Behavioral/Systems/Cognitive

Impaired Fear Extinction Learning and Cortico-Amygdala Circuit Abnormalities in a Common Genetic Mouse Strain

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Fear extinction is a form of new learning that results in the inhibition of conditioned fear. Trait deficits in fear extinction are a risk factor for anxiety disorders. There are few examples of naturally occurring animal models of impaired extinction. The present study compared fear extinction in a panel of inbred mouse strains. This strain survey revealed an impairment in fear extinction in 129/SvImJ (129S1). The phenotypic specificity of this deficit was evaluated by comparing 129S1 and C57BL/6J for one-trial and multitrial fear conditioning, nociception, and extinction of conditioned taste aversion and an appetitive instrumental response. 129S1 were tested for sensitivity to the extinction-facilitating effects of extended training, as well as D-cycloserine and yohimbine treatment. To elucidate the neural basis of impaired 129S1 fear extinction, c-Fos and Zif268 expression was mapped after extinction recall. Results showed that impaired fear extinction in 129S1 was unrelated to altered fear conditioning or nociception, and was dissociable from intact appetitive extinction. Yohimbine treatment facilitated extinction in 129S1, but neither extended extinction training nor D-cycloserine treatment improved 129S1 extinction. After extinction recall, 129S1 showed reduced c-Fos and Zif268 expression in the infralimbic cortex and basolateral amygdala, and elevated c-Fos or Zif268 expression in central nucleus of the amygdala and medial paracapsular intercalated cell mass, relative to C57BL/6J. Collectively, these data demonstrate a deficit in fear extinction in 129S1 associated with a failure to properly engage corticolimbic extinction circuitry. This common inbred strain provides a novel model for studying impaired fear extinction in anxiety disorders.

Key words: fear; extinction; anxiety; gene; prefrontal cortex; amygdala; intercalated cell masses

Introduction

Fear extinction is a form of learning in which the expression of a conditioned fear response is reduced after repeated experience of a conditioned stimulus in the absence of an unconditioned aversive stimulus (Pavlov, 1927). There is growing evidence that fear conditioning and fear extinction are independent forms of learning that are mediated by partially dissociable neural mechanisms (Kamprath and Wotjak, 2004; Quirk and Mueller, 2007; Myers and Davis, 2007). Fear conditioning and short-term extinction appear to be principally amygdala-mediated, whereas the ability to form and express extinction memory over the long term recruits the rodent ventromedial prefrontal cortex and homolo-

gous regions in the human brain (Maren and Quirk, 2004; Phelps et al., 2004).

At the molecular level, extinction acquisition deficits are caused, for example, by upregulation of cyclin-dependent kinase 5 (Sananbenesi et al., 2007), L-type voltage-gated calcium channel blockers (Cain et al., 2002), overexpression of type I adenylyl cyclase (Wang et al., 2004), metabotropic glutamate mGluR1 antagonism (Kim et al., 2007a), or cannabinoid receptor CB-1 inactivation (Marsicano et al., 2002; Chhatwal et al., 2005), whereas extinction facilitation is produced, for instance, by NMDA receptor (NMDAR) partial agonism (Davis et al., 2006), metabotropic glutamate mGluR7 activation (Fendt et al., 2008), histone deacetylase inhibition (Lattal et al., 2007), dopamine D₂ receptor antagonism (Ponnusamy et al., 2005), cyclin-dependent kinase 5 inactivation (Hawasli et al., 2007), protein kinase A inhibition (Isiegas et al., 2006), AMPA receptor potentiating (Zushida et al., 2007), or noradrenaline (Ouyang and Thomas, 2005; Berlau and McGaugh, 2006) or α2-adrenoreceptor antagonism (Cain et al., 2004). In addition, the mitogen-activated protein kinase (MAPK) signaling pathway is activated in the amygdala of rats and mice during fear extinction (Lu et al., 2001; Lin et al., 2003a,b; Chen et al., 2005; Herry et al., 2006), whereas preextinction intraamygdala administration of MAPK or inhibitors im-

pairs fear extinction (Lu et al., 2001; Lin et al., 2003b; Herry et al., 2006).

Fear extinction is readily quantifiable in laboratory rodents, providing important behavioral models for translational studies of anxiety disorders (Cryan and Holmes, 2005; Quirk and Beer, 2006). Such models can be applied in a variety of ways; for example, phenotypic surveys of mouse inbred strains provide a valuable approach to identifying genetically associated variation in behavior and neural circuitry (Wahlsten et al., 2006). To date, however, the majority of inbred mouse studies of fear extinction have used the commonly used C57BL/6 inbred strain or related substrains (Radulovic et al., 1998; Stiedl et al., 1999; Siegmund et al., 2005), and there has been limited study of potential strain differences in fear extinction (Falls et al., 1997; Stiedl et al., 1999; McCaughran et al., 2000; Waddell et al., 2004). Therefore, in the present study, we conducted a survey of fear extinction across a panel of inbred mouse strains. This identified the 129S1 strain as exhibiting a significant deficit in fear extinction. We went on to determine the behavioral specificity of this extinction deficit, and the sensitivity of this strain to putative extinction-facilitating behavioral and pharmacological interventions. Next, we sought to elucidate the neural basis of the fear extinction impairment in the 129S1 strain by quantifying extinction-induced activation of the immediate-early genes (IEGs) in a cortico-amygdala circuit mediating fear extinction across species (Paré et al., 2004; Phelps et al., 2004; Milad et al., 2006; Wellman et al., 2007). Both c-Fos and Zif268 were measured, given previous evidence that these IEGs show differential patterns of extinction-induced activation (Herry and Mons, 2004). The results reveal a model of impaired fear extinction in a common inbred strain associated with a failure to recruit the key neural circuitry necessary for extinction.

Materials and Methods

Subjects. Subjects were male 129S1/SvImJ, A/J, BALB/cByJ, C57BL/6J, DBA/2J, and FVB/NJ strains. These strains were chosen on the basis of their frequent use in behavioral neuroscience, including as genetic backgrounds for mutants and inclusion as "group A" priority strains in the Mouse Phenome Project, an international effort to provide the biomedical research community with phenotypic data on the most commonly used mouse strains (www.jax.org/phenome). For experiments conducted at the National Institutes of Health, mice were obtained at ~8 weeks of age from The Jackson Laboratory and housed (two to four per cage) side-by-side in a temperature (22 ± 1°C)- and humidity (45 ± 15%)-controlled vivarium under a 12 h light/dark cycle (lights on, 6:00 A.M.). The IEG activation experiments were conducted in Innsbruck. These mice were obtained from Charles River and housed (four to five per cage) side-by-side in a temperature (22 ± 2°C)- and humidity (50–60%)-controlled vivarium under a 12 h light/dark cycle (lights on, 7:00 A.M.). The number of mice used in each experiment is given in the figure legends. All experimental procedures were approved by the National Institute on Alcohol Abuse and Alcoholism Animal Care and Use and Austrian Ethical Committees on Animal Care and Use (Bundesministerium für Wissenschaft und Forschung) and followed the National Institutes of Health guidelines outlined in *Using Animals in Intramural Research* and the local animal care and use committees.

Strain survey of fear extinction. Fear extinction was assessed based on methods described previously (Izquierdo et al., 2006a). Mice were moved to a holding room adjacent to the test room and acclimated for 1 h before testing. Fear conditioning was conducted in a 27 × 27 × 11 cm chamber with transparent walls and a metal rod floor, cleaned with a 79.5% water/19.5% ethanol/1% vanilla extract solution to provide a distinctive olfactory cue. After 180 s, mice received three pairings (60–120 s variable interpairing interval) between a 30 s, 80 dB, 3 kHz tone [conditioned stimulus (CS)] and a 2 s, 0.6 mA scrambled footshock [unconditioned stimulus (US)], in which the shock was presented during the last 2 s of the CS. There was a 120 s no-stimulus consolidation period after the final

US–CS pairing before mice were returned to the home cage. Stimulus presentation was controlled by the MED Associates VideoFreeze system.

Twenty-four hours later, initial fear recall and within-session fear extinction were tested. Mice were placed in a novel context (black/white-checkered walls, solid-Plexiglas, opaque floor, cleaned with a 70% ethanol/30% water solution), housed in a different room from conditioning. After 180 s, there were 50 × 30 s CS presentations (5 s no-stimulus interval). Twenty-four hours later, extinction recall was tested using the same procedure as the previous day.

Freezing was measured as an index of fear (Blanchard and Blanchard, 1969), and manually scored every 5 s as no visible movement except that required for respiration and converted to a percentage [(number of freezing observations/total number of observations) × 100]. Freezing during conditioning was measured during the baseline period and each of the three CS presentations. Freezing during extinction and extinction recall was binned into 3× CS trial blocks.

Behavioral specificity of extinction deficit in the 129S1 strain. On the basis of the strain comparison data revealing a marked deficit in fear extinction in the 129S1 strain, we next assessed the behavioral specificity of the deficit in this strain.

