Prediction Error Governs Pharmacologically Induced Amnesia for Learned Fear

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in the cells, and both viruses also triggered the production of cGAMP that activated IRF3 (Fig. 3G, lower panel). Collectively, these results indicate that DNA transfection and DNA virus infections in human and mouse cells produced cGAMP, which led to IRF3 activation.

To determine whether cGAMP activates IRF3 through STING, we carried out three sets of experiments. First, we established a HEK293T cell line stably expressing STING, stimulated these cells with cGAMP, and then measured IFN-β induction by quantitative RT-PCR (Fig. 4A). HEK293T cells did not respond to cGAMP, likely because of absent or very low STING expression in these cells. The expression of STING in HEK293T cells rendered a high level of IFN-β induction by cGAMP. However, DNA did not stimulate HEK293T-STING cells to induce IFN-β, consistent with a defect of HEK293T cells in producing cGAMP in response to DNA stimulation. In contrast, L929 cells induced IFN-β in response to stimulation by either cGAMP or DNA. HSV-1 infection induced IRF3 dimerization in L929 cells but not in HEK293T or HEK293T-STING cells (Fig. 4B, upper panel), which suggests that the production of cGAMP is important for HSV-1 to activate IRF3 in cells. Indeed, extracts from HSV1-infected L929 cells, but not from HEK293T or HEK293T-STING cells, contained the cGAMP activity that led to IRF3 dimerization in permeabilized Raw264.7 cells (Fig. 4B, lower panel). These results indicate that the expression of STING in HEK293T cells installed the ability of the cells to activate IRF3 and induce IFN-β in response to cGAMP, but was insufficient to install the response to DNA or DNA viruses because of a defect of HEK293T cells in synthesizing cGAMP.

Second, we tested the response of L929 and L929-shSTING cells to cGAMP (Fig. 4C). Similar to ISD and c-di-GMP, cGAMP-induced IRF3 dimerization was dependent on STING. In contrast, poly(I:C) still induced IRF3 dimerization in the absence of STING. These results demonstrate that STING is necessary for cGAMP to activate IRF3.

Finally, we examined whether STING binds to cGAMP directly. Recombinant STING protein containing residues 139 to 379, which has been shown to bind c-di-GMP (*14*), was expressed and purified from *Escherichia coli* and then incubated with [^{32}P]cGAMP followed by ultraviolet (UV)–induced cross-linking (Fig. 4D). A radiolabeled band corresponding to a cross-linked STING-cGAMP complex was detected when both STING and [^{32}P]cGAMP were present. High concentrations of ATP or GTP did not compete with the formation of the STING-cGAMP complex. By contrast, the intensity of this band decreased as the concentrations of competing cold cGAMP, c-di-GMP, or c-di-AMP increased; this finding suggests that the cGAMP binding sites on STING might overlap with those that interact with c-di-GMP and c-di-AMP. Indeed, mutations of several residues that were recently shown to participate in the binding of STING to c-di-GMP (*14*), including Ser^{161} → Tyr, Tyr^{240} → Ser, and Asn^{242} → Ala, also impaired the binding of STING to cGAMP (fig. S5). Collectively, these results demonstrate that cGAMP is a ligand that binds to and activates STING.

Cyclic dinucleotides have been shown to function as bacterial second messengers that regulate a variety of physiological processes, including bacterial motility and biofilm formation (*15*). A recent report showed that c-di-GMP is produced in the protozoan *Dictyostelium* and functions as a morphogen to induce stalk cell differentiation (*16*). Our results identify cGAMP as a first cyclic dinucleotide in metazoa and show that cGAMP is a potent inducer of type I interferons. The role of cGAMP is similar to that of cyclic adenosine monophosphate (cAMP), the best-studied second messenger (*17*). Like cAMP, which is synthesized by adenylate cyclase upon its activation by upstream ligands, cGAMP is synthesized by a cyclase in response to stimulation by a DNA ligand (*18*). cAMP binds to and activates protein kinase A and other effector molecules. Similarly, cGAMP binds to and activates STING to trigger the downstream signaling cascades. As an endogenous molecule in mammalian cells, cGAMP may be used in immune therapy or as a vaccine adjuvant.

