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Awareness Predicts the Magnitude of Single-Cue Trace Eyeblink Conditioning

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ABSTRACT: Studies of differential eyeblink conditioning (CS^+ and CS^-) have demonstrated that successful conditioning requires awareness of the stimulus contingencies and that delay conditioning does not. Two experiments were carried out to determine whether awareness is also important for single-cue trace eyeblink conditioning. In experiment 1, participants who performed a secondary, attention-demanding task emitted significantly fewer conditioned eyeblink responses than participants who watched a silent movie during the conditioning session. In experiment 2, participants who became aware of the stimulus contingencies early in the conditioning session emitted significantly more conditioned responses during the remainder of the session than participants who became aware later in the session or who never became aware. These results indicate that awareness is important for single-cue trace eyeblink conditioning, just as it is for differential trace conditioning. The relationship between awareness and trace eyeblink conditioning is discussed in the light of these and other recent findings. *Hippocampus 2000;10:181–186.*

KEY WORDS: declarative memory; amnesia; hippocampus; classical conditioning

INTRODUCTION

Memory is composed of several different abilities that depend upon different brain systems (Squire, 1992; Schacter and Tulving, 1994). Declarative memory provides the capacity for conscious recollection of facts and events and is dependent on the hippocampus and related medial temporal lobe structures. Nondeclarative memory is expressed through performance as skills, habits, priming, and certain forms of classical conditioning and is independent of these brain structures.

Human eyeblink classical conditioning provides a useful paradigm for exploring the distinction between declarative and nondeclarative forms of memory. Typically, a tone (the conditioned stimulus; CS) is presented immediately before a puff of air (the unconditioned stimulus; US) is delivered to the eye. After repeated pairings of the CS and US, individuals begin to blink in response to the tone (the conditioned response; CR). In delay eyeblink conditioning, the tone remains on until the air puff has been delivered. Delay conditioning is nondeclarative and can be acquired by amnesic patients or experimental animals despite damage to the hippocampus and related structures (Norman et al., 1977; Weiskrantz and Warrington, 1979; Mauk and Thompson, 1987; Daum et al., 1989; Gabrieli et al., 1995; Clark and Squire, 1998). By contrast, in trace eyeblink conditioning, an empty interval separates the end of the tone from the onset of the air puff. Amnesic patients or experimental animals with damage to the hippocampus are unable to acquire trace conditioning when the trace interval is sufficiently long (McGlinchey-Berroth et al., 1997; Clark and Squire, 1998; Moyer et al., 1990; Solomon et al., 1986). In addition, in rabbits hippocampal lesions 1 day (but not 28 days) after trace conditioning abolish the conditioning (Kim et al., 1995).

Clark and Squire (1998) found that awareness of the stimulus contingencies was related to successful trace conditioning. At the end of the conditioning session (120 trials of differential conditioning with a CS^+ and a CS^-), participants were asked about the relationship between the CS and the US. Only those participants who had become aware of this relationship (i.e., that the CS predicted the US) exhibited successful conditioning. Awareness was unrelated to differential delay conditioning. These results suggested that trace conditioning might be dependent on the hippocampus, because declarative

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knowledge about the stimulus contingencies must be available during conditioning.

The study by Clark and Squire (1998) followed a differential conditioning procedure. Two auditory stimuli were presented (a CS^+ and a CS^-), and conditioning was measured as the percentage of CRs to the CS^+ minus the percentage of CRs to the CS^-. Yet, most previous human and animal studies of eyeblink conditioning have used single-cue conditioning. In single-cue conditioning, only one conditioned stimulus is used (a CS^+), and the level of conditioning is determined by the percentage of trials in which a CR occurs. It is not known whether the importance of awareness for trace conditioning extends to the single-cue paradigm. Indeed, it is possible that the extra demands of differential trace conditioning (processing two CSs and appreciating their relationship to the US) might require awareness, whereas the simpler single-cue trace conditioning procedure may not (LaBar and Disterhoft, 1998).

To determine whether awareness is an important factor for trace single-cue eyeblink conditioning, as it is for trace differential eyeblink conditioning, we carried out two experiments. The first experiment compared the magnitude of eyeblink conditioning in two groups of volunteers. One group watched a silent movie during the conditioning trials, and the other performed an attention-demanding, secondary task. If awareness of the stimulus contingencies is important for single-cue trace conditioning, then the group watching the movie should have become more aware and should have become conditioned to a greater extent than the group that was distracted. In the second experiment, all participants watched the silent movie and were asked about the relationship between the CS and the US early in the conditioning session, midway through the session, and at the end of the session. If awareness is important for single-cue trace conditioning, then those who become aware of the relationship between the CS and the US early in the session should condition to a greater extent than those who become aware later in the session or who never became aware.

