Supplemental Information: Nonconscious fear is quickly acquired but swiftly forgotten

Candace Raio, David Carmel, Marisa Carrasco, and Elizabeth A. Phelps

Inventory of Supplemental Information:

Nonconscious fear is quickly acquired but swiftly forgotten: Supplemental information

Supplemental Experimental Procedures

Experiment 1

Participants

Fifty-four healthy volunteers were divided into unaware (CFS) and aware (no CFS) groups. As in previous fear conditioning studies (S1,S2), participants who failed to show reliable skin conductance responses (see "Psychophysiological stimulation and assessment" below) were excluded prior to analysis. This resulted in the exclusion of seven participants in the aware group and ten in the unaware group. The remaining thirty-eight participants (18 females) had a mean age of 24.4 (range 22-34). Participants signed a consent form approved by New York University's Committee on Activities Involving Human Subjects (UCAIHS) and were compensated for their participation. Prior to the experiment, participants completed the State-Trait Anxiety Inventory (STAI) (S3).

Stimuli and Procedure

Stimuli were presented on a DELL PC monitor. A viewing distance of 45cm was maintained with a chin-rest. Observers viewed the display through a mirror stereoscope (Stereoaids, Australia) that presented a separate image to each eye. Textured black and white bars (2.54° width) were placed 5.72° on either side of a fixation cross presented to each eye, to facilitate binocular alignment.

A fear discrimination paradigm with partial reinforcement was used. Fear arousal was measured using skin conductance response (SCR). The unconditioned stimulus (US) was a mild electric shock, and conditioned stimuli (CSs) consisted of a male and female

fearful face (8.9°x8.9°; S4). These "prepared" stimuli (S5) were chosen because emotional faces preferentially engage the amygdala (S6), a region critical to the acquisition and expression of fear (S7), and have produced successful conditioning elsewhere (S1). CS contrast was set at a level that was clearly visible when viewed on its own (S8), but was easily suppressed with CFS.

We employed delay conditioning, in which one image (CS+) co-terminated with shock on 50% of trials (CS-US) while the other (CS-) was never paired with shock (total trials: 12 CS+, 12 CS-, 12 CS-US). Partial reinforcement was used so that unreinforced trials could be assessed for conditioning without contamination by distinct physiological responses to shock. Trial order was pseudorandomized: the first trial was always reinforced and no more than two of the same trial type ever occurred consecutively. CSs were counterbalanced across participants.

To suppress CSs from awareness, we used continuous flash suppression (CFS), a novel method in the context of fear conditioning. A number of previous studies (S9-S12) have attempted to investigate whether fear responses could be acquired nonconsciously by using a different method, backward masking, to suppress briefly-presented CSs from awareness. The efficacy of this method, however, has been called into question by later work showing that the stimulation parameters employed in these studies were unreliable in effectively suppressing stimuli from awareness (S13, S14). Furthermore, the awareness measures used in these studies were not sufficiently rigorous (S15). Finally, it was later shown (S16) that trial-order confounds could account for the acquisition effects found in many of these studies (S9-S11). We therefore chose to use CFS to suppress CSs from awareness. This allowed for suppression of long-duration (4 seconds) CSs, fully equating

the temporal parameters of learning with and without awareness and enabling verification of reliable suppression by both objective (chance identification performance) and subjective (reported guessing) measures.

On each trial, CSs were presented to participants' left eye, at fixation, for 4 s. For the unaware group, the right eye was additionally presented with "mondrians" – arrays (10.16°x10.16°) consisting of multi-colored, high contrast rectangles; the arrays alternated at 10 Hz. Such dynamic monocular stimulation is known to lead to effective suppression of images presented to the other eye (S17).

