DISCRIMINATIVE AVERSIVE OLFACTORY LEARNING INDUCES RAPID PHYSIOLOGICAL AND PERCEPTUAL PLASTICITY

By

MICHELLE C. ROSENTHAL

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ABSTRACT OF THE THESIS

Discriminative Olfactory Aversive Learning Induces Rapid Physiological and Perceptual Plasticity

by MICHELLE C. ROSENTHAL

Thesis Director:

John P. McGann, Ph.D.

Associative mechanisms allow organisms to learn which stimuli in the environment predict danger. Such learning allows the brain's sensory systems to increase their sensitivity to ecologically-critical stimuli or optimize discrimination between threat-predictive and neutral stimuli. Here, we used discriminative aversive conditioning in human subjects to explore these interactions between sensory processing and learning. Prior to conditioning we used a triangle task to assess each subject's ability to discriminate between a pair of very similar odorants and categorized them as baseline discriminators or non-discriminators. Each subject then underwent discriminative conditioning consisted of 8 trials of one of the odorants (the CS+) paired with a co-terminating mild wrist-shock and 8 trials of the other odorant (the CS-) presented alone. Odorants were counterbalanced across subjects and trials were presented in random

order. Odorant-evoked skin conductance responses (SCR) were recorded throughout conditioning. Subjects very quickly (within the first few trials) developed a preferential enhancement of the SCR evoked by the CS+ odorant, including the group of non-discriminators that performed poorly on the baseline olfactory assessment. Post-conditioning perceptual testing on a subset of these subjects revealed that these non-discriminators exhibited an impressive improvement in their ability to discriminate the two odorants compared to their own pre-conditioning baselines. Control groups receiving odors without shocks or shocks without odors showed no differential SCR and no improvements in perceptual discrimination. Interestingly, a subset of participants with relatively high levels of trait anxiety (assessed via the State-Trait Anxiety Inventory) exhibited much less difference in the SCR to the CS+ and CS- after conditioning compared to participants with normal levels of trait anxiety, which is consistent with previous reports. The results of this study highlight the capacity of the olfactory system for rapid plasticity in response to fear learning.

Keywords: olfactory discrimination, aversive olfactory conditioning, perceptual plasticity

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Introduction

The perception of an olfactory stimulus in the environment depends not only on the physical properties of the stimulus itself but also on prior experience with that stimulus. Even brief exposure to an odorant can induce adaptation at the level of sensory receptors ((T.-Y. Chen & Yau, 1994; Munger et al., 2001) and at higher levels of neural processing (Fletcher & Wilson, 2003), which in turn diminish the odor's perceptual salience. Long-term exposure to an odorant can induce dramatic changes in the peripheral olfactory system, including changes in receptor expression (Cadiou et al., 2014), olfactory sensory neuron physiology (Kass, Rosenthal, Pottackal, & McGann, 2013) and odor perception (Kass, Guang, Moberly, & McGann, 2016)). More recently it has been found that associative learning about the ecological significance of odorants can alter early sensory processing of those odors, including both aversive learning (Kass, Rosenthal et al. 2013) and appetitive learning (Abraham, Vincis, Lagier, Rodriguez, & Carleton, 2014)

Fear learning is increasingly appreciated to induce plasticity not only in the classical "emotional centers" of the brain (Cousens & Otto, 1998; LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998; LeDoux, Sakaguchi, & Reis, 1984; Phelps, Delgado, Nearing, & LeDoux, 2004), but also in sensory systems at both cortical and subcortical levels (Bieszczad & Weinberger, 2012; C.-F. F. Chen, Barnes, & Wilson, 2011; Cruikshank, Edeline, & Weinberger, 1992; Edeline & Weinberger, 1991). The impact of fear learning is particularly robust in the olfactory system, influencing every step in olfactory processing from the sensory neurons;(Jones, Choi, Davis, & Ressler, 2008; Kass et al., 2013). to the olfactory bulb (Fletcher, 2012)to the olfactory cortex (C.-F. F. Chen et al.,

2011), with effects that can even be passed on future generations (Dias & Ressler, 2014). This plasticity has been hypothesized to improve perceptual sensitivity and/or discrimination for threat-predictive stimuli, to enhance memory retrieval, or to facilitate attentional processing of key stimuli (McGann, 2015). However, these perceptual consequences have been difficult to assess in animal models.

In humans, several studies have assessed the effects of odor-cued aversive conditioning on olfactory perception. Aversive conditioning selectively lowers the absolute detection threshold for the threat predictive odor detection of an odor that predict a shock by lowering the absolute detection threshold for that odor (Åhs, Miller, Gordon, & Lundström, 2013), and that this perceptual threshold can be reduced further with repeated conditioning sessions over two weeks (Parma et al. 2015). Initially-indiscriminable enantiomer pairs (odorants with the same molecular structure except for opposite chirality) have been shown to become more perceptually different after discriminative conditioning, with corresponding changes in odorant-evoked activity in piriform cortex (Li, Howard, Parrish, & Gottfried, 2008). However, these sorts of experiments raise a "chicken and egg" question: how can the olfactory system know that there are two similar odorants being differentially reinforced if it doesn't discriminate between them in the first place? One clue would be how quickly the odorants become discriminable over the course of conditioning.

