

# Auditory aversive learning increases discrimination thresholds

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Animal studies of discriminative fear conditioning traditionally use stimuli that are distant in physical features and thus easily distinguished perceptually. Independently, human studies have shown that training mostly improves discrimination thresholds. We found that aversive learning actually induced an increase in discrimination thresholds in humans and that subjective aversion during conditioning predicted the individual threshold change. This counterintuitive performance deterioration occurred when using odors or sounds as aversive reinforcers and was not a result of attentional distraction or decision bias. In contrast, positive reinforcement or mere exposure induced the typically reported decrease in thresholds. Our findings indicate that aversive outcomes induce wider stimulus generalization by modulating perceptual thresholds, suggesting the engagement of low-level mechanisms. We suggest that for risk- or loss-related stimuli, less specificity could be a benefit, as it invokes the same mechanisms that respond quickly and efficiently in the face of danger.

It is crucial to distinguish dangerous from safe stimuli. In auditory fear conditioning, a procedure that has been intensively studied and for which neural mechanisms are relatively well understood<sup>1–3</sup>, findings have implicated auditory pathways and the amygdala in discrimination and generalization of aversive associations<sup>4–10</sup>. Behaviorally, it has been shown that discrimination improves with extended training<sup>9,11</sup>. However, discrimination in these studies is usually tested with stimuli that are far apart in their physical properties, such as pure tones with a frequency difference that is higher than the perceptual thresholds by orders of magnitude. This is probably a result of behavioral limitations with animals<sup>12,13</sup>, with the question of how aversive conditioning affects fine discrimination and perceptual thresholds being less extensively studied<sup>14,15</sup>.

An independent series of studies in humans found that perceptual discrimination usually improves with training and that the improvement is specific to the trained stimuli, suggesting that plasticity occurs in areas of the brain that have relevant information about low-level (for example, physical) properties of the stimuli<sup>16,17</sup>. It has been shown that attention and reward can modulate perceptual learning and influence its specificity<sup>18–20</sup>, but the effects of aversive versus rewarding or neutral stimuli have been less systematically studied. In the auditory domain, although numerous animal studies have used aversive or rewarding reinforcers and observed different physiological markers of plasticity<sup>3,21,22</sup>, it is still unclear how these modulations translate to actual performance and how they affect specificity, generalization and fine discrimination for the conditioned stimulus.

Should a highly aversive reinforcer result in better discrimination for the conditioned stimulus so that it can later be identified more accurately? A counter-intuitive hypothesis is that it could be useful to not discriminate stimuli that are very similar if one of them predicts an aversive outcome. According to this rationale, because the stimuli are close in physical properties, the chance of the new stimulus belonging

to the same set as the conditioned one is increased<sup>23,24</sup>, in the sense that there is a higher likelihood it will entail the same aversive outcome. If the conditioned stimulus elicits a fast and efficient defensive behavior, it is safer to have the new stimulus elicit the same response. The best way to do so is to not discriminate it at the perceptual level to begin with. We tested this hypothesis in humans using pure-tone frequency discrimination before and after tones were associated with either odors or sounds as pleasant or aversive reinforcers.

