Supplemental Materials

Supplemental Results

Experiment 1

Method

All techniques were conducted as described for Experiment 1 in the main text, except an additional two groups were exposed to fear conditioning. These groups are described below.

Fear Conditioning. Rats were handled at least 3 days prior to fear conditioning. Rats received fear conditioning in Context A with 5 pairings of tone (30 s, 80 db, 2 kHz) and foot shock (1 s, 0.7 mA); there was a 3 min period after each tone. One group of rats received "predictable" training (predictable-shock group), in which the offset of each tone triggered foot-shock delivery. In a second group of rats (the unpredictable, coterminating-shock/short-tone group), tone offset triggered foot-shock delivery, but the length of each tone was unpredictable (2, 4, 15, 30 or 40 s in duration).

Results

During fear conditioning, a similar elevation of freezing during the auditory cues was observed in the predictable-shock groups and the unpredictable-shock groups for the short-tone condition, $F(1,18) = 1.04$, $p = 0.32$ (Fig. S1a, left), and the long-tone condition, $F(1,24) = 2.31$, $p = 0.14$ (Fig. S1b, left). Although freezing during the tone presentations is depicted, the freezing levels reflect fear to both the tones and the conditioning box itself. Conditional freezing across the contextual fear memory test session also did not differ between the predictable-shock groups and the unpredictable-shock groups for the short-tone condition, $F(1,18) = 0.0004$, $p = 0.98$ (Fig. S1 a, middle), and the long-tone condition, $F(1,24) = 0.64$, $p = 0.43$ (Fig. S1b, middle).

The overall levels of conditional freezing to the context were lower than those evoked by the tone, but this likely reflects the fact that we selected fear conditioning parameters (long intertrial intervals, 210s, and lengthy CSs, 30s) which favored auditory fear over contextual fear. In contrast to the findings for contextual fear memory recall, the significant difference between the predictable-shock groups and the unpredictable-shock groups seen in during auditory fear recall (Fig. S1a,b, right panels) persisted throughout the full auditory fear memory test for the short-tone condition, $F(1,18) = 7.81$, $p = 0.012$ (Fig. S1a, right), and for the first four blocks (8 tone presentations) of the auditory fear memory test for long-tone condition, $F(1,24) = 4.68$, $p = 0.040$ (Fig. S1b, right).

To determine whether rats trained with unpredictably timed footshock displayed resistance to extinction, we compared extinction rates across the predictable-shock groups and the unpredictable-shock groups. Although successful extinction was observed within the tone extinction session to conditional freezing expressed during the tone, there were no differences in extinction rates between the predictable-shock groups and the unpredictable-shock groups. There were significant main effects of block for the short-tone condition (Fig. S1a, right, $F(9,162) = 6.74$, $p < 0.0001$) and the long-tone condition (Fig. S1b, right, block: $F(9,216) = 2.74$, $p = 0.005$), but no significant interaction of Training Type and Block for the short-tone condition (Fig. S1a, right, $F(9,162) = 0.95$, $p = 0.48$) or long-tone condition (Fig. S1b, right, $F(9,216) = 1.26$, $p = 0.26$). Thus, the differences in freezing levels that we observed cannot be attributed to impaired extinction in the unpredictable-shock groups.

It might be argued that presentations of the tone late in the extinction session could be impacted by second-order context conditioning to the novel extinction context because of presentation of the aversive tone. To determine whether second-order contextual conditioning

occurred, half of the rats in the short-tone condition were placed in the tone extinction context for a second extinction session (Fig. S1a, far right). Prior to the presentation of the tone, rats exhibited very little conditional freezing to the extinction context. In fact, the levels of freezing were not significantly different from those displayed on the previous extinction day. There was no main effect of day, $F(1,8) = 2.61$, $p = 0.15$, nor a Day X Group interaction, $F(1,8) = 0.003$, $p = 0.96$, suggesting that no significant second-order conditioning occurred in the extinction context. Despite this, the enhancement in fear memory was also observed during this second extinction day, $F(1,7) = 10.2$, $p = 0.015$ (Fig. S1a, far right). Thus, the freezing observed during auditory extinction can be attributed solely to the strength of the association between the tone and the aversive footshock, and not to the strength of contextual fear.

We also sought to determine whether the fear levels observed in the unpredictable-shock/short-tone group were impacted by the persistence of the CS beyond the footshock presentation on most trials (Fig. 1a), a factor that was not present for rats in the predictable-shock/short-tone group. To do this, we ran an additional group in which the timing of the footshock remained ambiguous from trial to trial, but the tone presentation co-terminated with each footshock (Fig. S2a). Despite an overall increase in ambiguity because neither the length of the cue nor the time of the footshock could be predicted, the ambiguity-related enhancement of fear was of similar magnitude to the previous experiment in which only the timing of the footshock was ambiguous, $F(1,18) = 7.81$, $p = 0.012$ (Fig. S2b). Interestingly, these data also suggest that the post-US time within each CS of Experiment 1 (Fig. 1a) does not act as an inhibitory safety signal (Williams, Johns, & Brindas, 2008) because freezing behavior was highly similar between the predictable-shock/short-tone group (post-US time within CS present)

and unpredictable, coterminating-shock/short-tone group (post-US time within CS absent) groups.