References and Notes

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Supplementary Materials

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Prediction Error Governs Pharmacologically Induced Amnesia for Learned Fear

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Although reconsolidation opens up new avenues to erase excessive fear memory, subtle boundary conditions put constraints on retrieval-induced plasticity. Reconsolidation may only take place when memory reactivation involves an experience that engages new learning (prediction error). Thus far, it has not been possible to determine the optimal degree of novelty required for destabilizing the memory. The occurrence of prediction error could only be inferred from the observation of a reconsolidation process itself. Here, we provide a noninvasive index of memory destabilization that is independent from the occurrence of reconsolidation. Using this index, we show in humans that prediction error is (i) a necessary condition for reconsolidation of associative fear memory and (ii) determined by the interaction between original learning and retrieval. Insight into the process of memory updating is crucial for understanding the optimal and boundary conditions on reconsolidation and provides a clear guide for the development of reconsolidation-based treatments.

A consolidated fear memory can enter a transient labile phase upon its reactivation. Pharmacological blockade of the subsequent protein synthesis–dependent restabilization (reconsolidation) produces a memory deficit in both animals (*1*) and humans (*2*). However, an independent measure for memory destabilization other than the occurrence of reconsolidation itself is not yet available. The functional role of reconsolidation might be to keep

memories up to date with new learning. Indeed, reconsolidation is triggered only when there is opportunity for new learning to take place during reactivation (*3*–*5*). Because associative learning requires prediction error (PE) (a discrepancy between actual and expected events) (*6*), reconsolidation might also be a PE-driven process. Even though it has frequently been suggested, there is no experimental evidence that PE is a necessary condition for reconsolidation. So far, PE could only be inferred from effective reconsolidation without an independent assessment of PE-driven relearning (*3*–*5*). Unveiling a crucial role for PE in reconsolidation of fear memory—which may serve as an index for memory destabilization independent from the process of reconsolidation itself—will provide a clear guide for developing treatments to permanently reduce unwanted and excessive fears (such as posttraumatic stress disorder).

General associative learning models (*6*) argue that PE is not determined by the mere co-occurrence of the conditioned stimulus (CS) and unconditioned stimulus (US), but by the discrepancy between what has already been learned (learning history) and what can be learned on a given trial. If memory retrieval follows a fully reinforced asymptotic learning episode, omission of a predicted reinforcement during reactivation (negative PE) (*7*) may destabilize a consolidated memory during its reactivation, whereas a reinforced reactivation trial would leave the memory intact given that PE would then be absent. In contrast, if memory retrieval follows a partially reinforced, non-asymptotic learning episode, a similar reinforced reminder trial (positive PE) (*7*) should generate additional learning and consequently be capable of inducing postretrieval plasticity because memory-strengthening through further learning also requires reconsolidation mechanisms (*8*).

The noradrenergic β-blocker propranolol administered either before or after memory retrieval eliminates affective responding (fear-potentiated startle) in human participants but leaves the predominantly cognitive component of fear (US-expectancy ratings or skin conductance response) intact (*5*, *9*, *10*). Given that propranolol does not affect declarative memory when reactivated with a single trial, online US-expectancy ratings during acquisition, retrieval, and test could serve as an independent measure to test whether PE-driven relearning during reactivation is essential for reconsolidation of affective fear memory. The current study had a threefold aim: (i) to examine the role of PE in reconsolidation of fear memory, (ii) to examine whether PE depends on the interaction between the available information during reactivation and the learning history, and (iii) to provide a measure for memory destabilization that is independent from the occurrence of reconsolidation itself.

In a human differential fear conditioning paradigm, we tested two groups in which fear acquisition was fully reinforced (100% of the trials) (Fig. 1, A and B). To ensure that asymptotic learning was indeed realized, the participants received explicit instructions regarding the contingencies between the CS and the US. On day 2, the memory was reactivated through either an unreinforced (negative PE group; $n = 15$ participants) or a reinforced (no PE group; $n = 15$ participants) reactivation trial, followed by administration of propranolol (40 mg) (Fig. 1, A and B). PE-driven cognitive relearning and corresponding reconsolidation should occur in the negative PE group but not in the no PE group. We tested a third group to examine whether PE depends on the interaction between the information presented during reactivation and the learning history. In this group, acquisition was partially reinforced (33% of the trials), and the memory was reactivated with a reinforced reminder trial (positive PE group; $n = 15$ participants), followed by administration of propranolol (40 mg) (Fig. 1, A and B). In contrast to the full-reinforcement condition, here the reinforced reminder trial should induce PE-driven additional learning given that a partial reinforcement schedule will not induce asymptotic learning. As such, a reinforced reactivation trial might also induce reconsolidation. Noradrenergic blockade after memory retrieval should disrupt reconsolidation—operationalized as a reduction in conditioned startle fear responding—in both the negative PE and positive PE group but not in the no PE group. On day 3, all groups underwent an extinction session followed by a reinstatement procedure to test to what extent the original fear memory trace was weakened (Fig. 1, A and B).

