Stress promotes generalization of older but not recent threat memories

Joseph E. Dunsmoor[a,1], A. Ross Otto[b], and Elizabeth A. Phelps[c,d]

[a]Department of Psychiatry, The University of Texas at Austin Dell Medical School, Austin, TX 78712; [b]Department of Psychology, McGill University, Montreal, QC, H3A 1G1 Canada; [c]Department of Psychology and Center for Neural Sciences, New York University, New York, NY 10009; and [d]Emotional Brain Institute, Nathan S. Kline Institute for Psychiatric Research, Orangeburg, NY 10962

Stress broadly affects the ability to regulate emotions and may contribute to generalization of threat-related behaviors to harmless stimuli. Behavioral generalization also tends to increase over time as memory precision for recent events gives way to more gist-like representations. Thus, acute stress coupled with a delay in time from a negative experience may be a strong predictor of the transition from normal to generalized fear expression. Here, we investigated the effect of a single-episode acute stressor on generalization of aversive learning when stress is administered either immediately after an aversive learning event or following a delay. In a between-subjects design, healthy adult volunteers underwent threat (fear) conditioning using a tone-conditioned stimulus paired with an electric shock to the wrist and another tone not paired with shock. Behavioral generalization was tested to a range of novel tones either on the same day (experiment 1) or 24 h later (experiment 2) and was preceded by either an acute stress induction or a control task. Anticipatory sympathetic arousal [i.e., skin conductance responses (SCRs)] and explicit measures of shock expectancy served as dependent measures. Stress administered shortly after threat conditioning did not affect behavioral generalization. In contrast, stress administered following a delay led to heightened arousal and increased generalization of SCRs and explicit measures of shock expectancy. These findings show that acute stress increases generalization of older but not recent threat memories and have clinical relevance to understanding overgeneralization characteristics of anxiety and stress-related disorders.

stress | Pavlovian conditioning | associative learning | generalization | memory

A number of anxiety and stress-related disorders can be characterized by an inability to discriminate threat from safety. For instance, a core symptom of trauma- and stress-related disorders [e.g., posttraumatic stress disorder (PTSD)] is persistent and widespread fear and avoidance of myriad harmless cues that act as reminders of the trauma, often referred to as overgeneralization (1). A key component of overgeneralization in anxiety and stress-related disorders may hinge on stress-induced changes in neural circuitry underlying the ability to discriminate threat from safety and regulate emotional responses (2–4). Behavioral generalization also tends to increase over time, as memory precision for recent events gives way to more gist-like representations (5, 6). It then follows that stress-induced impairments in discriminating threat from safety coupled with a loss of memory precision might jointly influence the transition away from normal fear (i.e., highly specific to a known threat) toward overgeneralized fear. Here, we investigate how acute stress and the time between learning and test impacts fear generalization in humans.

The effects of stress on the ability to regulate defensive behaviors have been detailed using Pavlovian threat (fear) conditioning tasks in humans and laboratory animals (7–9). Much of this research has focused predominately on how acute and chronic stress impairs threat extinction to conditioned stimuli (CS) or contexts via structural and functional changes in the neural circuitry involved in the learning (10), consolidation (11), and retrieval (12) of extinction memories (see ref. 13 for review). In humans, a single episode of acute stress can impair retrieval of cued threat extinction after a delay (14), consistent with studies of patients with PTSD showing deficits in the ability to retain extinction memories following standard extinction procedures (15). Notably, PTSD is also characterized by difficulty discriminating between a dangerous CS and a safe CS (16, 17) and overgeneralization of amygdala activity to a variety of cues that resemble a learned threat (18). Whether a single episode of acute stress affects the ability to behaviorally discriminate between threat cues and similar, but harmless cues—thereby leading to overgeneralization—has to our knowledge remained unexplored in humans and in other species (see ref. 19 for related work on contextual generalization in rats as a function of the number of electric shocks).

Another important factor that contributes to behavioral generalization is time from initial learning (5, 20). In the laboratory, time-dependent effects can be revealed by simply varying the duration between learning and test. Research in animal associative learning shows that as time from initial conditioning elapses, animals respond to a broader range of cues (e.g., different tones or colors) that decreasingly resemble the originally reinforced cue (21–24). Notably, much of the stimulus generalization research on delayed testing has involved instrumental appetitive conditioning; how delayed testing affects generalization of Pavlovian threat learning, per se, has been explored almost exclusively in the realm of context conditioning (1, 6), in which the animal learns to associate the environment itself—rather than a single cue—with an aversive unconditioned stimulus (US). In rodents, an escalation in the time between learning and test increases the likelihood of contextual generalization, indicated by increased freezing in novel environments (25, 26). One explanation for time-dependent behavioral generalization is that stimulus features or attributes of the originally reinforced stimulus or context are forgotten over time, while the acquired behavior persists in memory (5, 27). Thus, the range of stimuli that can elicit the conditioned response increases, reflected by a broadening (or flattening) of the stimulus generalization gradient. This loss of fear memory precision over time may be a contributing factor to overgeneralization in PTSD (1).

Significance

A hallmark of anxiety disorders is the tendency to overgeneralize fear-related behaviors toward harmless stimuli or situations. But little is known about the factors contributing to overgeneralization. Here we investigated the role of acute stress and consolidation in promoting fear overgeneralization. Acute stress resulted in broad generalization of a conditioned fear memory, but only if stress occurred at least 24 h after memory formation. This finding shows that remote fear memories are more sensitive to the effects of stress than newly formed fear memories. Susceptibility to stress for old memories may be a contributing factor to overgeneralization in a host of anxiety and stress-related disorders.