Variability in the timing of an aversive reinforcer could re-shape the temporal window over which conditional behavior is expressed (Balsam, Sanchez-Castillo, Taylor, Van Volkinburg, & Ward, 2009). Predictable cue to reinforcer intervals give rise to "inhibition of delay" (R.A. Rescorla, 1967), in which conditional behavior peaks at the expected time of footshock, whereas variable cue to reinforcer intervals lead to steady, invariant levels of conditional behavior. Thus, one might predict that predictable, or fixed, intervals would lead to lower average levels of conditional behavior than unpredictable intervals simply because behavior would be expressed over a shorter temporal window immediately surrounding the time of expected reinforcement. However, average conditional response rates, during the CS and especially during the post-CS intervals, are determined by the interval between reinforcer presentations (Kirkpatrick & Church, 2004), and not by the schedule of reinforcement (fixed versus variable) (Kirkpatrick & Church, 2003). Because the interval between reinforcers was virtually identical for rats in the PRED and UNPRED groups, one would not predict differences in conditional responding to arise.

Nonetheless, to explicitly address the issue of whether inhibition of delay contributed to differences in conditional freezing between rats in the predictable-shock/short-tone group and the unpredictable-shock/short-tone group, we performed a higher resolution analysis of conditional freezing during tone extinction (Fig. S3). Rats trained with unpredictably timed footshocks displayed significantly higher conditional freezing than rats trained with predictably timed footshocks, $F(1,18) = 8.35$, $p = 0.0097$, and there were significant changes in conditional freezing over the course of the tone presentation, $F(9,162) = 38.38$, $p < 0.0001$, with levels of conditional

freezing higher during the last three seconds relative to the first three seconds, $F(1,19) = 77.72$, $p < 0.0001$. This suggests that some degree of inhibition of delay was present in both groups. However, the predictable-shock/short-tone group and the unpredictable-shock/short-tone group did not differ in the rate at which conditional freezing changed across the presentation of the tone. There was no significant Group X Time interaction, $F(9,162) = 1.28$, $p = 0.25$), suggesting that inhibition of delay was similar across the two groups. This shows that differences in conditional freezing across the tone cannot be explained by differences in inhibition of delay.

Experiment 3

Results

Fear conditioning produced a significant enhancement of freezing across all groups; there was a significant main effect of training, $F(4,140) = 82.55$, $p < 0.0001$ (Fig. S4, left). Additionally, muscimol-infused rats displayed more freezing more than vehicle-infused rats; there was a significant main effect of drug, $F(1,35) = 10.31$, $p < 0.0028$ (Fig. S4, left), consistent with previous reports (Maren & Holt, 2004). Demonstrating the efficacy of the dorsal hippocampal inactivation procedure, contextual fear was eliminated in muscimol-infused rats; there was a significant main effect of infusion type, $F(1,35) = 17.08$, $p = 0.0002$ (Fig. S4, middle). We also observed a significant effect of hippocampal inactivation on auditory fear memory; there was a significant main effect of infusion type, $F(1,35) = 21.45$, $p < 0.0001$) (Fig. S4, right). However, the effects of hippocampal inactivation were different in the predictable shock and unpredictable shock groups; there was a significant Training Type X Infusion interaction, $F(1,35) = 7.77$, $p = 0.0085$ (Fig. S4, right).

Experiment 4

Results

A significant effect of infusion type was observed during the contextual fear memory test, $F(1,16) = 9.42$, $p = 0.0073$ (Fig. S5, left), however no differences were observed across the extinction session. There was no significant Infusion X Minute interaction, $F(9.144) = 1.63$, $p = 0.11$(Fig. S5, left).

Similarly, the drug infusion did not impact conditional freezing across the auditory extinction session; there was no significant Infusion X Minute interaction, $F(7,133) = 1.55$, $p = 0.15$ (Fig. S5, right). The reinforcement schedule also did not impact the expression of auditory fear memory across the extinction session; no significant Training Type X Minute interaction was observed, $F(7,133) = 0.34$, $p = 0.93$ (Fig. S5, right). Collectively, these findings support the claims that muscimol infusion into the dorsal hippocampus prior to fear conditioning eliminated contextual fear memory acquisition, and that unpredictable timing of footshock during fear conditioning does not impact subsequent within-session extinction.

Experiment 5

Method

Viruses. Some mice were infused with an AAV expressing the light-gated nonspecific cation channel Channelrhodopsin-2 (ChR2) in frame with GFP or an AAV expressing GFP alone; gene expression was regulated by the CAG promoter. All other aspects of the virus were identical to that described in the main text for Experiment 5.

Optical Stimulation of Hippocampal CA1. A 473 nm blue laser diode (300 mW; Shanghai Laser & Optics Century Co., Shanghai, China) was coupled to a 200 µm multimode silica-core optical fiber through an FC/PC adapter. The laser was attached to each mouse as described in the main text for Experiment 5. Each period of photostimulation (4 s) was applied in 3.5 ms 473 nm light pulses at 20 Hz (Takata et al., 2015; Zhang et al., 2013).

Results

Mice that received optogenetic silencing of the dorsal hippocampus during fear conditioning at times in which footshock was expected but not administered (unpredictable conditioning, light during negative prediction errors + ArchT group) exhibited significantly lower freezing than control mice (unpredictable conditioning, light during negative prediction errors + GFP group), $F(1,20) = 10.57$, $p = 0.0040$ (Fig. S6a). Auditory extinction rates were similar between the two groups; there was no significant Infusion X Trial interaction, $F(4,80)=1.50$, $p = 0.21$(Fig. S6a). This effect was not observed in the predictable conditioning, random light groups, $F(1,14) = 0.12$, $p = 0.73$ (Fig. S6b). Similarly, mice that received unpredictable fear conditioning and optogenetic silencing of dorsal hippocampus during random times (unpredictable conditioning, random light +ArchT group) displayed enhanced long-term auditory fear levels that were similar to mice that received unpredictable fear conditioning without silencing (unpredictable conditioning, random light +GFP group), $F(1,10) = 1.25$, $p = 0.29$ (Fig. S6c).