All three groups showed fear learning and memory reactivation on days 1 and 2, respectively, for the startle fear response and online US-expectancy ratings (data available in the supplementary materials).

Analyses of differential US-expectancy ratings (CS1 versus CS2) from the last trial of acquisition (day 1) to the first trial of extinction (day 3) revealed differences between the three groups [stimulus × trial × group, analysis of variance (ANOVA) $F_{2,42} = 25.44$, $P < 0.001$, $\eta^2_p = 0.55$]. Follow-up analyses of the differential US-expectancy ratings from the last trial of acquisition (day 1) to the first trial of extinction (day 3) revealed a decrease in the negative PE group (stimulus × trial × group, $F_{1,28} = 8.18$, $P < 0.008$, $\eta^2_p = 0.23$) and an increase in the positive PE group (stimulus × trial × group, $F_{1,28} = 42.98$, $P < 0.001$, $\eta^2_p = 0.61$) relative to the no PE group (Fig. 2). A non-reinforced reactivation trial resulted in a decrease in differential US-expectancy ratings from the last trial of acquisition (day 1) to the first trial of extinction (day 3), when acquisition was fully reinforced (negative PE) (stimulus × trial, $F_{1,14} = 18.46$, $P < 0.001$, $\eta^2_p = 0.57$) (Fig. 2A). Reinforcement of reactivation left the US-expectancy ratings unaffected in the no PE group (stimulus × trial, $F_{1,14} < 2.47$) (Fig. 2B). However, when acquisition had been partially reinforced, a similar reactivation trial resulted in an increase in US-expectancy ratings (positive PE group; stimulus × trial, $F_{1,14} = 31.72$, $P < 0.001$, $\eta^2_p = 0.69$) (Fig. 2C).

The three groups differed in startle responding on the first retention trial of extinction on day 3 (stimulus × group, $F_{2,42} = 6.49$, $P < 0.003$, $\eta^2_p = 0.24$). Propranolol reduced the differential startle response (CS1 versus CS2) on the first extinction

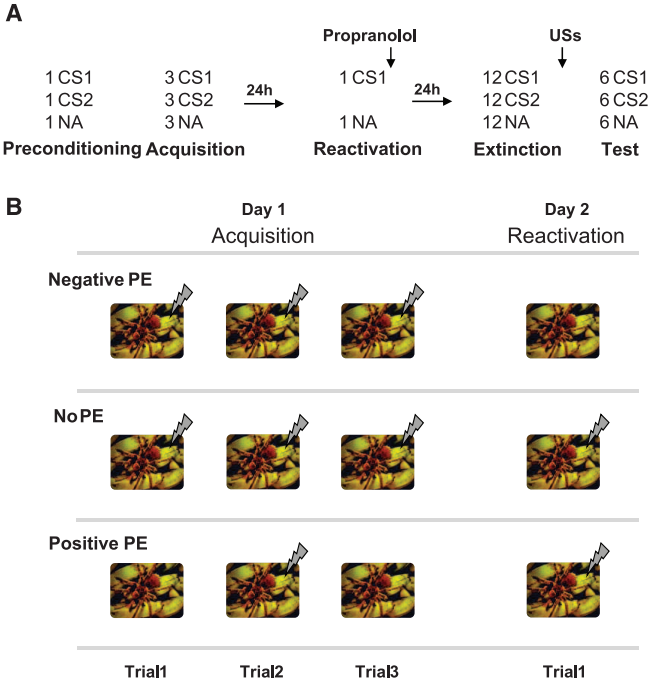

Fig. 1. (**A**) Schematic representation of the experimental design. (**B**) Reinforcement schedule of the CS1 during the acquisition and reactivation phase for the experimental groups. CS1 is depicted as one of the two images used as CSs. US (electrical stimulus) is depicted as a lightning bolt.