Here, we investigated the role of stress and delayed testing on threat (fear) generalization using a Pavlovian conditioning and generalization task in healthy adults. Participants all underwent an identical differential conditioning protocol in which one CS (CS+) was paired with an aversive electrical shock (US) to the right wrist, and a within-subjects control CS was unpaired (CS−). In experiment 1, conditioning was followed by stress induction or a control task on the same day, while in experiment 2 stress induction or control was administered 24 h after conditioning. For all participants, behavioral generalization was tested ≈15 min after the stress/control task and involved presentations of the CS+, CS−, and intermediate tones not paired with shock. We predicted an interactive effect of stress and the amount of time between conditioning and generalization testing. Specifically, we expected a recent threat memory (experiment 1) to be less sensitive to stress-induced overgeneralization than a temporally distant threat memory (experiment 2), as reflected through behavioral generalization to harmless cues that resemble a learned threat.

Results

Experiment 1: Effects of Stress at an Immediate Test of Generalization.
This experiment occurred across two phases, threat conditioning and a generalization test, separated by a stress manipulation [cold pressor task (CPT)] (28) or control task to manipulate cortisol levels before the start of generalization testing. During threat conditioning, subjects learned to discriminate between a 550- and 1,000-Hz tone that served as either the CS+, paired with an electrical shock to the wrist, or the CS− (counterbalanced across subjects). During the CPT, subjects placed their forearm in ice-cold water for 3 min; in the control task, the water was room temperature. In a subsequent generalization test, subjects were presented with the CS+, CS−, and eight intermediate tones [generalization stimuli (GS)] that varied in pitch between the CS+ and CS− frequency (600, 650, 700, 750, 800, 850, 900, and 950 Hz). Adjacent tone frequencies were collapsed to form four stimulus bins (e.g., 600 + 650 Hz = GS1, etc.) in keeping with prior research on perceptual-based generalization (29). This created a continuum of decreasingly similar tones that range from the GS4 (most similar to the CS+) to GS1 (most similar to the CS−). See *Materials and Methods* for further details on the experimental design.

Salivary Cortisol and Subjective Ratings of Stress and Fear.
Analysis of cortisol (Fig. 1A) across each time point (baseline, postthreat conditioning, 10 min following CPT/control, postgeneralization test) revealed a significant time × group interaction [$F(1.662, 56.496) = 14.422$, $P < 0.001$, $\eta^2 = 0.298$]. Self-reported levels of subjective stress were also greater immediately following the stress induction (mean ± SD: 7.1 ± 2.3) versus the control task (2.8 ± 1.8), $t(34) = 6.107$, $P < 0.0001$. Thus, the stress manipulation was successful at increasing cortisol levels and subjective reports of stress.

After the generalization test, subjects completed self-reports of subjective shock intensity ("How unpleasant did the shock feel?"), retrospective ratings of how much fear was experienced during the task, an estimate of how many shocks were received during the task, and they were asked to identify the CS+ among different tones that varied in frequency (*Materials and Methods*). Postexperimental ratings of subjective shock intensity ($P = 0.616$), retrospective fear ($P = 0.595$), and estimate of how many shocks were received ($P = 0.764$) were not different between groups, indicating that subjective appraisal of the aversive US itself and conscious feelings of fear were not affected by the stress manipulation. The percentage of subjects who correctly identified the CS+ also did not differ by group, $X^2(1, n = 36) = 1.003$, $P = 0.317$. Thus, stress induction did not enhance how intense the shock felt, how much fear subjects felt, estimates of how many aversive events (shocks) were experienced overall, or accuracy in retrospectively identifying the CS+ frequency.

Skin Conductance Responses.
Conditioning. Mean threat conditioning skin conductance responses (SCRs) (Fig. 2A) were characterized by a main effect of CS type (CS+, CS−), $F(1,34) = 43.287$, $P < 0.001$, $\eta^2 = 0.560$, with no effect of group ($P = 0.172$), and no CS type × group interaction, $P = 0.981$. As expected, given our subject inclusion criteria (*Materials and Methods*), mean SCRs were greater for CS+ than CS− trials in both groups [control: $t(18) = 5.196$, $P < 0.0001$; stress: $t(16) = 4.189$, $P = 0.001$]. The lack of any group effect on threat conditioning was also expected as it occurred before the stress manipulation.

Generalization. In experiment 1, behavioral generalization to novel stimuli was tested on the same day as fear conditioning, ≈15 min following administration of an acute stressor (CPT) or a control task. Analysis of behavioral generalization focused on early (first half of testing) and late (second half of testing) trials, because behavioral
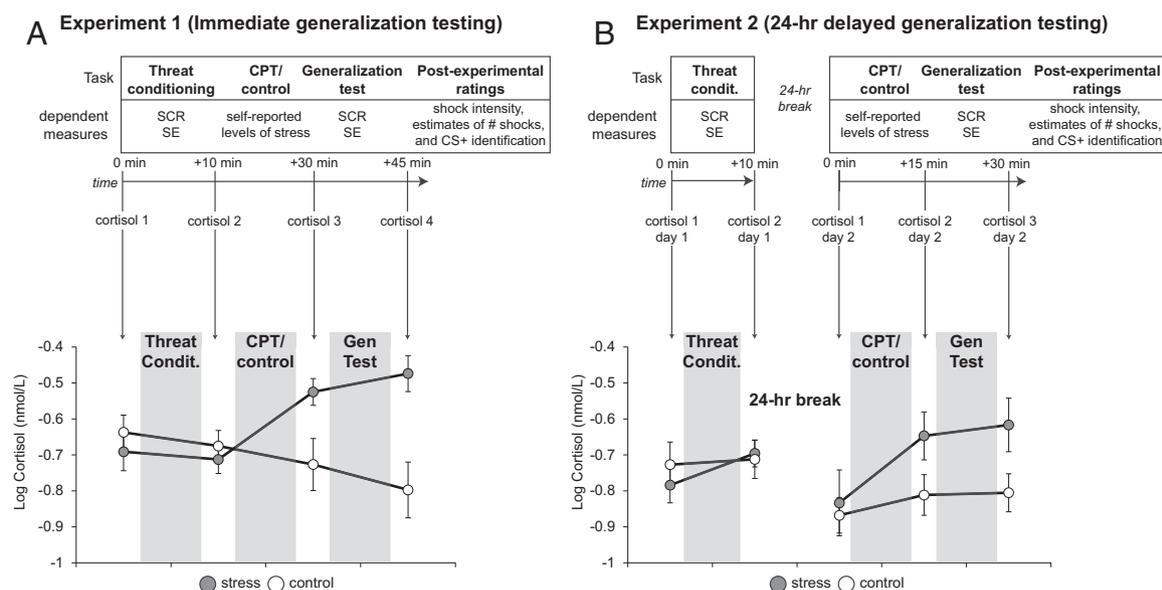