Because these results suggest that activity during putative aversive negative prediction errors enhanced long-term auditory fear memory, we next sought to determine whether false aversive negative prediction errors could be induced during temporally predictable auditory fear

conditioning using optogenetic stimulation. All mice received three trials of standard auditory delay conditioning, where footshock occurred at the end of the auditory cue presentation (Fig. S7a). Light (4 s duration for each period of stimulation) was administered at 5 s into the second tone presentation, and at 5 s and 17 s into the third tone presentation.

Long-term auditory fear memory recall was similar across the GFP and ChR2 groups; there was no main effect of infusion, $F(1,14) = 1.35$, $p = 0.26$ (Fig. S7b). This suggests that activity in CA1 of the dorsal hippocampus is not sufficient to induce aversive negative prediction errors capable of enhancing fear memory strength.

Supplemental Discussion

In several of our experiments, the average CS onset-to-US onset time was shorter in the groups that received unpredictable fear conditioning (18s) relative to the predictable fear conditioning (30s). One might hypothesize that the dorsal hippocampus is nonetheless preferentially recruited when fear conditioning paradigms with short intervals between the CS and US onsets are employed. However, this conflicts with an abundance of existing literature. Temporary inactivation of the dorsal hippocampus prior to fear conditioning does not affect long-term fear memory when using shorter CS onset to US onset intervals (10s) than those reported here (30s) (Esclassan, Coutureau, Di Scala, & Marchand, 2009; Maren & Holt, 2004). Additionally, studies using trace conditioning (Beylin et al., 2001; Chowdhury, Quinn, & Fanselow, 2005), where a temporal break is imposed between the termination of the CS and the initiation of the US, and delay conditioning (Beylin et al., 2001), reveal that the dorsal hippocampus is preferentially engaged by longer, not shorter, intervals between the CS and US . Finally, studies of aversive eyeblink conditioning find the dorsal hippocampus is not necessary

for delay conditioning using substantially shorter CS-US intervals than those employed here (Solomon, Vander Schaaf, Thompson, & Weisz, 1986). Thus, the special involvement of the hippocampus in our unpredictable conditioning as compared to the predictable conditioning can be attributed to the uncertainty in timing of the US, rather than the use of short CS to US intervals.

It is worthwhile to consider what is meant by the term "ambiguity" throughout this paper and how this is related to associative learning. We show that ambiguity in the timing and occurrence of aversive reinforcement enhances fear under conditions where auditory cues inform the subject about the occurrence of reinforcement (that is, where there is contingency between the cue and the reinforcer). We do not claim that all ambiguity enhances fear memory. For example, truly random reinforcement is perhaps maximally ambiguous in terms of the timing of its occurrence, and yet this typically produces impaired learning (R. A. Rescorla, 1968); however, this was observed in conditions where the cues and reinforcers had no contingent relationship. In experiments without contingency, ambiguity cannot enhance learning because no learning occurs.

It may seem surprising that we elected to perform our manipulations in dorsal, rather than ventral hippocampus, because the ventral hippocampus plays an important role in aversive memories and is known to regulate fear, whereas the dorsal hippocampus is associated with spatial and temporal processing (Fanselow & Dong, 2010). However, because variability in the timing of reinforcement is a process that requires temporal processing, we hypothesized that the dorsal hippocampus was more likely to be recruited than ventral hippocampus. Our data do not discount the possibility that the ventral hippocampus may also play a role in generating signals related to ambiguity in the timing of aversive footshock. However, because temporary

inactivation of ventral hippocampus impairs the acquisition of fear conditioning using temporally predictable footshock (Maren & Holt, 2004), it may be difficult to explore the role of ventral hippocampus specifically in fear conditioning with temporally unpredictable footshocks.

Historically, prediction errors are investigated as the absence of an expected outcome or the occurrence of an unexpected outcome [e.g. (Hollerman & Schultz, 1998)], rather than variability in the timing of a consistent outcome. Indeed, little is known about the neural substrates of temporally-based prediction errors. Contrary to what is reported here for an aversive Pavlovian task, dopaminergic reward prediction errors in an appetitive Pavlovian task were only weakly sensitive to unpredictably timed reward delivery (Fiorillo, Newsome, & Schultz, 2008). That is, dopaminergic reward prediction errors have a low precision when encoding the expected time of reward. In contrast, here, hippocampal aversive negative prediction errors are highly sensitive to the timing of footshock delivery.

At least two distinct processes may engage the dorsal hippocampus during computations of ambiguity in aversive outcomes. First, the dorsal hippocampus may calculate the passage of time. Our observation that CA1 photoinhibition during auditory fear conditioning at times other than expected reinforcement times within the predictive auditory cue does not impede the ability of ambiguity to enhance fear (Fig. 6g) suggests that extra-hippocampal structures may provide time-related information to the hippocampus to generate temporally-based aversive negative prediction errors. A second possibility is that the hippocampus computes the ambiguity of predictive cues. This is consistent with our finding that dorsal hippocampal inactivation during a non-temporal form of ambiguity, fear conditioning under a partial reinforcement schedule, also enhances fear memory. It is also consistent with the classic "comparator" mechanism (Gray, 1982) of the hippocampus, but our results suggest this mechanism must compare the expected

versus actual occurrence of stimuli *through time*. In such a model, CA1 cells may compare the representation of a currently experienced sequence of events, initiated by a predictive cue, with previously stored representations of the events following the cue. If the temporal sequence of events does not match what was expected, the hippocampus generates a signal, perhaps related to "mismatch" (Duncan, Curtis, & Davachi, 2009; Rutishauser, Mamelak, & Schuman, 2006; Wood, Dudchenko, & Eichenbaum, 1999) signals that have been observed in other studies, which is conveyed to the amygdala, a site of plasticity during associative fear learning (Goosens, Hobin, & Maren, 2003). If the temporal sequence of events matches the expected sequence, the hippocampus may generate a "match" signal (Duncan et al., 2009; Rutishauser et al., 2006; Wood et al., 1999), but any such signal does not seem to modulate fear memory, as dorsal hippocampal inactivation does not affect fear learning when aversive events are predictably timed following cue presentation.

