Cholinergic Blockade Frees Fear Extinction from Its Contextual Dependency

Moriel Zelikowsky, Timothy A. Hast, Rebecca Z. Bennett, Michael Merjanian, Nathaniel A. Nocera, Ravikumar Ponnusamy, and Michael S. Fanselow

Background: Fears that are maladaptive or inappropriate can be reduced through extinction training. However, extinction is highly context-sensitive, resulting in the renewal of fear after shifts in context and limiting the clinical efficacy of extinction training. Lesion and inactivation studies have shown that the contextualization of extinction depends on the hippocampus. Parallel studies have found that intrahippocampal scopolamine (Scop) blocks contextual fear conditioning. Importantly, this effect was replicated with a noninvasive technique in which a low dose of Scop was administered systemically. We aimed to transfer the effects of this noninvasive approach to block the contextualization of fear extinction.

Methods: Rats were tone fear conditioned and extinguished under various systemic doses of Scop or the saline vehicle. They were subsequently tested (off drug) for tone fear in a context that was the same (control subjects) or shifted (renewal group) with respect to the extinction context.

Results: The lowest dose of Scop produced a significant attenuation of fear renewal when renewal was tested either in the original training context or a novel context. The drug also slowed the rate of long-term extinction memory formation, which was readily overcome by extending extinction training. Scopolamine only gave this effect when it was administered during but not after extinction training. Higher doses of Scop severely disrupted extinction learning.

Conclusions: We discovered that disrupting contextual processing during extinction with the cholinergic antagonist Scop blocked subsequent fear renewal. Low doses of Scop might be a clinically promising adjunct to exposure therapy by making extinction more relapse-resistant.

Key Words: Anxiety, exposure therapy, extinction, fear conditioning, hippocampus, renewal, scopolamine

Fear orchestrates and organizes the way in which each species defends itself in a dangerous situation (1). However, when the mechanisms underlying fear go awry, the consequence is as detrimental as a functional fear system is beneficial. Fear can be maladaptive when elicited in nonthreatening situations or in excess, and the persistence of such inappropriate fears contributes to the formation of anxiety disorders and phobias (2).

Fear extinction, wherein a fear-provoking stimulus is repeatedly presented in the absence of an aversive consequence, provides one of the most effective ways of reducing fear (3). However, extinction does not erase the original fear memory formed during acquisition (4). Instead, extinction produces a new inhibitory memory that can serve to suppress or compete with the original fear (5). Importantly, extinction memories are more tenuous and sensitive to disruption in contrast to the resilient and enduring nature of fear memories. Indeed, after extinction, several factors cause fear to recover (6). In fear renewal, a rodent presented with an extinguished stimulus in a context that is different than the extinction context will show a renewal of the original fear response (7,8), demonstrating the context-dependency of extinction. This effect has been extended to human studies as well (9–11). Fear renewal poses a serious problem for the treatment of anxiety disorders in the clinic, because fears of patients are often extinguished in a context (e.g., office of therapist) that is different than the context in which they are likely to re-encounter extinguished fear stimuli (3).

Interestingly, the majority of experimental research on fear extinction and the development of techniques for the treatment of phobias and anxiety disorders have focused on methods of enhancing extinction or "erasing" the original fear memory rather than on reducing relapse or preventing fear renewal. Specifically, pharmacological studies tend to use methods to enhance learning during extinction (e.g., cognitive enhancers). One such example is the use of the partial *N*-methyl-D-aspartate agonist d-cycloserine to enhance extinction learning (12). Although some but not all investigations of this drug have shown that it increases the rate of extinction (12–16), there is considerable evidence that it does not change the nature of extinction (15) (i.e., extinction under d-cycloserine remains context-bound, and renewal is not attenuated [15,16]). Thus, although such approaches might enhance the rate of extinction, they importantly do not attenuate relapse effects such as renewal, which are perhaps a more clinically important consideration for the long-term effectiveness of exposure treatment.

Taking a different approach, other groups of researchers have attempted to erase the original fear formed, as opposed to enhancing extinction. Although fear extinction is thought to be a form of new inhibitory learning rather than an unlearning of the original fear (4,6), researchers have suggested that extinction immediately after a traumatic event (10 min) might serve to "erase" or cause the "unlearning" of fear, because extinction occurs before the trauma memory has consolidated (17). However, the translatability of this research is limited because exposure therapy must be given very soon after the trauma, which would be difficult to achieve in many situations.