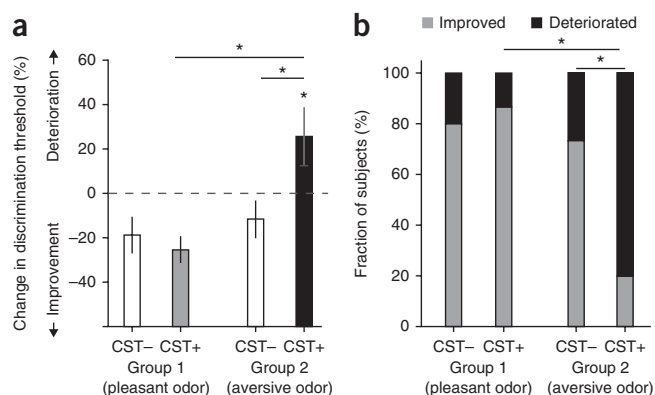
## RESULTS

In a classical auditory conditioning procedure, participants were subjected to pairing between a conditioned stimulus (CST+, a pure tone of 1 or 2 kHz, counterbalanced) and an odor (unconditioned stimulus, UST). Another tone (CST–, 2 or 1 kHz) was unpaired with odors (matched in number of presentations). In group 1 ( $n = 10$ ), the CST+ was paired with a pleasant odor, whereas it was paired with an aversive odor in group 2 ( $n = 15$ ). We used breathing suspension following the tone as the unconditioned response and as an indication for aversiveness<sup>25</sup>. Subjects held and delayed their next breath as measured by increased inter-breath interval (IBI) following the tone that predicted aversive odor ( $1.6 \pm 0.35$  and  $2.1 \pm 0.39$  s following CST– and CST+, respectively,  $P < 0.001$ , paired test), but not for the tone that predicted pleasant odor ( $1.82 \pm 0.38$  and  $1.78 \pm 0.36$ ,  $P > 0.1$ , paired test). Breathing suspension was significantly different between aversive and pleasant conditioning (IBI ratio for CST+/CST– of  $1.3 \pm 0.2$  in group 2 and  $0.97 \pm 0.1$  in group 1,  $P < 0.001$ ), indicating that subjective valence was indeed different between groups. To further verify valence, subjects rated the odors on an explicit scale, from 1 (highly pleasant) to 9 (highly aversive) (group 1, pleasant odor,  $4 \pm 1.4$ ; group 2, aversive odor,  $7.5 \pm 0.82$ ;  $P < 0.001$ ).

We tested the discrimination thresholds of each subject surrounding both tones, the CST+ and CST–, immediately before and after

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**Figure 1** Aversive tone-odor conditioning increases discrimination thresholds. **(a)** Subjects were tested for their frequency-discrimination thresholds before and after the tones were conditioned with a pleasant odor or an aversive odor. For the CST-, a control tone that was not conditioned, subjects in both groups showed a decrease, improvement, in their thresholds ( $\pm$  s.e.m.). Subjects that were conditioned with a pleasant tone showed improvement for the CST+ as well, but subjects in group 2, conditioned with an aversive odor, showed a decrease in their thresholds. **(b)** These threshold changes occurred in the majority of subjects, as most subjects improved on the CST, improved for the pleasantly conditioned tone (left), but deteriorated for the aversively conditioned tone (rightmost bar). The gray portion shows the fraction of the subjects that had a post-conditioning threshold that was lower than pre-conditioning threshold and the black portion shows the reverse (post > pre). \* denotes significant comparisons ( $P < 0.01$ ).

conditioning. Before conditioning, subjects showed a slight difference in threshold (measured as  $\Delta f/f$ ) for the two frequencies (1 or 2 kHz,  $2.6 \pm 1.8$  and  $1.8 \pm 1.0\%$ , respectively,  $P = 0.02$ ), but this was similar across the two experimental groups ( $P > 0.1$ , two-way ANOVA). We therefore focused on the change in discrimination thresholds before versus after conditioning as a function of valence and our task enabled this within-individual comparison (CST+ versus CST-) that overcomes variability in thresholds across individuals. A decrease in threshold is negative, although it is an improvement in performance, and an increase in threshold is positive, but is a deterioration in performance.

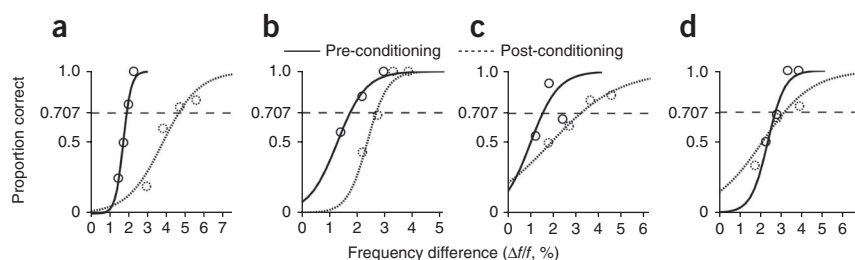
Subjects in both groups showed a significant improvement on the tone that was not reinforced (CST-; group 1,  $-18.8 \pm 8.3\%$  improvement,  $P < 0.001$ ; group 2,  $-11.6 \pm 8.5\%$  improvement,  $P < 0.01$ ; **Fig. 1a**). Thus, repeated exposure can induce rapid performance gains<sup>26,27</sup>. Moreover, performance gains were found in group 1 for the CST+ that was reinforced with a pleasant odor ( $-25.3 \pm 6.0\%$  improvement,  $P < 0.001$ ; **Fig. 1a**) and these gains were slightly higher when compared with the gains for the CST- ( $P < 0.05$ , paired test).

In contrast, the discrimination thresholds increased (decreased performance) for the CST+ that was reinforced with an aversive odor ( $+25.7 \pm 13.1\%$  deterioration,  $P < 0.01$ ; **Fig. 1a**) and this was even more evident when compared with the CST- in the same experimental group ( $P < 0.001$ , paired test; **Fig. 1a**) or when compared with the pleasant CST+ of group 1 ( $-25.3$  versus  $+25.7\%$ ,  $P < 0.0001$ ). In a combined model, we found a significant effect of interaction between valence (aversive/pleasant/neutral) and conditioning (pre/post,  $P < 0.001$ , two-way ANOVA). This effect occurred in the majority of tested individuals, for improvement after no reinforcement (CST-, 80 and 73.3% of the subjects in groups 1 and 2, respectively; **Fig. 1b**), improvement after positive reinforcement (86.7%, group 1) and deterioration after aversive conditioning (80%, group 2;  $P < 0.01$ , Fisher's exact test comparing CST+ between groups;  $P < 0.01$ , Fisher's exact test comparing CST+ and CST- in group 2; **Fig. 1b**). **Figure 2** shows four individual examples of full psychometric curves before and after aversive conditioning ( $P < 0.01$  for all comparing coefficients of logistic fits).