Associative learning-induced sensory improvements are presumably adaptive because they enhance detection of threat-predictive stimuli against a noisy background. However, enhancements in sensory sensitivity are not always a beneficial adaptation. In certain anxiety disorders, notably PTSD (Post Traumatic Stress Disorder), patients can

exhibit hypersensitivity to sensory stimuli (Yehuda, 2002) and sometimes exhibit distinctive changes in sensory processing (Felmingham, Rennie, Manor, & Bryant, 2011; Kleim, Ehring, & Ehlers, 2012; Morgan & Grillon, 1999; Neylan et al., 1999; Rothbaum, Kozak, Foa, & Whitaker, 2001), including a dramatic attentional bias, where unpleasant or trauma-related stimuli disproportionately attract and hold attention during search tasks. They also have unpleasant emotional and physiological responses to trauma-related stimuli and situations long after the trauma has passed. The development of anxiety disorders, including PTSD, is predicted by the exhibition of high trait anxiety (i.e. proclivity towards feeling apprehensive, hypervigilant and ruminating (Chambers, Power, & Durham, 2004; Sylvers, Lilienfeld, & LaPrairie, 2011; Weems et al., 2007). High levels of trait anxiety also predict a subject's tendency to inappropriately generalize fear to non-threatening CS- stimuli in discriminative aversive paradigms (Gazendam, Kamphuis, & Kindt, 2013; reviwed in Lissek et al., 2005). Taken together, these findings suggest that trait anxiety might influence sensory plasticity during aversive learning, such that more anxious subjects are less likely than controls to discriminate between similar stimuli when one predicts a shock and another stimulus does not.

In the present study we sought to replicate the previous finding that perceptual discrimination between chemically-similar odorants can be improved by pairing one odorant with shock and the other without. Previous work has employed pairs of almost universally indiscriminable enantiomers as odor cues (Åhs et al., 2013; Li et al., 2008). However, we sought to have a range of ambiguity between odorants, which would allow us to compare the time course of discrimination learning for subjects who initially discriminated between the odorants and those who did not. We thus exploited the natural

variability in the human ability to discriminate between the aldehydes Hexanal and Heptanal, a pair of aliphatic homologues that differ by one carbon in length. These odors are also difficult for mice to discriminate (Kass et al., 2016), presenting a future opportunity to compare plasticity in humans and animal models. Baseline and post-conditioning odor discrimination ability was assessed using a psychophysical discrimination task. Acquisition of discriminative conditioning was assessed by comparing the skin conductance response (SCR) evoked by each odorant over the course of conditioning. Trait anxiety was assessed via the State-Trait Anxiety Inventory (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983) a widely used (Chambers et al., 2004) and reliable measure of trait anxiety (Metzger, 1976). We predicted that most participants would rapidly develop an elevated CS+-evoked SCR and improved olfactory discrimination, but that those with very high levels of trait anxiety would exhibited less difference in the SCR to the CS+ and CS- after conditioning compared to participants with normal levels of trait anxiety (Gazendam et al., 2013; but see Torrents-Rodas et al., 2013).

Methods

Participants

Participants consisted of 82 Rutgers University students, recruited either via the Rutgers human subject pool system or via flyers placed around campus. Forty-four of these participants were women and the average age of all participants was 21.26 *(SD* = 4.67*).* Participation was limited to non-smokers with no respiratory issues that could

potentially affect olfactory processing during the experiment (e.g. significant nasal congestion, asthma). Participants were asked to consume nothing but water and not to chew gum one hour in advance of participation. All participants were instructed to avoid wearing scented products. Participants who were noticeably scented were excluded from the study. All experiments were conducted after acquiring written informed consent and in accordance with protocols approved by the Rutgers Institutional Review Board.

Olfactory Stimuli and Apparatus

Two aliphatic homologues, Hexanal (≥98% purity, Sigma-Aldrich) and Heptanal (≥95% purity, Sigma-Aldrich), differing only by one carbon in the length of their carbon chain, were used as olfactory stimuli for this experiment. These odors were selected based on animal data indicating they are perceptually difficult to discriminate (Kass et al., 2016). Odorant dilutions were corrected for the difference in vapor pressure to prevent discrimination on the basis of concentration. Odors were presented via a custom-built, 8 channel, computer-controlled olfactometer. Odors were delivered through a cylindrical glass tube approximately 5" long and 1" wide, at a rate of 1.5L/min in room air. The tube was positioned 2cm from the participants' nostrils to deliver odorant binostrally. A constant vacuum pulled the odor away from the odor delivery tube in between square shaped odor presentations. The olfactometer was calibrated daily prior to data collection each day via photoionization detector (ppBRae 3000, RaeSystems). Participants were positioned on an adjustable chin rest to ensure a constant distance between the odor delivery tube and the nose throughout the experiment. The odor presentation were controlled by custom programs written in PsychoPy (Peirce, 2007). Participants were

asked to breathe normally throughout the experiment, while respiration was monitored using a piezoelectric chest strap.