From the Departments of Psychology and Psychiatry and Biobehavioral Sciences, University of California, Los Angeles, Los Angeles, California.
Address correspondence to Michael S. Fanselow, Ph.D., Psychology Department, University of California, Los Angeles, 405 Hilgard Avenue, Los Angeles, CA 90095-1563; E-mail: fanselow@psych.ucla.edu.
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For example, in individuals that seek treatment for an anxiety disorder, the interval between symptom onset and treatment-seeking ranges between 9 and 23 years (18).

Other approaches have used a brief extinction ("reminder") session followed by either propranolol (19–22) or a longer extinction session (23,24) to cause a "deconsolidation" of the original fear memory. These methods have proved sometimes successful and sometimes not (25). Another limitation to this approach is that the mechanisms mediating this type of deconsolidation still seem to be time-limited, requiring therapy to occur shortly after trauma (26–28).

Behavioral strategies for overcoming the contextualization of extinction have attempted to extinguish in multiple contexts. These studies have also met with mixed results in human samples, with one research group showing a significant reduction of renewal (29) and another group failing to find such benefits (30). Importantly, Bouton *et al.* (31) have shown that extinction in four different contexts did not prevent renewal when rats were shifted either back to the original fear acquisition context or an entirely novel context. Another behavioral approach uses a cue as a reminder for extinction. Although this approach seems successful in highly controlled laboratory settings (32,33), it has produced only weak and nonreplicated results in a human anxious sample (34).

Recent investigations into the neural underpinnings of fear renewal offer important insights into the brain regions that must be targeted to specifically eliminate renewal. These studies have implicated the hippocampus (HPC) in the contextual regulation of extinction (35–40). These data show that lesions or temporary and reversible inactivation of the HPC before the acquisition and/or expression of fear extinction are able to disrupt fear renewal. Such findings are in line with a more general role for the HPC in the encoding and processing of integrated contextual representations (41,42). Collectively, these findings demonstrate that contextual processing during fear-related learning might be attenuated by manipulations that render the HPC dysfunctional.

Although interfering with hippocampal function during or shortly after extinction prevents fear renewal, the invasiveness of these experimental procedures makes them clinically unfeasible for the treatment of anxiety disorders. Therefore, we sought a readily translatable pharmacological approach to impair the HPC during extinction. Ideally such a treatment would have three characteristics: 1) it would be systemically deliverable; 2) it would have a history of clinical use in humans; and 3) it would preferentially target contextual learning. For insight, we turned to studies in contextual fear conditioning that used the muscarinic cholinergic antagonist scopolamine (Scop) to temporarily and selectively disrupt contextual fear conditioning in a dose-dependent, HPC-mediated manner (43,44).

Our prior work testing rats with a wide range of doses (.01–100 mg/kg) showed that low doses of the drug had selective effects on contextual learning and closely paralleled the behavioral effects of intrahippocampal Scop (45). Higher doses had a more general impact on learning and behavior. For example, the ED50 for HPC-independent tone fear conditioning was approximately 14 times higher than the ED50 for HPC-dependent context conditioning, even though this comparison is made off-drug and tone and context learning occurred simultaneously. Additionally, Scop has been used in humans to treat a variety of conditions, including seasickness (46), Parkinson's disease (47), and depression (48–50). Thus, Scop met the three aforementioned characteristics, indicating that it was a promising candidate for the decontextualization of extinction.

Rats were tone fear conditioned, extinguished under Scop or the vehicle saline, and subsequently tested for fear renewal, to examine the effects of Scop in reducing fear renewal after extinction. We used a design for which we have previously demonstrated a role for the HPC in renewal (35). Control experiments included the application of higher doses of Scop, post-injection administration of Scop, and variations in the experimental design.

Methods and Materials

Subjects

A total of 90 naïve, adult male Long-Evans rats, initially weighing 300 g, purchased from Harlan (Indianapolis, Indiana) were used. Housing, feeding, and handling involved standard procedures described in Supplemental Methods in Supplement 1.

Drugs

Scopolamine hydrobromide (Sigma-Aldrich, St. Louis, Missouri) was dissolved in .9% saline (vehicle) to obtain a concentration of .1 mg/mL. Doses of .5, 1.35, and 2.7 were also tested (Figure S1 in Supplement 1). Injections were made into the IP cavity. See Supplemental Methods in Supplement 1 for additional information.

Behavioral Testing Apparatus, Fear Conditioning, and Analyses

The behavioral testing apparatus consisted of three distinct physical "contexts" (counterbalanced) and modes of transport to these contexts (four experimental chambers per context). A detailed description of fear conditioning procedures and automated analysis of freezing behavior are provided in the Supplemental Methods in Supplement 1 (also see Figure 1A).