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Supplemental Figures

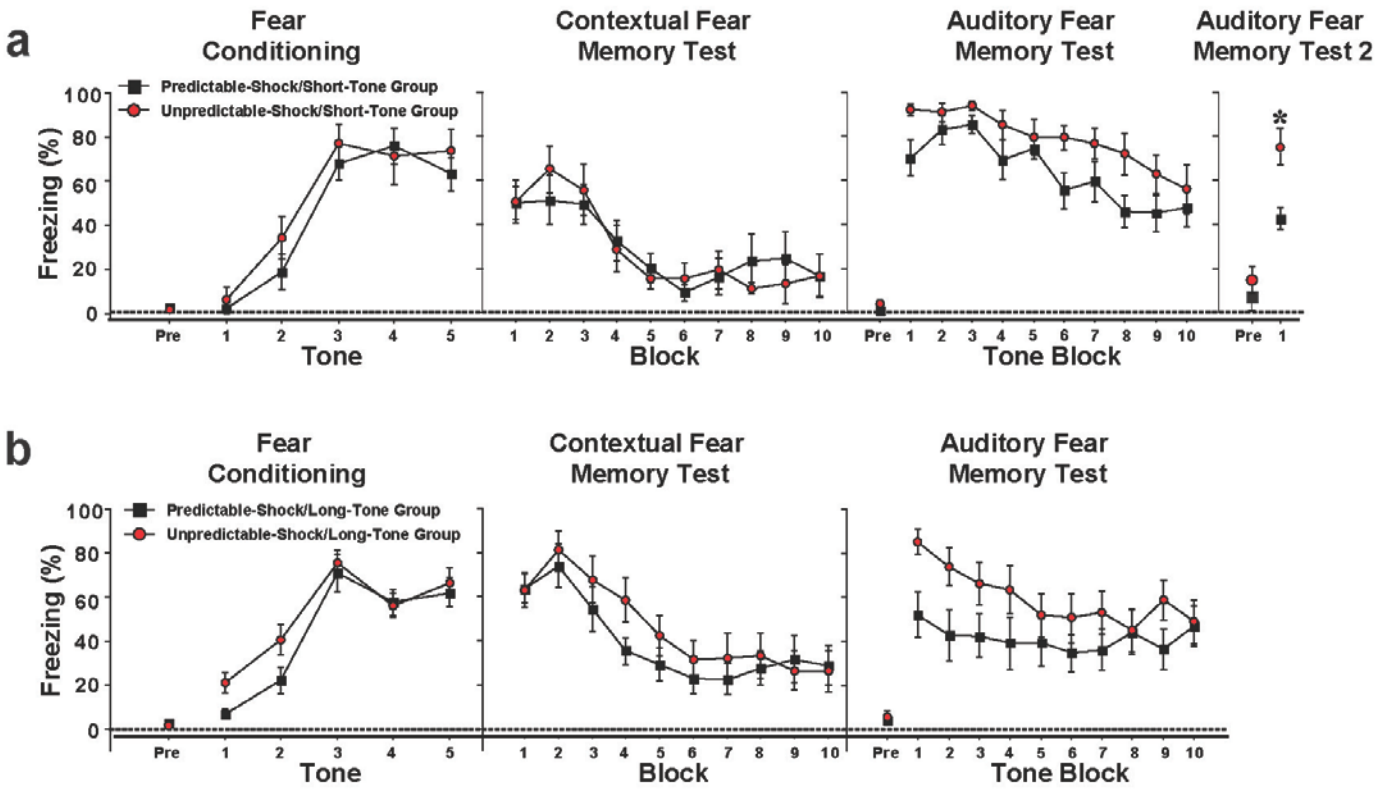

Figure S1. Temporal ambiguity of footshock does not alter conditional freezing during fear conditioning or fear extinction. a) All rats (n=10 per group) were fear conditioned with five tone (30s)-footshock (1s) pairings (210s intertrial interval). Under "predictable" training conditions, each 30s tone co-terminated with a footshock. Under "unpredictable" training conditions, each 30s tone was paired with a footshock that occurred pseudorandomly within the tone. b) All rats (n=13 per group) were fear conditioned with five tone (42 s)-footshock (1 s) pairings (210 s intertrial interval). Under "predictable" training conditions, the footshock was presented 17 s after the onset of each tone presentation (42 s). Under "unpredictable" training conditions, each 42s tone was paired with a footshock that occurred pseudorandomly within the tone. During the long-term memory tests, all rats received 10 min of context extinction one day and 20 trials of auditory fear extinction the next day. A second auditory fear extinction test was conducted 24h following the first auditory extinction session for a subset of rats (n = 5 per group). Each data point during the memory tests corresponds to the average conditional freezing during two consecutive trials (defined as one block) of context or tone presentation. Values are ±S.E.M. * $p < .05$

