Increased fear generalization results in reduced generalization of extinction

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Abstract

Generalization of conditioned fear is adaptive for survival. However, overgeneralization of fear from threat cues to loosely similar yet safe stimuli is a hallmark of anxiety-related disorders. Such overgeneralization may moreover impact other fear learning processes. In particular, broad fear generalization might limit the effectiveness of extinction training, which may further maintain anxiety.

Hypothesizing that increased generalization of conditioned fear might weaken the later generalization of fear extinction, we here compared rats conditioned with moderate- versus high-intensity foot shocks, the latter group expected to show stronger generalization of acquired fear than the former. Within each shock intensity group, we then conducted extinction training using the original conditioned stimulus (CS) for half of the animals and a generalization stimulus (GS) for the others. We found significantly less generalization of extinction to the CS if rats had initially been conditioned using the high-intensity foot shock than if they had been conditioned using the moderate-intensity foot shock, in line with our hypothesis.

In conclusion, increased generalization of acquisition from a CS to a GS appears to go hand in hand with weaker generalization of extinction from that GS back to the CS. This may be important to consider in extinction-based exposure therapy because patients often present with broadly generalized fears, and exposure is usually not performed with the exact cues or situations for which fear was initially acquired.

Keywords: generalization, extinction, fear, anxiety, rats, freezing, fear conditioning.

Introduction

Generalization of acquired fear is an adaptive phenomenon. In daily life, animals and humans may encounter threat-predictive stimuli in different forms and under different conditions, and learning to generalize from one cue for impending danger to another one can be vital for survival. However, overgeneralization, or the tendency to exhibit fear to essentially safe cues that merely resemble a genuine cue for danger, can interfere with adaptive functioning, and has been suggested to be a hallmark of anxiety and stress-related disorders (Cooper et al., 2022).

Research on fear generalization began over a century ago with the Little Albert experiment (Watson and Rayner, 1920). Building on this long tradition, in a landmark study in a non-clinical human sample of Lissek and colleagues (2008), Pavlovian fear conditioning was established using a small and a big circle, where one circle served as a threat cue (conditioned stimulus, CS+) that predicted the administration of an unpleasant electric stimulus (unconditioned stimulus, US), and the other circle was not paired with electrical stimulation and served as a safety cue (CS-). In a subsequent generalization test, 8 circles of intermediate sizes between the CS+ and CS- were presented (generalization stimuli, GSs). This allowed for measuring generalization of fear to stimuli on a gradient of similarity that ranges from an obvious cue for threat to a clear safety signal (Lissek et al., 2008). Similarly, rodent generalization studies also start with a fear acquisition phase, where a neutral stimulus (CS) is paired with an aversive foot shock (US). Later, animals are presented with different cues that resemble the original CS. The degree of defensive responding that generalizes to those cues depends on how perceptually similar they are to the CS (Asok et al., 2019; Luyten et al., 2016; Zhang et al., 2017). The relevance of studying fear generalization to improve our understanding of anxiety-related disorders is underlined by a recent meta-analysis of 16 studies, suggesting that patients with anxiety-related disorders show heightened fear generalization relative to non-anxious controls (Cooper et al., 2022).

Prior research has shown that while fear acquisition will generalize readily to new GSs, fear extinction with such a GS will leave fear responding to the original CS or other GSs relatively intact in humans (Vervliet et al., 2004, 2005; but see Struyf et al., 2018) and in rodents (Alfei et al., 2021, 2020; Boddez et al., 2012; Bouton and Bolles, 1979). Individuals with high trait anxiety show such lack of generalization of GS extinction to the original CS even more markedly (Wong and Lovibond, 2020). This observation has clinical relevance, because exposure therapy is often conducted using generalization cues (e.g., cars and traffic situations in general) rather than with the original cues that a patient acquired fear for (e.g., the car they had a crash in, the intersection they got run over at). At the same time, as fear generalizes further, the chances increase that a therapist will expose a patient to these generalized cues as they are fear-eliciting cues as well. Here, we studied whether heightened generalization of fear after acquisition would weaken the later generalization of fear extinction.

We performed fear conditioning using moderate- and high-intensity USs in male and female rats, in light of the evidence that high-intensity USs promote stronger fear generalization than moderate-intensity USs (Laxmi et al., 2003; Xuan et al., 2023). Subsequently, we conducted fear extinction using either the CS or a GS. We hypothesized that enhanced generalization of fear from CS to GS after acquisition would weaken the later generalization of fear extinction from GS to CS. Additionally, we expected female rats to show overall slower extinction than male rats (Baran et al., 2009; Fenton et al., 2016; Greiner et al., 2019) and more generalization of freezing to stimuli similar to the CS (Greiner et al., 2019; Keiser et al., 2017).

Methods

Preregistration and data availability

The design, procedures, sample size and analysis plan were preregistered on the Open Science Framework (OSF) prior to the start of data collection (https://osf.io/9yd83/). Additionally, data and scripts are available on OSF.

Subjects

The experiment was performed in accordance with Belgian and European laws (Belgian Royal Decree of 29/05/2013 and European Directive 2010/63/EU) and the ARRIVE 2.0 guidelines (Percie du Sert et al., 2020) and approved by the KU Leuven animal ethics committee (project license number 011/2019). The number of animals was calculated based on previous studies. The study was conducted in 8-week-old male (270-300 g at arrival) and female (200-230 g at arrival) rats (Janvier Labs, France). Animals were housed in groups of 4, on a 12-hour light-dark cycle (lights on at 7 am); experimental procedures were performed between 9 am and 5 pm. The cages had bedding and cage enrichment in the form of a hanging red polycarbonate tunnel. Water and food were available ad libitum for the entire experiment, except during behavioral testing. Animals were acclimated for one week to our facility and habituated to handling for 2 days before the start of the experiment.

Apparatus

Eight identical operant chambers (30.5 cm width, 25.4 cm depth and 30.5 cm height; Rat Test Cage, Coulbourn Instruments, Pennsylvania, USA) were used simultaneously and were enclosed in sound-attenuating boxes. The experiment was conducted using Graphic State 4 (Coulbourn Instruments) software with the house light off. Behavior was continuously recorded during the experimental sessions using an IP camera (Foscam C2M, Shenzhen, China).

Experimental design

Rats (n = 64) were evenly divided into four groups: moderate shock during fear conditioning and extinction with CS (MSCS), moderate shock during fear conditioning and extinction with GS (MSGS), strong shock during fear conditioning and extinction with CS (SSCS), strong shock during fear conditioning and extinction with GS (SSGS) (see Figure 1). Rats of both sexes were divided equally over the four groups, the final group sizes were: MSCS (n = 16; 8 females), MSGS (n = 16; 8 females), SSCS, (n = 16; 8 females), SSGS, (n = 16; 8 females). All sessions were scheduled 24h apart, except for the spontaneous recovery test, which was scheduled 7 days after the reinstatement test.

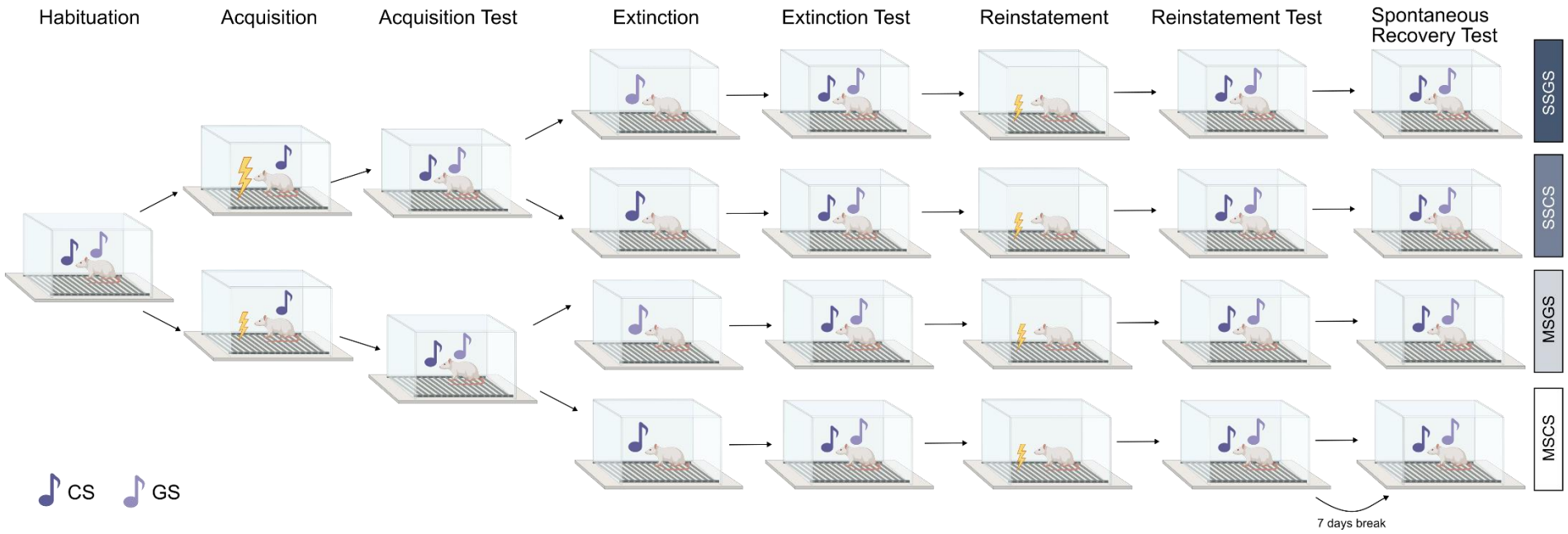

Figure 1. Experimental design. SSCS: strong shock acquisition and extinction with CS; SSGS: strong shock acquisition and extinction with GS; MSCS: moderate shock acquisition and extinction with CS; MSGS: moderate shock acquisition and extinction with GS. All sessions were scheduled 24h apart, except for the spontaneous recovery test, which was scheduled 7 days after the reinstatement test.