Statistical Analyses

Data were statistically analyzed with analyses of variance. After significant findings, pairwise comparisons with a Bonferroni correction were performed. See Supplemental Methods in Supplement 1 for additional details.

Results

Scop Alters Freezing During Extinction

Tone fear-conditioned rats were extinguished under the influence of Scop to investigate the effects of Scop-mediated extinction on subsequent fear renewal. In our first experiment, rodents were fear conditioned and given two sessions of extinction under a low systemic dose of Scop (.1 mg/kg intraperitoneal) (design depicted in Figure 1A).

Figure 1B shows the fear acquisition data. All rats acquired significant tone fear across acquisition trials [$F(3,84) = 140.9$, $p < .0001$]. Animals were equally split into groups (Same/Shifted and Saline/Scop) on the basis of their final levels of fear and rate of acquisition, ensuring that groups were balanced before extinction. Freezing during Extinction Session One is displayed in Figure 1C (left panel). There was an overall significant effect of drug condition [$F(1,150) = 4.59$, $p < .05$], which interacted with the rate of behavior change during extinction, such that animals extinguished under Scop showed a slower loss of freezing across trials compared with saline control subjects [$F(5,150) = 7.56$, $p < .0001$]. This was partially driven by an initial reduction in freezing in Scop compared with saline-treated animals extinguished in a novel context (shifted groups: Bin 1: $p < .001$; Bin 2: $p < .05$) and disappeared as saline control subjects extinguished to the same low levels of freezing as Scop rats (Bin: 6: $p > .05$).

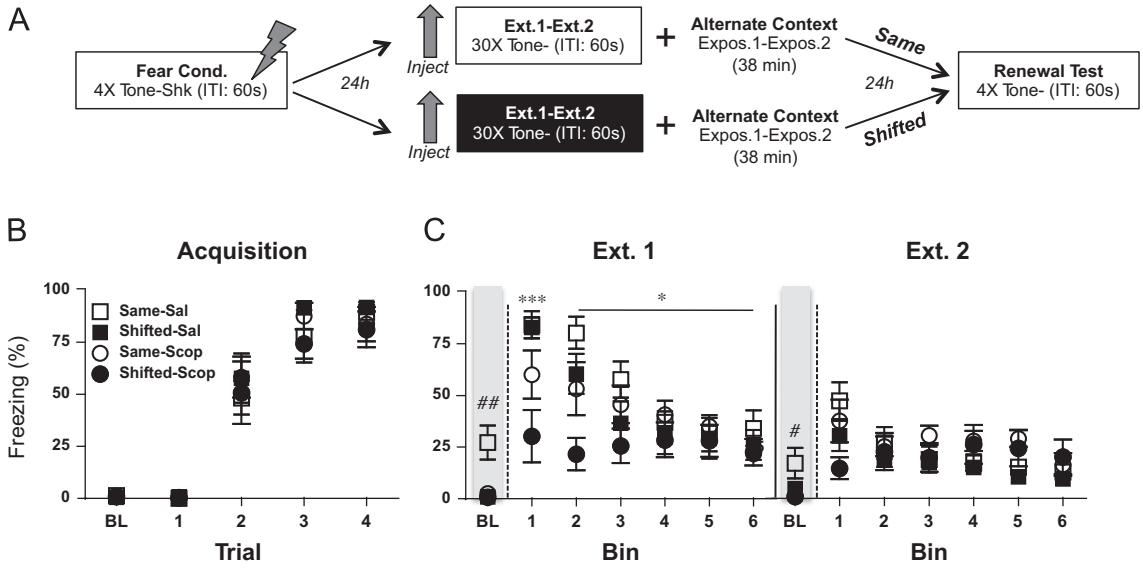

Figure 1. Effects of scopolamine (Scop) on fear extinction (Ext.) (two sessions). **(A)** Experimental design. Tone: 80 dB, 30 sec. Shock: .9 mA, 2 sec. Intertrial interval (ITI): 60 sec. **(B)** Fear acquisition. Mean (± SEM) percent baseline (BL) freezing and tone freezing during each tone-footshock trial. **(C)** Extinction. Mean (± SEM) percent freezing during BL and each bin of five tone-alone presentations for Ext. sessions 1–2. Rats were extinguished in the same context as acquisition/test (Same) or in a novel context (Shifted), under Scop or the vehicle saline (Sal) ($n = 8$). Freezing during the first and second bins in the novel context was reduced by Scop compared with Sal ($^{***}p < .001$; $^{*}p < .05$). Pre-Ext. BL fear was reduced for Scop-treated animals ($^{\#\#}p < .01$; $^{\#}p < .05$). Expos, exposure; h, hours; Shk, shock.