To measure both objective and subjective awareness (S18), participants answered two questions (presented for 1.5 s each) after each CS presentation: "Which seen?" for which participants reported which of the two faces had been shown by pressing a key to indicate its gender (1=male, 2=female); and "How confident?" for which they rated their confidence from 1 (guess) to 3 (sure). The questions were followed by an 8-10 s inter-trial interval (ITI), during which a central fixation cross was presented binocularly. Before the experiment began, participants performed four practice trials using grey squares instead of CSs to familiarize them with the trial sequence.

Psychophysiological stimulation and assessment

Shocks (200 ms; 50 pulses/s) were delivered with a stimulator bar electrode (Grass Medical Instruments) attached to participants' right inner wrists. A work-up procedure was employed to set individuals' shock intensity, beginning at a mild level and increasing incrementally either until participants found the shock "uncomfortable, but not painful", or to a maximum of 60v.

SCRs were sampled at 200 Hz using a BioPac system module connected to an Apple computer. SCRs were collected using shielded Ag-AgCl electrodes filled with standard NaCl electrolyte gel and applied to the middle phalanges of the second and third fingers of the left hand. The largest peak-peak amplitude response 0.5 to 4.5 s after stimulus onset was recorded for each trial. Responses lower than a pre-determined criterion of 0.02μS were recorded as zero. Participants lacking measurable SCR on >75% of unreinforced trials were classified as non-responders.

SCR Analysis

Individual's SCR data were preprocessed by low-pass filtering (cutoff frequency 25 Hz) and mean-value smoothing with a 3-sample window prior to analysis, using AcqKnowledge software (Biopac systems). Raw SCR amplitudes were square root transformed to reduce skewness and subsequently divided by individual mean US responses to account for individual differences in shock reactivity (S19, S20). We assessed conditioned responding by analyzing unreinforced trials. We normalized the differential SCR by dividing the difference between each participants' average CS+ and CS- responses by their sum ([CS+ minus CS-]/[CS+ plus CS-]). This ratio is analogous to those commonly used in neuroimaging and single-unit studies (S21,S22), and enables an assessment of conditioning strength on a standardized scale bound between -1 and 1. Analyses were performed separately for early (first half of trials) and late (second half of trials) acquisition, to assess how learning developed over time. Given that we expected conditioning to result in greater responses to the CS+ than the CS-, we employed one-tailed *t* tests for all difference-score comparisons with zero; for all remaining

comparisons we used two-tailed tests. An alpha level of 0.05 was used in all statistical comparisons.

Experiment 2

Methods were the same as in Experiment 1, except for the following differences.

Participants

Of thirty-one new healthy volunteers, seven were classified as non-responders and excluded; an additional three reported consistently seeing CSs during their first session, which was therefore terminated. The remaining twenty-one participants (9 females) had a mean age of 26.4 (range 18-63; the wide age range was due to the inclusion of two outliers participants, aged 50 and 63, without whom the range would have been 18-37. We include these participants in the analyses reported below, as removing them does not affect the pattern or significance of the results). One participant completed the first session, but did not return for the second session.

Stimuli and Procedure

The experiment comprised two sessions, 24 hours apart. CSs were suppressed from awareness using CFS for all participants (no aware group). To maximize learning, participants underwent only the early acquisition portion (first half) of the original experiment (total trials: 6 CS+, 6 CS-, 6 CS-US). Participants returned to the lab 24 hours later to test retention of conditioning. There was no reinforcement on day 2 (total trials: 1 grey square at the start of the experiment to absorb an orienting response, 6 CS+, 6 CS-);

any expression of conditioned fear would thus reflect acquisition from day 1.

Supplemental Results

Experiment 1

Objective and subjective measures of awareness

Participants in the aware group were near-perfect in the CS discrimination task (97%; an average of 1.05 errors in 36 trials, SD = 2.34). Confidence ratings for correct responses were high (M = 2.83) and differed significantly from confidence ratings for the rare incorrect responses (M = 1.81; sign test, $p = 0.016$).