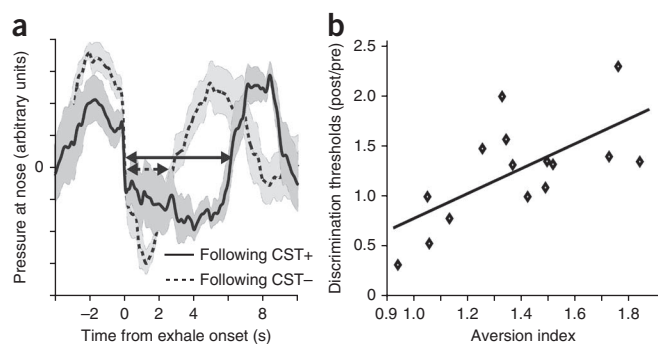
Furthermore, the individual subjective intensity of the aversive stimuli, as measured by the IBI during the conditioning session (**Fig. 3a**), reliably predicted the change in discrimination thresholds measured after it

( $r = 0.66$ , linear regression,  $F = 9.76$ ,  $P = 0.008$ ; **Fig. 3b**). To verify that aversive conditioning induced deterioration only in fine discrimination and that gross distinctions were still learned and made, we carried out an additional experiment ( $n = 15$ , group 8) and compared the IBI following a tone that was different from the CST+ by 1% (around the discrimination threshold) and that was never conditioned (that is, catch trials) to the CST- that was 100% away (IBI was  $1.66 \pm 0.08$  s for the CST- and  $1.9 \pm 0.1$  s for the altered CST+,  $P < 0.01$ , paired  $t$  test). Thus, although fine discrimination deteriorated after aversive conditioning, gross distinctions were still clearly made.

To determine whether this finding is specific to tone-odor interactions, we performed additional experiments (group 3,  $n = 15$ ; **Fig. 4**) using a highly unpleasant tone as the reinforcer (metal scraping on slate, 90 dB, rated  $8.1 \pm 1.2$ ). Subjects again showed an increase in discrimination thresholds for the CST+ ( $+36.7 \pm 20.3\%$  deterioration,  $P < 0.01$ , paired test; **Fig. 4a**), particularly when compared with the CST- ( $-18.7 \pm 7.2\%$  improvement,  $P < 0.001$  comparing CST+ to CST-, paired test). As with the odors, the effect was also evident at the population level (60.0 and 33.3% of subjects improved for CST- and CST+, respectively,  $P < 0.05$ , Fisher's exact test; **Fig. 4b**). In an additional experiment (group 4,  $n = 9$ ) that was designed to assure that the effect was not a result of auditory masking by the aversive sound, we compared the aversive CST+ to a pleasant CST+ delivered similarly at 90 dB (ocean sounds, rated  $2.1 \pm 1$ , median of 2; aversive sound,  $7.5 \pm 1.6$ , median of 7.5, for the aversive sound;  $P < 0.01$ , paired test). We obtained similar results ( $-37 \pm 17\%$  for the pleasant CST+, and  $+31 \pm 15\%$  for the aversive CST+,  $P < 0.001$ , paired tests; **Fig. 4d**). Moreover, even the two subjects that improved for the aversive CST+ did so less than for the pleasant CST+ (**Fig. 4c**). These findings suggest



**Figure 2** Psychometric curves before and after aversive conditioning. **(a-d)** Stimulus discrimination shown as the proportion of correct answers, as a function of frequency difference, presented as percentage from base frequency (that is,  $100 \times \Delta f/f$ , where  $f$  is the frequency of the CST+). Actual proportion is depicted in circles overlaid with logistic fits (Weibull fits produced highly similar results). Because we used an adaptive 'two-down, one-up' staircase converging procedure, there was no homogenous sampling of all  $\Delta f$  and we therefore used different binning (but always four bins) for each subject.



**Figure 3** Individual implicit aversiveness predicts change in discrimination thresholds. **(a)** The implicit measure relies on the IBI following the CST+ (solid line  $\pm$  s.e.m.) versus that following the CST- (dashed line). Subjects delayed their next breath following the tone that predicted release of aversive odor. The aversion index was calculated as the ratio of IBI following the CST+ (solid arrow) divided by the IBI following the CST- (dashed arrow). Shown is an example from one subject, averaged over the last 15 presentations. **(b)** The change in perceptual discrimination thresholds (post-conditioning divided by pre-conditioning), as a function of the implicit aversion index. Notice that all but one subject delayed their breathing following the CST+ and that the magnitude of this delay was significantly correlated with the increase in their perceptual threshold (linear regression,  $r = 0.66$ ,  $F = 9.76$ ,  $P < 0.001$ ). Notice that the IBI was measured during the conditioning, whereas the thresholds were measured after it, thus the correlation is a real prediction.

that the effect of aversive conditioning is not specific to tone-odor associations, but is general to negative valence.