Odor Discrimination

To test participants' ability to discriminate between Hexanal and Heptanal, participants performed a forced-choice triangular odor discrimination test (Laska & Teubner, 1999). This task consisted of six triangles, each composed of three sequential 6 sec odorant presentations, two of which were the same and one of which was different (**Fig 1**). The participant was asked to identify which odor was different from the others via keypress on a computer keyboard containing keys that were labeled 1, 2 and 3 representing each odorant presentation within a triangle. The order in which the odors were presented within each triangle was pseudo-randomized such that the participant was not presented the same arrangement of odor presentations more than once. The ISI (inter-stimulus interval) within triangles was 8 seconds between odor presentations and the ITI (inter-trial interval) between triangles was 30 seconds. Participants' reaction time was recorded and perceptual data from participants whose mean reaction times during the pre or post triangle tasks were more than 2.5 standard deviations above or below the overall mean were excluded from perceptual discrimination analysis. This excluded 5 participants who consistently answered too early or too late.

Electrical Stimulation

During aversive conditioning the participant received a mild electric shock delivered to the forearm via an SD9 stimulation unit from Natus Technologies (Phelps et al., 2004). Shocks were delivered via two point bar electrode positioned along the palmaris longus tendon. Microlyte gel was used to improve current stability. Shocks were delivered in 200 msec 50Hz trains of constant-voltage pulses delivered at least one minute apart. Shock voltage was determined for each subject individually before the experiment by increasing the voltage in 5V increments from a starting point of 20V until the participant deemed the shock to be "uncomfortable but not painful" (maximum 100V). The average voltage level across all participants was 35 volts. The perceptual experience of this electrical stimulation is comparable to the sudden static electric discharge occasionally experienced in daily life. Participants were not advised of the parameters surrounding the shock or how often the shock would be delivered. Participants in control conditions that did not include shock delivery during conditioning nonetheless underwent the same pre-experiment shock calibration as subjects in other groups.

Aversive Conditioning & Control Paradigms

During aversive conditioning participants in the odor-shock paired group received 8 trials of CS+ and 8 trials of CS-, each of which consisted of a 6 second odor presentation that co-terminated with a mild electric shock during the last 200ms of the CS+ trials (see **Fig. 1**). The odors Hexanal and Heptanal were used as stimuli, with which

odorant served as the CS+ counterbalanced across subjects. During aversive conditioning the duration of the ITIs varied pseudo-randomly from 45 seconds to 1 minute and 15 seconds (mean = 1 min). To control for the effects of odor exposure, we included an odor only group where participants underwent the same paradigm as participants in the paired group except no shocks were delivered. To control for any potential effects of repeated electrical stimulation, we also included a shock only control group, who underwent the same paradigm as participants in the paired group but no odors were presented during the "conditioning" paradigm.

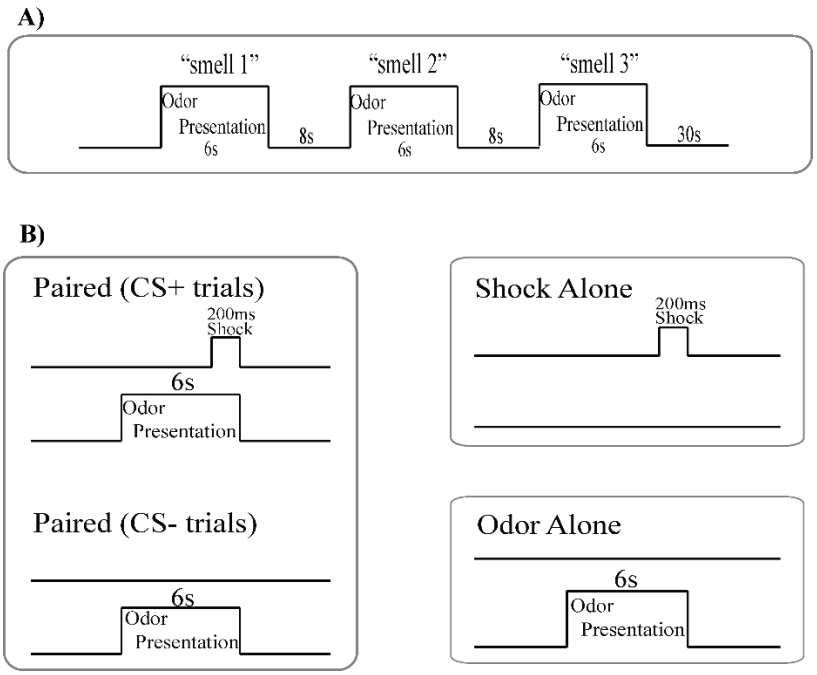

Figure 1. Experimental design and paradigms. **A)** Triangular discrimination task (one set of six). **1B)** Example trial from each group.