Participants in the unaware group reported very few instances of CSs escaping suppression. Nonetheless, to ensure only SCRs from suppressed trials were analyzed, we excluded any trials correctly identified with confidence ratings of 2 (M = 1.38 trials per participant, SD = 2.50) or 3 (M = 0.05 trials per participant, SD = 0.24; we note that this exclusion did not alter the general pattern of results). Accuracy before and after removal of these trials was slightly less than chance (46% and 44%, respectively). For included trials, participants reported very low confidence; furthermore, confidence ratings did not differ between correct (M = 1.09) and incorrect responses (M = 1.06; $p = 0.73$), indicating an absence of residual awareness. Thus, the aware group showed both objective and subjective awareness, while the unaware group exhibited neither.

Psychophysiological conditioning

Normalized SCR differences were entered into a two-way mixed model ANOVA

with a between-subject factor of group (aware, unaware) and within-subject factor of acquisition time (early, late). There was a significant main effect of group ($F_{(1,35)} = 4.41$, $p = 0.04$), no main effect of time ($F < 1$, *ns*), and a significant group*time interaction ($F_{(1,35)} = 13.88$, $p = 0.001$). Follow up *t* tests revealed a pattern of progressive learning for the aware group: early acquisition did not significantly differ from zero ($t_{(18)} = 0.91$ $p = 0.187$; one-sample *t* test), whereas late acquisition did ($t_{(18)} = 3.62$, $p < 0.001$). The unaware group showed an inverse pattern: acquisition significantly differed from zero during the early ($t_{(17)} = 3.26$, $p = 0.002$), but not late half of the session ($t_{(17)} = 1.02$, $p = 0.161$). Paired-sample *t* tests confirmed that for both groups, acquisition differed significantly between the early and late stages, albeit in opposite directions (aware: $t_{(18)} = 2.56$, $p = 0.010$; unaware: $t_{(17)} = 3.00$, $p = 0.004$). An independent-sample *t* test showed that the between-group difference between the stages of acquisition in which there was learning did not reach significance ($t_{(35)} = 1.76$, $p = 0.09$). **Figure S1** displays mean SCRs to the CS+ and CS- for each group **(Panel A)**, and also examines the normalized SCR differences on a finer-grained temporal scale **(Panels B and C)**.

As described above, CSs were reliably suppressed on non-reinforced trials in the unaware group. However, as fear can be acquired after even a small number of reinforced trials in which observers are aware of the CS-US pairing, it is important to rule out the possibility that participants in this group were aware, even weakly, of the CSs on any of the reinforced trials. If the learning we observed had been due to even weak conscious exposure, there would be a correlation between confidence ratings on correct reinforced trials and the normalized differential SCR responses. However, there was no such correlation (R = -0.07).

It remains possible, though, that a very small number of reinforced trials with awareness might lead to fear acquisition while still being insufficient for a measurable correlation to arise. To further rule this out, and to ensure that learning in the unaware group could not have arisen from even minimal awareness of the CS+ on any reinforced trials, we examined all reinforced trials and found a single participant who, on two reinforced trials, gave correct responses with high confidence (rating of 3). However, these two trials were among the last three reinforced trials and the participant showed no differential responses on any trial following them, but did show differential responses earlier in the experiment. Furthermore, removing this participant from the analysis did not alter the normalized SCR difference for early learning (before removal: M = 0.15, t = 3.34, p = 0.003; after removal: M = 0.16, t = 3.34, p = 0.003). Two other participants rated two and eight reinforced trials, respectively, with medium confidence (rating of 2), but the identification on all of these trials was incorrect. We therefore conclude that the significant group-level nonconscious fear acquisition we found could not be due to even a small number of trials in which participants were aware of the CS-US pairing.