Fear conditioning. Failure of 129S1 to show extinction could result from either an extinction deficit per se or an inability of an intact extinction memory to overcome an excessive fear memory. We therefore examined whether impaired fear extinction in 129S1 mice was dissociable from an increase in fear. In one experiment, 129S1 (and for comparison, C57BL/6J) mice were conditioned using the same three tone–shock protocol described above and, 24 h later, tested for fear recall via average freezing over three CS presentations (Yang et al., 2008). Because three tone–shock pairings during conditioning could conceivably produce ceiling levels of fear during recall, an additional experiment was conducted in the same manner with the exception that there was only one tone–shock pairing during conditioning.

Nociception. To exclude the possibility that increased pain perception could confound fear extinction, 129S1 (and for comparison, C57BL/6J) mice were tested on the hot plate and Von Frey tests. The hot plate test apparatus was a flat plate (Columbus Instruments) heated to 55°C on which the mouse was placed (Boyce-Rustay and Holmes, 2006). The latency to show a hindpaw shake or lick was manually timed, with a maximum response latency of 30 s. For the Von Frey test, the threshold value for hindpaw withdrawal in response to punctate mechanical stimulation was measured with graded Von Frey filaments (Stoelting) (Hasnie et al., 2007). Each mouse was given 2 min of acclimation to the wire mesh cage and then tested on the right hindpaw. Threshold response was defined by the filament that caused active paw withdrawal at least three times for every five applications. There were two trials separated by a 2 min interval (average = withdrawal threshold).

Extinction of appetitive instrumental response. We next evaluated extinction to a nonaversive, appetitively motivated form of learning. 129S1 (and for comparison, C57BL/6J) were tested for extinction of an instrumental response using a touchscreen-based operant system described previously [for details of apparatus, see Izquierdo et al. (2006b) and Brigman et al. (2008)]. Food-restricted mice were acclimated to the 14 mg pellet food reward and to eating the rewards from the pellet magazine. Mice then underwent pavlovian autoshaping during which variously shaped stimuli were presented in the touchscreen windows (one per window) for 10 s [intertrial interval (ITI), 15 s]. The disappearance of the stimuli coincided with provision of a single pellet food reward, and mice were required to eat the pellet for the next trial to commence. The criterion was eating 30 pellets in a 30 min session. There were then two stages of operant shaping: (1) respond to a (variously shaped) stimulus that appeared in one of the two windows (spatially pseudorandomized) and remained on the screen until a response was made, (2) same as (1), plus a new trial was initiated by a head entry into the pellet magazine. Criterion for each shaping stage was 90% responding in a 30 trial session (15 s ITI).

For the task proper, mice were required to respond to stimuli (1 × 2.8 cm² white square per window) to obtain reward. Stimuli remained on the screen until a response was made, with 30 trials (5 s ITI) per session. Criterion was performing 30 trials within 12.5 min on each of five con-

secutive sessions. Next, extinction of the response was tested in sessions during which responses to the stimulus were no longer rewarded. Criterion was a two session average of 75% or more response omissions. The dependent measure was the number of trials to reach the acquisition and extinction criteria.

Extinction of conditioned taste aversion. To assess whether impaired fear extinction occurred in another test of extinction to an aversive learning event, 129S1 (and for comparison, C57BL/6J) were tested for extinction of a conditioned taste aversion (CTA) as described previously (Jacobson et al., 2006). Singly housed mice were first water-deprived and habituated to drinking from two water-filled sipper tubes offered in the cage for 30 min twice per day (morning, evening) for 6 d. On day 7, mice were offered only one tube, containing 0.5% saccharin, during the morning presentation. Thirty minutes after the 30 min presentation, mice were given an intraperitoneal injection of 0.15 M LiCl in volume of 20 ml/kg (=0.30 M) and observed for signs of malaise. Malaise was defined as protracted periods of nonsleeping immobility, piloerection, contraction of the flanks, prostrate, flat belly, and elongated body posture (mice displaying malaise were given a score of 1). Mice were offered water during the 5:00 P.M. presentation to prevent excessive dehydration.

Extinction was assessed over 13 daily sessions. The procedure was the same as for habituation days, except that mice were offered two tubes, one containing 0.5% saccharin and one containing water, for 40 min during each twice daily presentation, with the left/right side of the water- versus saccharin-containing tubes counterbalanced across days and experimental groups. A daily aversion index was calculated as fluid consumed from water-contained tube/total fluid consumed from both tubes; with an index score closer to 1.0 indicating maximum aversion.

To assess unconditioned saccharin preference, an additional experiment was conducted in naive mice. Mice were tested as above with the exception that LiCl was replaced with saline, and saccharin preference was measured on days 8–10 only.

Behavioral and pharmacological influences on fear extinction in 129S1. We next conducted a series of experiments to test whether the extinction deficit in 129S1 mice was rescued by behavioral or pharmacological manipulations.

Extended massed-trial extinction. To examine whether 129S1 mice would demonstrate extinction over an extended massed-trial extinction protocol, mice were tested on a 100 trial extinction protocol followed by a 50 trial extinction recall protocol.

Effects of D-cycloserine treatment. Systemic treatment with the NMDAR partial agonist D-cycloserine facilitates fear extinction in rodents and has proven to be effective as an adjunct to exposure therapy in human anxiety disorders (Davis et al., 2006). We therefore assessed the ability of D-cycloserine to facilitate impaired extinction in 129S1 mice. 129S1 mice underwent three tone–shock conditioning as above and, 24 h later, treated with saline vehicle or 5, 15, or 30 mg/kg (injected intraperitoneally in a volume of 10 ml/kg body) D-cycloserine (Sigma-Aldrich) 30 min before 50 trial extinction testing. Mice were tested, drug-free, 24 h later for fear via average freezing over 3× tone presentations. Because the effects of D-cycloserine have not been demonstrated in our fear extinc-
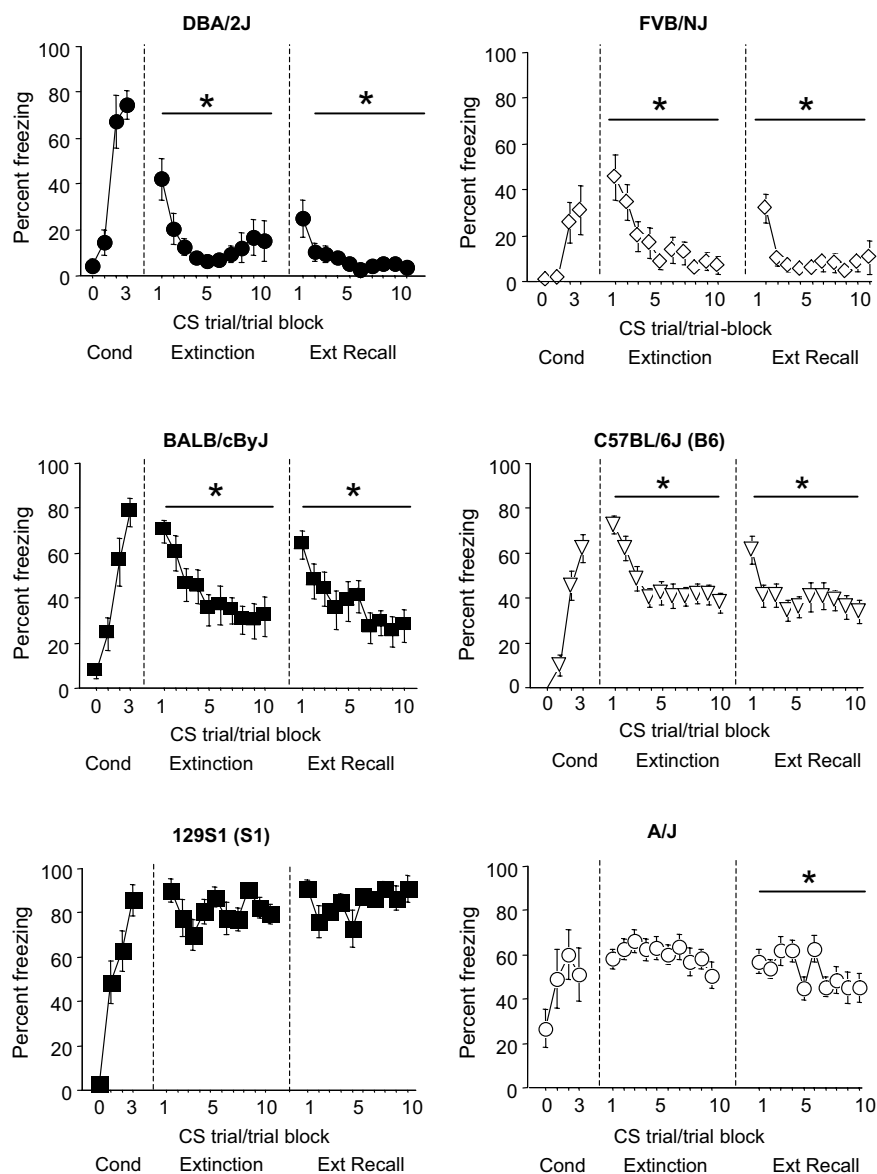
Figure 1. Survey of fear extinction across inbred strains. All strains except 129S1 showed a significant reduction in conditioned fear over at least one extinction session. Cond, Conditioning; Ext recall, extinction recall. n = 7–14/strain. *p < 0.05, significant change over trials. Data in Figures 1–7 are means ± SEM.

tion paradigm in C57BL/6J, we also tested the drug in this strain. Doses were chosen based on extinction facilitating doses of D-cycloserine in rats and humans (Walker et al., 2002; Ressler et al., 2004; Richardson et al., 2004).

