source of the malaise (in turn increasing the magnitude of the conditioning). A potential mechanism underlying this effect might be enhanced sucrose-induced acetylcholine (ACh) release: ACh levels in the *gustatory cortex* have been shown to be linked to taste novelty (Miranda et al. 2000), and to the role of novelty in CTA memory formation (Gutiérrez et al. 2003; Clark and Bernstein 2009; Neseliler et al. 2011). Perhaps experience with tastes up-regulates the later ACh response to novel tastes, thus enhancing learning.

This interpretation is not a perfect match for the data, however. A specific increase in the perceived novelty of sucrose

should, if anything, increase neophobia to sucrose (Lin et al. 2012). Previous studies have not reported this effect toward sucrose, however (Miller and Holzman 1981b), and there appeared to be no systematic trend in this direction in our data. Previous studies have shown that the innate palatability of sucrose is insensitive to initial reduced acceptability making it a perfect candidate as a conditioned stimulus in our experiments (Young and Greene 1953; Young and Asdourian 1957; Hammer 1967). Thus, while familiarization to a small array of tastes may have functioned to increase attention toward novel sucrose when it appeared for the first time in conditioning, it is not entirely clear that this increase—and the observed phenomenon—reflects an increase in perceived novelty per se.

Alternatively, experience might change learning via the novelty of the innocuous tastes themselves, through a mechanism known as "behavioral tagging." Recently, it has been postulated that the introduction of a novel behavioral experience within a certain temporal window before or after a learning trial can provide the necessary plasticity-related proteins to upgrade a nonlasting memory into a lasting one (Moncada and Viola 2007). Our paradigm is very different from that shown to cause behavioral tagging in a CTA context (Ballarini et al. 2009)—the delay between final preexposure and conditioning was longer in our experiments by a factor of >20, and our learning was significantly stronger—but we witnessed a similar effect in Experiment 4, in that a single session of preexposure of innocuous tastes enhanced a later CTA toward novel sucrose more than two sessions of preexposure. Tagging does not provide a full, compelling explanation of our observed phenomena, however: Figure 7 shows that three sessions of preexposure (i.e., further increase in familiarity/experience) strengthens CTA—a result that dovetails nicely with the other experiments. It appears that novelty and experience are two separate environmental mechanisms impacting learning.

Another possible explanation for the increase in CTA strength is that exposure to a diverse array of tastes essentially served as a form of environmental enrichment, the likes of which have been shown to cause neuroanatomical changes (van Praag et al. 2000), such as synaptogenesis (Globus et al. 1973), that increase the density of synaptic contacts (Altschuler 1979). Such increases in the numbers of dendritic spines (Rampon et al. 2000) are often associated with experience-dependent plasticity, and could support the enhanced learning observed in rats exposed to the small taste array (Sehgal et al. 2013). That is, it is plausible to speculate that experience with a small set of tastes can be understood as a form of environmental enrichment, enhancing the general capacity to learn. However, this theory of general increase in learning capacity is not necessarily congruent with the specificity and speed of the learning observed in our CTA procedures, and must be considered speculative in advance of further study.

Regardless of the precise mechanism that underlies the observed phenomena, our results highlight the importance of experience with diverse stimuli and the extent to which it can impact later learning. Specifically, even innocuous experiences with stimuli other than future conditioned stimuli can alter decisions regarding behaviors in response to such stimuli, a finding that, to our knowledge, has not yet appeared in the extensive literature related to factors that alter associative learning. This work should raise caution among researchers when generalizing the results of CTA studies to subjects beyond laboratory rats. Perhaps innocuous exposure to a small array of tastes prior to the study of other novel tastes should become standard adaptation procedure, to ensure a closer-to-optimal analog for experiments on human subjects with rich taste experiences.

Materials and Methods

Subjects

Adult female Long Evans rats ($N = 104$, 250–315 g at time of surgery) from Charles River Laboratories served as subjects for these experiments. All rats were housed individually in humidity and temperature-controlled cages and kept on a 12-h light–dark cycle. Animals had ad libitum access to food and water prior to all experiments. All procedures were conducted in accordance with the guidelines established by the Brandeis University Institutional Animal Care and Use Committee.

Surgery

Rats were anesthetized using a ketamine/xylazine mixture (1 mL ketamine, 0.05 mL xylazine per kilogram of body weight) delivered via intraperitoneal injection. The head was shaved and positioned into a stereotaxic device, after which the scalp was excised for insertion of four self-tapping support screws. Intraoral cannula (IOC), made of flexible hollow plastic tubing, were inserted parallel to the masseter muscle through the mouth posterolateral to the first maxillary molar (Phillips and Norgren 1970) on both sides of the mouth. A stable, rigid dental acrylic head cap was formed around the IOCs and skull screws.

Following surgery, rats were given post-operative analgesic (meloxicam 0.04 mg/kg), saline, and antibiotic (Pro-Pen-G 150,000 U/kg). Additional antibiotic and analgesic injections were delivered 24, 48, and 72 h later. The weight of each animal was recorded each day; any rat displaying lethargy, lack of grooming or weight loss >15% of presurgery weight were removed from the study. Rats were given 7 d to recover from surgery before the start of the experiment.