Anxiety scores

For the aware group, mean state and trait anxiety scores were 36.16 (range 24-50, SD = 8.00) and 38.68 (range 26-50; SD = 10.49), respectively. Participants in the unaware group had mean state and trait anxiety scores of 38.77 (range 27-66, SD = 8.00) and 38.11 (range 26-51; SD = 6.88), respectively. Anxiety scores did not differ between groups (state: $t_{(35)} = 0.87$, $p = 0.39$; trait: $t_{(35)} = 0.23$, $p = 0.82$).

Correlations between the normalized SCR differences and STAI scores were

calculated for both early and late acquisition. Outliers were removed using an iterative procedure in which each data point's Mahalanobis distance was calculated (S23,S24); points > 2 standard deviations from the mean distance were removed. For state anxiety, this resulted in the removal of one data point from each group during early acquisition, and two and four data points from the aware and unaware groups, respectively, during late acquisition. We found that the lower participants' state anxiety, the better their SCRs discriminated between the CS+ and CS-; this pattern, however, was limited to the period in which significant learning occurred. For the aware group, state anxiety was negatively correlated with the magnitude of differential conditioning during late (r = -0.559, p = 0.020), but not early acquisition (r = 0.154, p = 0.541). For the unaware group, state anxiety was negatively correlated with the magnitude of differential conditioning during early (r = -0.575, p = 0.016), but not late acquisition (r = 0.202, p = 0.488). No relation between learning and trait anxiety was found.

Experiment 2

Objective and subjective measures of awareness

Day1: As in Experiment 1, SCRs from trials correctly identified with a confidence rating of 2 (M = 1.33 trials per participant, SD = 2.33) were removed. No trials were identified with a confidence rating of 3. Participants' accuracy was at chance before and after trial removal (52% and 47%, respectively). Participants indicated low confidence, which did not differ between correct (M = 1.15) and incorrect (M = 1.17) trials (p = 0.58).

Day 2: SCR trials correctly identified with a confidence rating of 2 (M = 1.85 trials

per participant, SD = 2.37) were removed. No trials were identified with a confidence rating of 3. Participants' accuracy was 63% before trial removal (slightly higher than chance, 50%; $t_{(19)} = 3.60$, $p < 0.01$), and 56% after (marginally higher than chance, $t_{(19)} = 1.82$, $p = 0.08$). Nonetheless, participants indicated low confidence, and average confidence ratings did not differ between correct (M = 1.31) and incorrect (M = 1.17) trials ($p = 0.27$).

Psychophysiological conditioning

For day 1, normalized SCR differences revealed significant learning (M = 0.14, t = 2.15, p = 0.047), replicating Experiment 1. This learning, however, was not retained one day later (M = 0.03, t < 1, *ns*). **Figure S2** displays mean SCRs to the CS+ and CS- for each day.

There was no correlation between confidence on correct reinforced trials and normalized SCR differences (R = -0.06). Two participants rated one such trial each with high confidence, but the identification on both of these trials was incorrect. No other participants rated any reinforced trials with medium or high confidence.

Anxiety scores

STAI questionnaires revealed mean state and trait anxiety scores of 33.38 (range 20-46, SD = 8.05) and 34.52 (range 22-49; SD = 7.68), respectively, on day 1. On day 2, participants reported mean state and trait anxiety scores of 30.35 (range 20-49, SD = 8.00) and 33.10 (range 20-49; SD = 8.30), respectively. Neither state ($t_{(19)} = 1.50$, $p = 0.15$) nor trait ($t_{(19)} = 1.70$, $p = 0.10$) anxiety scores differed significantly between

sessions.

Following the same outlier elimination procedure used in Experiment 1 (n=5), state anxiety was again negatively correlated with magnitude of differential conditioning (r = -0.415, p =0.048). No relation was found with trait anxiety. Given that retention of conditioning was not demonstrated, correlations with anxiety were not calculated for day 2.