Effects of yohimbine treatment. Systemic treatment with the α2-adrenoreceptor antagonist yohimbine has been shown to improve fear extinction in mice (Cain et al., 2004). We therefore also tested the effects of this drug in 129S1 mice. The procedure was the same as for D-cycloserine. Doses of 2.5 and 5 mg/kg yohimbine (Sigma-Aldrich) were chosen based on extinction facilitating doses of the drug in C57BL/6J mice (Cain et al., 2004).

Extinction-related cortico-amygdala IEG activation. We next sought to identify neural correlates of the extinction deficit in 129S1 using IEG expression as a marker for neuronal activity (Singewald, 2007). The focus of this analysis was a prefrontal cortex–amygdala circuit that has been consistently shown to be recruited during extinction in rodents and humans (Paré et al., 2004; Phelps et al., 2004; Quirk et al., 2006). For these experiments, 129S1 were compared with C57BL/6J. Mice underwent fear conditioning in a 26 × 30 × 32 cm chamber with transparent walls and a metal rod floor. After 120 s, mice received five pairings (120 s interpair-

ing interval) between a 120 s, 80 dB white noise and a 2 s, 0.7 mA scrambled footshock, in which the shock was presented during the last 2 s of the tone. There was a 120 s no-stimulus consolidation period after the final tone–shock pairing before mice were returned to the home cage. A non-conditioned control group ("CS minus baseline") underwent the same procedure except there was no footshock exposure. Stimulus presentation was controlled by the Habitest operant system (Coulbourn Instruments). Twenty-four hours later, mice were placed in a novel context (26 × 20 × 13 cm cage, cleaned with a 100% ethanol, illuminated to 10 lux) for a 120 s acclimation period and then exposed to 15 × 120 s tone presentations (5 s no-stimulus interval). Twenty-four hours later, mice were returned to the novel context, exposed (after a 120 s acclimation period) to 15 × 120 s tone presentations, and killed for immediate-early gene analysis 2 h after the start of the session [time interval according to postextinction c-Fos and Zif268 data obtained by Herry and Mons (2004)].

For IEG analysis, mice were deeply anesthetized with an overdose of sodium pentobarbital (200 mg/kg) and transcardially perfused with 20 ml of 0.9% saline followed by 20 ml of 4% paraformaldehyde in 0.1 mol/L PBS, pH 7.4. Brains were then removed and postfixed at 4°C overnight in 4% paraformaldehyde in PBS. Coronal sections (50 μm) were cut with a vibratome (Ted Pella) and collected in immunobuffer. The sections were processed for (1) c-Fos immunoreactivity as described previously (Singewald et al., 2003), via incubation with a polyclonal primary antibody (1:20,000; sc-52; Santa Cruz Biotechnology), and (2) Zif268-like immunoreactivity via incubation with a polyclonal primary antibody (1:5000; sc-189; Santa Cruz Biotechnology) and a biotinylated goat anti-rabbit secondary antibody (1:200; Vector Laboratories). Cells containing a nuclear brown-black reaction product were considered to be c-Fos-positive or Zif268-positive cells, respectively. The anatomical localization of c-Fos-positive or Zif268-positive cells was aided by using the illustrations in a stereotaxic atlas (Paxinos and Franklin, 2001). Zif268-positive neurons in intercalated (ITC) cell masses were identified with reference to published studies in the rat and mouse (Millhouse, 1986; Berretta et al., 2005; Marowsky et al., 2005; Geracitano et al., 2007). One mass of ITC cells situated along the external capsule at the junction of lateral nucleus of the amygdala (LA) and the basolateral nucleus of the amygdala (BLA) was identified as lateral paracapsular ITC neurons (Ilp). A second mass of ITC cells was observed along the intermediate capsule at the junction of LA/BLA and lateral to the central nucleus of the amygdala (CeA) and was defined as the medial paracapsular ITC mass (Imp) (see Fig. 7A). Aiding the identification of ITC cell masses, the size of Zif268-labeled nuclei in the masses was considerably smaller than those in the adjacent BLA and CeA (ITCs, 21.9 ± 3.3 μm^2; BLA, 45.2 ± 3.0; CeA, 40.5 ± 3.8; one-factor ANOVA, $F_{(2,60)} = 102.98$, $p < 0.01$; Bonferroni's *post hoc* comparisons, $p < 0.01$). Unless otherwise stated, all c-Fos-positive or Zif268-positive cells that were distinguishable from background staining were bilaterally counted in each region of interest within a defined area (0.01 mm^2) averaging counts from two to four sections per mouse depending on the brain area under investigation.

Fear-related cortico-amygdala IEG activation. To test for possible strain differences in immediate-early gene activation after fear recall per se, an additional control experiment was conducted. 129S1 and C57BL/6J mice were conditioned as above for extinction-related immediate-early gene analysis and, 24 h later, exposed to 1 × 30 s tone presentation before being killed and processed for immediate-early gene analysis 2 h later, as above.

Statistics. The effects of strain × tone-trial/block, session, or day on freezing, instrumental responses, or taste aversion index were analyzed using two-factor ANOVA, with repeated measures for tone-trial/block, session, or day, followed by Bonferroni's *post hoc* analysis. In the presence of a significant interaction in this initial strain profile, within-session strain profiles were either analyzed via separate repeated-measures ANOVA. Effects of strain and condition on IEG expression after fear recall were analyzed using unpaired *t* tests. Effects of strain by condition on IEG expression after fear recall were analyzed using two-factor ANOVA followed by Bonferroni's *post hoc* analysis. Correlations between freezing during extinction recall and number of c-Fos and Zif268 cells were performed using the Spearman's coefficient test. The threshold
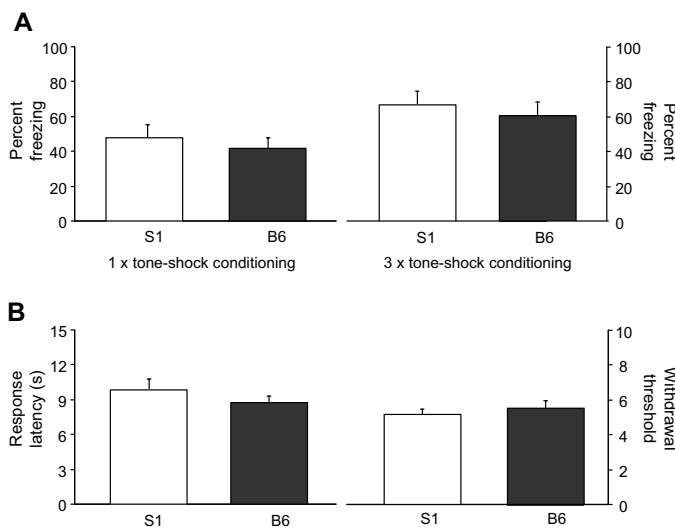
Figure 2. Fear conditioning and nociception in 129S1 mice. *A*, Fear recall was no different between 129S1 (S1) and C57BL/6J (B6) after either 1× tone–shock conditioning ($n = 15$/ strain) or 3× tone–shock conditioning ($n = 15$/strain). *B*, Nociceptive responses were no different between S1 and B6 in either the hot plate or von Frey tests ($n = 11$/strain).

for statistical significance was set at $p < 0.05$ (statistical results below this threshold are not described).

Results
Strain survey of fear extinction
There was a significant strain × tone-trial/block interaction for freezing during conditioning ($F_{(15,186)} = 3.43$; $p < 0.01$), extinction ($F_{(45,558)} = 2.67$; $p < 0.01$), and extinction recall ($F_{(45,558)} = 1.86$; $p < 0.01$) sessions. For clarity, the profiles of each of the six strains are presented separately in Figure 1. During conditioning, all strains showed a significant increase in freezing across trials (all $p < 0.01$) with the exception of A/J, which had high baseline freezing. During extinction, DBA/2J, FVB/NJ, BALB/cByJ, and C57BL/6J showed a significant decrease in freezing across trial-blocks (all $p < 0.01$). Neither A/J nor 129S1 showed a significant decrease in freezing during the extinction session. All strains (all $p < 0.01$) except 129S1 displayed a significant decrease in freezing during the extinction recall session.

Behavioral specificity of extinction deficit in the 129S1 strain
Fear conditioning
There were no differences between 129S1 and C57BL/6J in fear recall after either 3× tone–shock (Fig. 2A, right) or 1× tone–shock (Fig. 2A, left) conditioning protocols.