Fig. 1. Schematic of experimental procedure and timeline of cortisol measurements for experiment 1 (A) and experiment 2 (B). In both experiments, submerging their forearm in ice-cold water for 3 min (stress) raised subjects' cortisol levels from baseline and relative to a warm water (control) condition. Notably, cortisol levels did not change from before to after threat conditioning. Error bars are SEM; Nmol/L, nanomole/liter; SCR, skin conductance responses; SE, shock expectancy ratings.
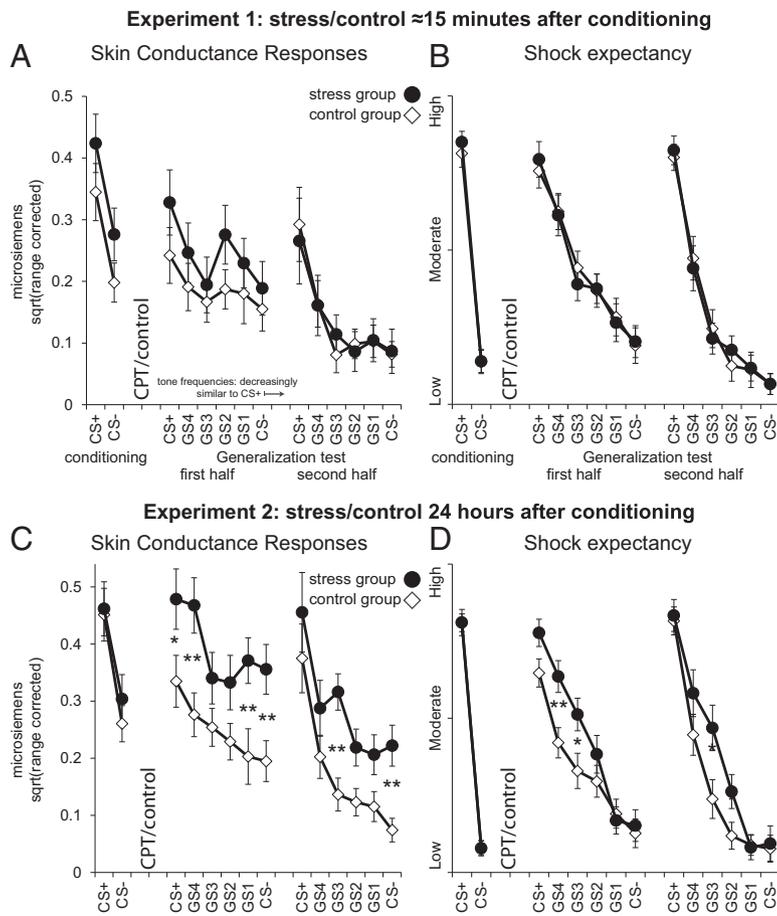
Experiment 1: stress/control ≈15 minutes after conditioning

A — Skin Conductance Responses

B — Shock expectancy

Experiment 2: stress/control 24 hours after conditioning

C — Skin Conductance Responses

D — Shock expectancy

Fig. 2. Subjects first underwent differential Pavlovian threat conditioning between two tones either paired (CS+) or unpaired (CS−) with an electrical shock. Threat conditioning was then followed by either a stress or control manipulation immediately after conditioning (experiment 1) or 24 h later (experiment 2), followed shortly thereafter by a generalization test consisting of tone frequencies between the CS+ and CS− frequency. In experiment 1, generalization of skin conductance responses (A) and expectancy (B) diminished as a function of similarity to the CS+, but there was no effect of stress. When stress or control were administered 24 h after threat conditioning (experiment 2), generalization of SCRs (C) and expectancy (D) were elevated following stress versus control. *$P < 0.05$; **$P < 0.01$; error bars are SEM.

generalization tends to diminish over the course of testing as subjects receive a number of unreinforced generalization trials (30). Note that there was no signaled break between the first and second halves of the generalization test. As shown in Fig. 2A, both groups exhibited a steep gradient of SCRs during early and late trials, with the maximal SCR to the CS+, and diminishing SCRs as similarity to the CS+ decreased. These generalization decrements resemble those observed in numerous animal stimulus generalization studies that test generalization shortly after conditioning (31, 32) and in human fear generalization studies that have almost universally tested generalization shortly after conditioning (33). Repeated-measures ANOVA incorporating stimulus (CS−, GS1, GS2, GS3, GS4, and CS+) and phase (early, late trials) as within-subjects factors and group as a between-subjects factor revealed main effects of stimulus [$F(2.729, 92.792) = 16.573$, $P < 0.0001$, $\eta^2 = 0.328$] with significant linear [$F(1,34) = 30.272$, $P < 0.0001$, $\eta^2 = 0.471$] and quadratic trends [$F(1,34) = 11.932$, $P < 0.0001$, $\eta^2 = 0.260$]. There were also main effects of phase (early, late testing) [$F(1,34) = 33.577$, $P < 0.0001$, $\eta^2 = 0.497$], and a stimulus × phase interaction [$F(2.729, 136.518) = 3.686$, $P = 0.009$, $\eta^2 = 0.095$]. Importantly, there was no effect of group ($P = 0.559$), no stimulus × group interaction ($P = 0.781$), and no stimulus × group × phase interaction ($P = 0.607$). The phase × group interaction was significant [$F(1,34) = 4.383$, $P = 0.044$, $\eta^2 = 0.114$]. This effect was driven by an overall increase in arousal in the stress group during the early phase of testing, but without any significant increase to a particular stimulus. In sum, we did not observe any meaningful or orderly effect of acute stress administration on the SCR gradient when testing, followed shortly after conditioning on the same day.