Supplemental References

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Supplemental Figures

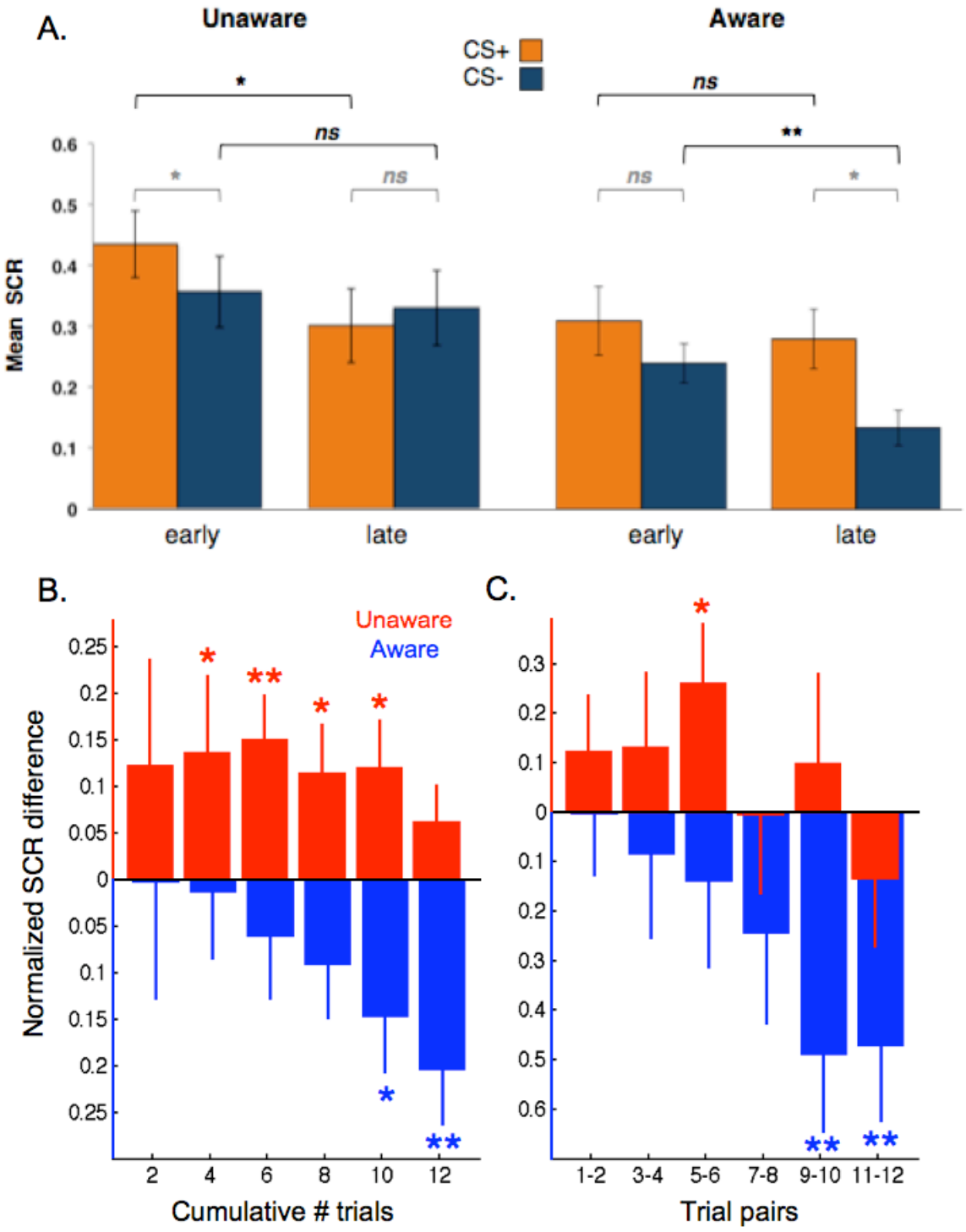

Figure S1. Experiment 1 – fear acquisition at different points in time. (A) Mean SCRs to the CS+ and CS- for early and late acquisition. Comparisons in black: For the unaware group, responses to the CS+ declined significantly between early and late