Nociception
Neither hot plate response latencies (Fig. 2B, left) nor Von Frey responses (Fig. 2B, right) differed between 129S1 and C57BL/6J.

Extinction of appetitive instrumental behavior
There was no significant effect of strain for trials to extinguish the instrumental behavior (129S1, 130 ± 18 trials; C57BL/6J, 120 ± 7 trials) (sessions 1–5 are shown in Fig. 3A). There was a significant effect of strain for trials to acquire an appetitively driven instrumental response behavior ($F_{(1,18)} = 4.70$; $p < 0.05$) because of faster learning in 129S1 (8.7 ± 1.4 trials to criterion) than C57BL/6J (13.4 ± 1.6 trials to criterion).

Extinction of conditioned taste aversion
There was a significant strain by day interaction for aversion index during CTA extinction ($F_{(12,192)} = 2.92$; $p < 0.01$). The

A

B
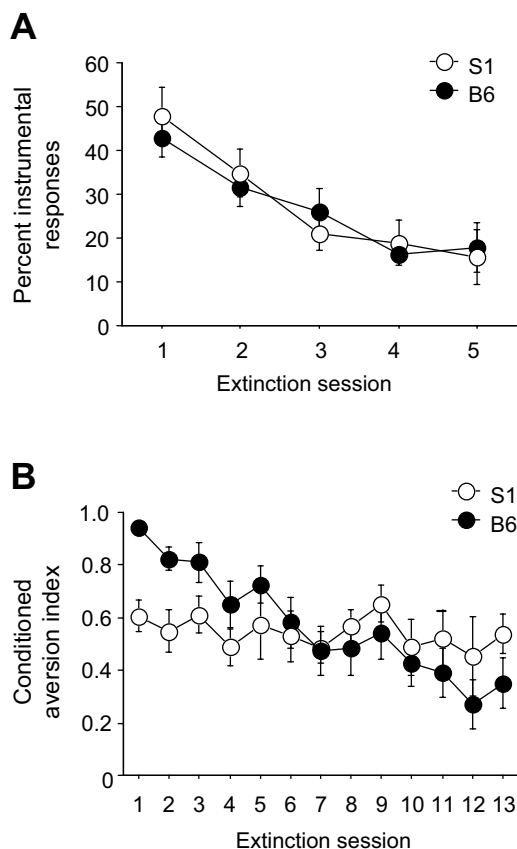
Figure 3. Extinction of an appetitive instrumental behavior and extinction of conditioned taste aversion in 129S1 mice. **A**, 129S1 (S1) and C57BL/6J (B6) showed similar rates extinction of an appetitively motivated instrumental response (*n* = 9 – 11/strain). **B**, 129S1 showed lesser initial expression of conditioned taste aversion and no reduction in the aversion over extinction sessions, compared with B6 (*n* = 9/strain).

aversion index in C57BL/6J was significantly higher than in 129S1 during the first two sessions ($p < 0.01$), and there was a significant decrease in the aversion index across sessions in C57BL/6J ($F_{(12,96)} = 17.26; p < 0.01$) but not 129S1 (Fig. 3*B*). Malaise scores were no different between strains (129S1 = 0.58 ± 0.11; C57BL/6J = 0.47 ± 0.12). Unconditioned C57BL/6J showed a clear preference for saccharin over water (80 ± 4.4% preference), whereas unconditioned 129S1 showed no preference (49 ± 5.9% preference) ($n = 7$ /strain).

Behavioral and pharmacological influences on fear extinction in 129S1
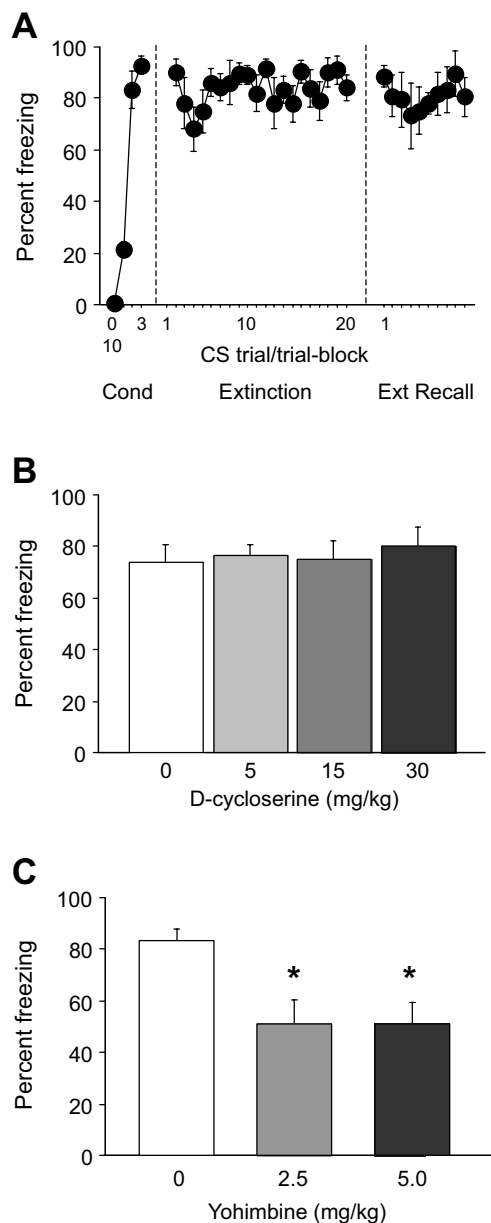
Extended massed-trial extinction
129S1 showed no decrease in freezing over two sessions in an extended massed-trial protocol (Fig. 4*A*).

Effects of D-cycloserine
There was no significant effect of D-cycloserine treatment on freezing during extinction recall in 129S1 mice (Fig. 4*B*). D-Cycloserine treatment significantly reduced freezing during extinction recall in C57BL/6J mice ($F_{(3,49)} = 2.80, p = 0.05$; vehicle, 72.0 ± 6.2%; 5 mg/kg, 50.7 ± 6.4%; 15 mg/kg, 52.6 ± 4.8%; 30 mg/kg, 53.1 ± 6.0%; $p < 0.05$, all doses vs vehicle; $n = 12$ –14/dose).

Effects of yohimbine
There was a significant effect of yohimbine treatment on freezing during extinction recall ($F_{(2,26)} = 5.16; p < 0.05$). Mice previ-

A

B

C

Figure 4. Effects of behavioral and pharmacological manipulations on fear extinction in 129S1 mice. **A**, Extended massed-trial extinction did not reduce conditioned fear (*n* = 7). **B**, D-Cycloserine treatment during extinction did not reduce conditioned fear during extinction recall (*n* = 9/dose). **C**, Yohimbine treatment during extinction significantly reduced conditioned fear during extinction recall relative to vehicle treatment (*n* = 9 –10/dose). *$p < 0.05$ versus vehicle.

ously treated with either 2.5 or 5.0 mg/kg showed significantly less freezing than vehicle-treated controls (Fig. 4*C*).