Caution should be exercised in interpreting extinction data while rats are receiving drug, because Scop is known to affect behavioral performance (45). Drug, context, and interaction effects disappeared by the second session of extinction (Figure 1C) (F values < 1). Interestingly baseline context fear before each extinction session (collapsed across contexts) was also reduced by Scop [Extinction 1: $F(1,28) = 8.50$, $p < .01$; Extinction 2: $F(1,28) = 6.33$, $p < .05$], further suggesting that Scop might interfere with the processing of contextual cues ("BL" shown in left panel of Figures 1B and 1C).

Extinction Under Scop Attenuates Renewal

After extinction, rats were tested for tone fear renewal in the same context as extinction ("AAA" design) or shifted out of the extinction context ("ABA" renewal). Baseline freezing to the context before tone onset (Figure 2A) was similarly negligible between groups (F values < 1), ensuring that tone fear expression was not due to an interaction between tone and context fear (51). In the critical tone test after extinction (Figure 2B), the saline-treated shifted rats froze more than the saline-treated same-context rats, demonstrating standard fear renewal in saline control subjects ($p < .01$). Scopolamine-treated rats froze similarly in the same and shifted conditions, indicating that the drug abolished renewal ($p > .05$)—providing evidence that intact cholinergic transmission during extinction is required for later fear renewal. This finding is consistent with those obtained when the HPC is inactivated during extinction (40), suggesting that cholinergic antagonism disrupts the ability for the HPC to function normally with respect to contextual encoding during extinction and subsequent contextual modulation of extinction memory retrieval. It should be noted that this effect was unique to this low dose of Scop, because extinction under higher doses of the drug had the effect of hindering extinction recall in a dose-dependent fashion (Figure S1 in Supplement 1).

Although Scop completely blocked renewal, the difference between the saline-shifted and Scop-shifted groups fell short of statistical significance ($p < .05$). This might have been caused by a modest attenuation of extinction in both Scop-treated groups. We hypothesized that because animals might have learned extinction in a changed, HPC-independent-like manner, extinction memories

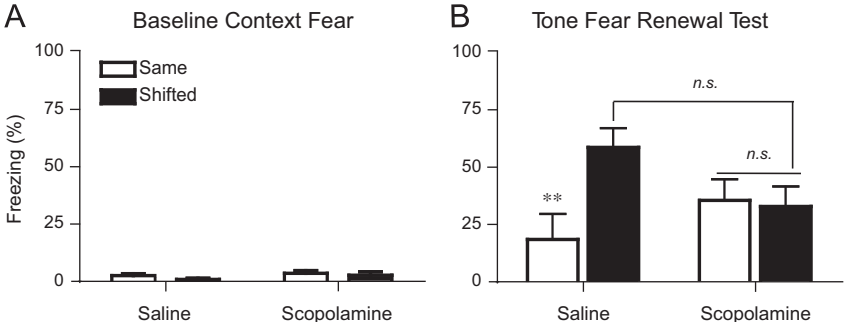

Figure 2. Fear renewal after two Ext. sessions. **(A)** Mean (± SEM) percent freezing during 180-sec BL period preceding tone onset. **(B)** Mean (± SEM) percent freezing to four, 30-sec tone presentations (ITI: 60 sec) in either the Same or Shifted context during test. Saline control subjects showed significant renewal (Same vs. Shifted: $^{**}p < .01$), which was blocked in animals that had been extinguished under the influence of Scop (n.s., $p > .05$). Abbreviations as in Figure 1.