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trial in both the negative PE (stimulus × group, $F_{1,28} = 10.76$, $P < 0.003$, $\eta^2_p = 0.28$) and positive PE group (stimulus × group, $F_{1,28} = 7.89$, $P < 0.009$, $\eta^2_p = 0.22$) as compared with the no PE group. Indeed, propranolol completely erased differential responding on the first extinction trial in the negative PE (main effect stimulus, $F_{1,14} < 1$) (Fig. 3A) and positive PE group (main effect stimulus, $F_{1,14} < 1$) (Fig. 3C). In contrast, this propranolol-induced amnesia was not observed when reactivation was devoid of new learning, as indicated by the differential startle response that was still present on the first extinction trial in the no PE group (main effect stimulus, $F_{1,14} = 13.47$, $P < 0.003$, $\eta^2_p = 0.49$) (Fig. 3B). Given that propranolol eliminated differential responding in the reactivation conditions under which new learning occurred, the three groups differed over the course of extinction learning (trial 1 versus trial 12) (stimulus × trial × group, $F_{2,42} = 5.23$, $P < 0.009$, $\eta^2_p = 0.20$) (follow-up analyses are available in the supplementary materials). Thus, propranolol affected startle fear responding only in those groups (negative PE and positive PE) in which the reactivation trial resulted in changes in cognitive learning, be it incremental or decremental.

Differences in startle fear responding (CS1 versus CS2) between the three groups on the reinstatement test trial approached significance (stimulus × group, $F_{2,42} = 2.25$, $P < 0.118$, $\eta^2_p = 0.10$). This small effect can be attributed to a general increase in startle responding from the end of extinction (trial 12) to the test trial in the no PE group (main effect trial, $F_{1,14} = 10.20$, $P < 0.006$, $\eta^2_p = 0.42$), which is typically observed after unpredictable shocks following fear extinction (*2*). Reanalyzing the differential startle response to the test trial with the noise alone (NA) trial as the control stimulus (CS1 versus NA) revealed, however, a significant difference between the three groups (stimulus × group, $F_{2,42} = 5.57$, $P < 0.007$, $\eta^2_p = 0.21$). Follow-up analyses revealed significantly more differential responding in the no PE group as compared with both the negative PE (stimulus × group, $F_{1,28} = 13.25$, $P < 0.001$, $\eta^2_p = 0.32$) and positive PE group (stimulus × group, $F_{1,28} = 7.38$, $P < 0.011$, $\eta^2_p = 0.21$). The startle response indeed recovered in the no PE group as indicated by stronger conditioned responding to the CS1 as compared with the NA (main effect stimulus, $F_{1,14} = 24.01$, $P < 0.001$, $\eta^2_p = 0.63$) (Fig. 3B), whereas no return of fear was observed in either the negative PE (main effect stimulus, $F_{1,14} < 1.44$) (Fig. 3A) or the positive PE group (main effect stimulus, $F_{1,14} < 1$) (Fig. 3C). Affective fear memory was only disrupted when actual learning took place during memory retrieval, showing that postretrieval plasticity depends on PE-driven relearning. Fear memory destabilization was not necessarily triggered by the absence of US-reinforcement (an extinction trial) but was also induced by a reinforced retrieval trial when fear learning on the previous day involved a partial reinforcement schedule. PE was determined by the interaction between the learning history