Extinction-related cortico-amygdala IEG activation
129S1 showed significantly more freezing than C57BL/6J over the extinction recall session (129S1, 65.3 ± 1.6%; C57BL/6J, 4.5 ± 0.9%; $t_{(13)} = 12.58; p < 0.01$) and the first tone presentation per se (129S1, 74.0 ± 5.4%; C57BL/6J, 23.8 ± 5.1%; $t_{(13)} = 6.72; p < 0.01$). There was a significant strain by condition interaction for the number of c-Fos-positive cells in the infralimbic cortex (IL) (1.78 mm from bregma, $F_{(1,19)} = 34.55, p < 0.01$; 1.54 mm from bregma, $F_{(1,19)} = 9.47, p < 0.01$), the basolateral ($F_{(1,19)} = 38.43; p < 0.01$) and central nucleus ($F_{(1,19)} = 15.72; p < 0.01$) of the amygdala. Although all three regions show higher c-Fos expres-
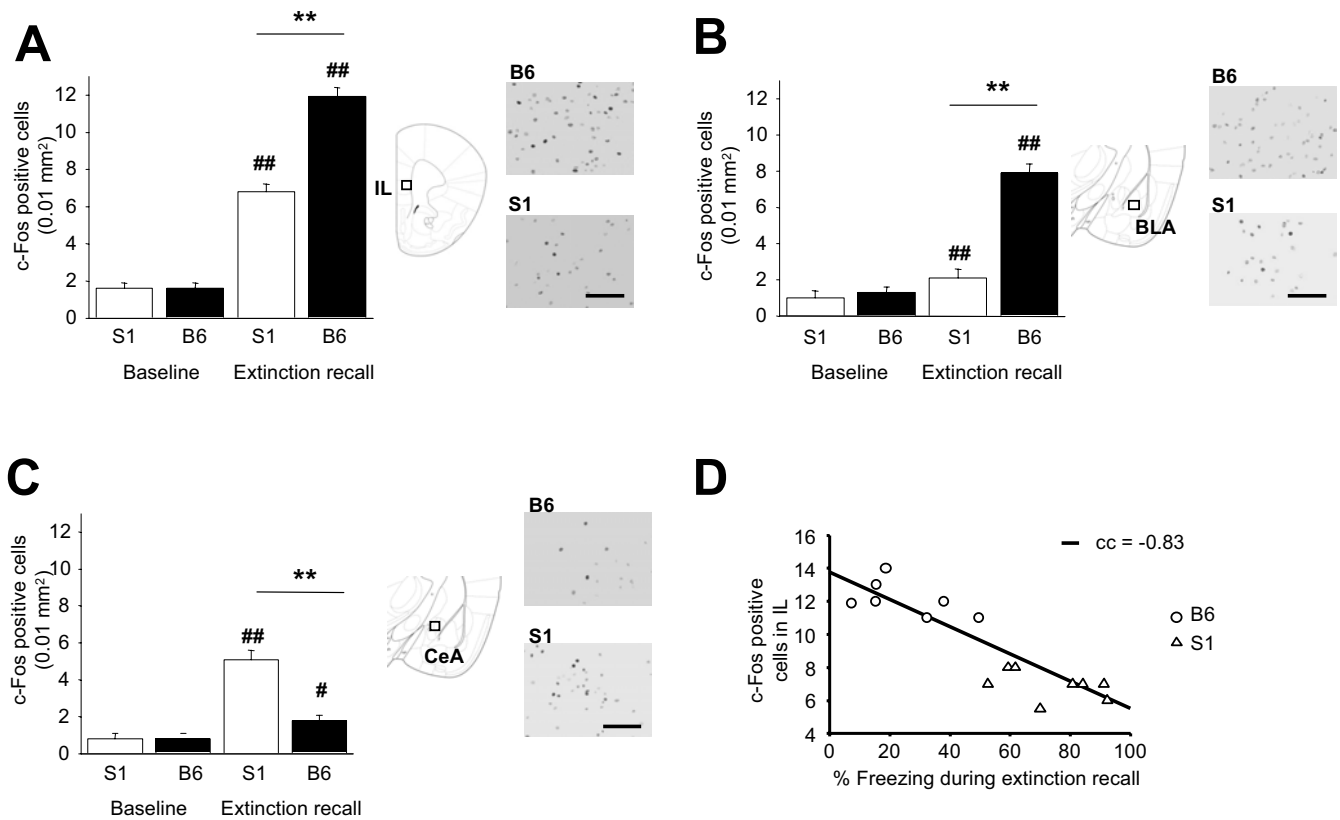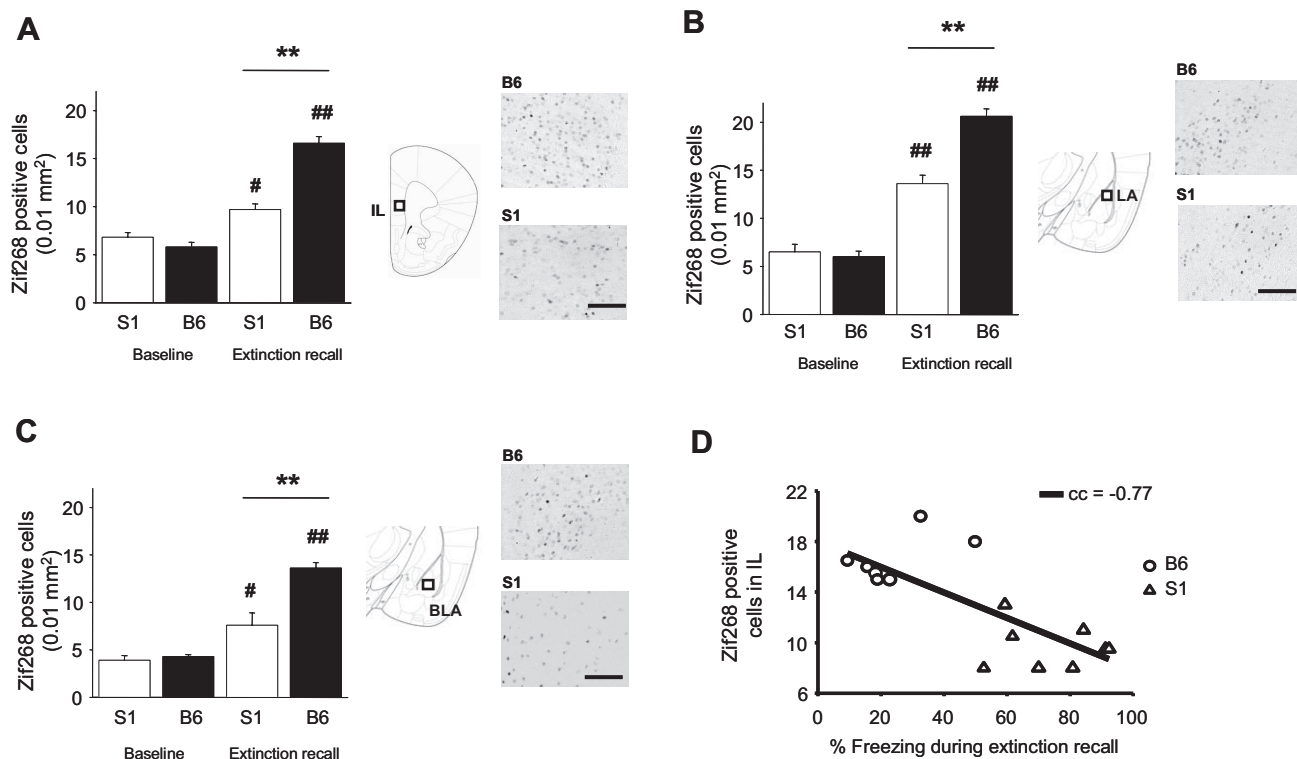
Figure 5. c-Fos expression after extinction recall. *A*, 129S1 (S1) mice showed a lesser increase (relative to CS minus baseline) in c-Fos-positive cells in the IL than C57BL/6J (B6) after extinction recall. *B*, S1 mice showed a lesser extinction-related increase in c-Fos-positive cells in the BLA than B6 after extinction recall. *C*, S1 mice showed a greater increase in c-Fos-positive cells in CeA than B6 after extinction recall. *D*, The number of c-Fos-positive cells in IL (but not BLA or CeA) (see Results) was highly negatively correlated with freezing during extinction recall. $n = 7$–8/strain for extinction recall; $n = 4$/strain for baseline. cc, Correlation coefficient. Scale bars, 100 μm. **$p < 0.01$ 129S1 versus B6; ##$p < 0.01$, #$p < 0.05$ versus baseline.

sion after extinction recall relative to CS minus baseline control levels in both strains, after extinction recall 129S1 mice exhibited fewer c-Fos-positive cells in the infralimbic cortex (Fig. 5*A*) and basolateral amygdala (Fig. 5*B*), and more positive cells in the central amygdala (Fig. 5*C*) than C57BL/6J. The cingulate cortex (1.78 mm from bregma, $F_{(1,19)} = 68.24$; $p < 0.01$; 1.54 mm from bregma, $F_{(1,19)} = 177.56$, $p < 0.01$; 1.10 mm from bregma, $F_{(1,19)} = 143.48$, $p < 0.01$), prelimbic cortex (1.78 mm from bregma, $F_{(1,19)} = 220.43$, $p < 0.01$; 1.54 mm from bregma, $F_{(1,19)} = 149.06$, $p < 0.01$), lateral amygdala ($F_{(1,19)} = 35.77$; $p < 0.01$), medial posterodorsal ($F_{(1,19)} = 91.49$; $p < 0.01$), medial posteroventral ($F_{(1,19)} = 58.73$; $p < 0.01$), anterior cortical ($F_{(1,19)} = 47.57$; $p < 0.01$), and posterolateral cortical ($F_{(1,19)} = 58.88$; $p < 0.01$) regions all expressed significantly more c-Fos-positive cells after extinction recall than after CS minus baseline conditions but did not differ between strains (Table 1). No other brain regions examined were significantly affected by condition or strain (Table 1).

There was a significant strain by condition interaction for the number of Zif268-positive cells in the infralimbic cortex (1.78 mm from bregma, $F_{(1,19)} = 30.74$, $p < 0.01$; 1.54 mm from bregma, $F_{(1,19)} = 14.32$, $p < 0.01$), the lateral ($F_{(1,19)} = 17.22$; $p < 0.01$) and basolateral nuclei of the amygdala ($F_{(1,19)} = 33.97$; $p < 0.01$), and the Imp ITC mass ($F_{(1,19)} = 23.80$; $p < 0.01$). Although these five regions show higher Zif268 expression after extinction recall relative to CS minus baseline control levels in both strains, after extinction recall 129S1 mice exhibited fewer Zif268-positive cells in the infralimbic cortex (Fig. 6*A*) and lateral amygdala (Fig. 6*B*), and more positive cells in Imp (Fig. 7*B*) than C57BL/6J. The

secondary motor ($F_{(1,19)} = 153.97$; $p < 0.01$), cingulate cortex (1.78 mm from bregma, $F_{(1,19)} = 109.96$, $p < 0.01$; 1.10 mm from bregma, $F_{(1,19)} = 8.88$, $p < 0.01$), prelimbic cortex (1.78 mm from bregma, $F_{(1,19)} = 97.41$, $p < 0.01$; 1.54 mm from bregma, $F_{(1,19)} = 77.67$, $p < 0.01$), central nucleus ($F_{(1,19)} = 72.11$; $p < 0.01$), Ilp ITC mass ($F_{(1,19)} = 34.25$; $p < 0.01$), medial posterodorsal ($F_{(1,19)} = 155.19$; $p < 0.01$), medial posteroventral ($F_{(1,19)} = 62.88$; $p < 0.01$), and posterolateral cortical ($F_{(1,19)} = 49.56$; $p < 0.01$) regions all expressed significantly more Zif268-positive cells after extinction recall than after CS minus baseline but did not differ between strains (Table 2). No other brain regions examined were significantly affected by condition or strain (Table 2).