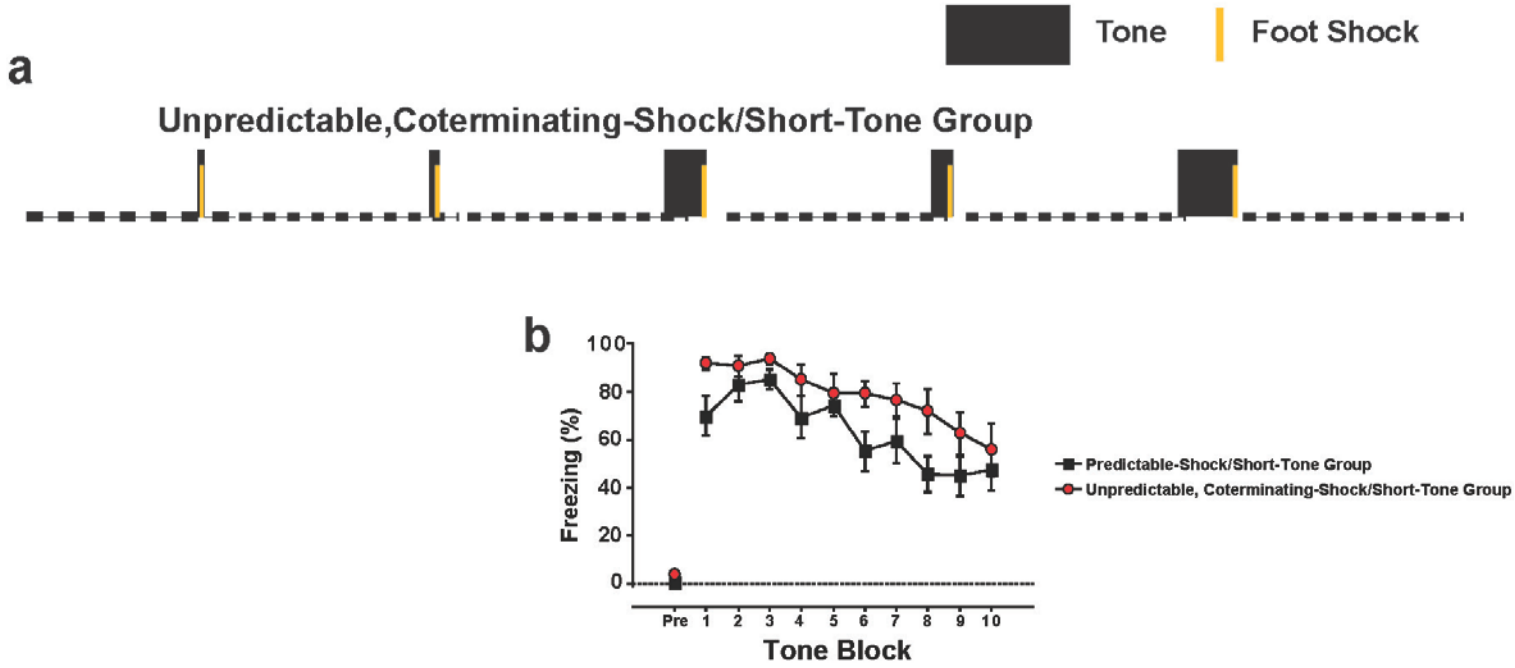

Figure S2. Ambiguity in the duration of the predictive tone does not enhance long-term fear memory. An additional group of rats (n = 10 per group) was fear conditioned (a) with five tone-footshock pairings in which tones of variable length (2-40s; 18.2s average) were co-terminated with a footshock (unpredictable, coterminating-shock/short-tone group). Thus, the duration of the predictive stimulus was ambiguous, as was the precise timing of the aversive footshock. Two days later, all rats received 20 trials of auditory fear extinction. Each data point (b) corresponds to the average conditional freezing during two consecutive trials (one block) of tone presentation. Values are ±S.E.M.