Stimuli

Taste solutions used in the experiments consisted of 0.01 M sodium chloride (N), 0.02 M citric acid (C), and distilled water (W). Distilled water was used in all taste stimuli to limit potential taste impact of the inorganic minerals, metals, and chemicals often found in tap water (Pfaffmann et al. 1954; Hoehl et al. 2010). A novel 0.2 M sucrose (S) solution was used as the conditioned stimulus in all experiments.

Experimental design

Experiments were conducted at the same time daily, following a 21-h water deprivation period that increased incentive to drink. All experiments occurred in a Plexiglas experimental chamber ($8.5 \times 9.5 \times 11.5$ in) separate from the rats' home cages.

The entire experimental protocol spanned 4–5 d total (one session per day)—2 to 3 d of exposure to a taste array (preexposure), 1 d of conditioning and 1 d of testing (Fig. 2). The experimental chamber and bottles were rinsed and sterilized before use for each animal and session.

General session properties

Adaptation sessions

For 2 d prior to the start of the experiment, rats were given access to distilled water through a bottle in the experimental chamber for ~30 min to ensure familiarization with the testing environment. No water deprivation occurred during familiarization with the testing environment. Each adaptation session lasted ~30 min.

Non–CS stimuli preexposure

Once accustomed to the experimental chamber, rats were given non-CS taste preexposure sessions involving both bottle and IOC delivery of fluids. Each session began with 5 min of bottle access to a control substance (the average milliliters of fluid consumed across all sessions provided a consumption baseline for

each animal); after a 5-min intermission, rats received 15 min of fluid delivery through the IOC—brief openings of a solenoid valve caused small (40 μL) aliquots of fluid to be ejected (under slight nitrogen pressure) onto the tongue via dedicated polyimide tubes inserted as a manifold into the IOC. Overall, 60 deliveries of tastes (pseudorandomly order) were ejected to the rat's oral cavity at 15-sec inter-trial-intervals, for a total of 2.4 mL of fluid. The IOC was used for delivery of the stimulus battery during preexposure because it ensured experimenter control over the volume of each fluid to which the rats were exposed; of particular importance, it ensured that rats consumed equal volumes of aversive C and palatable N.

Conditioning session

The conditioning session, which took place in the same experimental chamber, involved exposure to sucrose CS immediately followed by a subcutaneous injection of lithium chloride (LiCl, 0.3 M, 0.5% of current bodyweight) administered to induce malaise as the unconditioned stimulus (US). Research investigating the effectiveness of LiCl administration has reported no difference in the effectiveness of interperitoneal and subcutaneous LiCl deliveries; therefore, the less invasive subcutaneous method was used here (Nachman and Ashe 1973). The concentration of LiCl used in all experiments was lower than that typically injected, ensuring that CTA learning would be submaximal, allowing us to observe any possible enhancements of learning (Nachman and Ashe 1973; Stone et al. 2005). Control experiments were identical except that the subcutaneous injections consisted of saline. The US/control injections were administered in a location distinct from both testing chamber and home cage, in order to reduce the association of malaise with a specific context. After receiving injections, rats were returned to their home cages.

Just as with preexposure sessions, we tested the generalizability of the phenomenon by manipulating variables within the conditioning session. To test whether the impact of innocuous preexposure was robust to CS delivery method, animals received the CS either via IOC or a lick spout and IOC. Rats receiving the CS via the lick spout had access to sucrose for 5 min during the conditioning session followed by 2.4 mL of sucrose infused via the IOC. Animals receiving CS via the IOC alone received pulses of infusion (100–50 μL aliquot deliveries of S with 15-sec inter-delivery-intervals resulting in 5 mL total). The amount of CS received was equated to the mean amount consumed by the lick spout group during the 5-min conditioning session.

In addition, we tested whether the results were robust to interference, by offering W, N, and C within the conditioning session, in addition to the CS, to a subset of rats. Thus, overall, there was a three-dimensional matrix of conditions (taste preexposure vs. not, multiple tastes in conditioning session vs. one, and IOC delivery of CS vs. lick spout delivery). Data were collected to fill each cell of this matrix save two—there would be little value to delivering the CS via IOC in the same preexposure (nonconditioning) session in which non-CS tastes were also delivered via IOC, as this would likely have obliterated conditioning entirely due to exposure to the CS prior to conditioning (latent inhibition; Lubow 1973).

Testing session

Twenty-four hours following exposure to the CS, animals were returned to the experimental chamber. Testing sessions differed from previous session types in that stimuli were delivered solely via lick spout. The rats were first presented with S via a CS designated bottle for 5 min and following a 5-min pause, were then presented with W within a different control fluid designated bottle for 5 min.

Consumption analysis

Milliliters of S consumed on test day via lick spout were divided by the amount of S given on conditioning day (i.e., $C_{\text{STestConsumed}}$ mL/$C_{\text{SConditioningConsumed}}$ mL) to provide a normalized assessment of CTA ("aversion index"). Smaller aversion indices imply stronger conditioning (i.e., animals drink a smaller proportion of the CS available to them on test day). All results were analyzed using SPSS and Matlab. We note that for rats that received S through an IOC during training, test day training consumption was capped; nonetheless, this index allowed us to compare across all groups.

Competing interest statement

The authors declare no competing financial interests.

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