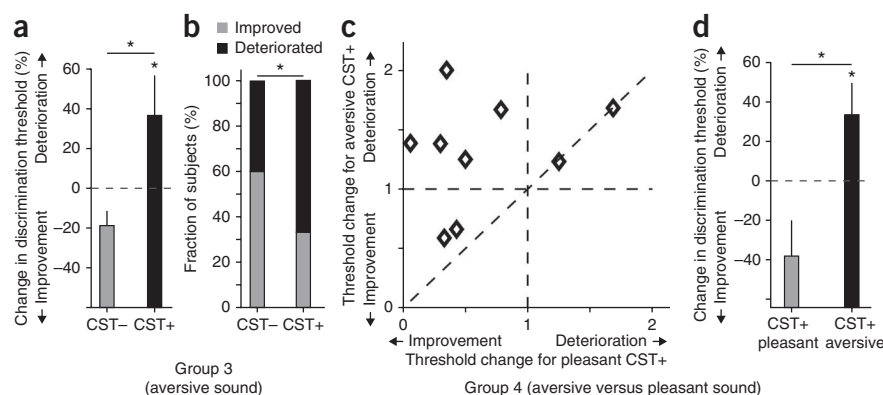
We also tested whether this effect is only a within-session short-lived effect that could result from adaptation or a longer-lasting effect that reflects learning about the stimulus. To do so, we repeated the experiments with an additional group (group 5, aversive sounds,  $n = 9$ ), re-called the subjects 24 h later and subjected them again to the threshold discrimination tests. The results replicated the original effect and showed that it persists after 24 h (CST+:  $+27.5 \pm 15\%$ , 6 of 9 subjects, deterioration post-conditioning; after 24 h,  $+90 \pm 35$ , 7 of 9; CST-:  $-24.3 \pm 15$ , 7 of 9, improvement post-conditioning; after 24 h,  $-29 \pm 12\%$ , 8 of 9; **Fig. 5a**). The differences between the CST+ and the CST- were significant both following the session and after 24 h ( $P < 0.01$  for both, paired  $t$  tests). We repeated the experiment with aversive odors (group 6,  $n = 13$ ) and compared the effect in a full model (**Fig. 5b**). We found a significant factor of time ( $P < 0.001$ , two-way ANOVA; *post hoc*  $t$  tests showing  $P < 0.001$  for post-conditioning versus pre-conditioning and  $P < 0.02$  for 24 h versus post-conditioning), but no significant effect of reinforcer (odors versus sounds,  $P > 0.1$ ). However, we are very careful in any interpretation here and our only conclusion is that the effect persists after 24 h and thus reflects a perceptual learning process.

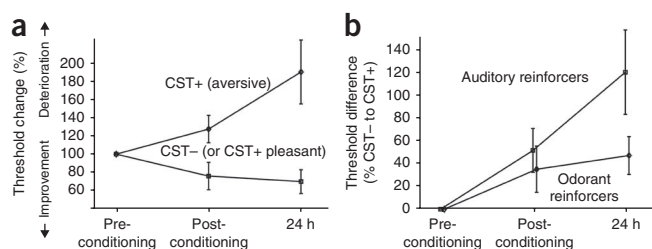
We explored the possibility that the change is a result of distracted attention following stimuli that predict aversive outcomes. To assess this, we performed the experiments in which each comparison in the two-interval discrimination task occurs twice: when the target tone comes first of the two compared tones, and when it comes second (order randomized across trials). We found that the distribution of choices between the two options was unchanged before and after conditioning (pre-conditioning, 56 and  $44 \pm 7.6\%$  when the target tone was presented first or second, respectively; post-conditioning,

49 and  $51 \pm 5.6\%$ ;  $P > 0.1$ , two-way ANOVA; **Fig. 6a**). Another possible contribution is a decision bias in which subjects actively prefer to not discriminate between similar tones, that is, the bias that subjects might have toward choosing 'Yes' after aversive conditioning. However, the choice ratio was virtually unchanged when compared to the CST- before and after conditioning (pre-conditioning,  $24.5 \pm 3.8$  and  $23.1 \pm 4.0\%$  for CST- and CST+, respectively; post-conditioning,  $23.1 \pm 3.9$  and  $26.3 \pm 4.1\%$ ;  $P > 0.1$  for comparing CST+ pre- versus post-conditioning,  $P > 0.1$  for comparing CST+ to CST- post-conditioning, two-way ANOVA; **Fig. 6b**). Moreover, we assessed discrimination thresholds in a new experimental group (group 7,  $n = 10$ ) with a two-alternative forced choice (2AFC) task, a procedure that discourages bias<sup>28</sup>, and obtained similar results ( $-40.8\%$  decrease in threshold for the CST-,  $+48.2\%$  increase for the CST+,  $P < 0.001$ , paired test).