EXPERIMENT 1, METHODS

Participants

The participants (10 men, 14 women) were volunteers or employees at the San Diego Veterans Affairs Medical Center. They averaged 69.1 years of age (range, 47–78) and had an average of 16.3 years of education. They obtained WAIS-R Information and Vocabulary subscale scores of 23.8 and 60.3, respectively.

Apparatus and Procedure

Participants were assigned to one of two groups. One group (n = 14) watched a silent movie (*The Gold Rush*) during conditioning. The other group (n = 10) performed an attention-demanding, secondary task. They watched digits appearing on a computer screen (once every 1.5 s for a 1-s duration) and pressed a button whenever three odd digits appeared consecutively (Mulligan and Hartman, 1996).

Participants were told that they were taking part in a study of how distraction affects learning and memory and that they would be distracted by tones and air puffs. After giving informed consent, participants were seated in a comfortable chair in a darkened room, approximately 0.7 m from either a television monitor (movie group) or a computer monitor (distraction task group). One hundred twenty trace conditioning trials were then administered, with an intertrial interval of 10–15 s. The CS was an 85-dB, 1-kHz tone, 250 ms in duration, delivered through earphones; 1,000 ms after termination of the CS, the US was delivered. The US was a 100-ms, 3-psi airpuff delivered to the left eye through specially designed goggles. The goggles also included an infrared reflective sensor for recording eyeblinks (Clark and Squire, in press).

Following the conditioning session, participants were given a true or false questionnaire that asked about aspects of the conditioning session. For the Movie group, 10 questions concerned the content of the silent movie that participants watched during conditioning. The remaining questions were identical for both groups. Six questions asked participants to identify what stimuli had been presented, four questions asked how participants had responded to the US, and four questions asked how participants had responded to the CS. Finally, the seven critical questions asked about the relationship between the CS and the US (Appendix A).

EXPERIMENT 1, RESULTS

Figure 1 shows the percentage of CRs emitted by each group during the first 10 trials of conditioning (Fig. 1A) and across 6 blocks of 20 trials (Fig. 1B). CRs were evident during the first few trials of conditioning, as reported previously in human volunteers for both single-cue delay conditioning (Gabrieli et al., 1995, their Fig. 1; Carrillo et al., 1997, their Fig. 2) and single-cue trace conditioning (Woodruff-Pak and Papka, 1996, their Fig. 1; McGlinchey-Berroth et al., 1997, their Fig. 3; Woodruff-Pak, 1999, his Fig. 1). Performance did not noticeably improve after the first few trials. This finding also matches what has been reported previously in humans for single-cue conditioning (Gabrieli et al., 1995, her Fig. 1; Carrillo et al., 1997, their Fig. 2; Woodruff-Pak and Papka, 1996, their Fig. 1; McGlinchey-Berroth et al., 1997, their Fig. 3). Finally, participants in the Movie group emitted more CRs than participants in the Distraction group. This difference was apparent during the first 10 trials of the session (46.9 ± 4.8% vs. 27.8 ± 5.8% CRs for the Movie and Distraction groups, respectively; $t(22) = 2.53$, $P < 0.05$), and also across all 120 trials of conditioning (45.3 ± 3.8% vs. 30.4 ± 4.6% CRs; $t(22) = 2.5$, $P < 0.05$).

Figure 1C shows the mean awareness scores, based on the seven critical questions that asked about the relationship between the CS and US. Participants in the Distraction group scored lower than participants in the Movie group (4.50 ± 2.4 vs. 6.1 ± 2.2), but