First, all rats underwent a habituation session during which the CS and GS were presented twice. Half of the rats received the tone presentations in the sequence CS, GS, CS, GS, while the other half received them in the sequence GS, CS, GS, CS. The CS and GS consisted of a pure 3-kHz tone and a pure 7-kHz tone, counterbalanced between animals. After 3 min of acclimation, each tone was presented for 30 s, with an average ITI of 300 s (270-330 s). After the last tone, the rats remained in the testing box for one additional minute.

The next day, rats underwent a conditioning session. After 3 minutes of acclimation, nine CS-US pairings were presented, with an ITI averaging 180 s (150-210 s). Half of the rats (n = 32) were trained using a moderate foot shock US of 0.4 mA and 1 s duration. The other half (n = 32) were trained using a strong foot shock US of 1 mA and 2 s duration. The 30-s CS co-terminated with the US in both conditions. After the last CS-US pairing, rats remained in the testing box for an additional minute. One day later, an acquisition retention test was performed, during which the CS and GS were each presented twice, with the same parameters as in the habituation session.

The extinction session consisted of 18 CS or GS presentations, after 3 min of acclimation, and with the ITI averaging 180 s (150-210 s). After the last tone presentation, rats remained in the testing box for an additional minute. Four rats per group, 2 of each sex, were perfused 90 min after the extinction test to dissect their brains for immunohistochemical staining. The next day, an extinction retention test was performed, during which the CS and GS were each presented twice, with the same parameters as the habituation session.

A reinstatement manipulation took place one day later. After 3 min of acclimation, a single foot shock of 0.4 mA and 1 s duration was delivered, followed by 2 more minutes in the testing box. The next day, a reinstatement test session was conducted, consisting of 2 CSs and 2 GSs, as in the habituation session. Two rats per group, one of each sex, were perfused 90 min after the reinstatement test.

Seven days after the reinstatement test, a spontaneous recovery test took place with 2 CSs and 2 GSs, using the same parameters as the habituation session. Four rats per group, two of each sex, were perfused 90 min after the spontaneous recovery test.

Due to problems with the tissue quality after freezing the specimens, the immunohistochemical stainings were not performed.

Behavior

Freezing during CS presentations was scored manually from the videos as a percentage of CS duration. Freezing was defined as full immobility except for minimal movements associated with breathing.

A freezing discrimination index was calculated for sessions where both CSs and GSs were presented. It was calculated as the difference in freezing between the two different tones (i.e., CS versus GS) divided by the sum of freezing to both tones (Xu and Südhof, 2013).

Data analyses

All statistical analyses were preregistered and any deviations are specified below. Data were analyzed using t-tests and one-way, two-way, repeated-measures or mixed ANOVAs, as appropriate. Post-hoc tests with corrections for multiple testing followed if significant main effects or interactions were found. If assumptions were violated, nonparametric statistical analyses were performed. For the nonparametric mixed RM ANOVAs, we applied an aligned rank transform ANOVA using the ARTool package (Elkin et al., 2021) in R (R Core Team, 2022) and R Studio (RStudio Team, 2021). All other statistical analyses were performed using performance (Lüdecke et al., 2021), afex (Singmann et al., 2023), emmeans (Lenth et al., 2024) and effectsize (Ben-Shachar et al., 2024) R packages. Data were processed and plotted using the tidyverse (Wickham and RStudio, 2022) and zoo (Zeileis and Grothendieck, 2005) R packages.

Habituation and acquisition session analyses were not preregistered. Habituation was analyzed with a non-parametric mixed ANOVA with shock intensity as a between-subjects variable and stimulus (i.e., CS or GS) as a within-subjects variable. The acquisition retention test was analyzed with a two-way ANOVA with shock intensity and sex as between-subjects factors.

Acquisition retention test, extinction retention test, reinstatement test and spontaneous recovery test were analyzed with sex and either shock intensity or experimental group as between-subjects factors and stimulus as within-subjects factor. If effects of sex were not significant, both sexes were collapsed in the mixed ANOVA as preregistered. As a deviation from the preregistration, we did not directly compare retention test sessions (i.e., acquisition test versus extinction test) within one mixed ANOVA.

Differences in freezing during extinction training were analyzed using a mixed ANOVA with trial as a within-subjects factor and sex and experimental group (i.e., MSCS, MSGS, SSCS and SSGS) as between-subjects factors. In a separate mixed ANOVA, to assess changes in freezing, the first three tones (i.e., early block) were compared to the last three tones (i.e., late block) and included as a within-subjects factor, with group and sex as between-subjects factors.

Results

The aim of this experiment was to test whether increased generalization of acquisition would reduce the generalization of extinction learning. To evaluate this, we needed to manipulate the generalization of acquisition. We therefore divided our sample in two groups, one that received a moderate intensity foot shock as US during acquisition and one that received a strong intensity foot shock as US, as higher US intensity has been shown to promote increased conditioned fear generalization (Dunsmoor et al., 2017; Laxmi et al., 2003; Xuan et al., 2023). After a test of generalization of acquisition, we conducted extinction training with either the CS or a GS and performed an extinction retention test 24 hours later

to test our hypothesis. Finally, we performed exploratory tests of reinstatement and spontaneous recovery.

Manipulation checks

In an initial habituation session, all rats exhibited uniformly low levels of freezing ($F(1, 62) = 1.58$, $p = 0.213$, $\omega_p^2 < -0.01$, see further analyses in Table 1), with no differences in discrimination between CS and GS ($t(31) = 0.65$, $p = 0.520$, $d = 0.12$) (see Figure 2). In acquisition, we did not observe differences in freezing between rats that experienced moderate shock compared to strong shock ($F(1, 60) = 1.58$, $p = 0.213$, $\omega_p^2 < -0.01$), but males showed significantly higher freezing than females ($F(1, 60) = 10.00$, $p = 0.002$, $\omega_p^2 = 0.12$). In the acquisition test, there was a significant interaction between shock intensity group and stimulus ($F(1, 62) = 5.22$, $p = 0.026$, $\omega_p^2 = 0.02$). Specifically, the moderate US group showed significantly higher freezing to the CS than the GS ($t(124) = 2.63$, $p = 0.01$, $d = 0.66$), while the strong US group showed no significant difference in freezing between the stimuli ($t(124) = 0.43$, $p = 0.669$, $d = 0.11$), suggesting stronger generalization in this group, although the freezing discrimination index did not differ significantly between both groups ($t(62) = 1.465$, $p = 0.148$).