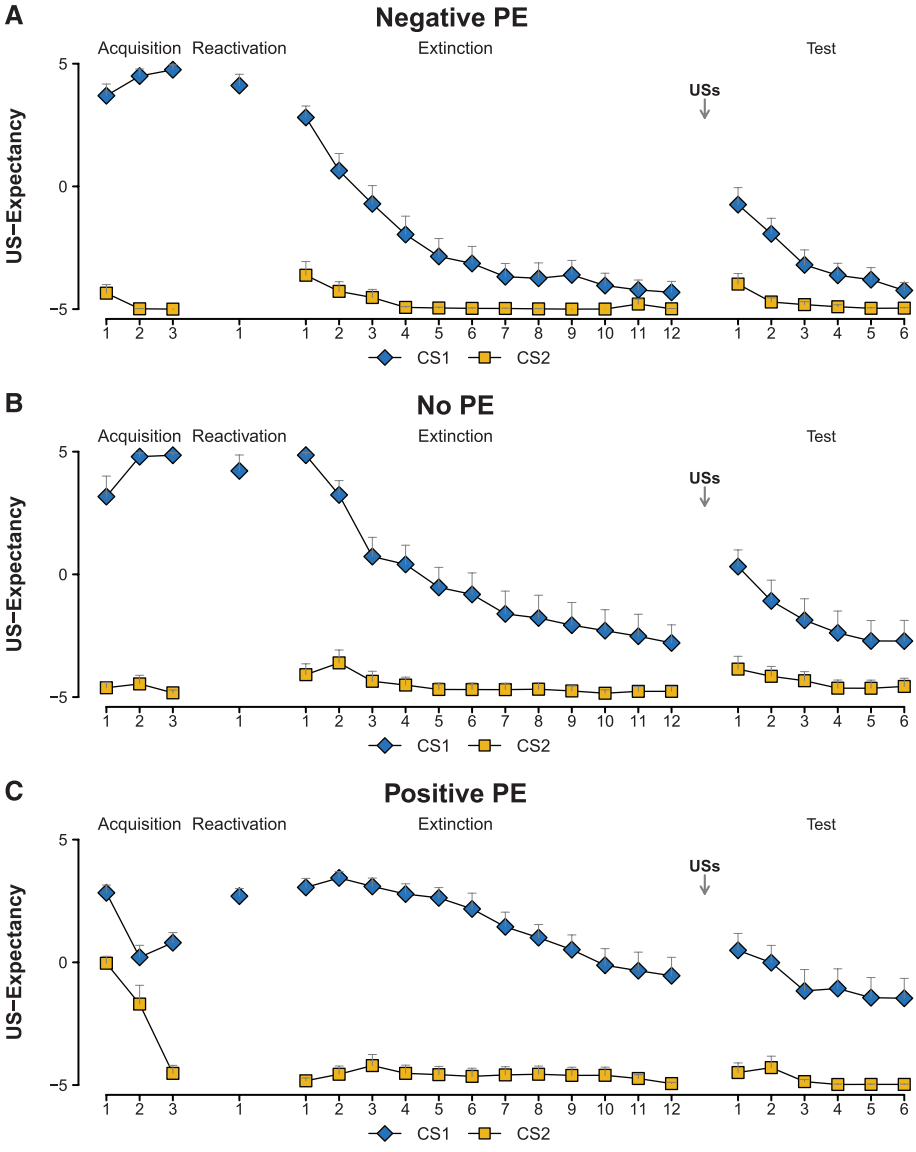

Fig. 2. Online US-expectancy ratings provide a measure of PE-driven learning. (**A** to **C**) Mean US-expectancy ratings to the CS1 and CS2 trials during acquisition, reactivation, extinction, and reinstatement test. US-expectancy ratings (A) decreased from the end of acquisition to the beginning of extinction in the negative PE group (n = 15 participants), (B) remained similar in the no PE group (n = 15 participants), and (C) increased in the positive PE group (n = 15 participants). Error bars represent SEM.

and the retrieval session. PE-driven learning—operationalized by a change in US-expectancy from the end of acquisition (day 1) to the beginning of memory testing (day 3)—may be used as a noninvasive index for memory destabilization.

The application of postretrieval amnesic agents is considered to be a highly promising procedure to target excessive emotional memories typically observed in patients suffering from psychiatric disorders (such as posttraumatic stress disorder or addiction). However, the feasibility of disrupting reconsolidation may also be criticized given the subtle boundary conditions under which the amnesic agents do not affect memory (*11*). Reconsolidation is supposed to occur when the retrieval experience is similar but not identical (*12*) to the original learning. Yet, a retrieval session that is too different from the original learning procedure might not cause destabilization of the original memory trace (*13*) but instead initiate the formation of a new memory trace, such as in extinction learning (*14*). Without an independent index of memory destabilization other than the memory-enhancing or amnesic effects of the manipulations themselves, determining the degree of similarity (or dissimilarity) between learning and retrieval presents a problem for empirical falsifiability (*15*).

Criteria for optimal similarity (or dissimilarity) cannot be inferred from the expression of the target memory itself during memory retrieval because the mechanisms that mediate memory destabilization are independent from the behavioral