Correlational analysis of the whole sample (i.e., both strains) revealed a significant negative correlation between percentage freezing during first tone presentation during extinction recall and the number of c-Fos-positive (-0.83, $p < 0.01$, 1.74 mm from bregma; -0.75, $p < 0.01$, 1.54 mm from bregma) (Fig. 5*D*) and Zif268-positive (-0.77, $p < 0.01$, 1.74 mm from bregma; -0.80, $p < 0.01$, 1.54 mm from bregma) (Fig. 6*D*) cells in the infralimbic cortex. Significant negative correlation was also observed between extinction recall freezing and c-Fos (-0.75; $p < 0.01$)- and Zif268 (-0.77; $p < 0.01$)-positive cells in the basolateral amygdala, as well as Zif268-positive cells in the lateral amygdala (-0.79; $p < 0.01$). Finally, there was a significant positive correlation between extinction recall freezing and the number of c-Fos-positive cells in the central amygdala (0.87; $p < 0.01$) and the number of Zif268-positive cells in the medial paracapsular

Figure 6. Zif268 expression after extinction recall. *A*, 129S1 (S1) mice showed a lesser increase (relative to CS minus baseline) in Zif268-positive cells in the IL than C57BL/6J (B6) after extinction recall. *B*, S1 mice showed a lesser increase in Zif268-positive cells in LA than B6 after extinction recall. *C*, S1 mice showed a lesser increase in Zif268-positive cells in the BLA than B6 after extinction recall. *D*, Across strains, the number of Zif268-positive cells in IL (but not BLA or CeA) (see Results) was negatively correlated with freezing during extinction recall. $n = 7-8$/strain for extinction recall; $n = 4$/strain for baseline. cc, Correlation coefficient. Scale bar, 100 μm. **$p < 0.01$ 129S1 versus B6; ##$p < 0.01$, #$p < 0.05$ versus baseline.

ITC (0.80; $p < 0.01$) (Fig. 7*C*, Tables 1, 2). No other brain region correlated significantly with freezing during extinction recall.

Fear-related cortico-amygdala IEG activation
129S1 and C57BL/6J did not significantly differ in freezing during fear recall (129S1, 55.2 ± 3.5%; C57BL/6J, 51.6 ± 5.2%; NS). There was no significant effect of strain on the number of either c-Fos- or Zif268-positive cells in any brain region examined (Table 3) (all NS) after fear recall.

Discussion
The major finding of the present study was the identification of a significant and selective impairment in fear extinction in a common inbred mouse strain, 129S1/SvImJ (129S1). This phenotype was associated with abnormal activation of a key prefrontal-amygdala pathway mediating fear extinction.

Our initial strain survey was the broadest conducted to date and revealed marked differences in fear extinction across inbred strains. Although not the focus of our study, we observed strain differences in the acquisition and expression of conditioned fear per se, in line with previous reports comparing various strains (Paylor et al., 1993, 1994; Fordyce et al., 1995; Caldarone et al., 1997; Owen et al., 1997; Wehner et al., 1997; Gerlai, 1998; Valentinuzzi et al., 1998; Stiedl et al., 1999; Nguyen et al., 2000; Bolivar et al., 2001; Balogh et al., 2002; Cook et al., 2002; Holmes et al., 2002; Balogh and Wehner, 2003; Bothe et al., 2005). In terms of extinction, DBA/2J, FVB/NJ, BALB/cByJ, and C57BL/6J exhibited significant within-session extinction, albeit to varying degrees. Of note, within-session extinction learning and between-session extinction recall was modest and incomplete in the reference strain, C57BL/6J, under these current test condi-

tions. This rate of extinction is comparable with that seen in other laboratories using a similar 30 s tone massed fear conditioning protocol (Herry et al., 2006) or a fear-potentiated startle paradigm (Waddell et al., 2004), although lesser than that seen with other conditioning protocols (Siegmund et al., 2005) and other test conditions in our hands (Norcross et al., 2008).

Of the strains currently tested, the most striking extinction profile was exhibited by the 129S1 strain. 129S1 showed no apparent short-term extinction learning over 2 × 50 trial massed extinction sessions and no long-term extinction recall. Although A/J also showed poor within-session extinction, as previously reported (Owen et al., 1997), these mice exhibited heightened unconditioned freezing that confounded interpretation of their extinction profile. In contrast, impaired extinction in 129S1 was not an artifact of increased pain perception (assayed via hot plate and Von Frey tests) or increased fear conditioned fear per se. This was demonstrated by a lack of fear recall differences between 129S1 and C57BL/6J on a multiple tone–shock paradigms and, to circumvent potential ceiling levels of freezing, a one tone–shock paradigm. Normal fear conditioning in 129S1 is consistent with the majority of previous reports comparing C57BL/6J and various 129 substrains, including 129S1 (Owen et al., 1997; Nguyen et al., 2000; Bolivar et al., 2001; Holmes et al., 2002; Balogh and Wehner, 2003; Bothe et al., 2005; Schimanski and Nguyen, 2005). Current data also showed that impaired fear extinction deficit in 129S1 did not extend to an appetitively driven conditioned instrumental response on touchscreen-based instrumental task (Izquierdo et al., 2006b; Brigman et al., 2008). On another measure of extinction to an aversive event (CTA) 129S1 again showed poor extinction, but a low level of acquisition and an uncondi-

Table 1. Strain differences in c-Fos expression in cortical and amygdaloid regions after extinction recall

	Baseline		Extinction recall	
	129S1	C57BL/6J	129S1	C57BL/6J
Cortical regions				
M1 (1.78 mm)	0.3 ± 0.2	0.1 ± 0.1	0.6 ± 0.3	0.4 ± 0.2
M2 (1.78 mm)*	0.9 ± 0.2	0.8 ± 0.4	0.8 ± 0.2	0.6 ± 0.2
Cg1 (1.78 mm)	2.0 ± 0.3	0.6 ± 0.3	9.8 ± 1.1	9.3 ± 0.5
Cg1 (1.54 mm)*	2.3 ± 0.3	0.8 ± 0.3	9.7 ± 0.7	9.4 ± 0.5
Cg2 (1.10 mm)*	3.1 ± 0.5	2.0 ± 0.5	12.3 ± 0.9	11.3 ± 0.7
PrL (1.78 mm)*	1.5 ± 0.3	1.1 ± 0.3	9.3 ± 0.8	10.0 ± 0.5
PrL (1.54 mm)*	2.0 ± 0.3	1.0 ± 0.2	10.9 ± 0.8	11.6 ± 0.3
IL (1.78 mm)*	1.6 ± 0.3	1.6 ± 0.3	6.8 ± 0.4	11.9 ± 0.5**
IL (1.54 mm)*	1.9 ± 0.6	2.1 ± 0.4	6.0 ± 0.4	9.7 ± 0.7**
Amygdala nuclei (all −1.58 mm)				
LA*	0.9 ± 0.3	0.8 ± 0.3	2.7 ± 0.5	2.8 ± 0.3
BLA*	1.0 ± 0.4	1.3 ± 0.3	2.1 ± 0.5	7.9 ± 0.5**
CeA*	0.8 ± 0.3	0.8 ± 0.3	5.1 ± 0.5	1.8 ± 0.3**
Imp[a]				
Ilp[a]				
MePD*	1.4 ± 0.5	1.0 ± 0.4	5.6 ± 0.5	6.7 ± 0.5
MePV*	4.8 ± 0.5	4.8 ± 0.3	8.3 ± 0.5	8.3 ± 0.4
ACo	5.9 ± 1.1	6.3 ± 0.7	10.6 ± 0.6	10.0 ± 0.6
PLCo*	2.9 ± 0.6	3.3 ± 0.9	8.1 ± 0.7	7.4 ± 0.4

129S1 mice had fewer c-Fos-positive cells in IL and BLA and more c-Fos-positive cells in CeA than C57BL/6J after extinction recall. No other cortical or amygdaloid region examined differed between strains ($n = 8$ per strain). CS minus baseline c-Fos expression did not differ between strains baseline in any brain region examined ($n = 4$ per strain). Data are mean $\pm$ SEM c-Fos-positive cells per 0.01 mm^2. M1, Primary motor; M2, secondary motor; Cg1, cingulate area 1; Cg2, cingulate area 2; PrL, prelimbic; MePD, medial, posterodorsal; MePV, medial, posteroventral; ACo, anterior cortical; PLCo, posterolateral cortical.