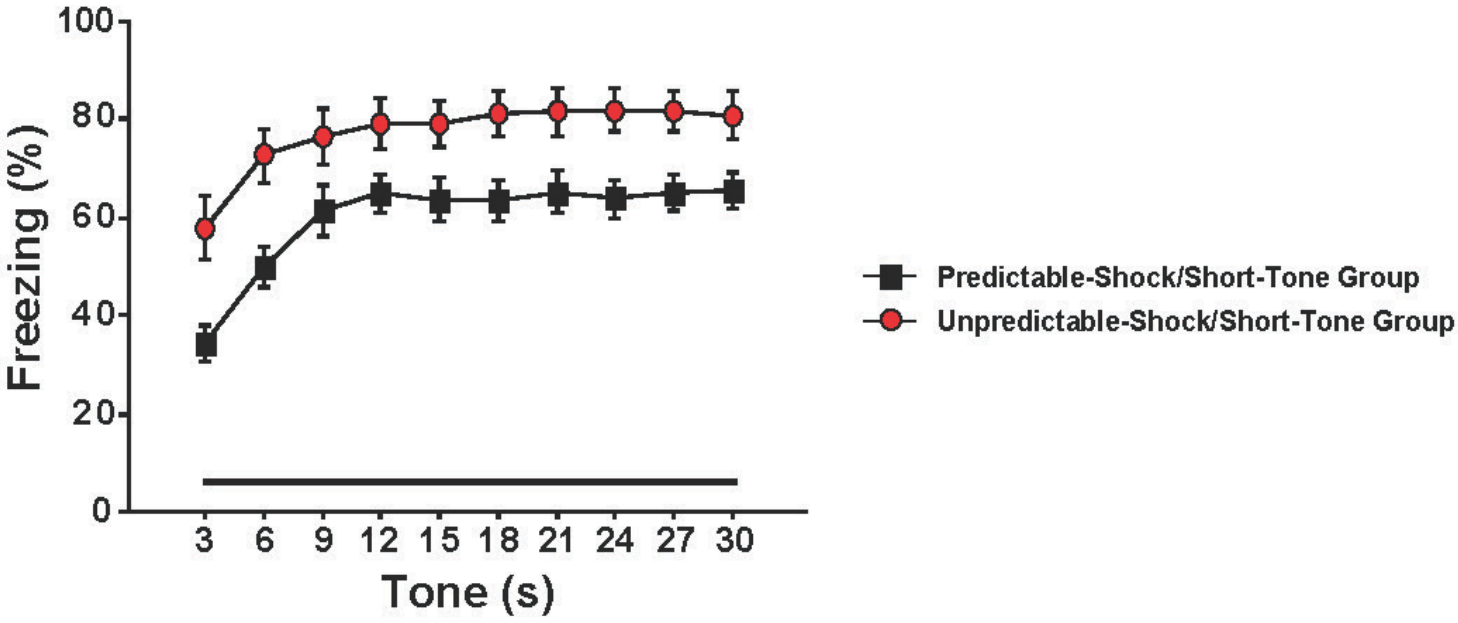

Figure S3. Temporal ambiguity of footshock does not alter inhibition of delay. Freezing during the tone presentations of auditory extinction was measured in 3s bins for rats that received temporally predictable (n = 10) or unpredictable (n = 10) fear conditioning. Each data point represents conditional freezing for a 3s bin averaged over 20 trials of tone presentation during extinction. Values are ±S.E.M.

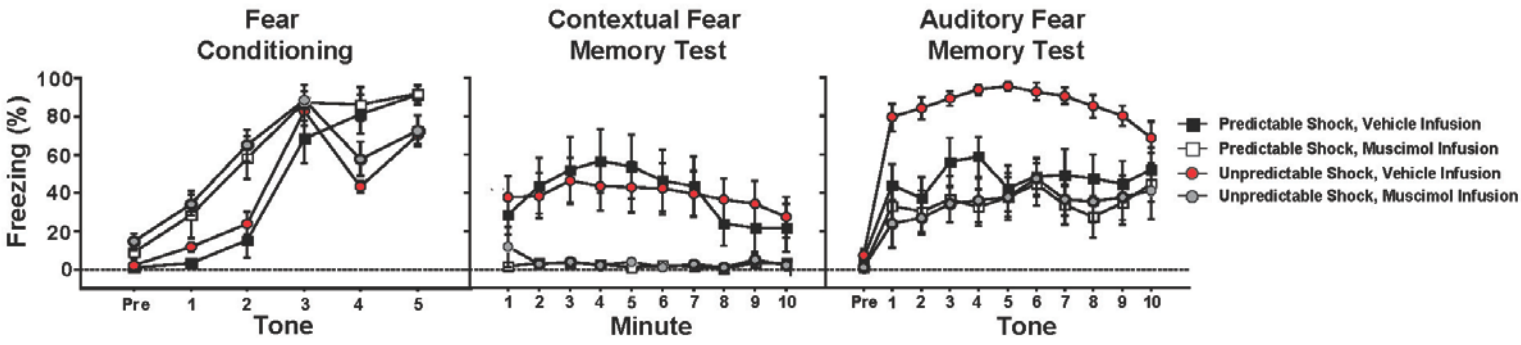

Figure S4. Dorsal hippocampal inactivation prevents the enhancement of fear by unpredictable timing of aversive events during fear conditioning. Freezing during fear conditioning is depicted (left). Trial-by-trial conditional freezing during the context extinction session (middle) or the auditory fear extinction session (right) is also shown. Values are ±S.E.M.

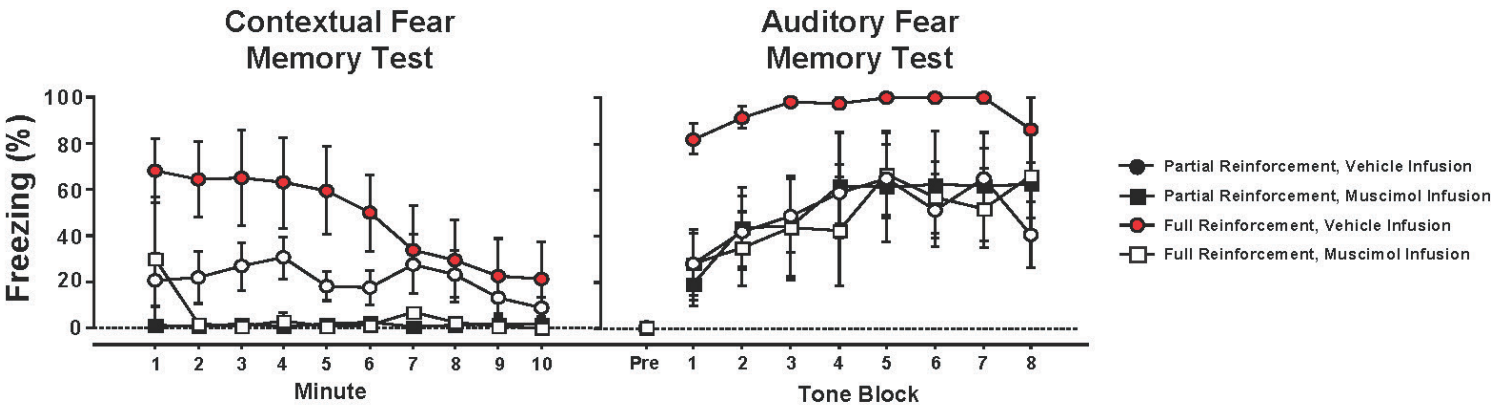

Figure S5. The dorsal hippocampus is necessary for the enhancement of fear by a partial reinforcement conditioning schedule. Conditional freezing across the contextual fear memory text (left) and auditory fear memory test (right) are shown. For the contextual fear memory test, each data point represents average freezing across 1 min periods. For the auditory fear memory test, each data point represents average freezing across two consecutive tone presentations. Because of a software error, video was not saved for four rats during the contextual fear memory test. The number of rats per group in the contextual fear memory test were as follows: Full Reinforcement, Vehicle Infusion, n = 2, Full Reinforcement, Muscimol Infusion, n = 3, Partial Reinforcement, Vehicle Infusion, n = 6, Partial Reinforcement, Muscimol Infusion, n = 5. For the auditory fear memory test, the group size was as follows: Full Reinforcement, Vehicle Infusion, n = 4, Full Reinforcement, Muscimol Infusion, n = 5, Partial Reinforcement, Vehicle Infusion, n = 6, Partial Reinforcement, Muscimol Infusion, n = 5. Values are ±S.E.M.