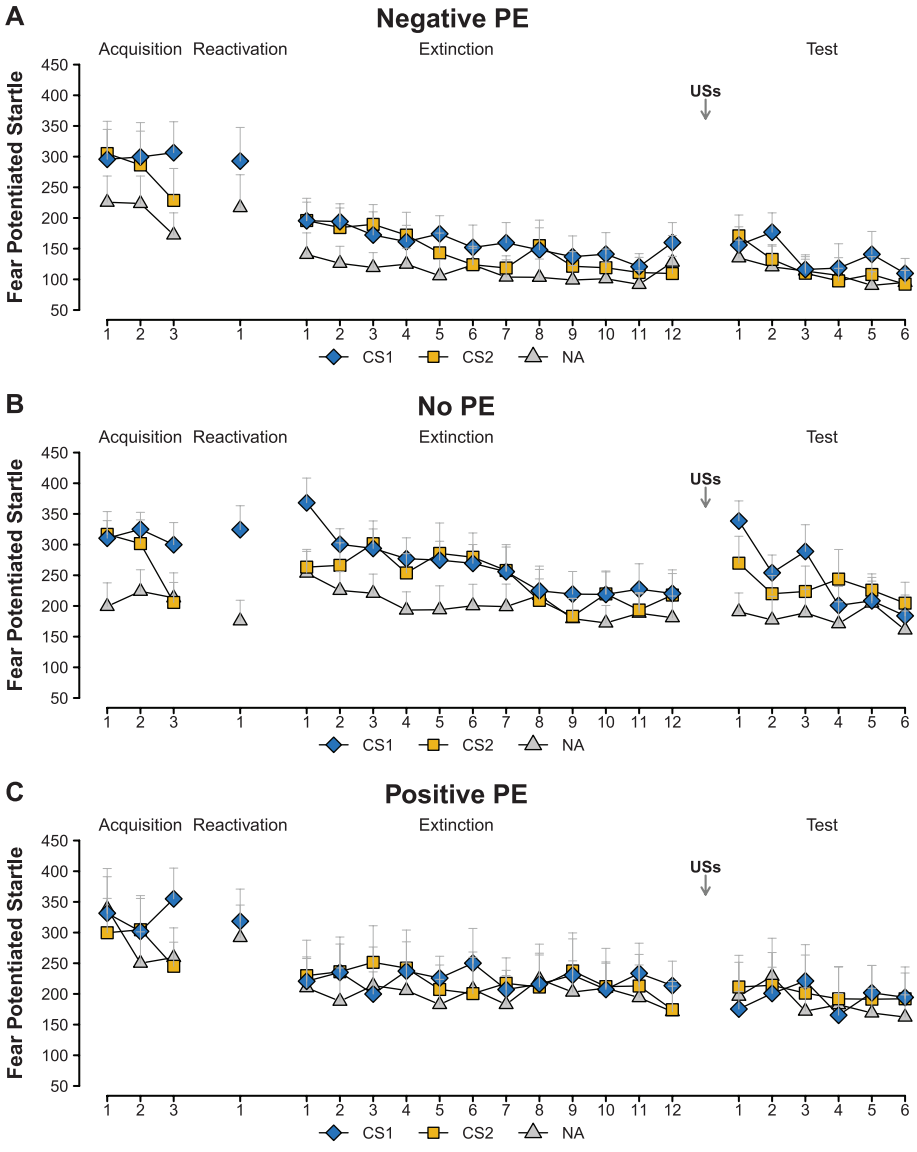

Fig. 3. PE is a necessary condition for reconsolidation. (**A** to **C**) Mean startle responses to the CS1 and CS2 trials during acquisition, reactivation, extinction, and reinstatement test. Propranolol affected the startle response in both (A) the negative PE group (n = 15 participants) and (C) the positive PE group (n = 15 participants) but not in (B) the no PE group (n = 15 participants). Error bars represent SEM.

fear expression (*5*, *16*). In addition, a certain reactivation procedure may induce plasticity after one but not another learning procedure. We have demonstrated that PE can be used as an independent measure of memory destabilization. When there was no modification of CS-US expectancies from acquisition to test, the memory trace was not updated. We believe that at least in the current protocol, it would be difficult to assess PE-driven learning at the moment of reactivation given that a small decrease in CS-US expectancies during the memory retrieval session itself would already induce extinction learning. Then, updating may no longer affect the original memory trace but—as a result of the small degree of similarity between acquisition and retrieval—instate the formation of a new extinction memory. Because reconsolidation of memory traces corresponding to different response systems (amygdala-dependent startle potentiation and hippocampal-dependent declarative memory) calls for different reactivation conditions (*9*, *10*), we are now capable of independently assessing the prerequisite for fear memory destabilization in humans in a noninvasive manner. Conditions that were previously regarded as constraints on reconsolidation (such as too little or too much similarity) may be resolved by taking into account PE during memory retrieval. The assessment of PE provides a feasible tool to develop and optimize reconsolidation-based treatments for patients suffering from chronic relapsing disorders such as anxiety disorders and substance-abuse disorders.

References and Notes

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Supplementary Materials

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