Skin Conductance Responses

Participants' odor-evoked, anticipatory skin conductance response (SCR) was measured via two 10mm Ag/AGCl electrodes. placed on the middle phalange of the first

and second finger of the subject's non-dominant hand (Phelps et al., 2004). A bead of Microlyte gel was applied to the recess in the electrodes to improve signal stability. A 0.5V signal was passed between the two electrodes and the conductance was measured using a Coulbourn, LabLinc V SCR module. SCR signals were digitized at a sampling rate of 100 kHz using a CED micro1401 and recorded using Spike2 software. Anticipatory odor-evoked SCR amplitudes were quantified by measuring the peak deviation from a 4 sec pre-stimulus baseline during the first 5.8 seconds of the odor presentation (the final 200 ms were excluded from all trials because a shock was sometimes concurrently presented).

State and Trait Anxiety Assessment

To assess the influence of state and trait anxiety on emotional learning and changes in perception, prior to the experiment, participants completed the State-Trait Anxiety Inventory (Spielberger et al., 1983). Each section of the questionnaire is comprised of 20 statements with which participants self-report their level of agreement on a scale of 1 to 4. Higher scores which can range from a minimum of 20 to a maximum of 80 points predict higher levels of anxiety. This inventory assesses transient anxiety at the time of participation (i.e. state anxiety) as well as more persistent proclivities towards being anxious (i.e. trait anxiety).

Environmental Controls

The experiment was conducted in a 9ft by 8ft room where temperature was maintained between 24-28 degrees Celsius to limit variation in skin conductance and

vapor pressure. Humidity was recorded at the start of each experiment. Participants were required to wear noise cancelling headphones that presented white noise for the duration of the experiment to muffle the sounds of the olfactometer and other ambient noises. The experiment was conducted in a dark environment illuminated only by a computer monitor 22 inches in front of the subject. All participants were asked to remove their watches and turn their phones off after filling out their consent form.

Data Quantification and Analysis

SCR

Discriminative aversive conditioning. Since the first shock occurred at the end of the first CS+ trial, trials 2 through 8 were the first trials in which a conditioned response could be assessed. SCRs evoked by the CS+ and CS- were averaged separately and then the SCR evoked by the CS- was subtracted from the SCR evoked by the CS+ to quantify the differential SCR for each acquisition trial. This metric demonstrates the discrimination between the CS+ and CS-. To exclude participants whose SCR reflected suspected motion artifacts during conditioning, three participants with a mean SCR differential during the acquisition trials that was outside the range of the mean plus or minus 2.5 *SD* for their group (1 participants in the paired group and 2 participants in the odor alone group) and were excluded from the SCR analysis. The effects of aversive conditioning on anticipatory SCR differential assessed via one-way ANOVA with group (paired, odor only, shock only) as a between-subjects variable. Planned comparisons were conducted, including using Bonferroni adjustment for multiple comparisons. Welch's *F* adjustment

are reported for any one-way ANOVA's where the homogeneity of variance assumption is violated. The significance threshold for this and all subsequent analysis was set at $p < 0.05$. All analyses were conducted using SPSS and plots constructed using Sigma Plot.

Perceptual Discrimination

Perceptual discrimination between odorants was expressed as the percentage of correct answers on the 6-question triangle test, with a chance level of 33%. . We collected perceptual data both before and after conditioning for a subset of participants ($N = 55$), while a minority of participants only received pre-conditioning perceptual testing. The effects of aversive conditioning on perceptual discrimination were assessed via a mixed-design ANOVA with group (paired, odor only, shock only) as a between-subjects variable and session (pre conditioning and post conditioning) as a within groups variable. Groups were compared using Bonferroni adjusted planned pairwise post-hoc tests.

Anxiety

For the state and trait anxiety measures (STAI) we dichotomized these variables at the value corresponding to the 85^{th} percentile of scores for state and trait anxiety, yielding two groups: anxious (score in the top 15%, raw score ≥ 51) and normal (score below 85% percentile, raw score ≤ 51). Independent samples t-tests were employed to investigate differences in anticipatory odor evoked mean SCR differential between anxious and normal participants.