Shock Expectancy Ratings.

Conditioning. Trial-by-trial subjective shock expectancy ratings (Fig. 2B) were collected throughout acquisition and generalization using a three-response scale (corresponding to "no risk," "moderate risk," or "high risk") for receiving the electrical shock (based on ref. 34). In the acquisition phase, we found a main effect of CS type [$F(1,34) = 267.808$, $P < 0.001$, $\eta^2 = 0.887$], but no effect of group ($P = 0.613$) and no CS type × group interaction ($P = 0.672$). Planned t tests confirmed greater shock expectancy ratings on CS+ versus CS− trials in both groups [control: $t(18) = 10.325$, $P < 0.0001$; stress: $t(16) = 13.757$, $P < 0.001$].

Generalization. During the generalization test (Fig. 2B), both groups showed gradients of shock expectancy ratings characterized by a main effect of stimulus [$F(2.852, 96.984) = 90.591$, $P < 0.0001$, $\eta^2 = 0.727$], with significant linear [$F(1,34) = 170.366$, $P < 0.0001$, $\eta^2 = 0.834$] and quadratic trends [$F(1,34) = 49.558$, $P < 0.0001$, $\eta^2 = 0.593$]. There was also a main effect of phase (early, late testing) [$F(1,34) = 32.721$, $P < 0.0001$, $\eta^2 = 0.490$], and a stimulus × phase interaction [$F(3.246, 110.375) = 4.999$, $P = 0.002$, $\eta^2 = 0.128$]. There was no effect of group ($P = 0.983$) and no interactions with group. Thus, acute stress did not have any detectable effect on shock expectancy, mirroring the pattern of physiological responses.

Experiment 2: Effects of Stress on a Delayed Test of Generalization.
Threat conditioning and generalization testing were separated by 24 h in experiment 2. A CPT or control task was used to manipulate acute stress before the start of generalization testing on day 2.

Salivary Cortisol and Subjective Ratings of Stress and Fear.
Analysis of cortisol (Fig. 1B) on day 1 using time point (before and after fear conditioning) and group as factors, showed no change in cortisol ($P = 0.227$), no effect of group ($P = 0.292$), and no time × group interaction ($P = 0.465$). On day 2, there was a significant time point × group interaction [$F(0.589, 58.795) = 3.613$, $P = 0.043$, $\eta^2 = 0.089$]. Self-reported levels of stress were also greater

immediately following the stress (mean ± SD: 8.4 ± 1.3) versus control task (1.8 ± 2.1), $t(38) = 11.362$, $P < 0.0001$. Consistent with experiment 1, postexperimental ratings of electrical shock intensity ($P = 0.345$), self-reported fear ($P = 0.711$), estimates of how many shocks were received overall ($P = 0.865$), and accuracy in retrospectively identifying the CS+ $X^2(1, n = 39) = 1.008$, $P = 0.292$ were not different between groups. To test whether accuracy at identifying the correct CS+ was affected over a delay, we compared results from experiment 1 (immediate testing) to experiment 2 (delayed testing). The proportion of subjects from experiment 1 who correctly identified the CS+ (50%) was significantly greater than the proportion of subjects from experiment 2 (23%), $X^2(1, n = 75) = 5.889$, $P = 0.015$, suggesting that subjects had a less precise memory for which stimulus had been paired with shock after a 24-h delay.

Skin Conductance Responses.

Conditioning. Successful threat conditioning in experiment 2 (Fig. 2C) was confirmed by a main effect of CS type (CS+, CS−), $F(1,39) = 45.374$, $P < 0.001$, but no effect of group ($P = 0.644$) and no CS type × group interaction, $P = 0.531$. Planned t tests confirmed greater mean SCRs on CS+ than CS− trials in both groups [control: $t(20) = 5.044$, $P < 0.001$; stress: $t(19) = 4.468$, $P < 0.001$].

Generalization. In experiment 2, behavioral generalization was tested 24 h after threat conditioning, ≈15 min following administration of an acute stressor or a control task. Following the 24-h delay, both groups continued to discriminate between the CS+ and CS− during the first half of the generalization test [control: $t(20) = 3.753$, $P = 0.001$; stress: $t(19) = 2.974$, $P = 0.008$]. Both groups also exhibited an incremental decline of SCRs during early and late trials, with the maximal response to the CS+. However, the shape of the SCR gradient was noticeably different between groups: whereas the control group exhibited a sharply decremented (i.e., narrow) gradient that clearly peaks at the CS+, the stress group exhibited heightened and widespread arousal to stimuli along the continuum (i.e., overgeneralization). Again, we used a repeated-measures ANOVA to characterize the effect of stress on the generalization gradient. Similar to the results from experiment 1, we detected a main effect of stimulus [$F(2.399,93.554) = 25.927$, $P < 0.0001$, $\eta^2 = 0.399$], with significant linear [$F(1,39) = 39.269$, $P < 0.001$, $\eta^2 = 0.502$] and quadratic trends [$F(1,39) = 28.218$, $P < 0.001$, $\eta^2 = 0.420$]. There was also a main effect of phase [$F(1,39) = 23.523$, $P < 0.0001$, $\eta^2 = 0.376$] and a stimulus × phase interaction [$F(5,195) = 5.812$, $P < 0.001$, $\eta^2 = 0.130$]. Importantly, unlike the results from experiment 1, there was an effect of group [$F(1,39) = 7.376$, $P = 0.01$, $\eta^2 = 0.159$], whereby subjects in the stress condition exhibited overall enhanced SCRs compared with subjects in the control condition and a significant stimulus × phase × group interaction [$F(5,195) = 2.515$, $P = 0.031$, $\eta^2 = 0.061$].