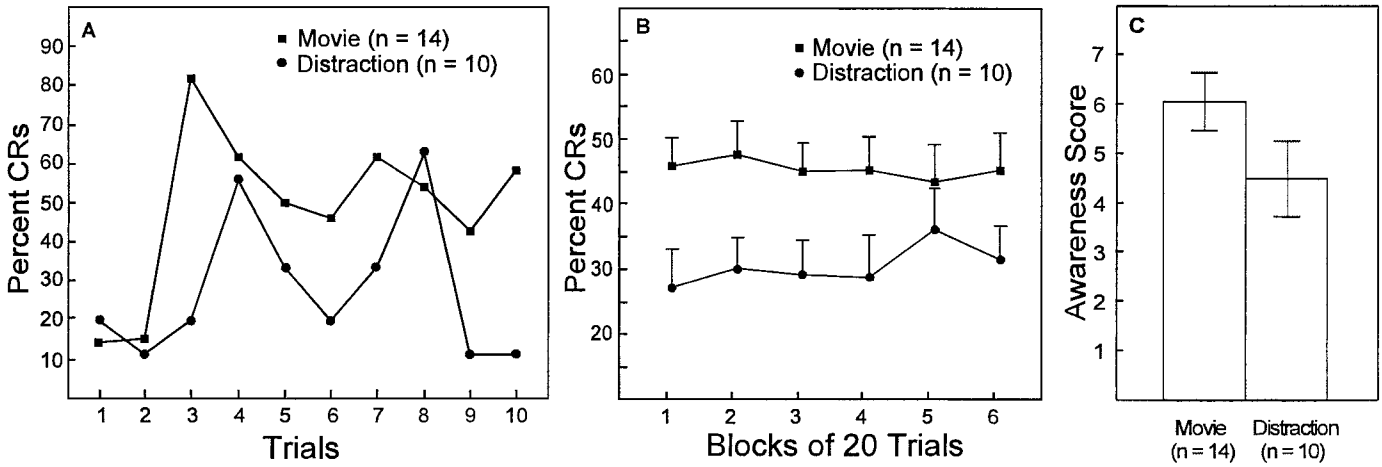

FIGURE 1. A: Percentage of participants in each group who emitted CRs during first 10 trials. B: Percent CRs for each group across 6 blocks of 20 conditioning trials. Brackets show SEM. C: Mean score obtained by each group on seven true or false questions that asked about the relationship between the CS and the US. Brackets show SEM.

this difference did not reach significance ($t(22) = 1.67$, $P = 0.11$). This nonsignificant difference was due to two participants in the Movie group who apparently reversed the relationship between the CS and the US and scored only 1 out of 7 correct on the seven critical questions. Still, the awareness score for the Movie group was significantly above chance ($t(13) = 4.44$, $P < 0.01$), whereas the awareness score for the Distraction group was not ($t(9) = 1.31$, $P = 0.22$). Moreover, 11 of the 14 participants in the Movie group obtained a perfect score of 7, whereas only 3 of the 10 participants in the distraction group obtained a score of 7 ($X^2 = 3.84$, $P = 0.05$).

Participants in the Movie group performed well on the 10 questions concerning the content of the movie (mean number of correct answers = 9.7 ± 0.2). Participants in the Distraction group performed well on the secondary distraction task (mean percent of strings correctly identified = 97.5 ± 0.6). Both groups were able to identify the CS and the US (5.9 and 5.6 correct out of 6 questions for the Movie and Distraction groups, respectively), and both groups acquired knowledge about how they had responded to the US (3.1 ± 0.2 and 2.9 ± 0.3 correct out of 4 questions; $P < 0.05$). Finally, neither group acquired significant knowledge about how they had responded to the CS (2.0 ± 0.1 and 1.8 ± 0.2 correct out of four questions).

The findings of experiment 1 suggest that awareness of the relationship between the CS and the US is important for single-cue trace conditioning, just as in the case of differential trace conditioning (Clark and Squire, 1998, 1999). Engaging participants in an attention-demanding task inhibited the acquisition of single-cue trace conditioning (Fig. 1A,B) and reduced awareness of the relationship between the CS and US.

Despite the difference between the two groups in performance, most of the participants did acquire considerable awareness about the relationship between the CS and US (16 of the 24 participants scored 6 or 7 correct on the 7 critical questions, including 4 of the 10 participants in the Distraction group). However, awareness was assessed only at the completion of all 120 conditioning trials. Accordingly, it was not possible to determine when in the conditioning session awareness developed. Some participants could have become aware towards the end of the 120 trials. These individuals would have been identified as aware but would not in fact have been aware during the course of the conditioning session.

The second experiment sought to determine the importance of awareness during the conditioning process itself. Conditioning trials were presented while participants watched a silent movie, as in experiment 1, but awareness was tested after 10 conditioning trials, after 60 trials, and after 120 trials. The question of interest was whether participants who became aware early in the conditioning session would produce more conditioned responses than participants who became aware only later in the session or who never became aware at all.

EXPERIMENT 2, METHODS

Participants

Seven men and 13 women who did not participate in experiment 1 were recruited for experiment 2. Their average age was 66.2 years (range, 51–75), and they averaged 14.9 years of education. Their scores on the Information and Vocabulary subscales of the WAIS-R averaged 20.1 and 53.9, respectively.