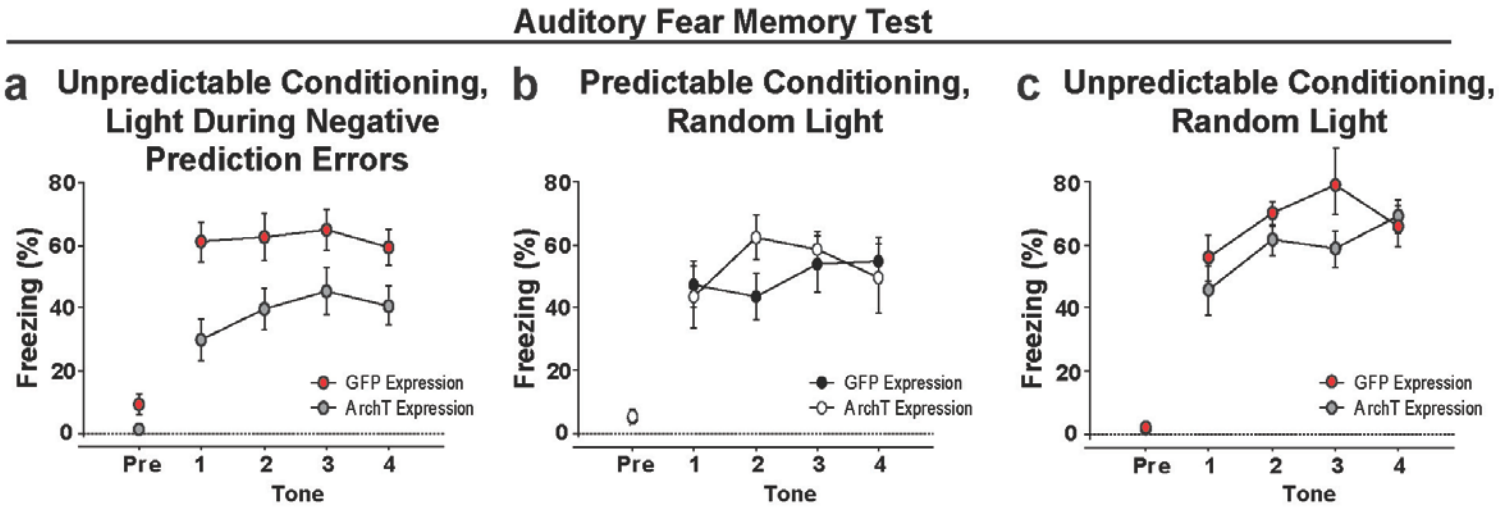

Figure S6. Photoinhibition of CA1 in dorsal hippocampus during putative aversive negative prediction errors eliminates the enhancement of fear by temporal ambiguity of footshock. Trial-by-trial conditional freezing during the long-term auditory fear recall session (right) is shown for the six groups of mice in Experiment 5. Values are ±S.E.M.