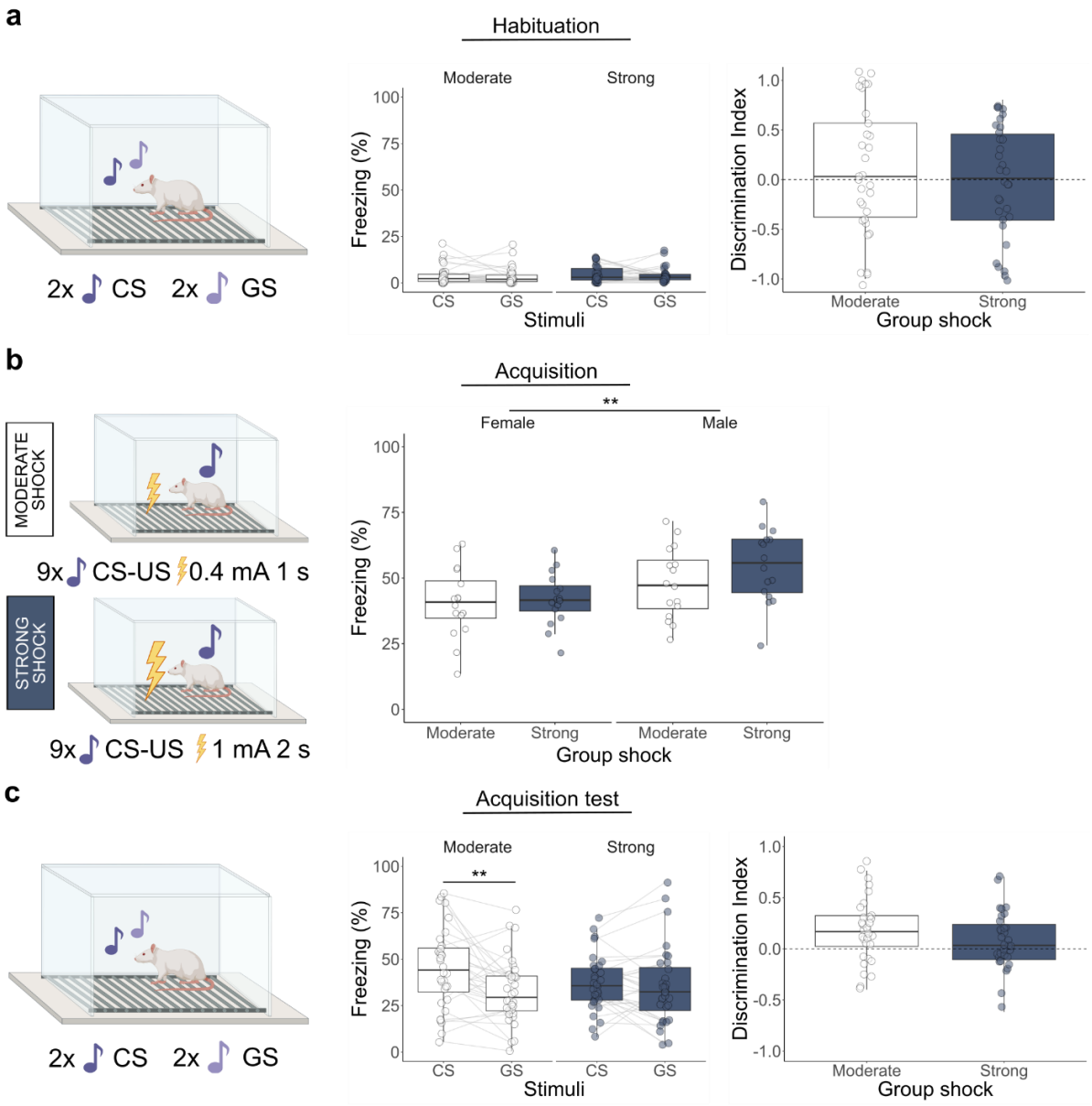

Figure 2. Freezing to the tones during habituation, acquisition and the acquisition retention test. Freezing boxplots represent average freezing to each stimulus, expressed as % of tone duration. Discrimination boxplots represent the average freezing discrimination index per group. Discrimination indices were calculated as the difference between freezing during the presentation of the CS and the GS, divided by the sum of freezing to both tones. **a.** Graphical representation and results of the habituation session. **b.** Graphical representation and results of the acquisition session. Males show higher freezing than females during acquisition ($F(1, 60) = 10$, $p = 0.002$). **c.** Graphical representation and results of the acquisition retention test. An interaction between shock intensity group and freezing ($F(1, 62) = 5.22$, $p = 0.026$)

reflected the fact that there were significant differences between CS and GS in the moderate shock group ($t(124) = 2.63$, $p = 0.01$), but not in the high shock group ($t(124) = 0.43$, $p = 0.669$).

In extinction training, we observed a reduction of freezing over trials ($F(17, 1020) = 16.06$, $p < 0.001$, $\omega_p^2 = 0.13$), and overall differences in freezing levels between groups ($F(3, 60) = 8.54$, $p < 0.001$, $\omega_p^2 = 0.24$) (see Figure 3). In particular, the SSCS group exhibited significantly higher levels of freezing than the SSGS ($t(60) = 3.39$, $p = 0.006$, $r = 0.32$) and MSGS groups ($t(60) = -4.92$, $p < 0.001$, $r = -0.43$).

Comparing freezing levels between an early block and a late block of the extinction training session, we found that males froze significantly more than females ($F(1, 56) = 4.66$, $p = 0.035$, $\omega_p^2 = 0.05$) and that freezing was lower in the late block than in the early block, as expected ($F(1, 56) = 93.47$, $p < 0.001$, $\omega_p^2 = 0.37$). Additionally, significant differences in freezing levels were detected between groups ($F(3, 56) = 8.43$, $p = 0.001$, $\omega_p^2 = 0.22$). The freezing levels differed between the two moderate shock conditions, with rats undergoing extinction with a CS (MSCS) exhibiting higher freezing compared to MSGS rats ($t(56) = 2.7$, $p = 0.044$, $r = 0.27$). Similarly, in the strong shock groups, SSCS rats showed elevated freezing levels compared with SSGS rats ($t(56) = 3.02$, $p = 0.019$, $r = 0.33$).

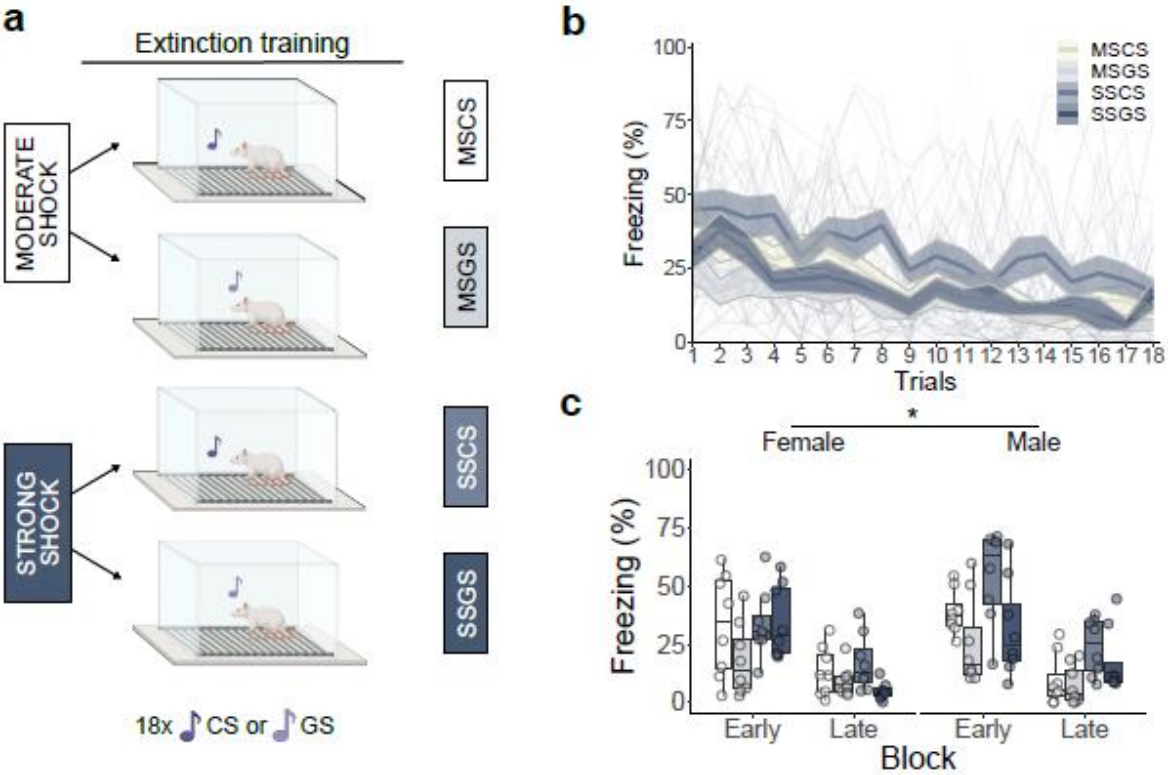

Figure 3. Freezing during extinction training, expressed in % of tone duration. **a.** Graphical representation of the extinction training session. **b.** Bold lines represent the mean and the surrounding shaded area the standard error of the mean. A reduction in freezing behavior is observed during extinction training ($F(17, 1020) = 16.06$, $p < 0.001$), along with significant differences between groups ($F(3, 60) = 8.54$, $p < 0.001$). **c.** Boxplots represent the average of the first three (i.e., early) and last three (i.e., late) tone presentations of the extinction session. While a general reduction in freezing was observed between the early and late block ($F(1, 56) = 93.47$, $p < 0.001$), males had higher freezing levels than females ($F(1, 56) = 4.66$, $p = 0.035$) and significant differences between groups were found ($F(3, 56) = 8.43$, $p = 0.001$).