acquisition ($t_{17)} = 2.60$, $p = 0.006$), while responses to the CS- did not differ throughout the learning session ($t_{(17)} = 0.37$, $p = 0.36$). Conversely, for the aware group, mean SCR to the CS+ did not differ between early and late acquisition ($t_{(18)} = 0.70$, $p = 0.49$), but responses to the CS- did ($t_{(18)} = 3.97$, $p = 0.0009$). Comparisons in grey: For the unaware group, SCR differences between the CS+ and CS-were significant for early, but not late acquisition; for the aware group these differences only reached significance during late acquisition. **(B) and (C) Temporal dynamics of fear acquisition with and without awareness.** Rather than dividing Experiment 1 arbitrarily into halves, in these panels we examine fear acquisition on a finer temporal scale. Trials are collapsed into pairs to compensate for trial-order randomization. The red and blue portions of the vertical axis indicate positive values for the unaware and aware groups, respectively, with negative values for each group indicated by crossing over to the other side (bars are slightly jittered to prevent overlap where this occurs). **Panel B shows cumulative normalized SCR differences:** Each bar shows the mean differential fear response when data is added gradually, taking into account first two trials of each type (CS+ and CS-), then four, and so on. The aware group shows a pattern of gradually increasing fear responses, which only reaches significance when ten trials are included. Conversely, the unaware group shows significant learning when only four trials are included; this learning peaks when six trials are taken into account, then plateaus and finally decreases. **Panel C shows normalized SCR differences for individual trial pairs.** Examining specific points in time reveals the reason for the pattern revealed in panel B: in the unaware group, normalized SCR differences increase gradually until the third pair (trials 5-6), and then drop back to around zero. For the aware group, normalized SCR differences continue to increase until the final trials. Error bars indicate standard errors. Asterisks in Panel A represent differences between indicated bars; asterisks in Panels B and C represent significant differences from zero. * $p < 0.05$; ** $p < 0.01$; *ns*, not significant.

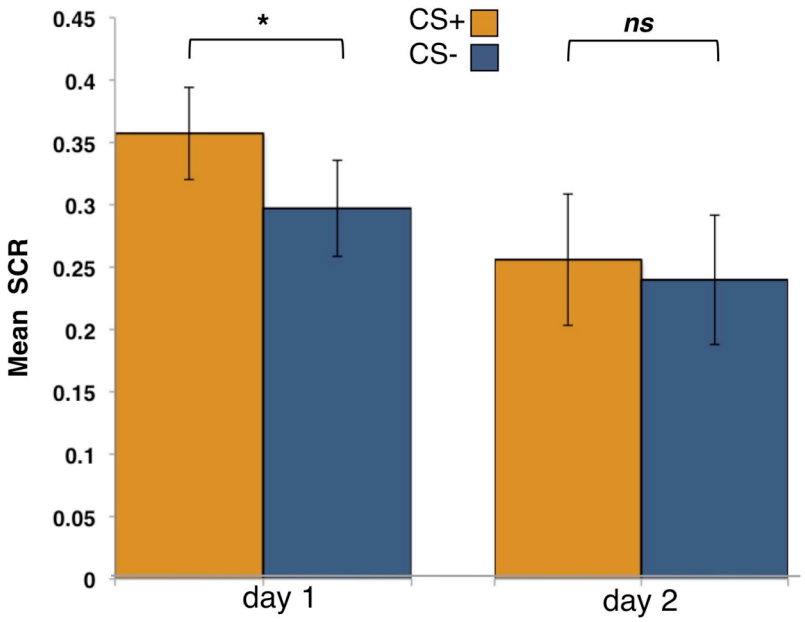

Figure S2. Experiment 2 – mean SCRs for day 1 (acquisition) and day 2 (retention).
* $p < 0.05$; *ns*, not significant.