As a final control, we added another condition in which the two-interval discrimination for the CST- was presented immediately following the CST+. That is, three tones were presented, but the subject was asked only about the two last tones. If the CST+ induced a transient reduction in sensory or attentional gain, it would harm discrimination in this condition as well. This additional test was performed both pre- and post-conditioning and in addition to the standard tests, allowing direct within-subject comparison. Subjects improved in discrimination for the CST- in this new condition, that is, even when it was preceded by the task-relevant CST+ ( $n = 8$ ,  $-45 \pm 14$ ; **Fig. 6c,d**), and this was directly compared to the reduced performance that was replicated for the CST+ ( $+31 \pm 15\%$ ,  $P < 0.01$ , paired  $t$  test). Moreover, the improvement on the CST- that followed the CST+ (the new test) was comparable to the standard improvement on the CST-, tested as before ( $-37 \pm 14\%$ ,  $P = 0.6$ , paired  $t$  test).

**Figure 4** Aversive tones also cause increase in thresholds. **(a,b)** Data are presented as in **Figure 1a,b**. Tones that were conditioned with a highly aversive tone induced a decrease in performance, that is, increase in threshold (black bar), and this was even stronger when compared with the CST- in the same group (gray bar) that induced improvement. The effect was seen for the majority of subjects **(b)**. **(c,d)** To ensure that this was not an auditory masking effect, the CST- was conditioned with a pleasant sound at 90 dB (hence a pleasant CST+) and compared to the aversive CST+ ( $n = 9$ ). The relative change in threshold for all subjects is presented in **c** and summarized in **d**.  $*P < 0.01$ . Error bars represent  $\pm$  s.e.m.





**Figure 5** Discrimination thresholds persist after 24 h. **(a)** Change in discrimination thresholds for the aversive CST+ (upper line showing an increase, hence a deterioration) and for the pleasant CST+ (lower line, showing a decrease, an improvement) following conditioning in the same session and after 24 h, that is, the following day. **(b)** Comparing sounds ( $n = 9$ ) to odors ( $n = 13$ ) as aversive reinforcers, by normalizing the difference between the change in the CST- to the change in the CST+ (y axis,  $\pm$  s.e.m.). A significant factor of time was found ( $P < 0.001$ , two-way ANOVA) but not of reinforcer ( $P > 0.1$ ).

## DISCUSSION

We found that aversive conditioning induced deterioration in auditory performance, measured as an increase in discrimination thresholds. On the one hand, these findings could be seen as being counter-intuitive, as one could speculate that predictors of aversive stimuli should be better discriminated to allow more accurate identification. On the other hand, it might be useful to not discriminate stimuli that are very similar and predict highly aversive outcomes, and thus allow brain mechanisms that are specialized for signaling fear and danger to respond fast and efficiently. In another study, we found that monetary loss induces a bias for wider generalization curves, in the sense that tones that are further away from a tone that was associated with loss are judged similarly<sup>15</sup>. Here we expand on this, finding that negative valence, at least in auditory aversive conditioning, can modulate finer perceptual processes and affect discrimination thresholds.

We used odors or sounds as reinforcers for auditory stimuli and our results could therefore imply that the two modalities are prepared for interactions. Odors and sounds might interact at early stages<sup>29</sup>, and a natural candidate for such convergence is the amygdala, which receives direct auditory and olfactory inputs and mediates fear and danger to produce fast defensive responses<sup>1,3,30</sup>. The shorter latencies for auditory responses in the amygdala that come by direct inputs from the auditory thalamus (the nonlemniscal pathway) versus those that come via the auditory cortex (the lemniscal pathway) could be highly crucial in scenarios of threats to survival<sup>3,31,32</sup>, and such threats are often reported by

olfactory and auditory signals in animals, as with predators, bad food or volatile chemicals. Thus, failure to discriminate two signals in the amygdala could result in producing a similar fast defensive response by its projections to brainstem mechanisms<sup>30</sup> and this can save crucial response time. Recent studies have addressed discrimination and generalization of aversive conditioned stimuli in networks of the amygdala<sup>4–10,33</sup> and more work is required to determine whether this is a modulatory role that in turn induces plasticity in modality-specific sensory areas.