Effects of generalization of acquisition on generalization of extinction

In the extinction retention test, we detected a group by sex interaction ($F(3, 56) = 3.01$, $p = 0.038$, $\omega_p^2 = 0.09$, see further analyses in Table 2) and a group by stimulus interaction ($F(3, 56) = 4.13$, $p = 0.01$, $\omega_p^2 = 0.06$) (see Figure 4). Further investigation of the group by stimulus interaction, zooming in on the degree of generalization of extinction to the

stimulus *not* presented during extinction training, revealed that rats in the MSGS group showed significantly lower freezing to the CS than rats in the SSGS group ($t(112) = -3.41$, $p = 0.005$, $d = -1.21$), whereas rats from the MSCS group and rats from the SSCS group did not display significant differences in freezing to the GS ($t(112) = -1.38$, $p = 0.514$, $d = -0.49$). Of note, rats in the MSCS group did show significantly less freezing to the CS than SSCS rats ($t(112) = -0.54$, $p = 0.01$, $d = -1.13$). Group differences were detected in freezing discrimination indices as well ($F(3, 60) = 10.89$, $p < 0.001$, $\omega^2 = 0.32$). The MSCS group showed a significantly lower discrimination index than the MSGS group ($t(60) = -5.47$, $p < 0.001$, $d = -1.94$) and the SSGS group ($t(60) = -3.24$, $p = 0.01$, $d = -1.15$). Further investigation of the significant sex by group interaction revealed differential freezing between sexes in the SSCS group only ($t(112) = -3.49$, $p < 0.001$, $d = -1.23$), with males showing higher freezing than females.

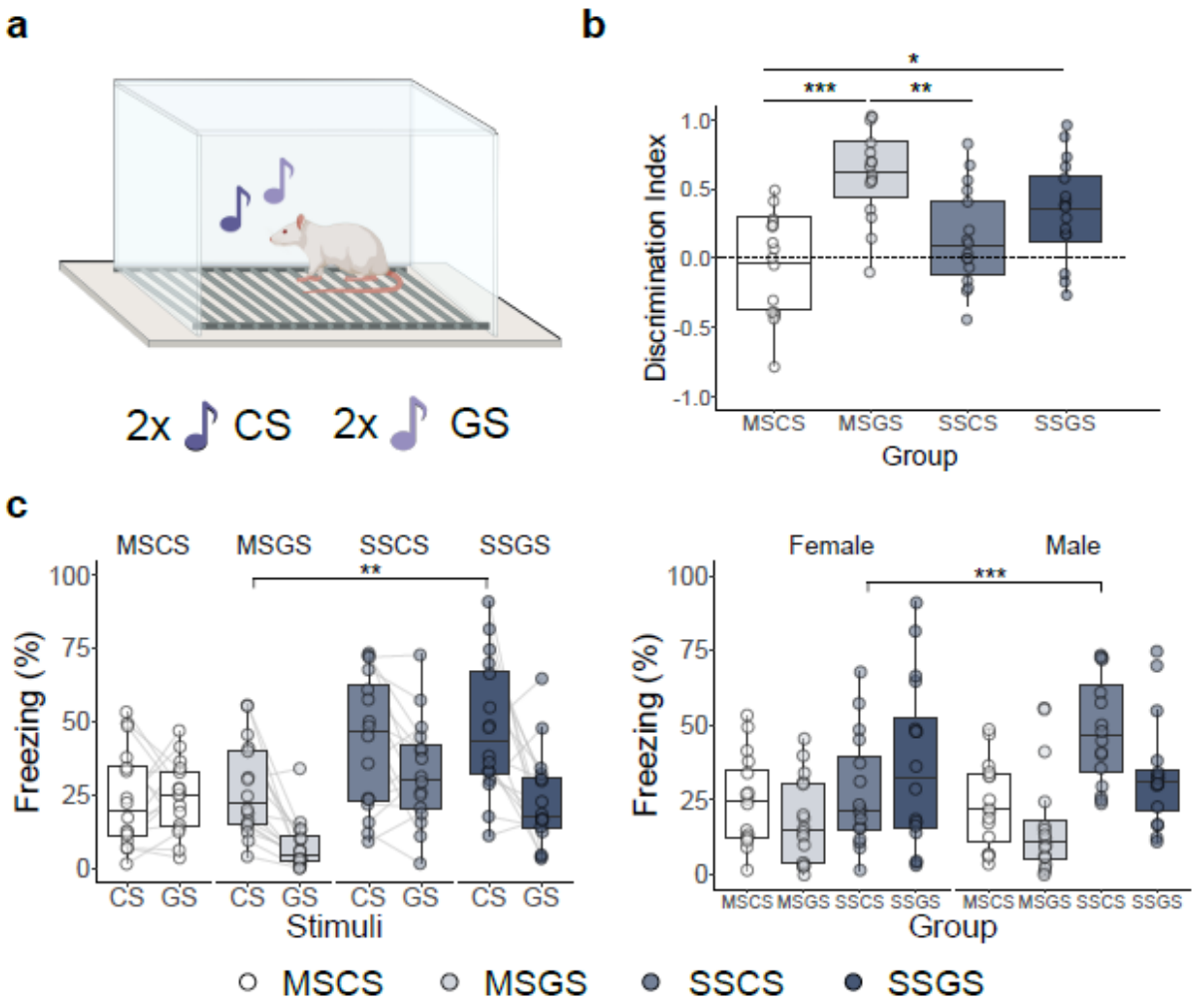

Figure 4. Results of the extinction retention test session. **a.** Graphical representation of the extinction retention test. **b.** The discrimination index boxplot represents the average of the freezing discrimination index. Discrimination indices were calculated as the difference in freezing to the different tones (CS versus GS), divided by the sum of freezing to both tones. Significant differences in the discrimination index between groups were present ($F(3, 60) = 10.89$, $p < 0.001$). **c.** The freezing boxplots represent the average percentage of freezing to each stimulus type, expressed in % of tone duration. Interactions between group and sex ($F(3, 56) = 3.01$, $p = 0.038$) and between group and stimulus ($F(3, 56) = 4.13$, $p = 0.01$) were found in freezing behavior. Rats in the MSGS group showed significantly lower freezing to the CS than rats in the SSGS group ($t(112) = -3.41$, $p = 0.005$). In the SSCS group, males showed higher freezing than females ($t(112) = -3.49$, $p < 0.001$).

Return of fear tests

We presented unsignaled USs, followed by a reinstatement test session 24 hours later (see Figure 5). While we did not observe significant group differences ($F(3, 45) = 1.48$, $p = 0.231$, $\omega_p^2 = 0.03$, see further analyses in Table 3), we observed significantly higher freezing to the CS than to the GS ($F(1, 45) = 18.1$, $p < 0.001$, $\omega_p^2 = 0.07$). No group differences were found in freezing discrimination indices either ($F(3, 45) = 1.62$, $p = 0.199$, $\omega^2 = 0.04$).

Seven days later, we tested differences in spontaneous recovery, and found higher freezing to the CS than to the GS ($F(1, 36) = 10.54$, $p = 0.003$, $\omega_p^2 = 0.06$), in the absence of group differences ($F(3, 36) = 0.4$, $p = 0.757$, $\omega_p^2 =-0.05$) (see Figure 5) We did find group differences in the freezing discrimination index ($F(3, 36) = 3.77$, $p = 0.019$, $\omega^2 = 0.17$). Specifically, rats in the MSGS group showed a significantly higher discrimination index than those in the MSCS group ($t(36) = -2.78$, $p = 0.041$, $d = -1.28$) and the SSCS group ($t(36) = 2.99$, $p = 0.024$, $d = 1.31$).