Results

Effects of discriminative aversive conditioning on SCR

To assess the effects of aversive conditioning on olfactory discrimination, subjects underwent a baseline olfactory discrimination test, followed by discriminative olfactory conditioning, followed by a second administration of the perceptual discrimination test. During conditioning, one odorant (CS+) was presented for 8 trials of 6 sec odorant followed by a 0.2 sec co-terminating wrist shock while a similar odorant (CS-) was presented on 8 interleaved trials but without the shock. SCR was measured throughout to assess the physiological response to odorants. Averaging across trials 2-8, participants who underwent discriminative olfactory conditioning with odor-shock pairing exhibited larger differential SCR (M =.06, SD = .08, N=37) than participants in the odor only (M = -.008, SD =.01, N=19) and shock only (M =.004, SD =.03, N=23) control groups (see **Fig 2**). This difference was statistically significant as revealed by a One-Way ANOVA using group as a factor, *Welch's F* (2, 47.37) = 10.62, p = 0.000. Planned post-hoc comparisons confirmed that the anticipatory SCR differential for the paired group was significantly enhanced compared to the odor only (p = .001, d = 1.58*)* and shock only (p =.006, d = 1.29) groups, which did not differ from each other (p > 1.000). While on average, participants in the shock only group had mean SCR differentials participants that did not differ from zero (p = .557) participants in the odor only group, on average showed a slightly negative SCR differential (p = .042). These results demonstrate that on average participants in the paired group developed a differential anticipatory autonomic response during discriminative conditioning, as expected.

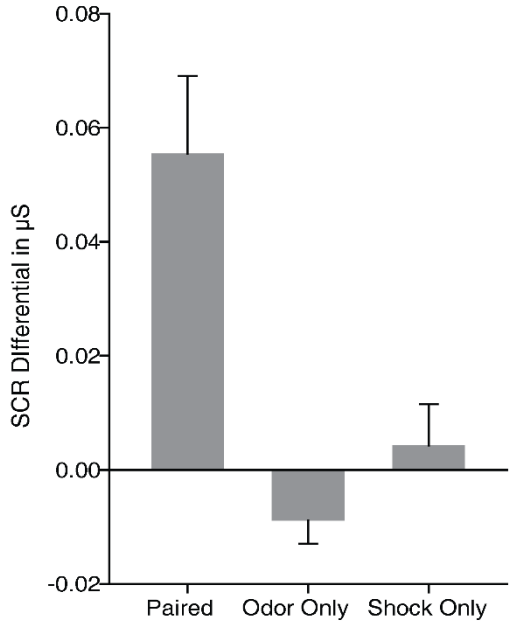

Figure 2. Mean SCR differential (CS+ minus CS-) of trials 2-8. Error bars represent 1 standard error of the mean.

Effects of discriminative aversive conditioning on odor perception

Prior to aversive conditioning, participants in this experiment exhibited a wide range of individual ability to perceptually discriminate between Hexanal and Heptanal, with a mean score of 64% and standard deviation of 26% on the baseline triangle test. Of 55 participants who received the discrimination test both before and after conditioning, 20 discriminated between the two odors with accuracy of 83% or better (5 or 6 correct out of 6 triangles; less than 1% probability of this result through guessing) at baseline. These participants were classified as "baseline discriminators." The remaining 35 participants, whose baseline discrimination accuracy ranged from 0 to 4 correct out of 6 triangles (recall that chance performance is 2 out of 6) were classified as "baseline non-

discriminators" (M = .48, SD = .20). To test the hypothesis that discriminative aversive conditioning improved the perceptual discrimination of threat-predictive odorants, we compared the before-conditioning and after-conditioning discrimination scores of participants classified as non-discriminators across all three experimental groups (paired, odor-alone, and shock-alone). Prior to aversive conditioning, there were no differences in perceptual accuracy between participants assigned to the paired acquisition group (N = 8), the odor only (N = 14) and shock only groups (N = 13), $p > 1.000$. However, as hypothesized there was a significant group x time point interaction, $F(2, 32) = 8.29$, p = .001. As shown in Fig. 3, the baseline non-discriminators in the paired group significantly improved their scores on the discrimination test ($p = .002$), while participants in the odor only group (p = .562) did not show any significant changes from their pre-conditioning baseline. Participants in the shock only group (p = .033) exhibited a small decrease in discrimination from their pre-conditioning baseline, a possible effect of habituation. After aversive conditioning, participants in the paired group performed significantly better on the odor discrimination compared to the odor only (p = .007) and shock only (p = .001) groups, which did not significantly differ from each other (p = .874).