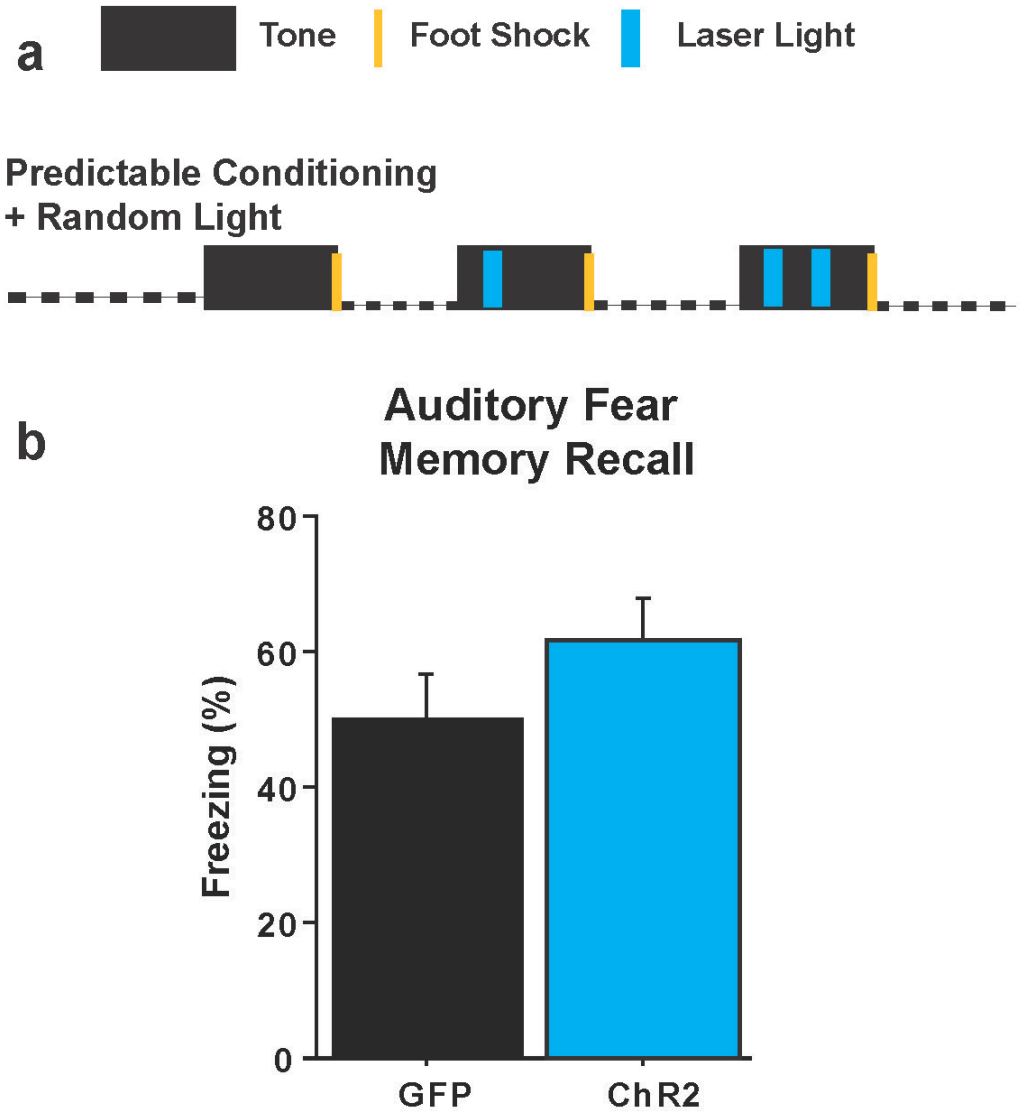

Figure S7. Photostimulation of CA1 in dorsal hippocampus is not sufficient to generate aversive negative prediction errors that enhance fear memory. a) Mice were exposed to three trials of fear conditioning with predictably timed footshocks (Predictable Conditioning + Random Light + GFP, n = 10; Predictable Conditioning + Random Light + ChR2, n = 6). Mice received intra-hippocampal delivery of blue light (λ = 473 nm) during 4 s periods at times at which footshock had never been administered on previous trials, with an additional second of light delivery prior to and following the time of the previous footshock. b) Auditory fear recall during a subsequent laser-free extinction session assessing auditory fear memory strength is shown. Each bar represents conditional freezing averaged over the first four auditory CS presentations. Values are ±S.E.M.

Figure Number	Main effect of replicate		Replicate X Factor Interaction	
	F-Value	p-value	F-Value	p-value
1b, Context Fear	F(1,16) = 0.018	p = 0.90	Replicate X Group; F(1,16) = 0.062	p = 0.81
1b, Auditory Fear	F(1,16) = 0.22	p = 0.65	Replicate X Group; F(1,16) = 0.043	p = 0.84
1d, Context Fear	F(1,22) = 0.22	p = 0.64	Replicate X Group; F(1,22) = 0.81	p = 0.38
1d, Auditory Fear	F(1,22) = 4.31	p = 0.05 *	Replicate X Group; F(1,22) = 0.10	p = 0.76
2	F(1,16) = 2.92	p = 0.11	Replicate X Group; F(1,16) = 0.71	p = 0.41
3	F(2,10) = 1.90	p = 0.20	Replicate X Group; F(4,10) = 1.76	p = 0.21
4c, Context Fear	F(2,27) = 0.74	p = 0.49	Replicate X Training Type X Infusion; F(2,27) = 0.35	p = 0.71
4c, Auditory Fear	F(2,27) = 1.67	p = 0.21	Replicate X Training Type X Infusion; F(2,27) = 0.58	p = 0.57
4d	F(2,9) = 0.47	p = 0.64	Replicate X Training Type X Infusion; F(2,9) = 3.47	p = 0.08*
5b, Context Fear	F(1,12) = 0.017	p = 0.90	Replicate X Training Type X Infusion; F(1,12) = 2.17	p = 0.17
5b, Auditory Fear	F(1,15) = 2.45	p = 0.14	Replicate X Training Type X Infusion; F(1,15) = 0.38	p = 0.55
6b	F(1,9) = 0.052	p = 0.83	Replicate X Group; F(1,9) = 1.64	p = 0.23
6e	F(2,16) = 0.38	p = 0.69	Replicate X Training Type; F(2,16) = 0.45	p = 0.65
6f	F(2,10) = 1.68	p = 0.23	Replicate X Training Type; F(2,10) = 0.36	p = 0.71
6g	F(2,6) = 0.60	p = 0.94	Replicate X Training Type; F(2,6) = 0.048	p = 0.95

* = trend towards significance ($0.05 < p < 0.10$)

Table S1. Inter-replicate reliability. ANOVA was used to determine whether repeated experiments yielded different results. The F-Values and associated p-values for the main effect of replicate and the Replicate X Measurement interaction are reported for all primary figures. No significant effects were observed, and thus, replications were collapsed into a single data set for each analysis.

Conditioning Paradigm	Interstimulus Interval (ISI; from tone onset to footshock onset)				Cycle Time (C/T)
	Average (s)	Minimum (s)	Maximum (s)	Median (s)	
PRED	30	30	30	30	7
UNPRED-short	18	6	30	18	11.92
PRED	17	17	17	17	13.05
UNPRED-long	29	17	41	29	7.81

Table S2. Parameters of auditory fear conditioning under temporally predictable and unpredictable footshock. The values for different parameters used in Experiment 1 are depicted. Pink boxes indicate a value that is greater for the PRED condition compared to the UNPRED condition. Blue boxes indicate a value that is greater for the UNPRED condition compared to the PRED condition. White boxes indicate a value that is equivalent across the PRED and UNPRED conditions. Note that the relationship between the PRED and UNPRED groups is systematically reversed between the UNPRED-short and UNPRED-long groups.