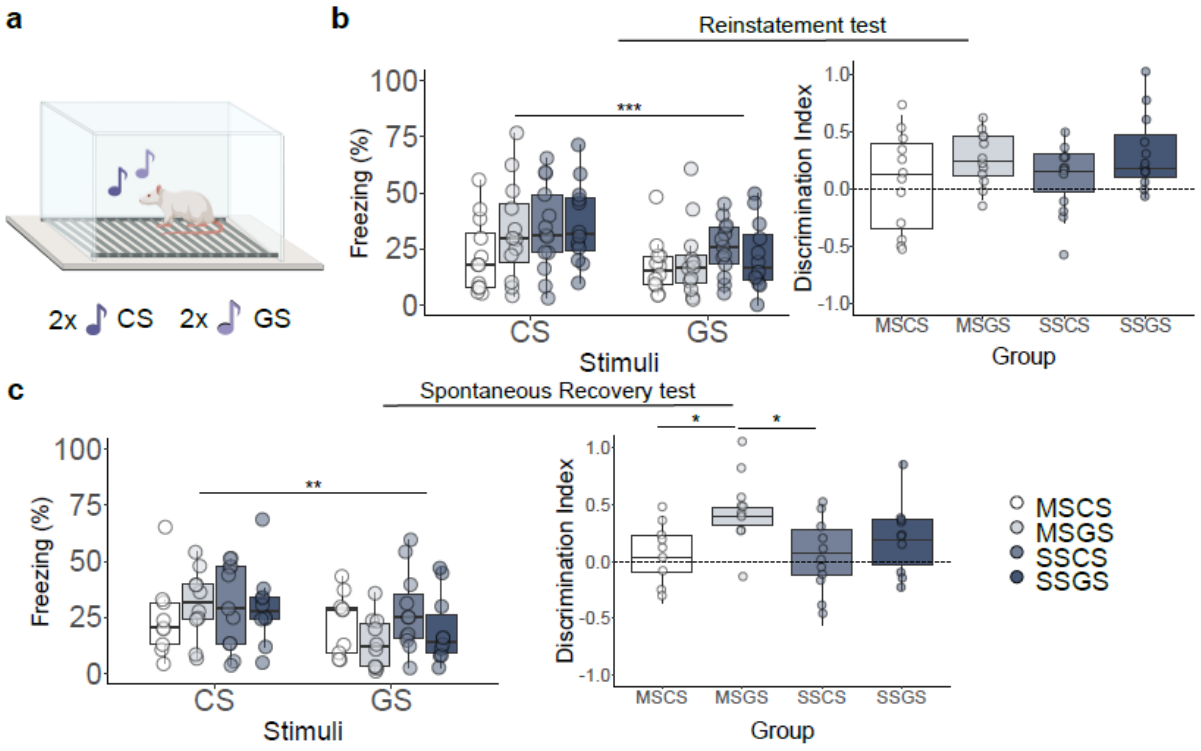

Figure 5. Freezing during reinstatement test and spontaneous recovery test. The freezing boxplots represent the average of each stimulus type and results are expressed in % of tone duration. The discrimination index boxplots represent the average of the freezing discrimination index calculation. Discrimination indices were established by calculating the difference in freezing to two different tones (CS versus GS) and dividing by the sum of freezing to both tones. Discrimination indices were calculated by taking the difference between freezing during the presentation of the CS and the GS and divided by the sum of freezing to both tones. **a.** Graphical representation of the reinstatement test, and spontaneous recovery test session. **b.** Results of the reinstatement test session. Significant differences between CS and GS ($F(1, 45) = 18.1$, $p < 0.001$) in freezing behavior, but no significant differences in discrimination index between groups were found ($F(3, 45) = 1.62$, $p = 0.199$). **d.** Results of the spontaneous recovery test session. Significant differences between CS and GS in freezing behavior ($F(1, 36) = 10.54$, $p = 0.003$) and in discrimination index between groups ($F(3, 36) = 3.77$, $p = 0.019$).

Discussion

Here we investigated how increased generalization of acquisition affects the generalization of extinction in a Pavlovian fear conditioning task.

To achieve differences in generalization of acquisition, in line with previous literature (Laxmi et al., 2003; Xuan et al., 2023), we subjected rats to cued fear conditioning with strong versus moderate intensity shocks as US. While rats that received moderate USs during acquisition discriminated between the CS and a GS on a subsequent retention test, rats that received strong USs did not and showed similar levels of freezing to both tones.

We compared freezing between an early extinction block (i.e., first three tones) and a late block (i.e., last three tones), which revealed that male rats showed higher freezing in early and late trials than female rats during those same trials. Such generally reduced freezing in female rats during extinction training has been reported previously in other related tasks (Shanazz et al., 2022). Additionally, while we expected slower extinction in female rats based on prior literature (Baran et al., 2009; Fenton et al., 2016; Graham et al., 2009; Greiner et al., 2019), we found no significant Sex x Block interaction, indicating similar extinction rates in both sexes.

In the extinction test session, we confirmed some of our preregistered hypotheses. In the moderate shock groups, we observed the expected asymmetry: in the MSGS group, we observed a high discrimination index, with higher freezing to the CS than to the GS, meaning that extinction did not strongly generalize from GS to CS, while in the MSCS group, the discrimination index was low, in other words, in the MSCS group extinction generalized strongly from the CS to the GS, yielding similar freezing for both tones. In the strong shock groups, we expected that the enhanced generalization of acquisition would further reduce the generalization of extinction, yielding larger discrimination at test between CS and GS particularly in the SSGS group. In support of our hypothesis, we found that the SSGS group showed significantly higher freezing to the CS than the MSGS group. Note that we did not

observe higher freezing to the GS in the SSCS group than in the MSCS group, which suggests that enhanced generalization of acquisition reduced the later generalization of extinction from an extinguished GS back to the CS but not from the extinguished CS to a GS. It is noteworthy that males showed higher freezing than females in the SSCS group only. Females in the SSCS group seemed to exhibit a pattern in line with our overall hypothesis, showing weaker generalization of GS extinction to the CS than their female counterparts in the MSCS group. Surprisingly, males in the SSCS group showed high freezing to the CS despite having undergone extinction training with that same CS in the preceding session. This result is puzzling given that their extinction training results look as expected, exhibiting a gradual reduction in freezing over extinction trials.

The lack of sex differences during the extinction test was unexpected, as we anticipated lower discrimination indices in female rats, in line with previous reports (Greiner et al., 2019; Keiser et al., 2017). Note that Keiser et al. (2017) tested generalization of contextual rather than cued fear. While Greiner et al. (2019) found a lack of extinction to a conditioned fear cue and a lack of discrimination between a conditioned fear cue and a conditioned safety cue in females when assessing freezing behavior. Females did show differential darting behavior in response to a safety versus a fear cue.

Regarding the return of fear tests, we did not find group differences in the reinstatement test. However, we found that rats showed increased CS freezing compared to GS freezing, indicating a selective return of fear to the CS, regardless of initial US intensity or the nature of the stimulus used for extinction training. One week later, we performed a spontaneous recovery test and observed a similar increase in CS-elicited freezing compared to the GS. Not all of our preregistered differences were confirmed here. One possible reason could be that we had less power than in the extinction test session, as some animals were euthanized to collect their brain tissue.

It is important to note that the results for the discrimination indices should be interpreted with caution. Rats that underwent GS extinction had mostly received reinforced

CS presentations, while the GS was never paired with shock. However, in the groups that underwent CS extinction, the CS became effectively ambiguous, as the CS was paired with shock in acquisition and then not followed by shock during extinction training. Thus, after extinction training, the results of discrimination indices may be biased towards increased discrimination in the MSGS and SSGS groups compared to the MSCS and SSCS groups.

Our data suggest that rats with higher generalization of acquisition from CS to GS exhibited less generalization of extinction from GS back to CS, as hypothesized. However, this effect did not extend to rats with increased generalization that underwent CS extinction, as their freezing towards the GS in an extinction test was the same as moderate shock rats that underwent CS extinction. While future studies need to replicate and generalize these results to humans, it would also be worthwhile to investigate the neurobiological mechanisms involved in and constraining the generalization of extinction. Whereas proactively limiting the generalization of fear after a threatening event might be difficult to achieve, finding ways to improve the generalization of extinction might be more feasible. This could prove beneficial then as an adjunct to exposure therapy. As one avenue for further exploration, previous research has shown that vagus nerve stimulation during extinction can increase generalization of extinction in male rats (Noble et al., 2019; Souza et al., 2020).

In conclusion, we observed that increased generalization of acquisition resulted in lower generalization of extinction to the original fear-eliciting cue. This may be important to consider in extinction-based exposure therapy, as patients frequently exhibit broadly generalized fears, and the exposure typically involves cues or situations different from those where the fear was originally acquired.

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Declaration of competing interests

The authors declare no conflicts of interest.

References

Alfei, J.M., De Gruy, H., De Bundel, D., Luyten, L., Beckers, T., 2021. Apparent reconsolidation interference without generalized amnesia. Progress in Neuro-Psychopharmacology and Biological Psychiatry 108, 110161. https://doi.org/10.1016/j.pnpbp.2020.110161

Alfei, J.M., Ferrer Monti, R.I., Molina, V.A., De Bundel, D., Luyten, L., Beckers, T., 2020. Generalization and recovery of post-retrieval amnesia. Journal of Experimental Psychology: General 149, 2063–2083. https://doi.org/10.1037/xge0000765

Asok, A., Kandel, E.R., Rayman, J.B., 2019. The neurobiology of fear generalization. Frontiers in Behavioral Neuroscience 12, 329. https://doi.org/10.3389/fnbeh.2018.00329

Baran, S.E., Armstrong, C.E., Niren, D.C., Hanna, J.J., Conrad, C.D., 2009. Chronic stress and sex differences on the recall of fear conditioning and extinction. Neurobiology of Learning and Memory 91, 323–332. https://doi.org/10.1016/j.nlm.2008.11.005

Ben-Shachar, M.S., Makowski, D., Lüdecke, D., Patil, I., Wiernik, B.M., Thériault, R., Kelley, K., Stanley, D., Caldwell, A., Burnett, J., Karreth, J., Waggoner, P., 2024. effectsize: Indices of Effect Size.