There are a number of possible ways to approach the post hoc analysis of SCRs within and between the control group and stress group. We first focus on the early phase of the generalization test, as it is during this period in which we expect to see the most generalized arousal (30); that is, before overall levels of arousal habituate and/or discrimination learning overtakes generalization. Planned independent-samples t tests comparing mean SCRs to each stimulus, between groups, revealed overall enhanced arousal in the stress group versus control group for the CS+ ($P = 0.0244$), GS4 ($P = 0.0018$), GS1 ($P = 0.0055$), and CS− ($P = 0.0051$). These results survived correction for multiple comparisons ($\alpha = 0.05/6 = 0.0083$) for the G4, GS1, and the CS−, but not for the CS+. Next, we assessed the point at which SCRs significantly diminished from the actual threat (the CS+) in each group during early generalization testing. The control group showed an immediate decrease in SCRs between the CS+ and most similar tone (GS4) ($P = 0.0296$). In contrast, the stress group showed a difference in SCRs between the CS+ and GS3 ($P = 0.001$), but SCRs between the CS+ and GS4 were not different ($P = 0.7876$), suggesting near complete generalization between the CS+ and GS4 (i.e., physiological arousal to the CS+ and GS4 is undifferentiated). During the late phase of generalization testing, planned comparisons revealed enhanced

arousal in the stress versus control group evoked by the GS3 ($P = 0.0065$) and CS− ($P = 0.00089$), suggesting that arousal remained selectively elevated to unreinforced cues in the stress versus control group. Interestingly, delayed testing by itself was not sufficient to produce overgeneralization profiles: a post hoc analysis of the no-stress control group from experiment 1 and no-stress control group from experiment 2 did not show an effect of group ($P = 0.373$) or a stimulus by group interaction ($P = 0.314$).

Shock Expectancy Ratings.

Conditioning. Analysis of shock expectancy ratings from threat conditioning (Fig. 2D) revealed a main effect of CS type [$F(1,39) = 680.474$, $P < 0.001$, $\eta^2 = 0.946$], but no effect of group ($P = 0.996$) and no CS type × group interaction ($P = 0.968$). Planned t tests confirmed greater shock expectancy ratings on CS+ versus CS− trials in both groups [control-immediate: $t(20) = 25.008$, $P < 0.0001$; stress-immediate: $t(19) = 15.068$, $P < 0.001$].

Generalization. During the generalization test, the stress and control groups both exhibited gradients of shock expectancy (Fig. 2D) characterized by a main effect of stimulus [$F(2.927,114.139) = 91.301$, $P < 0.0001$, $\eta^2 = 0.701$], with significant linear [$F(1,39) = 180.497$, $P < 0.0001$, $\eta^2 = 0.822$] and quadratic trends [$F(1,39) = 21.747$, $P < 0.0001$, $\eta^2 = 0.358$], such that test stimuli more similar to the CS+ elicited a larger expectancy. There was also a main effect of phase (early, late testing) [$F(1,39) = 6.503$, $P = 0.015$, $\eta^2 = 0.143$] and a stimulus × phase interaction [$F(3.557,138.737) = 6.747$, $P < 0.002$, $\eta^2 = 0.147$]. Importantly, unlike experiment 1 there was a main effect of group [$F(1,39) = 4.550$, $P = 0.039$, $\eta^2 = 0.105$] and a stimulus × group interaction [$F(2.927,138.737) = 2.704$, $P = 0.022$, $\eta^2 = 0.065$]. Shock expectancy was elevated on CS+ ($P = 0.04$), GS4 ($P = 0.004$), and GS3 ($P = 0.024$) trials in the stress versus control group during the early phase of testing, and on GS3 ($P = 0.021$) and GS2 ($P = 0.046$) trials during the late phase of testing. These shock expectancy ratings more or less mirror the physiological results, with the exception that subjects in the stress and control groups maintained equally low shock expectancy for the learned safety stimulus, CS−.

Discussion

There is increasing interest in understanding the multiple ways stress can affect emotion and cognition (35). This interest derives in large measure from the clinical implications of this research, as stressful life events are a risk factor for developing a number of psychopathologies (36). While the effects of acute and chronic stress on acquisition and extinction of threat (fear) conditioning has been explored across species, the role of stress on stimulus generalization has received extremely limited attention, especially in humans. Moreover, the question of whether delayed testing promotes generalization of emotional learning has until now remained largely unexamined in humans. Here, we found that a single episode of acute stress administered almost immediately after an aversive learning experience did not alter behavioral generalization in healthy adults. In contrast, an acute stress episode 24 h after learning did increase autonomic arousal (and explicit ratings of expectancy) to a learned threat and to harmless stimuli that resembled a learned threat. Put another way, more temporally distant (older) threat memories appear to be more sensitive to the effects of stress than recently formed memories.

Neuroscience research on acute and chronic stress has focused largely on feedback projections between the hypothalamic–pituitary–adrenal axis and prefrontal and subcortical regions involved in emotion and cognition (37). Components of this circuitry are important for the acquisition and, in the case of the prefrontal cortex and hippocampus, the regulation of emotional behaviors. The role of stress on extinction (and emotion regulation more generally) is complex, as glucocorticoid activity is in some cases associated with stronger within-session extinction and may improve treatment for PTSD and some anxiety disorders (38). But cortisol is also associated with impaired extinction learning (39), and chronic stress is linked with a number of anxiety disorders and compromises the structure

and function of the hippocampus. Impaired hippocampal function may contribute to overgeneralization in stress-related disorders by affecting pattern separation processes (1, 2). This model proposes that stress directly impairs the pattern separation function of the dentate gyrus, thereby impacting the ability to discriminate between past threats and the presently encountered stimulus or situation. A failure of pattern separation provides a reasonable account of the present results, but future neuroimaging evidence is necessary to claim that stress-induced impairments in dentate gyrus function promote behavioral overgeneralization in humans.

Intriguingly, a complementary neurobiological account (40) posits that stress-induction promotes enhanced neural plasticity in lateral amygdala neurons, which may contribute to overgeneralization. For instance, increases in shock intensity can alter the tuning profiles of lateral amygdala neurons in rats, causing a switch from specific to generalized fear in response (41). This neural model is supported by observations from fMRI investigations in patients with PTSD showing amygdala hyperactivity in response to emotional stimuli (3, 4). On the basis of this work, a plausible underlying mechanism supporting behavioral overgeneralization in experiment 2 involves a constellation of factors including a loss of memory precision over time, impairments in pattern separation processes in the hippocampus, and enhanced neuronal excitability and hyperactivity in the amygdala following stress.