[a]No detectable expression.

*$p < 0.01$, extinction recall versus baseline; **$p < 0.01$, C57BL/6J versus 129S1/same condition.

tioned aversion to saccharin prevented clear interpretation of CTA extinction. Together, these data demonstrate a selective deficit in the formation of fear extinction memory in 129S1.

Fear extinction in 129S1 was not improved by providing additional massed extinction trials. Nor was it facilitated by treatment with the partial NMDAR agonist D-cycloserine. This differs from the extinction facilitating effects of D-cycloserine in conditioned freezing or fear-potentiated startle paradigms in C57BL/6J mice (Tomilenko and Dubrovina, 2007; current study), rats (Walker et al., 2002; Richardson et al., 2004), as well human phobics (Ressler et al., 2004). The inefficacy of D-cycloserine in 129S1 could further reflect usually strong resistance to extinction in this strain and/or different molecular mechanisms driving the behavior across different mouse stains. In this context, yohimbine, a compound with antagonist properties at α2-adrenoreceptors among other effects, significantly improved long-term extinction in 129S1, as previously seen in rats and C57BL/6J mice (Cain et al., 2004; Morris and Bouton, 2007). This demonstrates that impaired extinction in 129S1 can be at least partially reversed, although the molecular mechanisms involved remain to be determined.

Deficient fear extinction in 129S1 was associated with a striking alteration in the activation profile of a key prefrontal-amygdala circuit mediating fear extinction. This was assayed via expression of the IEG c-Fos, a surrogate marker for neuronal activation (Singewald, 2007) and Zif268, an IEG that acts as a transcription factor subserving fear memory (re)consolidation (Davis et al., 2003; Lee et al., 2004). Consistent with the absence of behavioral differences after fear recall or nonconditioned baseline, IEG activation in IL (or any region examined) did not differ between strains. In contrast, C57BL/6J showed significantly greater extinction-related IEG activation than 129S1 in IL, and IL activation showed a high correlation (-0.86) with freezing during initial extinction recall. This suggests an extinction-related failure to recruit IL in 129S1 mice, consistent with the important

role ascribed to IL in rodent extinction (Herry and Mons, 2004; Quirk et al., 2006) and homologous regions in human extinction (Phelps et al., 2004; Kalisch et al., 2006; Milad et al., 2007).

Impaired fear extinction in 129S1 was not associated with blunted IL activation alone, but instead reflected a circuit level failure. 129S1 showed lesser IEG expression than C57BL/6J in BLA (but not medial or cortical amygdala subnuclei) after extinction. They also showed lesser induction of Zif268, but not c-Fos, in LA. Although the functional significance of this subnuclei dissociation between the two IEGs is not clear, it is in full agreement with the finding of low Zif268, not c-Fos, expression in the LA of poor extinguishing C57BL/6J mice after extinction recall (Herry and Mons, 2004) (see also Santini et al., 2004). More generally, although LA and BLA drive conditioned fear via CeA, the contribution of these regions to extinction learning is only now being uncovered. For example, whereas BLA lesions do not affect extinction learning (Sotres-Bayon et al., 2004; Anglada-Figueroa and Quirk, 2005), a subclass of LA/BLA neurons are active and depotentiated during extinction (Repa et al., 2001; Kim et al., 2007b). Furthermore, blockade of NMDARs or MAPK signaling in BLA impairs fear extinction in rats and C57BL/6J mice (Herry et al., 2006; Sotres-Bayon et al., 2007), and depotentiation of conditioning-induced long-term potentiation in BLA predicts successful extinction (for review, see Barad et al., 2006). Together with current findings, this supports a role for LA/BLA, likely working in concert with IL, in the acquisition and expression of fear extinction (see Note added in proof). As such, possible abnormalities in BLA neuronal depotentiation (BLA long-term potentiation appears normal in these mice) (Schimanski and Nguyen, 2005) or signaling in 129S1 will be an interesting avenue for future study.

The ITC cell masses of the amygdala are posited to be another important component of the extinction circuit (Paré et al., 2004). These GABAergic neurons serve as an intra-amygdala relay station exerting feedforward inhibition over CeA amygdala output (Paré et al., 2004) (see Note added in proof). BLA and IL send projections to the ITC cell masses (McDonald et al., 1996; Royer et al., 1999; Vertes, 2002; Berretta et al., 2005). In the rat, IL stimulation increases ITC c-Fos expression (Berretta et al., 2005) and produced an associated inhibition of CeA neurons (Quirk et al., 2003). In our mouse extinction paradigm, extinction was associated with strong Zif268 expression in the Imp and Ilp ITC masses, and this correlated with levels of freezing during extinction. To our knowledge, this is the first demonstration of extinction induced ITC activation. However, unlike Berretta et al. (2005) had, we did not see ITC c-Fos expression, possibly because of relatively weaker IL stimulation by extinction than chemical stimulation, or a species difference. Zif268 expression in ITCs differed between strains. Curiously, however, there was relatively greater activation of Imp (but not Ilp) in 129S1 than C57BL/6J. Moreover, and as expected given the high fear during extinction recall in 129S1, CeA c-Fos expression was markedly higher in these mice relative to C57BL/6J. As such, the strain differences in
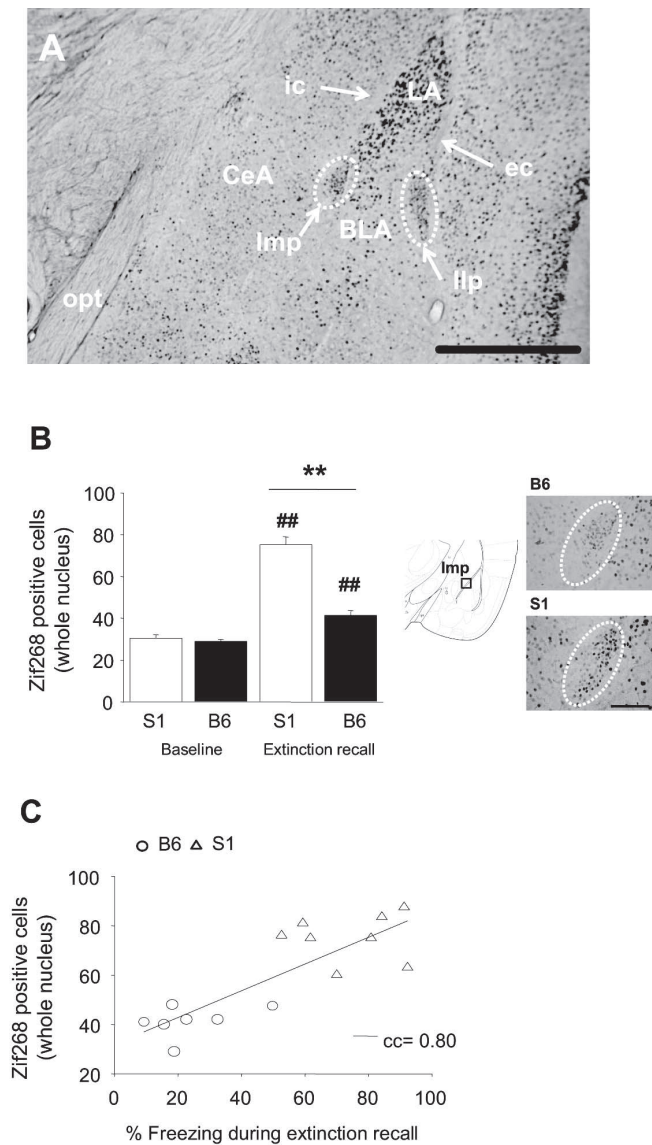
Figure 7. Zif268-positive cells identified in the ITC masses of the amygdala after extinction recall. Representative illustration of anatomical definition of ITC mass nuclei labeled with Zif268 immunoreactivity. ***A***, Anatomical definition of Zif268-expressing medial paracapsular ITC mass (Imp) and lateral paracapsular ITC mass (Ilp). Scale bar, 500 μm. ***B***, 129S1 (S1) mice showed a greater increase (relative to CS minus baseline) in Zif268-positive cells in Imp (but not Ilp) (Table 1) than C57BL/6J (B6) after extinction recall. Scale bar, 100 μm. ***C***, Across strains, the number of Zif268-positive cells in Imp was positively correlated with freezing during extinction recall. $n =$ 7– 8/strain for extinction recall; $n =$ 4/strain for baseline. cc, Correlation coefficient; ic, internal capsule; LA, lateral nucleus; ec, external capsule; CeA, central nucleus; BLA, basolateral nucleus; opt, optic tract; Imp, medial paracapsular nucleus; Ilp, lateral paracapsular nucleus. ***p < 0.01 129S1 versus B6; ##p < 0.01 versus baseline.