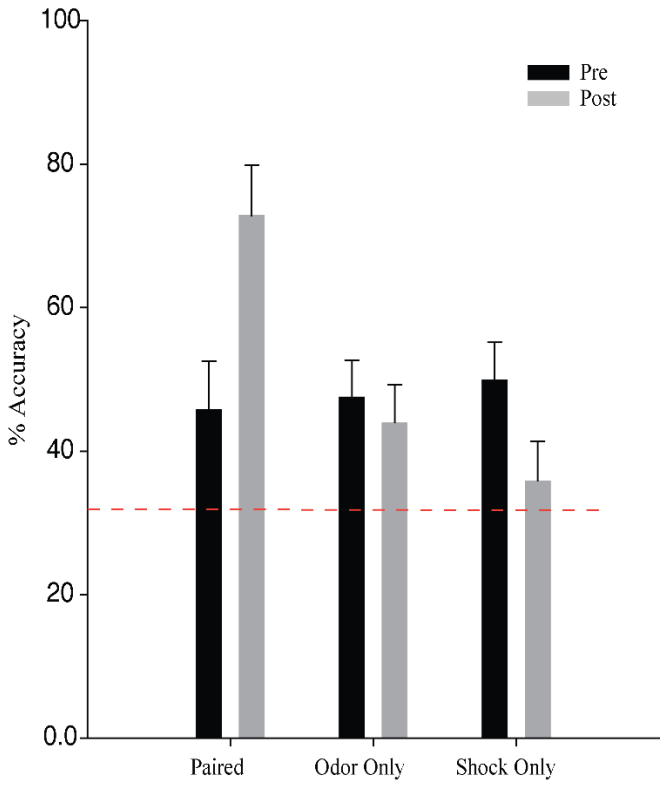

Figure 3. Effects of aversive conditioning on perceptual discrimination for baseline non-discriminators. Means represent percent accuracy. Error bars are 1 standard error of the mean.

Given the robust change in perceptual ability to discriminate between heptanal and hexanal with relatively few number of discriminative aversive conditioning trials (i.e. 8 CS+ and CS-), we investigated how many trials it took participants to developed a CR to the CS+ odor during the acquisition phase of the experiment, measured via SCR. To this end, we conducted a rm-ANOVA with acquisition-phase trial number (1 through 8) and CS type (CS+ and CS-) as within subjects variable for all participants who underwent acquisition in the paired group (N = 37); Greenhouse-Geisser adjusted degrees of freedom were reported where the assumption of sphericity was violated. As shown in Fig. 4 the results revealed that participants SCR increased with the number of trials,

$F(7,131.06) = 5.87$, $p = .000$, $\eta_{p2} = .140$. As expected, participants displayed larger anticipatory SCR to the CS+ ($M = .18$) than to the CS- ($M = .14$), $F(1, 36) = 11.75$, $p = .002$, $\eta_{p2} = .245$. Furthermore, there was a significant trial number x CS type interaction $F(1, 36) = 6.23$, $p = .017$, $\eta_{p2} = .246$. As shown in Fig. 4A, Bonferroni adjusted pairwise post-hoc tests revealed that by the second CS+ trial, participants had already developed a significantly higher anticipatory SCR to the CS+ compared to the one evoked during the first CS+ trial (i.e. before any shock was experienced), $p = .008$, whereas no significant increases in anticipatory SCR developed over trials to the CS- odor, compared to the first CS- trial, all $p > 1.000$, Fig. 4b. To assess whether baseline perceptual ambiguity between the odors modulated the speed of development of an anticipatory SCR to the CS+, we conducted the same analysis described above for non-discriminators exclusively ($N = 21$). The results of the rm-Anova revealed that for this subgroup of participants there was also an increase in SCR as trials progressed $F(7,67.541) = 4.12$, $p = .007$, $\eta_p^2 = .171$. Non discriminators displayed larger CS+ anticipatory SCR ($M = .16$, $SD = .12$), than they did to the CS- ($M = .11$, $SD = .16$), $F(1, 36) = 11.75$, $p = .002$, $\eta_p^2 = .245$. We also observed a significant trial number x CS type interaction $F(1, 36) = 6.23$, $p = .017$, $\eta_p^2 = .246$. As shown in **Fig. 4C,** Bonferroni adjusted pairwise post-hoc tests, revealed that baseline non-discriminators required 5 trials of the CS+ before developing a significantly larger anticipatory SCR on trial 6, compared to the first CS+ trial, $p = .022$. Baseline discriminators did not develop significant increases in anticipatory SCR over trials to the CS- odor, compared to the first CS- trial, all corrects $p > 1.000$ **Fig. 4D**.