While the results from experiment 2 are consistent with overgeneralization, it is worth considering the role of response sensitization. Response sensitization occurs when a cue elicits a behavior by mere fact that an aversive event has occurred, and not as a result of the learned CS–US association (42). Sensitization is considered a nonassociative form of expression, whereas generalization is the byproduct of associative learning (43, 44). That the gradient of SCRs and expectancy declined as similarity to the CS+ diminished suggests associative overgeneralization (33, 45) rather than sensitization, per se. However, one limitation of the present design is that we did not include an entirely novel stimulus from a different modality—such as a visual cue—to test for differences in purely nonassociative sensitization between groups.

An alternative account for how stress may operate on older threat memories is by enhancing the overall amount of physiological arousal, which increases responding to the learned threat itself and selectively sensitizes cues that may likely portend threat as well (compare ref. 46). That is, because the memory representation of the original CS+ is less precise over the passage of time, stress might promote a bias to overgeneralize to stimuli that are most similar to the CS+, while also maintaining (slightly less) heightened arousal to stimuli that are less similar. This view is also in line with an anxiety conservation (47) or "better-safe-than-sorry" approach to defensive responding. In other words, if stress impairs the ability to inhibit arousal to safe stimuli, and subjects can no longer remember precisely which stimulus is dangerous over a delay, they may be more inclined to react to a wider range of stimuli. By this account, a failure to remember the precise details of the CS+ is revealed by overall heightened arousal across the stimulus dimension, while stimuli bearing the closest resemblance to a learned threat remain the most likely to elicit the strongest behavioral response.

In the extant research on how stress affects conditioned learning, stress is typically induced either before initial training to examine the effects on learning, after training to examine the effects of consolidation, or before a test to examine effects on expression or retrieval (9). The present design can be considered a test of expression or retrieval. As stress has been shown to modulate retrieval of hippocampal (48) and amygdala-dependent (40) memories, one possibility is that stress-induction impaired retrieval of the precise CS+ memory in experiment 2, thereby altering the generalization gradient.

Given the strong clinical relevance, it is surprising that research on whether stress induced at a variety of different times differentially affects behavioral threat generalization is so limited. The present results provide evidence in humans that older threat memories are more sensitive to the effects of stress than recent memories when testing shortly follows an acute stress

episode. Because stress can also enhance consolidation (40), it will be of interest for future investigations to examine, for example, whether elevations of cortisol immediately after encoding strengthens the memory representation of the CS+ memory, thereby leading to less behavioral generalization after a delay. Further investigations like these are warranted to examine how stress impacts neurobehavioral correlates of fear generalization across species, as this knowledge could ultimately be applied to better understand stress-related psychopathologies.

Materials and Methods

Participants. These experiments were approved by the New York University Committee on Activities Involving Human Subjects. A total of 89 healthy adults provided written informed consent. Six subjects were excluded based on technical problems with either the psychophysiological equipment or stimulus presentation software. An additional six subjects were removed from the final analysis due to failure to detect differential SCRs between the CS+ and CS− (greater than 0) during the second half of threat conditioning. Removing these subjects is justified because the primary analysis was on generalization of aversive learning, which is predicated on successful acquisition of conditioned learning. The final sample included 36 subjects in experiment 1 (control: $n = 19$, 11 female, mean age = 23.6; stress: $n = 17$, 6 female, mean age = 22.2) and 41 subjects in experiment 2 (control: $n = 21$, 12 female, mean age = 20; stress: $n = 20$, 13 female, mean age = 23).

Threat Conditioning and Tests of Behavioral Generalization. Discriminative threat conditioning included a 550-Hz and a 1,000-Hz tone that served as either the CS+ or CS− (counterbalanced across subjects). Stimuli consisted of pure tone sine waves presented binaurally at a moderate volume (<60 decibels) through headphones (Sennheiser HD-280 PRO) for 2.5 s each and separated by a 7- to 8-s waiting period between trials that contained a fixation cross on a blank screen. Threat conditioning included 12 presentations each of unpaired CS+ and CS−. An additional eight CS+ trials were paired with the US (40% reinforcement rate). Because CS duration was short, all CS+ trials paired with shock were excluded from analysis to mitigate potential confounds introduced by the shock.

After fear conditioning, volunteers were presented with eight novel tones of increasing frequency ranging between the CS+ and CS−. For analysis, adjacent tone frequencies (e.g., 600 and 650 Hz) were collapsed to form a generalization stimulus class (GS1–GS4). During the generalization test, the CS+, CS−, and each GS class were presented eight times. An additional four CS+ trials paired with shock were included during the generalization test to prevent extinction and habituation over the course of the lengthy generalization test (steady-state generalization testing; see also refs. 30 and 49). These paired CS+ trials were not included in the analysis.

Subjects rated shock expectancy on every trial, but were instructed that their button presses did not affect the outcome on a trial to mitigate the potential for volunteers to attribute the outcome to their choice or reaction times. Volunteers were told to pay attention and try to learn the association between the tones and the shock, but no explicit information was given regarding the CS–US contingencies. Presentation was pseudorandomized so that no more than three presentations of the same tone occurred in a row. After generalization testing, subjects underwent a hearing test, which validated that all subjects had normal hearing and the capacity to discriminate between each tone frequency used in the experiment. Stimulus presentation was controlled using E-Prime 2.0 (Psychology Software Tools).

Psychophysiology and Shock. The electrical shock was a 200-ms pulse delivered to the right wrist using disposable pregelled electrodes connected to a Grass Medical Instruments stimulator. Shocks were calibrated using an ascending staircase procedure starting with a low voltage setting near a perceptible threshold to reach a level deemed "highly annoying but not painful."