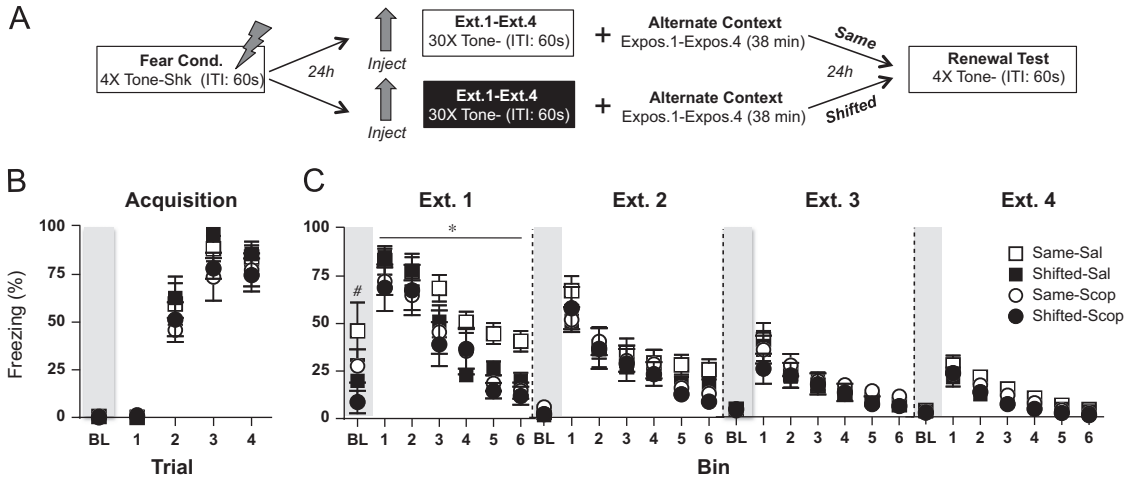

Figure 3. Effects of Scop on four sessions of fear Ext. **(A)** Experimental design. Tone: 80 dB, 30 sec. Shock: .9 mA, 2 sec. Intertrial interval: 60 sec. **(B)** Fear acquisition. Mean (± SEM) percent BL freezing and tone freezing during each tone-footshock trial. **(C)** Extinction. Mean (± SEM) percent freezing during BL and each bin of five tone-alone presentations for Ext. sessions 1–4. Rats were extinguished in the same context as acquisition/test (Same) or in a novel context (Shifted), under Scop or the vehicle Sal ($n = 7$–8). Freezing during Ext. 1 was reduced by Scop ($^{*}p < .05$). Pre-Ext. BL fear was reduced for Shifted animals ($^{\#}p < .05$). Abbreviations as in Figure 1.

might have taken longer to acquire. Thus, we asked whether additional sessions of extinction under Scop could produce a greater reduction in context fear for shifted rats.

Extinction Under Scop Strengthens with Additional Sessions

To try to enhance the degree of extinction under Scop we conducted a longer four-session extinction phase to strengthen extinction memory recall in both the same and shifted conditions. Rats were fear-conditioned, extinguished, and tested with a procedure identical to that used in the two-session extinction experiment, with the exception that they received four sessions of extinction and context exposure (see Figure 3A for design).

All rats showed significant fear acquisition [$F(3,78) = 146.3$, $p < .0001$] (Figure 3B**)**. Similar to the previous results, the data for Extinction Session 1 (Figure 3C) reveal an overall significant amount of extinction across tone bins [$F(5,140) = 73.64$, $p < .0001$], with a significant effect of drug such that Scop-extinguished animals showed lower fear expression during extinction compared with saline control subjects [$F(1,140) = 4.73$, $p < .05$]. Baseline freezing to the context before each extinction session revealed an overall increase in freezing for rodents extinguished in the acquisition context [Extinction 1: $F(1,26) = 4.27$, $p < .05$] that did not interact with drug condition ($F < 1$). Freezing differences during baseline and extinction disappeared by Extinction 2.

When animals were tested for tone fear renewal, we found again minimal levels of baseline freezing (Figure 4A). Average freezing to the tone at test (Figure 4B) demonstrated fear renewal in saline control subjects ($p < .001$), which was blocked by Scop ($p > .05$). This replicated our previous finding, providing further support for the idea that intact cholinergic transmission during extinction is required for fear renewal. Moreover, we saw a significant attenuation of tone freezing in the shifted condition for Scop, contrasted with saline rats ($p < .05$). Thus, although the formation of extinction memories under cholinergic antagonism might require more extinction training, what is learned seems to be more resilient to fear relapse effects such as renewal.