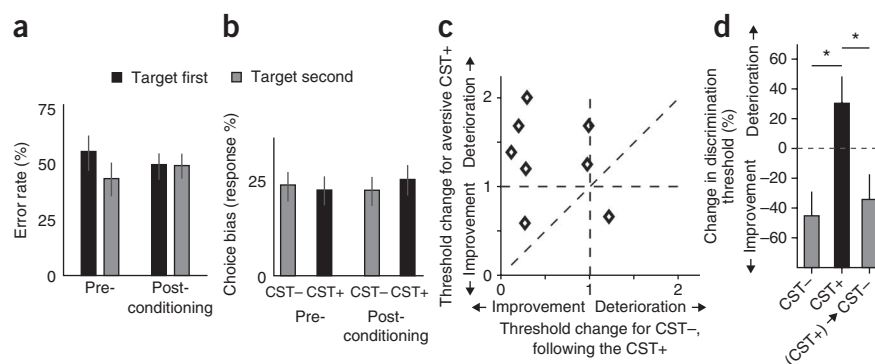
Indeed, studies have described changes in the responses of neurons in auditory pathways following training and conditioning and different markers of plasticity have been reported<sup>3,21,22,34,35</sup>. Although these neural changes are sometimes assumed to induce higher sensitivity, the same described physiological changes can also induce a reduction in sensitivity (but one that is specific to the reinforced tone), depending on the network implementation<sup>36</sup>. The differential effects of positive versus negative conditioning on physiological properties and plasticity (for example, tuning curves and receptive fields) are less clear<sup>13</sup>. Plasticity that results from aversive training most likely includes the auditory thalamus, the amygdala and the auditory cortex<sup>3,22</sup>, and the exact balance between these structures remains unknown. If the effect of valence on perceptual thresholds that we observed is general and modality independent (for example, found for visual conditioned stimulus), then areas such as the amygdala, prefrontal cortex or association cortices are involved to a larger extent.

Another hint to the location of plasticity can be found in the number of trials during learning. We used relatively few trials during the acquisition stage (21). This is in contrast with most protocols of perceptual training, which traditionally use hundreds of trials<sup>17</sup>, and more similar to fear-conditioning protocols, which use few trials. It is thought that short training protocols result in wider generalization and less specificity in fear conditioning<sup>9,11</sup> and in perceptual tasks<sup>37,38</sup>, whereas it has been suggested that prolonged training induces plasticity in early sensory areas and therefore specificity to the trained properties that are encoded in these regions<sup>19</sup>. The combination of aversive reinforcers and few trials that we used support the idea that representation is first mediated by areas such as the amygdala and the thalamic pathway that leads to it, where auditory tuning curves are more variable and wider<sup>31,39</sup> and therefore result in less discrimination and wider generalization.

A recent study found improvement in olfactory discrimination following aversive conditioning<sup>14</sup>. The discrepancy in findings could be a result of the fact that the studies used different modalities both for the conditioned stimulus (odors versus tones) and for the UST

**Figure 6** The potential contribution of distracted attention and decision bias to the change in discrimination thresholds. **(a)** Fraction of errors when the target tone was either the first or the second of the two tones in the two-interval comparison task. There was no significant difference both before or after conditioning. **(b)** The total fraction of 'Yes' choices (meaning the two tones are judged the same) indicate the decision bias. No significant difference was found before or after conditioning for both CST- and CST+.

**(c,d)** In another control experiment ( $n = 8$ ), the aversive CST+ preceded each trial of the 2AFC task (that is, there were three tones, but the subject was asked only about the last two). The threshold change for the CST- in this condition (tested both pre- and post-conditioning) was almost always improved when compared to the change for the CST+ (c) and was comparable to the improvement for the CST- in the standard test (that is, when not preceded by the CST+, d). Hence, the aversive CST+ did not induce a transient reduction in attention or sensory gain. \* $P < 0.01$ . Error bars represent  $\pm$  s.e.m.





(electric shocks versus odors) and therefore likely involve different brain mechanisms. Notably, we tested threshold discrimination on a continuous axis (tone frequency) using protocols that systematically vary this parameter and test perceptual sensitivity (or the just noticeable difference)<sup>28</sup>. This is harder to test for olfaction where a continuous space is not easily defined<sup>40</sup>. In fact, it seems reasonable that some stimuli should be better discriminated following aversive learning, such as the smell of a cat versus that of a lion<sup>14</sup>, whereas others are better off not discriminated, such as the roar of one lion versus that of another. Both aspects most likely coexist: a coarser discrimination after learning by a fast, responsive, possibly amygdalar, system<sup>1,3</sup> and an active decision-making process for finer discrimination by a more complex, possibly slower, cortical system<sup>13,14,22</sup>.