Boddez, Y., Callaerts-Vegh, Z., Vervliet, B., Baeyens, F., D'Hooge, R., Hermans, D., Beckers, T., 2012. Stimulus generalization and return of fear in C57BL/6J mice. Frontiers in Behavioral Neuroscience 6. https://doi.org/10.3389/fnbeh.2012.00041

Bouton, M.E., Bolles, R.C., 1979. Contextual control of the extinction of conditioned fear. Learning and Motivation 10, 445–466. https://doi.org/10.1016/0023-9690(79)90057-2

Cooper, S.E., van Dis, E.A.M., Hagenaars, M.A., Krypotos, A.-M., Nemeroff, C.B., Lissek, S., Engelhard, I.M., Dunsmoor, J.E., 2022. A meta-analysis of conditioned fear generalization in anxiety-related disorders. Neuropsychopharmacol. 47, 1652–1661. https://doi.org/10.1038/s41386-022-01332-2

Dunsmoor, J.E., Kroes, M.C.W., Braren, S.H., Phelps, E.A., 2017. Threat intensity widens fear generalization gradients. Behavioral Neuroscience 131, 168–175. https://doi.org/10.1037/bne0000186

Elkin, L.A., Kay, M., Higgins, J.J., Wobbrock, J.O., 2021. An Aligned Rank Transform Procedure for Multifactor Contrast Tests, in: The 34th Annual ACM Symposium on User Interface Software and Technology. Presented at the UIST '21: The 34th Annual ACM Symposium on User Interface Software and Technology, ACM, Virtual Event USA, pp. 754–768. https://doi.org/10.1145/3472749.3474784

Fenton, G.E., Halliday, D.M., Mason, R., Bredy, T.W., Stevenson, C.W., 2016. Sex differences in learned fear expression and extinction involve altered gamma oscillations in medial prefrontal cortex. Neurobiology of Learning and Memory, MCCS 2016 135, 66–72. https://doi.org/10.1016/j.nlm.2016.06.019

Graham, L.K., Yoon, T., Lee, H.J., Kim, J.J., 2009. Strain and sex differences in fear conditioning: 22 kHz ultrasonic vocalizations and freezing in rats. Psychology & Neuroscience 2, 219–225. https://doi.org/10.3922/j.psns.2009.2.015

Greiner, E.M., Müller, I., Norris, M.R., Ng, K.H., Sangha, S., 2019. Sex differences in fear regulation and reward-seeking behaviors in a fear-safety-reward discrimination task. Behavioural Brain Research 368, 111903. https://doi.org/10.1016/j.bbr.2019.111903

Gruene, T.M., Flick, K., Stefano, A., Shea, S.D., Shansky, R.M., 2015. Sexually divergent expression of active and passive conditioned fear responses in rats. eLife 4, e11352. https://doi.org/10.7554/eLife.11352.001

Keiser, A.A., Turnbull, L.M., Darian, M.A., Feldman, D.E., Song, I., Tronson, N.C., 2017. Sex Differences in Context Fear Generalization and Recruitment of Hippocampus and Amygdala during Retrieval. Neuropsychopharmacol 42, 397–407. https://doi.org/10.1038/npp.2016.174

Laxmi, T.R., Stork, O., Pape, H.C., 2003. Generalisation of conditioned fear and its behavioural expression in mice. Behavioural Brain Research 145, 89–98. https://doi.org/10.1016/S0166-4328(03)00101-3

Lenth, R.V., Bolker, B., Buerkner, P., Giné-Vázquez, I., Herve, M., Jung, M., Love, J., Miguez, F., Piaskowski, J., Riebl, H., Singmann, H., 2024. emmeans: Estimated Marginal Means, aka Least-Squares Means.

Lissek, S., Biggs, A.L., Rabin, S.J., Cornwell, B.R., Alvarez, R.P., Pine, D.S., Grillon, C., 2008. Generalization of conditioned fear-potentiated startle in humans: Experimental validation and clinical relevance. Behaviour Research and Therapy 46, 678–687. https://doi.org/10.1016/j.brat.2008.02.005

Lüdecke, D., Ben-Shachar, M.S., Patil, I., Waggoner, P., Makowski, D., 2021. performance: An R Package for Assessment, Comparison and Testing of Statistical Models. Journal of Open Source Software 6, 3139. https://doi.org/10.21105/joss.03139

Luyten, L., Schroyens, N., Luyck, K., Fanselow, M.S., Beckers, T., 2016. No effect of glucose administration in a novel contextual fear generalization protocol in rats. Transl Psychiatry 6, e903–e903. https://doi.org/10.1038/tp.2016.183

Mitchell, J.R., Trettel, S.G., Li, A.J., Wasielewski, S., Huckleberry, K.A., Fanikos, M., Golden, E., Laine, M.A., Shansky, R.M., 2022. Darting across space and time: parametric modulators of sex-biased conditioned fear responses. Learn Mem 29, 171–180. https://doi.org/10.1101/lm.053587.122

Noble, L.J., Meruva, V.B., Hays, S.A., Rennaker, R.L., Kilgard, M.P., McIntyre, C.K., 2019. Vagus nerve stimulation promotes generalization of conditioned fear extinction and reduces anxiety in rats. Brain Stimulation 12, 9–18. https://doi.org/10.1016/j.brs.2018.09.013

Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M.T., Baker, M., Browne, W.J., Clark, A., Cuthill, I.C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S.T., Howells, D.W., Karp, N.A., Lazic, S.E., Lidster, K., MacCallum, C.J., Macleod, M., Pearl, E.J., Petersen, O.H., Rawle, F., Reynolds, P., Rooney, K., Sena, E.S., Silberberg, S.D., Steckler, T., Würbel, H., 2020. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS Biol 18, e3000410. https://doi.org/10.1371/journal.pbio.3000410

R Core Team, 2022. R: A language and environment for statistical computing (manual). R Foundation for Statistical Computing, Vienna, Austria.

RStudio Team, 2021. RStudio: Integrated development environment for R (manual). RStudio, PBC, Boston, MA.

Shanazz, K., Dixon-Melvin, R., Nalloor, R., Thumar, R., Vazdarjanova, A.I., 2022. Sex Differences In Avoidance Extinction After Contextual Fear Conditioning: Anxioescapic Behavior In Female Rats. Neuroscience 497, 146–156. https://doi.org/10.1016/j.neuroscience.2022.06.031

Singmann, H., Bolker, B., Westfall, J., Aust, F., Ben-Shachar, M.S., Højsgaard, S., Fox, J., Lawrence, M.A., Mertens, U., Love, J., Lenth, R., Christensen, R.H.B., 2023. afex: Analysis of Factorial Experiments.

Souza, R.R., Robertson, N.M., Mathew, E., Tabet, M.N., Bucksot, J.E., Pruitt, D.T., Rennaker, R.L., Hays, S.A., McIntyre, C.K., Kilgard, M.P., 2020. Efficient parameters of vagus nerve stimulation to enhance extinction learning in an extinction-resistant rat model of PTSD. Progress in Neuro-Psychopharmacology and Biological Psychiatry 99, 109848. https://doi.org/10.1016/j.pnpbp.2019.109848

Struyf, D., Hermans, D., Vervliet, B., 2018. Maximizing the generalization of fear extinction: Exposures to a peak generalization stimulus. Behaviour Research and Therapy 111, 1–8. https://doi.org/10.1016/j.brat.2018.09.005

Trott, J.M., Hoffman, A.N., Zhuravka, I., Fanselow, M.S., 2022. Conditional and unconditional components of aversively motivated freezing, flight and darting in mice. eLife 11, e75663. https://doi.org/10.7554/eLife.75663

Vervliet, B., Vansteenwegen, D., Baeyens, F., Hermans, D., Eelen, P., 2005. Return of fear in a human differential conditioning paradigm caused by a stimulus change after extinction. Behaviour Research and Therapy 43, 357–371. https://doi.org/10.1016/j.brat.2004.02.005

Vervliet, B., Vansteenwegen, D., Eelen, P., 2004. Generalization of extinguished skin conductance responding in human fear conditioning. Learning and Memory 11, 555–558. https://doi.org/10.1101/lm.77404

Watson, J.B., Rayner, R., 1920. Conditioned emotional reactions. Journal of Experimental Psychology 3, 1–14. https://doi.org/10.1037/h0069608

Wickham, H., RStudio, 2022. tidyverse: Easily Install and Load the “Tidyverse.”