Scop Before But Not After Extinction Prevents Renewal in a Novel Context

In the first three experiments (Figures 1 and 3; and Figure S1 in Supplement 1) we examined the renewal that occurs when testing is in the original fear acquisition context (ABA renewal). Renewal also occurs when testing is in a novel context (ABC renewal [5]). ABC renewal might be more clinically relevant than ABA renewal, because return to the same context as fear acquisition is unlikely. Therefore, the final experiment used an ABC renewal design, to test the generality of our findings. The renewal condition used a different context for acquisition (A),

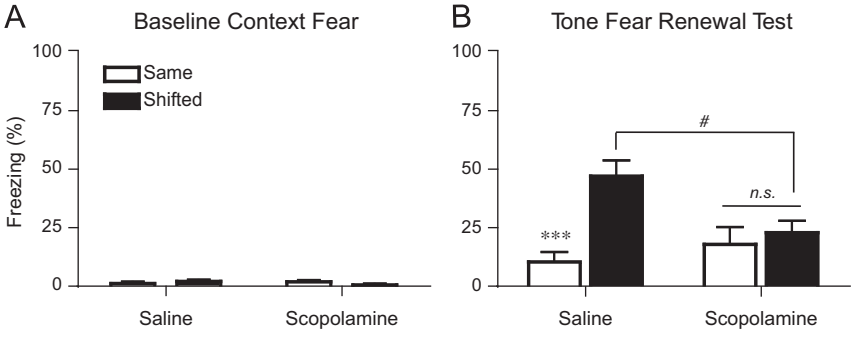

Figure 4. Fear renewal after four sessions of Ext. **(A)** Mean (± SEM) percent freezing during 180-sec BL period preceding tone onset. **(B)** Mean (± SEM) percent freezing to four 30-sec tone presentations (ITI: 60 sec) in either the Same or Shifted context at test. Saline control subjects showed significant renewal ($^{***}p < .001$), which was blocked by pre-Ext. Scop (n.s., $p > .05$). Scopolamine-extinguished rats showed a significant decrease in freezing in the Shifted condition ($^{\#}p < .05$). Abbreviations as in Figure 1.

extinction (B) and renewal (C). The control subjects had acquisition in one context (A), whereas both extinction and testing occurred in the same context (C).

Our interpretation of the influence of Scop on renewal is that extinction training becomes more general when context processing is disrupted during extinction. This interpretation predicts that Scop must be present during extinction to influence later renewal of fear. However, the prior experiments did not rule out the possibility that simply receiving Scop influenced later performance, because control subjects never received the drug. Therefore, rather than a vehicle-only control, the present experiment injected the drug 15 min after extinction in the control condition. In this way all animals were equated for drug exposure; the difference was whether or not the drug was given in conjunction with extinction.

Although Scop made extinction more generalizable, it is possible that it diminished the robustness of extinction. For example, extinction memory acquired under Scop might not last as long as drug-free extinction. Therefore, we tested renewal 3 days rather than 1 day after extinction.

Our ABA extinction design equated exposure to both the extinction and alternate contexts (Figures 1A and 3A; Figure S1A in Supplement 1). This procedure equates all groups for exposure to the various contexts. It is also a necessary feature of an ABA design, because it reduces baseline context fear before testing, which is required for auditory fear conditioning data to be interpretable (51). By testing renewal in a novel context, the ABC design obviates the need for extinguishing fear of the test context. During exposure therapy clients are unlikely to have anything equivalent to our "alternate" context exposure; so the ABC design again makes this procedure more analogous to a typical exposure treatment.

Fear acquisition and extinction proceeded similarly to our previous experiments (Figures 1 and 3). Seventy-two hours after the final extinction session, animals were tested for tone fear in either a novel context ("shifted" rats) or the context in which they were extinguished ("same" rats). Baseline freezing (Figure 5B) before presentation of the tone was low and similar across groups ($F < 1$).

Postextinction injected animals displayed significant fear renewal; the shifted rats froze more than nonshifted rats (Figure 5C) ($p < .05$). Pre-extinction injected rats did not show renewal; average freezing to the tone at test demonstrated no differences in freezing between shifted and same animals (Figure 5C) ($p > .05$). This blockade replicated our previous findings, extending our attenuation of renewal effect to renewal in a novel context and to fear recovery after the passage of time. Importantly, pre-extinction Scop-extinguished rats showed a significant decrease in freezing in the shifted condition ($p < .05$) compared with shifted rats injected postextinction. Thus, Scop must be present during extinction to have its effects; simply receiving the drug does not impact later performance. In addition, the tone-fear extinction memory was still robust 3 days after extinction.