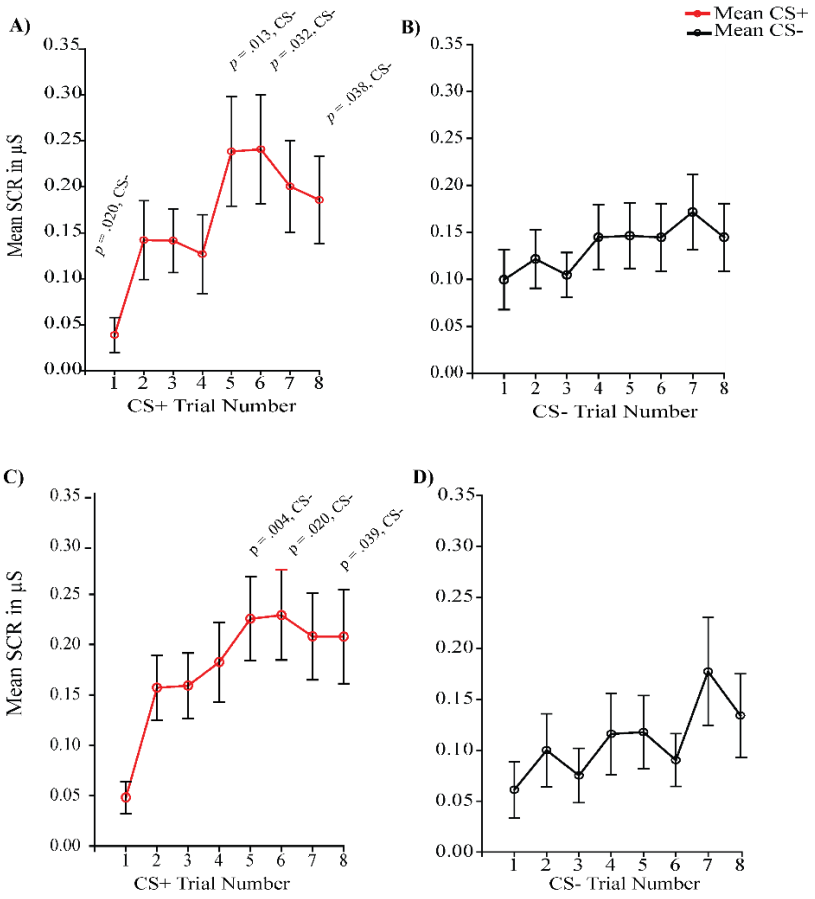

Figure 4. Mean SCR over trials. **A)** CS+ trials for all participants (*N* = 37). **B).** CS- trials for all participants (*N* = 37). **C)** CS+ trials for non-discriminators (*N* = 21). **D)** CS- trials for non-discriminators (*N* = 21). *p* values indicate Bonferroni corrected significance level of CS+ and CS- trials comparisons.

Anxiety

Pooling across all subjects (both discriminators and non-discriminators) that received paired odor-shock conditioning (*N* = 37) revealed no significant difference in anticipatory SCR between subjects scoring highly for trait anxiety on the STAI (*M* = .02, *SD* = .05) and those with normal trait anxiety (*M* = .06, *SD* = .09) after conditioning *t*(35) = 1.35, *p* = .264). However, among the baseline non-discriminators, we observed that while those with normal levels of trait anxiety developed a pronounced differential

SCR to the two odors during conditioning (*N* = 16, *M*=.08, *SD*=.08), the five non-discriminators with high levels of trait anxiety exhibited no significant differential SCR at all (*N=5*, *M*=.01, *SD*=.01; *t* (5) = .93, *p* = .394 compared to zero). This SCR difference between normal and anxious participants was statistically significant according to an independent samples t-test (corrected for unequal variances between the groups, *F(*1,19*)=* 5.23, *p* = .004), *t* (17.80) = 1.96, *p* = .002 (see **Fig. 5**). There was no difference in the shock levels selected by these participants or the SCR evoked by these shocks (*p* = .479), suggesting that the difference was not related to differences in unconditioned stimulus processing. The mean absolute amplitude of anticipatory odor evoked SCR (for both CS+ and CS- combined) during acquisition trials was on average smaller for participants with high levels of trait anxiety (*M* = .03, *SD* = .05) compared to those with normal levels of trait anxiety (*M* = .17, *SD* = .21), *t* (334) = .9.79, *p* = .000. This result is consistent with previous reports of high state anxiety individuals having lower amplitude levels of SCR evoked by neutral or aversive stimuli (Naveteur & Baque, 1987). This result suggests a possible interaction between trait anxiety and perceptual ambiguity, such that highly anxious subjects are selectively impaired in their ability to learn when stimuli are perceptually similar. We did not have enough discriminators with high levels of trait anxiety in our sample (*N* = 2) to assess the influence of trait anxiety on the acquisition of differential SCR for participants who were able to perceptually discriminate at baseline.

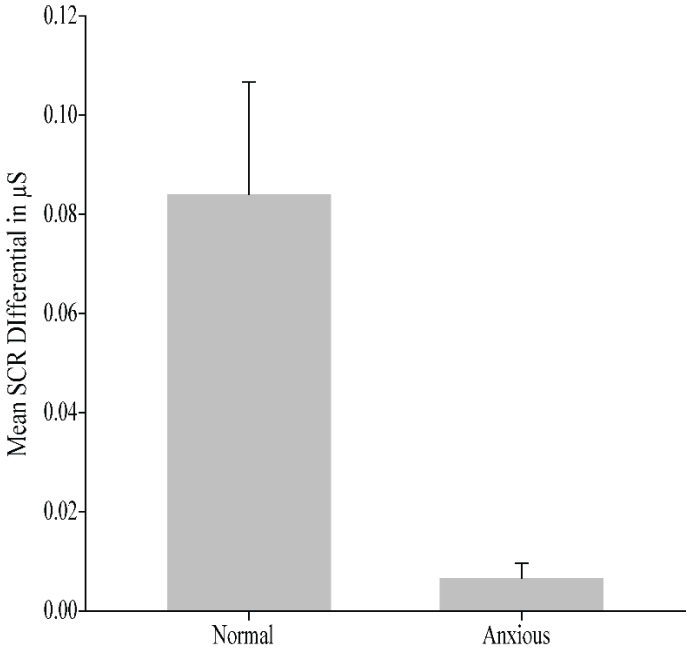

Figure 5. Mean differential SCR during acquisition trials (2-8) by trait anxiety status. Error bars represent one standard error of the mean.