SCRs were acquired from the hypothenar eminence of the left palmar surface using disposable pregelled snap electrodes connected to the MP-100 BIOPAC System (BIOPAC Systems). Analysis of SCRs has been described elsewhere (50). In brief, an SCR was considered related to CS presentation if the trough-to-peak deflection occurred 0.5–3 s following CS onset, lasted between 0.5 and 5.0 s, and was greater than 0.02 microsiemens (µS). Responses that did not fit these criteria were scored as zero. SCR values were obtained using a custom Matlab (The Mathworks, Inc.) script that extracts SCRs for each trial using the above criteria (51). Raw SCR scores were range corrected by dividing each value by the subject's maximum response during that phase of the task (either conditioning or the generalization test) evoked in most cases by the shock. Range-corrected values were square-root transformed before statistical

analysis (52). Results were analyzed separately for threat conditioning and the generalization test by repeated measures ANOVA incorporating stimulus as a within-subjects factor and group as a between-subjects factor, and followed where necessary by paired-samples t tests or independent-samples t tests. Greenhouse–Geisser correction was used when assumption of sphericity was not met. All analyses were considered significant at $\alpha <0.05$, two tailed.

Stress Induction. Stress induction involved a CPT wherein subjects submerged their right hand to the elbow in 0–4° ice water for 3 min (28). The control groups submerged their right hand in room temperature water for 3 min. Afterward, subjects rated how stressful they found the water bath from 1 (not at all stressful) to 10 (extremely stressful).

Salivary Cortisol. Saliva samples were collected at various time points to assess the effects of the stress induction versus the control task on cortisol concentrations. Samples were collected using absorbent swabs placed under the tongue for 2 min. Time points for cortisol collection for experiment 1 were before and after fear conditioning, +10 min, and +20 min after the stress/control task. For experiment 2, salivary cortisol was assessed before and after fear conditioning on day 1, and at time 0, +10, and +20 min after stress/control task on day 2. To control for diurnal rhythms in cortisol levels, all subjects completed the task between 12:00 PM and 5:00 PM. After collection, samples were stored in a freezer and shipped to

Salimetrics for analysis using high-sensitivity enzyme immunoassay kits. Data were log transformed before analysis to normalize the distribution.

Retrospective Ratings of Fear of the Shock. At the conclusion of the study, subjects self-reported how intense the shock had felt on a scale from 1 (not at all unpleasant) to 9 (extremely unpleasant), and how much fear they had felt during the experiment from 1 (not at all afraid) to 9 (extremely afraid). They also estimated how many shocks they had received during the entire experiment (including during conditioning and the generalization test, but not counting during the calibration phase), and retrospectively identified which tone (among nine different tones) was paired with shock. For the retrospective CS+ identification, subjects heard the CS+ and eight other tones that varied in frequency by ±50, ±150, ±250, and ±350 Hz relative to the CS+. Tones were played in a random order and subjects responded whether each tone was paired with the shock and their level of confidence. Accuracy was calculated as a high-confidence correct response to the CS+. Postexperimental data were lost for two subjects from the no-stress control group from experiment 2.