The deterioration in performance that we observed cannot be attributed to context mechanisms elicited by the aversive odors or sounds, as we observed improvement on the CST– that was randomly interleaved in the same sessions. A more specific contribution to the deterioration can be distracted attention. In this scenario, the conditioned stimulus that is now associated with an aversive outcome distracts attention implicitly when it is heard. This in turn harms either the processing of the CST– in the two-interval task or the maintenance of the first tone in working memory for comparison. Although attention could be involved<sup>41</sup>, it cannot account completely for our findings. First, we would expect some deterioration on the pleasant conditioning as well, at least when compared to the CST–, but found the reverse instead. Second, the discrimination task was performed after removal of the odor-delivery mask and after subjects physically moved to another desk; thus, it was very clear that this is a different context without odors. Finally, we tested this option directly by manipulating the order of presentation of the target tone and by using a three-tone task when the CST+ precedes the CST– test. Even if attentional mechanisms are involved in this auditory task, our results indicate that they are selective for aversive rather than pleasant factors. This entails differential involvement of brain regions and a similar end result for the organism, less discrimination for aversive outcomes.

Finally, failures to discriminate could be involved in the reported behaviors for post-traumatic stress disorder (PTSD). People who experienced trauma respond fast, many times subconsciously, with physiological markers of threat and stress, to stimuli that more or less resemble the original experience<sup>42</sup>. It has been shown that individuals with PTSD have wider stimulus generalization<sup>43,44</sup>, but the contribution of perceptual mechanisms versus higher cognitive processes remains unknown. This wider generalization in individuals with traumatic behavior is also consistent with the suggestion that the encoding of the conditioned stimulus is a result of one or few exposures, aversively reinforced, and hence a 'protocol' that results in plasticity in areas with wider tuning properties. One additional implication of exposure therapy, besides extinction training, could be that repeated exposures induce re-encoding of the original stimulus in sensory areas with finer tuning properties and therefore allow a more specific representation. Later on, fewer stimuli would evoke the original emotional memory.

## METHODS

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/natureneuroscience/>.

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## AUTHOR CONTRIBUTIONS

R.P. conceived the hypothesis. R.P., N.S. and J.R. designed experiments. J.R. performed the experiments. R.P. and J.R. performed the data analyses and wrote the manuscript.

## COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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## ONLINE METHODS

**Subjects.** We recruited 96 healthy human participants (aged 20 to 30 years, median age of 25 years) through advertisement and paid a constant amount for their participation. All experiments were conducted under a Helsinki protocol approved for R.P. and with signed consent by the participants. Four subjects were excluded because they had abnormally high discrimination thresholds, indicating that they did not understand the task. Subjects were randomly divided into eight experimental groups, four (groups 1, 2, 6 and 8) reinforced with odors and four (groups 3–5 and 7) with sounds.

**Experimental procedure.** Each participant first performed a discrimination task to find perceptual thresholds for 1-kHz and 2-kHz pure tones. Immediately after, participants were subjected to the conditioning session and then they immediately re-performed the discrimination task again for the two pure tones. Groups 5 and 6 were also re-called after 24 h (that is, 1 d later) and they then repeated the discrimination task.

**Conditioning session.** The 1-kHz and 2-kHz tones were assigned as CST+ and CST– (randomly counterbalanced across subjects). Each trial started with a conditioned stimulus presentation, followed immediately (zero delay and zero overlap) by delivery of a pleasant odor (in group 1 after a CST+), an aversive odor (in groups 2, 6 and 8 after a CST+), an aversive sound (in groups 3–5 and 7 after a CST+), nothing (after a CST– in all but one group) or a pleasant sound (after the second tone in group 4, hence a pleasant CST+). There were 21 CST+ trials and 21 CST– trials, randomly interleaved with a random intertrial interval with an average of 35 s.

Learning to differentiate between the CST+ and the CST– was assessed in a separate group (group 8,  $n = 15$ ) by introducing catch trials of a tone that was different from the CST+ by 1% (around or below the discrimination threshold in most of our subjects). This tone was never conditioned or followed by an aversive odor and we compared the IBI following it to that following the CST– to verify that gross distinctions between tones are learned.