ITC recruitment are not easily reconciled with the aforementioned circuit model of extinction and raise the question of why CeA activity in 129S1 is high in the face of both high ITC and low BLA input. One possibility is that there may be other sources of excitatory input to CeA that could be functionally aberrant in 129S1 mice (e.g., from posterior thalamic nucleus). Another possibility is that there is a network of functionally diverse ITC cells, some signaling fear and others inhibiting CeA, and we are sampling the former subpopulation. [For further discussion of these possibilities, see Paré et al. (2004).] Clearly, additional studies are needed to fully elucidate the precise nature of the strain differences in extinction-related cortico-amygdala activation. Not-

withstanding, the main conclusion from the current data are that impaired extinction in 129S1 is associated with a failure to properly recruit prefrontal and amygdala circuitry mediating this behavior.

In summary, through a strain survey, we identified an inbred mouse strain, 129S1, with a marked deficit in fear extinction. The extinction deficit in this strain was dissociable from fear conditioning and extinction of an instrumental appetitive behavior, both of which were normal in 129S1. 129S1 were resistant to the extinction facilitating effects of extended massed training and treatment with D-cycloserine, but yohimbine treatment improved long-term extinction. Analysis of neural activation after extinction recall revealed hypoactivation of IL and BLA, differential activation of the ITCs, and hyperactivation of CeA in 129S1 relative to C57BL/6J. Although previous studies have produced fear extinction deficits by neuroanatomical lesions, gene mutations, and selected breeding, the 129S1 mouse is a naturally occurring example of impaired fear extinction and cortico-amygdala dysfunction in an animal model. This provides an opportunity to study sources of genetic variation driving differences in extinction. More generally, given the increasing convergence of rodent and human studies of emotional disorders and extinction in particular (Holmes and Hariri, 2003; Phelps et al., 2004; Milad et al., 2006; Cryan and Slattery, 2007; Ji and Maren, 2007), 129S1 mice could provide a useful model for studying the pathophysiology and therapeutic alleviation of impaired fear extinction in anxiety disorders such as phobias and posttraumatic stress disorder.

Note added in proof. At the time the current article was going to press, two important studies were published further describing the role of the amygdala and ITC masses in rodent fear extinction (Herry et al., 2008; Likhtik et al., 2008).

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Table 2. Strain differences in Zif268 expression in cortical and amygdaloid regions after extinction recall

	Baseline		Extinction recall	
	129S1	C57BL/6J	129S1	C57BL/6J
Cortical regions				
M1 (1.78 mm)	12.3 ± 0.8	11.9 ± 0.5	14.1 ± 0.5	13.6 ± 0.7
M2 (1.78 mm)*	12.6 ± 0.7	12.2 ± 0.7	22.8 ± 0.6	23.9 ± 0.8
Cg1 (1.78 mm)	14.3 ± 0.6	13.6 ± 0.9	22.0 ± 0.8	23.6 ± 0.8
Cg1 (1.54 mm)*	13.3 ± 0.8	14.4 ± 0.3	13.8 ± 0.6	13.9 ± 0,6
Cg2 (1.10 mm)*	17.4 ± 1.6	16.5 ± 0.9	20.7 ± 0.8	20.1 ± 0.3
PrL (1.78 mm)*	10.8 ± 0.6	11.3 ± 0.9	21.1 ± 1.2	23.1 ± 0.8
PrL (1.54 mm)*	11.3 ± 0.6	13.1 ± 0.6	23.1 ± 1.1	22.3 ± 0.6
IL (1.78 mm)*	6.8 ± 0.5	5.8 ± 0.5	9.7 ± 0.6	16.6 ± 0.7**
IL (1.54 mm)*	7.3 ± 0.6	6.1 ± 0.5	12.9 ± 0.9	21.2 ± 1.3**
Amygdala nuclei (all −1.58 mm)				
LA*	6.5 ± 0.8	6.0 ± 0.6	13.6 ± 0.9	20.6 ± 0.8**
BLA*	3.9 ± 0.5	4.3 ± 0.2	7.6 ± 1.3	13.6 ± 0.6**
CeA*	1.5 ± 0.3	1.7 ± 0.6	3.1 ± 0.1	2.9 ± 0.4
Imp[a]	30.5 ± 1.7	29.0 ± 0.9	75.1 ± 3.9	41.4 ± 2.4**
Ilp[a]*	21.1 ± 5.1	18.5 ± 1.8	41.3 ± 3.9	41.2 ± 2.6
MePD*	2.9 ± 0.4	2.7 ± 0.6	9.8 ± 0.6	10.4 ± 0.5
MePV*	10.8 ± 0.8	10.5 ± 0.8	13.8 ± 1.2	13.8 ± 0.9
ACo	11.0 ± 1.1	10.8 ± 0.9	11.3 ± 0.9	11.8 ± 0.8
PLCo*	9.4 ± 1.1	10.3 ± 1.2	16.8 ± 0.6	18.0 ± 1.0

129S1 mice had fewer Zif268-positive cells in IL, LA, BLA, and Imp than C57BL/6J after extinction recall. No other cortical or amygdaloid region examined differed between strains ($n = 7–8$ per strain). Baseline Zif268 expression did not differ between strains in any brain region examined ($n = 4$ per strain). Data are mean ± SEM Zif268-positive cells per 0.01 mm^2. For each region, the abbreviation and plane of section relative to bregma is given in parentheses. M1, Primary motor; M2, secondary motor; Cg1, cingulate area 1; Cg2, cingulate area 2; PrL, prelimbic; MePD, medial, posterodorsal; MePV, medial, posteroventral; ACo, anterior cortical; PLCo, posterolateral cortical.

[a]Whole nucleus counted.

*$p < 0.01$, extinction recall versus CS minus baseline; **$p < 0.01$, C57BL/6J versus 129S1/same condition.

Table 3. Absence of strain differences in c-Fos or Zif268 expression in cortical and amygdaloid regions after fear recall

	c-Fos		Zif268	
	129S1	C57BL/6J	129S1	C57BL/6J
Cortical regions				
M1 (1.78 mm)	0.1 ± 0.1	0.1 ± 0.1	25.8 ± 1.0	26.0 ± 1.0
M2 (1.78 mm)	4.9 ± 0.4	4.6 ± 0.4	28.9 ± 1.0	27.9 ± 1.2
Cg1 (1.78 mm)	7.5 ± 0.7	7.6 ± 0.6	28.6 ± 0.9	29.1 ± 0.7
Cg1 (1.54 mm)	9.5 ± 0.7	9.4 ± 0.6	29.3 ± 1.1	30.2 ± 0.8
Cg2 (1.10 mm)	13.0 ± 0.8	12.5 ± 0.4	36.5 ± 1.3	35.9 ± 0.7
PrL (1.78 mm)	11.3 ± 0.9	11.6 ± 0.6	27.4 ± 0.7	27.7 ± 1.0
PrL (1.54 mm)	13.0 ± 1.0	13.7 ± 0.7	31.4 ± 1.3	33.6 ± 1.0
IL (1.78 mm)	2.9 ± 0.5	2.4 ± 0.4	9.1 ± 0.5	9.7 ± 0.6
IL (1.54 mm)	4.2 ± 0.3	3.8 ± 0.3	11.0 ± 0.7	11.6 ± 0.6
Amygdala nuclei (all −1.58 mm)				
LA	2.7 ± 0.3	2.5 ± 0.3	32.4 ± 1.3	32.0 ± 0.7
BLA	7.6 ± 0.5	6.6 ± 0.3	15.2 ± 0.8	14.1 ± 0.8
CeA	4.9 ± 0.4	5.1 ± 0.3	8.0 ± 0.3	8.2 ± 0.4
Imp[a]	ND	ND	39.0 ± 1.2	36.9 ± 1.2
Ilp[a]	ND	ND	38.4 ± 1.1	37.2 ± 1.1
MePD	4.8 ± 0.6	4.9 ± 0.5	15.0 ± 0.4	14.2 ± 0.6
MePV	8.8 ± 0.5	8.0 ± 0.4	22.2 ± 1.1	19.6 ± 0.5
ACo	9.4 ± 0.8	8.6 ± 0.6	26.2 ± 0.9	26.3 ± 1.3
PLCo	10.9 ± 1.1	10.6 ± 0.8	27.4 ± 1.3	28.2 ± 1.1

129S1 and C57BL/6J mice did not differ in c-Fos- or Zif268-positive cells in any brain region examined ($n = 10$/strain). Data are mean ± SEM c-Fos- and Zif268-positive cells per 0.01 mm^2. For each region, the abbreviation and plane of section relative to bregma is given in parentheses. M1, Primary motor; M2, secondary motor; Cg1, cingulate area 1; Cg2, cingulate area 2; PrL, prelimbic; MePD, medial, posterodorsal; MePV, medial, posteroventral; ACo, anterior cortical; PLCo, posterolateral cortical; ND, no detectable expression.

[a]Whole nucleus counted.

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