Discussion

In the data reported here, we examined the effects of administering Scop before or after extinction training on fear renewal. We found that when extinction training occurred under Scop, a significant attenuation in fear renewal could be observed. Indeed, extinction memories formed under Scop were long-lasting and more resilient to shifts in context. This reduced

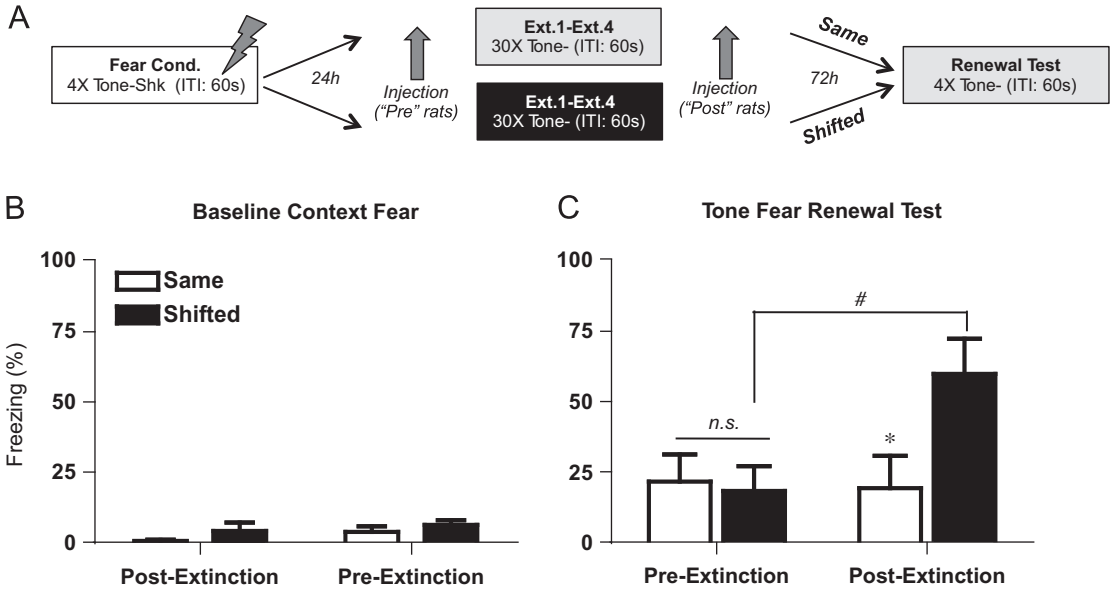

Figure 5. Effects of pre- or post-Ext. Scop on fear renewal in a novel context and recovery after the passage of time. **(A)** Experimental design. Tone: 80 dB, 30 sec. Shock: .9 mA, 2 sec. Intertrial interval: 60 sec. Note that the tone test occurred in a third novel context. **(B)** Mean (± SEM) percent freezing during 180-sec BL period preceding tone onset. **(C)** Mean (± SEM) percent freezing to four 30-sec tone presentations (ITI: 60 sec) in either the Same or Shifted context at test. Pre-Ext. Scop-injected animals showed no fear renewal, because freezing levels between the Same and Shifted groups were not significantly different (n.s., $p > .05$). Conversely, animals injected with Scop subsequent to Ext. sessions showed significant renewal ($^{*}p < .05$). Pre-Ext. Scop-extinguished rats showed a significant decrease in freezing in the Shifted condition ($^{\#}p < .05$), compared with Shifted rats injected post-Ext. ($n = 7–8$). Abbreviations as in Figure 1.

sensitivity to context shift made the rats less susceptible to relapse effects such as renewal. Furthermore, we showed that the effect on renewal is an interaction between extinction training and drug; the drug only works this way when given in conjunction with extinction. Collectively, these findings suggest that cholinergic blockade during extinction produces learning that is changed in nature: the safety memory is more slowly acquired and resilient to shifts in context. Importantly, the slowing of long-term memory formation is readily overcome by additional extinction training (compare Figures 2B and 4B).

Extinction Memories Formed Under Scop Are Slower to Acquire

The rate at which a fear behavior is reduced across repeated exposures to a feared stimulus is often taken as an indication of successful fear extinction. Importantly, facilitating or increasing the rate of extinction performance has been used as an indirect marker of enhanced extinction. The data presented here suggests that not only is extinction rate a poor indicator of whether a fear is actually being extinguished but slower extinction might in some cases be indicative of extinction memories that are resilient to relapse.

In other words, although the presence of Scop during extinction makes freezing behavior during extinction or "within-session extinction" difficult to interpret, the requirement for additional sessions of extinction (four as opposed to two sessions) to reduce tone fear at test for Scop-treated rats demonstrates that extinction learning under Scop requires more sessions to be learned. Indeed, when Scop is administered during or after a single session of extinction, animals fail to retain those extinction memories (52). In that study, Santini *et al.* reported that a systemic Scop dose 15 times greater than our .1-mg/kg dose reduced extinction when only a single extinction session was used. They attributed this effect to an action on infralimbic cortex, because a direct cortical infusion had similar effects. Thus, it is possible that in our experiments the ability of the drug to slow the formation of the extinction memory was mediated by the infralimbic cortex, whereas the ability of the drug to enhance generalization of the extinction memory involved the HPC (see following text). It is also possible that Santini *et al.* would have seen similar effects had they used a more robust extinction training procedure, as was used here.