Discussion

The present study aimed to replicate a previous finding that discriminative aversive conditioning to initially indiscriminable odorants improves discrimination between these odorants in humans (Åhs et al., 2013; Li et al., 2008). We also sought to extend these results to consider differences in learning rates as a function of stimulus ambiguity and potential mediation of learning by anxiety.

Consistent with an earlier report using enantiomer pairs, we observed that 16 trials of discriminative aversive conditioning induced a dramatic improvement (~59% performance increase) in perceptual discrimination between the aliphatic aldehydes

heptanal and hexanal for baseline non-discriminators. Odor exposure alone had no effect on non-discriminators, showing that mere exposure to these odorants does not induce perceptual change. Unexpectedly, participants in the shock alone control group exhibited a small but significant decrease in their discrimination performance. Because these subjects encountered the odorants during psychophysical testing before and after a period of unsignaled shocks, this decrease could perhaps reflect learning that both odorants equally constituted a safety signal. The improvement in perceptual discrimination between heptanal and hexanal for baseline non-discriminators further substantiates the flexible nature of the olfactory system.

In addition to the perceptual plasticity, we investigated the time course with which participants developed odor-evoked autonomic responses during the conditioning phase of this experiment. This allowed us to assess how quickly subjects developed conditioned responses (by comparing SCR to the pre-conditioning baseline) and whether and when they exhibited discriminative learning (by comparing responses to the CS+ and CS-). A key question was whether baseline non-discriminators would differ from discriminators on these metrics, since at least initially they could presumably have perceived the paradigm as a single CS odorant being paired with shock only 50% of the time. This might be expected to produce slower conditioning, possibly reaching a lower conditioned SCR asymptote, and at least initially to produce conditioned increases in the SCR to both odorants, which might become more different over the course of conditioning. Remarkably, we observed one-trial learning, such that after a single CS+-shock pairing, subjects overall displayed a significantly increased SCR on the second presentation of the CS+ odorant. A very similar increase in response was observed for the

subset of subjects that could not discriminate the CS+ and CS- at baseline, suggesting that these subjects learned the CS-US contingency just as quickly as those who could discriminate between the odorants. Moreover, these non-discriminators reached a similar asymptotic level of SCR across conditioning trials. However, a key comparison was whether baseline non-discriminators would be more likely than discriminators to respond to the CS-. Surprisingly, there was no significant difference in CS- evoked SCR between discriminators and non-discriminators on any trial. These data suggest that even though non-discriminators performed poorly on the explicit discrimination task, their autonomic nervous system implicitly discriminated between the odorants from the very beginning of conditioning.

How is it possible for the olfactory system to seemingly develop new perceptual acuity within a brief conditioning session, possibly as quickly as one trial? The olfactory epithelium is famously plastic, constantly replacing neurons and dynamically changing receptor expression levels based on sensory experience, and experience-dependent changes in OSN signaling have been linked to improved behavioral acuity in mice (Kass et al. 2016). However, new neurons require days or weeks to mature, and olfactory plasticity studies have not yet reported peripheral effects with less than several days of experience. A more likely explanation is that the olfactory system was always capable of discriminating hexanal and heptanal in the non-discriminators, but was nonetheless treating them as the same odorant for perceptual purposes. Outside the laboratory, naturally-occurring odors are almost always complex mixtures of many volatile odorants, and part of the olfactory system's main function is to bind mixtures of co-occurring into a single synthetic percept (Kadohisa & Wilson, 2006; Stevenson & Wilson, 2007). Odors

that fall into the same functional category are likely grouped together perceptually, but when they begin to predict different ecological outcomes the system shifts the category boundary to separate the CS+ and CS- instead of combining (Bao, Raguet, Cole, Howard, & Gottfried, 2016; Cleland et al., 2012). The improved explicit discrimination for non-discriminators may reflect such a categorical shift, which could be reinforced or even caused by the difference in autonomic response to the odorants.

Generalized fear learning has been demonstrated to be a characteristic of high trait anxiety individuals, though it has been principally assessed in other sensory modalities (Gazendam et al., 2013; reviewed in, Lissek et al., 2005). Here, we found that non-discriminators who reported high levels of trait anxiety did not develop a differential SCR to the CS+ and CS- odorants, in contrast to the rapid autonomic discrimination developed in anxiety-typical participants. Instead, they exhibited large conditioned SCRs to both odorants. This is consistent with previous reports that high trait anxiety individuals tend to overgeneralize among stimuli specifically when the conditioned stimuli are perceptually ambiguous (Torrents-Rodas et al., 2013). We did not have enough high-anxiety participants to fully compare discriminators and non-discriminators or to compare perceptual data among them. However, the data we do have suggests that further work with patient populations may reveal intriguing new details about how anxiety interacts with ambiguity and perceptual change.

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