1. Besnard A, Sahay A (2016) Adult hippocampal neurogenesis, fear generalization, and stress. *Neuropsychopharmacology* 41:22–44.
2. Kheirbek MA, Klemenhagen KC, Sahay A, Hen R (2012) Neurogenesis and generalization: A new approach to stratify and treat anxiety disorders. *Nat Neurosci* 15:1613–1620.
3. Pitman RK, et al. (2012) Biological studies of post-traumatic stress disorder. *Nat Rev Neurosci* 13:769–787.
4. Shin LM, Liberzon I (2010) The neurocircuitry of fear, stress, and anxiety disorders. *Neuropsychopharmacology* 35:169–191.
5. Jasnow AM, Cullen PK, Riccio DC (2012) Remembering another aspect of forgetting. *Front Psychol* 3:175.
6. Wiltgen BJ, Tanaka KZ (2013) Systems consolidation and the content of memory. *Neurobiol Learn Mem* 106:365–371.
7. Raio CM, Phelps EA (2014) The influence of acute stress on the regulation of conditioned fear. *Neurobiol Stress* 1:134–146.
8. Maren S, Holmes A (2016) Stress and fear extinction. *Neuropsychopharmacology* 41:58–79.
9. Rodrigues SM, LeDoux JE, Sapolsky RM (2009) The influence of stress hormones on fear circuitry. *Annu Rev Neurosci* 32:289–313.
10. Izquierdo A, Wellman CL, Holmes A (2006) Brief uncontrollable stress causes dendritic retraction in infralimbic cortex and resistance to fear extinction in mice. *J Neurosci* 26:5733–5738.
11. Pugh CR, Tremblay D, Fleshner M, Rudy JW (1997) A selective role for corticosterone in contextual-fear conditioning. *Behav Neurosci* 111:503–511.
12. Zhou M, Kindt M, Joëls M, Krugers HJ (2011) Blocking mineralocorticoid receptors prior to retrieval reduces contextual fear memory in mice. *PLoS One* 6:e26220.
13. Wolf OT, Atsak P, de Quervain DJ, Roozendaal B, Wingenfeld K (2016) Stress and memory: A selective review on recent developments in the understanding of stress hormone effects on memory and their clinical relevance. *J Neuroendocrinol*, 10.1111/jne.12353.
14. Raio CM, Brignoni-Perez E, Goldman R, Phelps EA (2014) Acute stress impairs the retrieval of extinction memory in humans. *Neurobiol Learn Mem* 112:212–221.
15. Milad MR, et al. (2009) Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. *Biol Psychiatry* 66:1075–1082.
16. Jovanovic T, Kazama A, Bachevalier J, Davis M (2012) Impaired safety signal learning may be a biomarker of PTSD. *Neuropharmacology* 62:695–704.
17. Grillon C, Morgan CA, 3rd (1999) Fear-potentiated startle conditioning to explicit and contextual cues in Gulf War veterans with posttraumatic stress disorder. *J Abnorm Psychol* 108:134–142.
18. Morey RA, et al.; VA Mid-Atlantic MIRECC Workgroup (2015) Fear learning circuitry is biased toward generalization of fear associations in posttraumatic stress disorder. *Transl Psychiatry* 5:e700.
19. Poulos AM, et al. (2016) Conditioning- and time-dependent increases in context fear and generalization. *Learn Mem* 23:379–385.
20. Riccio DC, Joynes RL (2007) Forgetting of stimulus attributes: Some implications for hippocampal models of memory. *Learn Mem* 14:430–432.
21. Thomas DR, Burr DE (1969) Stimulus generalization as a function of the delay between training and testing procedures: A reevaluation. *J Exp Anal Behav* 12:105–109.
22. Thomas DR, Lopez LJ (1962) The effects of delayed testing on generalization slope. *J Comp Physiol Psychol* 55:541–544.
23. Thomas DA, Riccio DC (1979) Forgetting of a CS attribute in a conditioned suppression paradigm. *Anim Learn Behav* 7:191–195.
24. Desiderato O, Butler B, Meyer C (1966) Changes in fear generalization gradients as a function of delayed testing. *J Exp Psychol* 72:678–682.
25. Biedenkapp JC, Rudy JW (2007) Context preexposure prevents forgetting of a contextual fear memory: Implication for regional changes in brain activation patterns associated with recent and remote memory tests. *Learn Mem* 14:200–203.
26. Wiltgen BJ, Silva AJ (2007) Memory for context becomes less specific with time. *Learn Mem* 14:313–317.
27. Riccio DC, Ackil J, Burch-Vernon A (1992) Forgetting of stimulus attributes: Methodological implications for assessing associative phenomena. *Psychol Bull* 112:433–445.
28. McRae AL, et al. (2006) Stress reactivity: Biological and subjective responses to the cold pressor and trier social stressors. *Hum Psychopharmacol* 21:377–385.
29. Lissek S, et al. (2008) Generalization of conditioned fear-potentiated startle in humans: Experimental validation and clinical relevance. *Behav Res Ther* 46:678–687.
30. Dunsmoor JE, Mitroff SR, LaBar KS (2009) Generalization of conditioned fear along a dimension of increasing fear intensity. *Learn Mem* 16:460–469.
31. Ghirlanda S, Enquist M (2003) A century of generalization. *Anim Behav* 66:15–36.
32. Honig WK, Urcuioli PJ (1981) The legacy of Guttman and Kalish (1956): Twenty-five years of research on stimulus generalization. *J Exp Anal Behav* 36:405–445.
33. Dymond S, Dunsmoor JE, Vervliet B, Roche B, Hermans D (2015) Fear generalization in humans: Systematic review and implications for anxiety disorder research. *Behav Ther* 46:561–582.
34. Coelho CA, Dunsmoor JE, Phelps EA (2015) Compound stimulus extinction reduces spontaneous recovery in humans. *Learn Mem* 22:589–593.
35. Phelps EA, Lempert KM, Sokol-Hessner P (2014) Emotion and decision making: Multiple modulatory neural circuits. *Annu Rev Neurosci* 37:263–287.
36. Kessler RC, Petukhova M, Sampson NA, Zaslavsky AM, Wittchen HU (2012) Twelve-month and lifetime prevalence and lifetime morbid risk of anxiety and mood disorders in the United States. *Int J Methods Psychiatr Res* 21:169–184.
37. Arnsten AF (2009) Stress signalling pathways that impair prefrontal cortex structure and function. *Nat Rev Neurosci* 10:410–422.
38. de Quervain DJ, Margraf J (2008) Glucocorticoids for the treatment of post-traumatic stress disorder and phobias: A novel therapeutic approach. *Eur J Pharmacol* 583:365–371.
39. Merz CJ, Hermann A, Stark R, Wolf OT (2014) Cortisol modifies extinction learning of recently acquired fear in men. *Soc Cogn Affect Neurosci* 9:1426–1434.
40. Roozendaal B, McEwen BS, Chattarji S (2009) Stress, memory and the amygdala. *Nat Rev Neurosci* 10:423–433.
41. Ghosh S, Chattarji S (2015) Neuronal encoding of the switch from specific to generalized fear. *Nat Neurosci* 18:112–120.
42. Groves PM, Thompson RF (1970) Habituation: A dual-process theory. *Psychol Rev* 77:419–450.
43. Hull CL (1943) *Principles of Behavior* (Appleton-Century-Crofts, New York).
44. Shepard RN (1987) Toward a universal law of generalization for psychological science. *Science* 237:1317–1323.
45. Laufer O, Israeli D, Paz R (2016) Behavioral and neural mechanisms of over-generalization in anxiety. *Curr Biol* 26:713–722.
46. Lovibond PF, Siddle DAT, Bond NW (1993) Resistance to extinction of fear-relevant stimuli: Preparedness or selective sensitization? *J Exp Psychol Gen* 122:449–461.
47. Solomon RL, Wynne LC (1954) Traumatic avoidance learning: The principles of anxiety conservation and partial irreversibility. *Psychol Rev* 61:353–385.
48. de Quervain DJ, Roozendaal B, McGaugh JL (1998) Stress and glucocorticoids impair retrieval of long-term spatial memory. *Nature* 394:787–790.
49. Blough DS (1975) Steady-state data and a quantitative model of operant generalization and discrimination. *J Exp Psychol* 104:3–21.
50. Dunsmoor JE, Campese VD, Ceceli AO, LeDoux JE, Phelps EA (2015) Novelty-facilitated extinction: Providing a novel outcome in place of an expected threat diminishes recovery of defensive responses. *Biol Psychiatry* 78:203–209.
51. Green SR, Kragel PA, Fecteau ME, LaBar KS (2013) Development and validation of an unsupervised scoring system (Autonomate) for skin conductance response analysis. *Int J Psychophysiol* 91:186–193.
52. Lykken DT, Venables PH (1971) Direct measurement of skin conductance: A proposal for standardization. *Psychophysiology* 8:656–672.

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