Wong, A.H.K., Lovibond, P.F., 2020. Generalization of extinction of a generalization stimulus in fear learning. Behaviour Research and Therapy 125, 103535. https://doi.org/10.1016/j.brat.2019.103535

Xu, W., Südhof, T.C., 2013. A neural circuit for memory specificity and generalization. Science 339, 1290–1295. https://doi.org/10.1126/science.1229534

Xuan, S.-M., Su, Y.-W., Liang, Y.-M., Gao, Z.-J., Liu, C.-Y., Fan, B.-F., Shi, Y.-W., Wang, X.-G., Zhao, H., 2023. mGluR5 in amygdala modulates fear memory generalization. Frontiers in Behavioral Neuroscience 17, 1072642.

Zeileis, A., Grothendieck, G., 2005. zoo: S3 infrastructure for regular and irregular time series. Journal of Statistical Software 14, 1–27. https://doi.org/10.18637/jss.v014.i06

Zhang, W.-H., Zhou, J., Pan, H.-Q., Wang, X.-Y., Liu, W.-Z., Zhang, J.-Y., Yin, X.-P., Pan, B.-X., 2017. δ Subunit-containing GABAA receptor prevents overgeneralization of fear in adult mice. Learn. Mem. 24, 381–384. https://doi.org/10.1101/lm.045856.117

Tables

Table 1. Statistical test results of the manipulation check

Phase	Measured outcome	Statistical test	Result	Effect size
Habituation	Freezing	Non-parametric mixed ANOVA	Group shock: F(1, 62) = 1.58, p = 0.213 Stimuli: F(1, 62) = 0.23, p = 0.63 Gs*S: F(1, 62) = 0.11, p = 0.737	$\omega_p^2 < -0.01$ [0,1] $\omega_p^2 < -0.01$ [0,1] $\omega_p^2 < -0.01$ [0,1]
Habituation	Discrimination index	Paired t-test	Stimuli: t(31) = 0.65, p = 0.52	d = 0.12 [-0.24, 0.47]
Acquisition	Freezing (trials)	Two-way ANOVA	Group shock: F(1, 60) = 1.58, p = 0.213 Sex: F(1, 60) = 10, **p = 0.002** Gs*S: F (1, 60) = 0.74, p = 0.393	$\omega_p^2 < -0.01$ [0,1] $\omega_p^2 = 0.12$ [0.02,1] $\omega_p^2 < -0.01$ [0,1]
Acquisition test	Freezing	Mixed ANOVA	Group shock: F(1, 62) = 0.46, p = 0.502 Stimuli: F(1, 62) = 10.08, **p = 0.002** Gs*S: F(1, 62) = 5.22, **p = 0.026**	$\omega_p^2 < -0.01$ [0,1] $\omega_p^2 = 0.03$ [0,1] $\omega_p^2 = 0.02$ [0,1]
Acquisition test	Freezing	Simple effects by group shock with Tukey correction	Moderate – Stimuli: t(124) = 2.63, **p = 0.01** Strong – Stimuli: t(124) = 0.43, p = 0.669	d = 0.66 [0.16, 1.16] d = 0.11 [-0.39, 0.6]
Acquisition test	Discrimination index	T-test	Group shock: t(62) = 1.46, p = 0.148	d = 0.37 [-0.13, 0.86]
Extinction training	Freezing (trials)	Non-parametric mixed ANOVA	Group: F(3, 60) = 8.54, **p < 0.001** Trial: F(17, 1020) = 16.06, **p < 0.001** G*T: F(51, 1020) = 1.32, p = 0.068	$\omega_p^2 = 0.24$ [0.07,1] $\omega_p^2 = 0.13$ [0.09,1] $\omega_p^2 < -0.01$ [0,1]
Extinction training	Freezing (trials)	Pairwise comparisons with Tukey correction	MSCS – MSGS: t(60) = 2.58, p = 0.059 MSCS – SSCS: t(60) = -2.34, p = 0.1 MSCS – SSGS: t(60) = 1.05, p = 0.719 MSGS – SSCS: t(60) = -4.92, **p < 0.001** MSGS – SSGS: t(60) = -1.52, p = 0.43 SSCS – SSGS: t(60) = 3.39, **p = 0.006**	r = 0.18 [0.09, 0.27] r = -0.24 [-0.33, -0.15] r = 0.07 [-0.03, 0.16] r = -0.43 [-0.5, -0.35] r = -0.14 [-0.23, -0.05] r = 0.32 [0.23, 0.40]
Extinction training	Freezing (early vs late block)	Non-parametric mixed ANOVA	Sex: F(1, 56) = 4.66, **p = 0.035** Group: F(3, 56) = 8.43, **p = 0.001** Block: F(1, 56) = 93.47, **p < 0.001**	$\omega_p^2 = 0.05$ [0,1] $\omega_p^2 = 0.22$ [0.06,1] $\omega_p^2 = 0.37$ [0.21,1]

			S*G: F(3, 56) = 1.75, p = 0.168 S*B: F(1, 56) = 1.52, p = 0.223 G*B: F(3, 56) = 2.39, p = 0.078 S*G*B: F(3, 56) = 2.19, p = 0.099	$\omega_p^2 < 0.01$ [0,1] $\omega_p^2 < 0.01$ [0,1] $\omega_p^2 < -0.01$ [0,1] $\omega_p^2 = 0.02$ [0,1]
Extinction training	Freezing (early vs late block)	Pairwise comparisons with Tukey correction	MSCS – MSGS: t(56) = 2.7, **p = 0.044** MSCS – SSCS: t(56) = -2.27, p = 0.12 MSCS – SSGS: t(56) = 0.76, p = 0.874 MSGS – SSCS: t(56) = -4.97, **p < 0.001** MSGS – SSGS: t(56) = -1.95, p = 0.221 SSCS – SSGS: t(56) = 3.02, **p = 0.019**	r = 0.27 [-0.01, 0.51] r = -0.26 [-0.5, 0.02] r = 0.05 [-0.23, 0.33] r = -0.56 [-0.72, -0.33] r = -0.22 [-0.47, 0.06] r = 0.33 [0.06, 0.56]

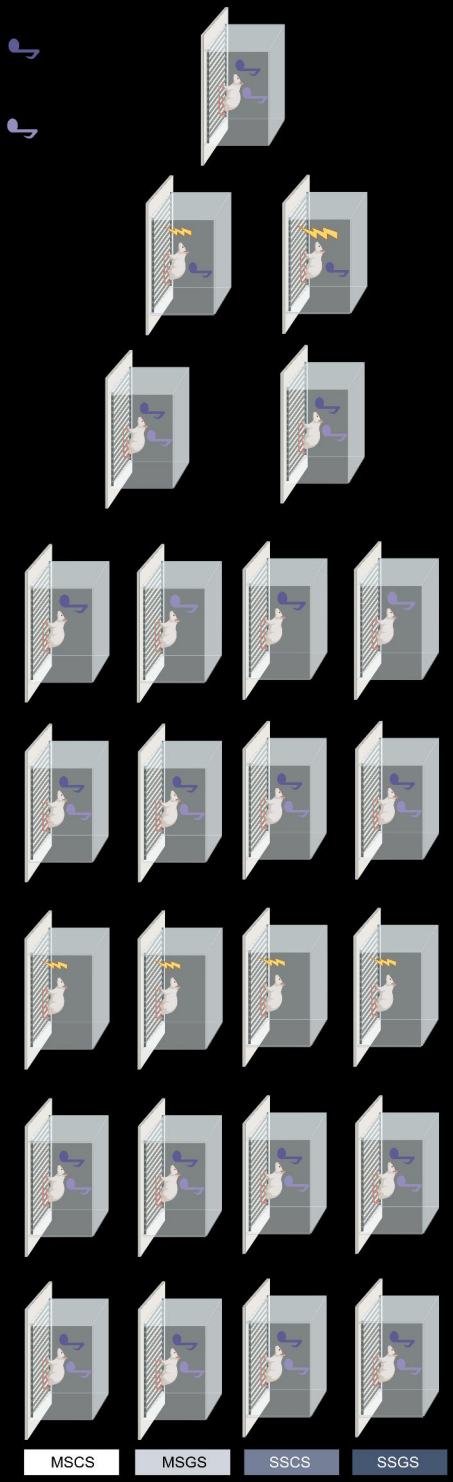
Table 2. Statistical test results for the effects of generalized acquisition on generalization of extinction