Thus, Scop might disrupt some of the processes underlying extinction such that additional sessions are required to retain extinction memories; however, the memories that are eventually formed possess qualities that contribute to its resiliency. This is consistent with the idea that the rate of within-session extinction is a poor indicator of whether an extinction memory will be retained at test (53–56).

Decontextualizing Extinction

Another key characteristic of extinction is its sensitivity to the context in which it occurs (6). This contextualization of extinction lies at the heart of relapse, because extinction—no matter how strong or how weak—remains dependent on context specificity for expression. The experiments presented here directly address this issue in that they specifically target the contextual encoding that occurs during extinction with a pharmacological agent previously shown to block contextual processing mediated by the dorsal HPC (43,45).

The approach taken here is not the first to specifically target the context-dependency of renewal; however, the method implemented to do so is. Previous researchers have focused on attenuating renewal by making extinction learning more generalizable across contexts. Notably, experiments in both rodents and human subjects alike have attempted to make extinction more generalizable and less susceptible to renewal by extinguishing in multiple contexts or using reminder cues from the extinction session to bridge the gap between extinction and test contexts (9,31,34,57). These studies have resulted in mixed findings, with some obtaining benefits of such behavioral protocols and others not.

Our approach similarly aimed to reduce renewal but instead aimed to remove the contextual encoding that occurs during extinction itself by using a low dose of Scop, a cholinergic antagonist that has been shown to interfere with contextual processing and exert its effect via the dorsal HPC (44). This approach took advantage of the known research with regard to the role of the hippocampal formation in both context fear and fear renewal after extinction. Indeed, the HPC has been repeatedly implicated in the formation of contextual representations (41,42) and the contextual encoding and/or retrieval required to drive renewal (35–40,58,59). By administering Scop before extinction, we were able to mimic the attenuation of renewal observed when the HPC is directly inactivated or lesioned during extinction. Thus, our data extend the notion that the HPC is normally involved in the context-bound nature of extinction and reveal a noninvasive, temporary, and translatable pharmacological manipulation that serves to de-contextualize extinction learning.

Cholinergic Contributions to Contextual and Cued Learning

Cholinergic transmission has been repeatedly implicated in fear conditioning paradigms thought to involve the HPC, such as contextual and trace fear conditioning (43–45,60–62). A number of microinfusion studies further localize the role of muscarinic acetylcholine receptors to areas including the dorsal HPC, entorhinal cortex, and perirhinal cortex (44,63–65). The data presented here extend the role of cholinergic processing from these contextual fear effects to context-sensitive fear extinction. This suggests that the role of the dorsal HPC in contextual fear conditioning and fear extinction is similar (35), with both effects dependent on intact cholinergic transmission.

Systemic administration of Scop has been shown to also effect delay auditory fear conditioning, provided high doses are administered (45,62). Interestingly, we found that, at higher doses of Scop, animals failed to appropriately extinguish their tone fear at all, consistent with the blockade of processing auditory associations. Moreover, it has been suggested that high doses of Scop (8 mg/kg), administered before testing for fear after extinction might serve to reverse extinction that has already accrued to the conditional stimulus (66). Collectively, these findings are in line with the idea that low doses of Scop effect hippocampal but not amygdala processing, whereas higher doses might additionally disrupt functioning within the amygdala.

Clinical Implications

These findings support the idea that disrupting hippocampal functioning during extinction produces a changed, context-free extinction. These results have implications for the treatment of phobias and anxiety disorders in the clinic. Currently championed pharmacological adjuncts to exposure therapy have focused on cognitive enhancers (12,14), which—although speeding up extinction—do not change its fundamental, context-dependent nature. In contrast, we used a compound that is known to impair cognition, and although this manipulation slowed the rate of extinction memory formation (requiring more training sessions),

it made extinction more robust. Moreover, Scop is a drug that is easily translatable to the clinic, because it is already approved for several uses in humans (see introductory text).

The clinical translatability of Scop, combined with the finding that extinction memories formed under this compound, although slower to acquire, demonstrate a changed nature—resilient against shifts in the environment—suggests that clinical-based treatments might benefit by incorporating Scop into exposure therapy.

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Supplementary material cited in this article is available online.

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