**Discrimination tasks.** All frequency-discrimination thresholds were converted to percentage of the original frequency for each subject, that is,  $\Delta f/f$ , where  $f$  is the base frequency (that is, 1 or 2 kHz) and  $\Delta f$  is the frequency difference at which a tone of  $f + \Delta f$  is correctly discriminated from  $f$  at 70.7% level in the discrimination task, by an adaptive two-down, one-up staircase converging procedure<sup>45</sup>. The task was a two-interval comparison procedure in groups 1–3 and 8 and a 2AFC in groups 4–7. In each step, the two tones ( $f$  and  $f + \Delta f$ ) were presented in a randomized order with 1,000-ms interval and the subjects were asked, “Are these two tones the same? (yes or no)” (groups 1–3 and 8) or “Which tone had a higher pitch, first/second?” (groups 4–7). No feedback was supplied. Although different procedures can result in different thresholds<sup>28</sup>, they reflect improvements in performance that result from perceptual learning<sup>46</sup>. We used a comparison procedure because it could be more readily implemented in an instrumental task with animals for future studies probing the neural correlates<sup>12</sup>. We used a 2AFC task because it is a procedure that discourages bias<sup>28</sup>. To further reduce decision bias, the mask through which the odor is delivered was removed and subjects moved to a different physical desk to perform the post-conditioning discrimination task. Thus, it was very clear that aversive (or pleasant) odors could no longer occur. We further randomized the order of presentation of the conditioned (CST+,  $f$ ) and comparison ( $f + \Delta f$ ) tone even in the comparison procedure so that we could compare the ratio of yes (same) and no (different) responses (see Results). We repeated all analyses using the reciprocal of the threshold, defined as  $f/\Delta f$  and referred to as sensitivity, and obtained similar results; that is, improvement for CST– in all groups and significant blocking of improvement for all aversive CST+.

Although the use of a staircase converging algorithm limits the homogenous sampling required to obtain full psychometric curves, we could still do so by optimal binning (that is, dividing the range of individually sampled  $\Delta f$  into four equal bins) and calculating the psychometric curves (Fig. 2). We then fitted

logistic regressions (for the comparison procedure) or Weibull functions (for the 2AFC) and compared the coefficients, which were found to be significantly different across the conditions, aversive versus neutral or pleasant ( $P < 0.01$  for all, paired  $t$  tests).

**Stimuli.** The auditory stimuli were pure tones of either 1 kHz or 2 kHz with duration of 250 ms and onset/offset ramps of 5 ms, for a total of 260 ms. Tones were delivered through headphones (Creative HQ1400) at 70 dB SPL. The two pure tones (1/2 kHz) were counterbalanced in each group of subjects as CST+/CST–. There was no significant difference for the effect of aversive reinforcers between the subjects who encountered 1 kHz or 2 kHz as the CST+ ( $P > 0.1$ ). The UST was initiated immediately following the conditioned stimulus, that is, zero delay and zero overlap.

The unconditioned odor stimuli (groups 1, 2, 6 and 8) was delivered through a nose mask, produced via a custom-made olfactometer<sup>47</sup>, and controlled by LabView software (National Instruments). On the basis of previous results, we used a highly unpleasant odor blend (‘corpse’, Sensale) at a concentration of 12.5% as the aversive UST, and a pleasant odor blend (‘herbal essence’) at a concentration of 12.5% served as the positive UST<sup>48</sup>. We verified that these stimuli were rated as aversive/pleasant in our subjects by asking the subjects to rate the odors on an analog scale of 1 (very pleasant) to 9 (very unpleasant). The aversive odor was rated with a median of 7 (mean of  $7.5 \pm 0.82$ ) and the pleasant odor was rated with a median of 4 (mean of  $4 \pm 1.4$ ). These explicit measures were supplemented by an implicit measure of IBI<sup>25</sup> (Fig. 3a). Subjects were monitored using a pressure sensor and recorded on a standard computer using Chart software (ADInstruments). Breath onset was detected offline by a standard custom-made threshold algorithm implemented in Matlab (MathWorks) and IBIs were calculated following each tone presentation. The IBIs were averaged over the 6–21 tone presentations and compared across the CST+ and the CST– in each individual.

The unconditioned auditory aversive stimulus (groups 3–5 and 7) was a ‘metal scraping on slate’ click of ~90 dB delivered through the headphones for duration of 3,000 ms. This stimulus was found to be highly aversive and comparable to other aversive unconditioned stimuli such as electric shocks<sup>49</sup>, and rated highly unpleasant by our subjects as well (median = 9, mean of  $8.1 \pm 1.2$  by group 3,  $7.5 \pm 1.6$  by group 4 and  $8.0 \pm 1$  by group 7). The pleasant sound that we used as a comparison in group 4 was ‘ocean sounds’, delivered at 90 dB similarly to the aversive sound, and was found to be highly pleasant in previous studies<sup>50</sup> and by our subjects (median = 2, mean =  $2.1 \pm 1$ ). All sounds were generated via Matlab (MathWorks) on standard PC/Mac computers.

**Statistical tests.** We conducted all statistical tests twice, once with a parametric  $t$  test, either paired when performed within group or unpaired when comparing across groups, and once with a nonparametric Wilcoxon test, either signed rank test for paired within group comparisons or rank sum test for unpaired test across groups. Unless otherwise mentioned, all  $P$  values were obtained using both parametric and nonparametric tests; that is, both tests had to be significant. Fisher’s exact test was used to compare proportions of subjects and two-way ANOVA was used to test interactions. We used Matlab glmfit/nlinfit functions to perform logistic or Weibull regressions for the psychometric curves.

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