Extinction test	Freezing	Mixed ANOVA	Group: F(3, 56) = 8.45, **p < 0.001** Sex: F(1, 56) = 1.39, p = 0.243 G*Se: F(3, 56) = 3.01, **p = 0.038** Stimuli: F(1, 56) = 25.24, **p < 0.001** G*St: F(3, 56) = 4.13, **p = 0.01** Se*St: F(1, 56) = 0.02, p = 0.883 G*Se*St: F(3, 56) = 0.81, p = 0.493	$\omega_p^2 = 0.27$ [0.1,1] $\omega_p^2 < 0.01$ [0,1] $\omega_p^2 = 0.09$ [0,1] $\omega_p^2 = 0.15$ [0.03,1] $\omega_p^2 = 0.06$ [0,1] $\omega_p^2 < -0.01$ [0,1] $\omega_p^2 < -0.01$ [0,1]
Extinction test	Freezing	Simple effects by stimuli with Tukey correction - CS	MSCS – MSGS: t(112) = -0.54, p = 0.949 MSCS – SSCS: t(112) = -3.20, **p = 0.01** MSCS – SSGS: t(112) = -3.96, **p < 0.001** MSGS – SSCS: t(112) = -2.65, **p = 0.044** MSGS – SSGS: t(112) = -3.41, **p = 0.005** SSCS – SSGS: t(112) = -0.76, p = 0.872	d = -0.19 [-0.89, 0.51] d = -1.13 [-1.85, -0.41] d = -1.4 [-2.12, -0.67] d = -0.94 [-1.65, -0.23] d = -1.21 [-1.93, -0.49] d = -0.27 [-0.97, 0.43]
Extinction test	Freezing	Simple effects by stimuli with Tukey correction - GS	MSCS – MSGS: t(112) = 2.73, **p = 0.036** MSCS – SSCS: t(112) = -1.38, p = 0.514 MSCS – SSGS: t(112) = 0.19, p = 0.997 MSGS – SSCS: t(112) = -4.11, **p < 0.001** MSGS – SSGS: t(112) = -2.54, p = 0.06 SSCS – SSGS: t(112) = 1.58, p = 0.397	d = 0.97 [0.25, 1.68] d = -0.49 [-1.19, 0.21] d = 0.07 [-0.63, 0.77] d = -1.45 [-2.18, -0.73] d = -0.9 [-1.61, -0.19] d = 0.56 [-0.15, 1.26]

Extinction test	Freezing	Simple effects by group with Tukey correction	MSCS – Sex: t(112) = 0.23, p = 0.822 MSGS – Sex: t(112) = 0.15, p = 0.882 SSCS – Sex: t(112) = -3.49, **p < 0.001** SSGS – Sex: t(112) = 0.53, p = 0.593	d = 0.08 [-0.62, 0.78] d = 0.05 [-0.65, 0.75] d = -1.23 [-1.95, -0.51] d = 0.19 [-0.51, 0.89]
Extinction test	Discrimination index	One-way ANOVA	Group: F(3, 60) = 10.89, **p < 0.001**	ω^2 = 0.32 [0.14, 1]
Extinction test	Discrimination index	Pairwise comparisons with Tukey correction	MSCS – MSGS: t(60) = -5.47, **p < 0.001** MSCS – SSCS: t(60) = -1.65, p = 0.358 MSCS – SSGS: t(60) = -3.24, **p = 0.01** MSGS – SSCS: t(60) = 3.82, **p = 0.002** MSGS – SSGS: t(60) = 2.23, p = 0.127 SSCS – SSGS: t(60) = -1.59, p = 0.39	d = -1.94 [-2.7, -1.17] d = -0.58 [-1.29, 0.12] d = -1.15 [-1.87, -0.42] d = 1.35 [0.6, 2.1] d = 0.79 [0.07, 1.51] d = -0.56 [-1.28, 0.15]

Table 3. Statistical tests for return of fear

Reinstatement test	Freezing	Non-parametric mixed ANOVA	Group: F(3, 45) = 1.48, p = 0.231 Stimuli: F(1, 45) = 18.1, **p < 0.001** G*S: F(3, 45) = 1.44, p = 0.244	ω_p^2 = 0.03 [0,1] ω_p^2 = 0.07 [0.01,1] ω_p^2 = -0.02 [0,1]
Reinstatement test	Discrimination index	One-way ANOVA	Group: F(3, 45) = 1.62, p = 0.199	ω^2 = 0.04 [0, 1]
Spontaneous recovery test	Freezing	Mixed ANOVA	Group: F(3, 36) = 0.4, p = 0.757 Stimuli: F(1, 36) = 10.54, **p = 0.003** G*S: F(3, 36) = 1.99, p = 0.133	ω_p^2 = -0.05 [0,1] ω_p^2 = 0.06 [0,1] ω_p^2 = 0.02 [0,1]
Spontaneous recovery test	Discrimination index	One-way ANOVA	Group: F(3, 36) = 3.77, **p = 0.019**	ω^2 = 0.17 [0, 1]
Spontaneous recovery test	Discrimination index	Pairwise comparisons with Tukey correction	MSCS – MSGS: t(36) = -2.78, **p = 0.041** MSCS – SSCS: t(36) = 0.07, p = 0.999 MSCS – SSGS: t(36) = -1.2, p = 0.631 MSGS – SSCS: t(36) = 2.99, **p = 0.024** MSGS – SSGS: t(36) = 1.62, p = 0.378 SSCS – SSGS: t(36) = -1.33, p = 0.549	d = -1.28 [-2.26, -0.3] d = 0.03 [-0.88, 0.94] d = -0.55 [-1.49, 0.39] d = 1.31 [0.37, 2.25] d = 0.73 [-0.2, 1.65] d = -0.58 [-1.48, 0.31]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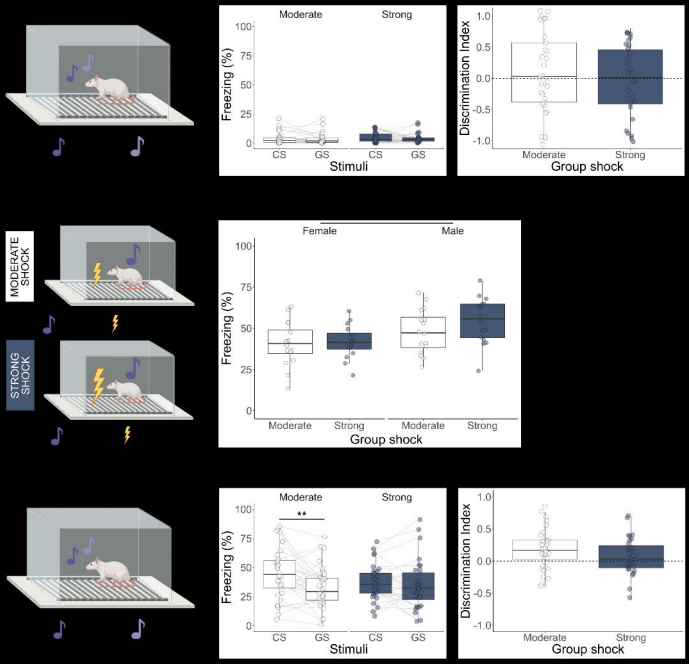
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Group shock
MODERATE SHOCK
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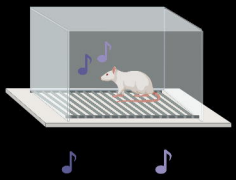

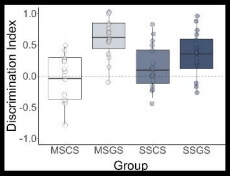
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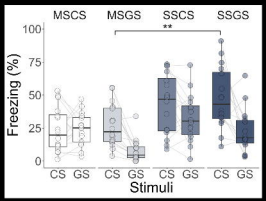
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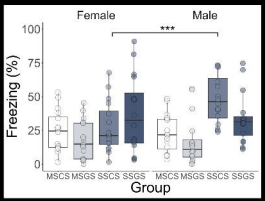
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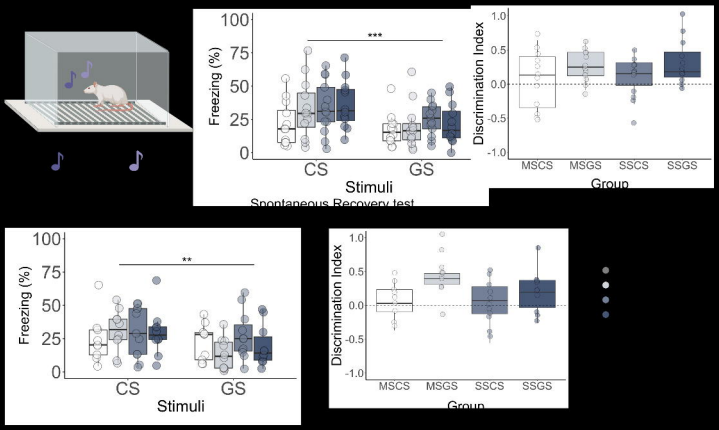
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