Apparatus and Procedure

The procedure was the same as for experiment 1, except that all participants watched the silent movie (*The Gold Rush*). Additionally, the seven critical true or false questions from experiment 1

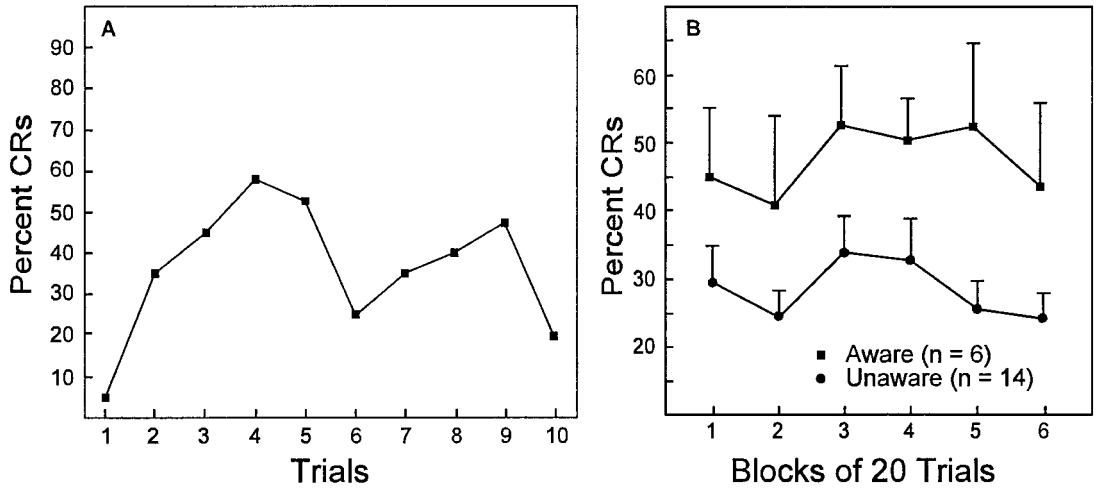

FIGURE 2. A: Percentage of participants who emitted CRs during of the first 10 trials. B: Percent CRs across 6 blocks of 20 trials by participants who were classified as aware or unaware on the basis of their answers to the seven true or false questions given after the first 10 trials. Brackets show SEM.

(Appendix A) were given without forewarning after the first 10 conditioning trials, again after 60 trials, and finally after all 120 trials were completed. At the end of the session, 17 of the 20 participants were also given the 10 true or false questions about the content of the movie from experiment 1.

EXPERIMENT 2, RESULTS

Figure 2A shows the percentage of CRs on each of the first 10 trials of conditioning. As in experiment 1, conditioning was evident during the first 10 trials (35.8 ± 4.9% CRs). Figure 2B shows the percentage of CRs emitted across all 6 blocks of 20 trials by participants who were classified after 10 trials as either aware or unaware. Participants classified as aware (n = 6) were those who answered correctly all 7 of the true or false questions given after the first 10 trials. Participants classified as unaware (n = 14) were those who answered fewer than 7 questions correctly (mean number of correct answers = 4.9). Note that on a seven-item true or false test, a score of 7 correct is significantly above chance (binomial test, $P < 0.05$), but a score less than 7 is not. The 6 participants who were designated as aware at the end of 10 trials emitted more CRs across all 120 conditioning trials than the 14 unaware participants (47.4 ± 9.7% CRs vs. 28.3 ± 3.4% CRs, respectively; $t(18) = 2.36$, $P < 0.05$). Figure 3 shows for all 20 participants the relationship between the awareness scores obtained after 10 conditioning trials and the mean percent CRs across all 120 trials. The awareness score after the first 10 conditioning trials correlated significantly with the strength of conditioning across the 120 trials ($r = 0.49$, $P < 0.05$).

A numerical difference in % CRs between the aware and unaware groups first appeared during trials 6–10 (trials 1–5, 40.0% vs. 38.2%; trials 6–10, 46.7% vs. 27.1%), but the difference was not significant ($t(18) = 1.44$, $P > 0.10$). After the first 10 conditioning trials, the difference in performance between those who had been designated as aware and those who had been designated as unaware remained about the same during the remainder of the conditioning session (trials 11–60; 45.8% vs. 28.3%, $P = 0.06$; trials 61–120, 48.1% vs. 27.4%, $P < 0.05$). For all participants, awareness scores increased a modest amount as conditioning progressed (number of correct answers out of 7 was 5.2 ± 0.4, 6.4 ± 0.2, and 6.6 ± 0.2 after 10, 60, and 120 trials, respectively). Finally, all participants

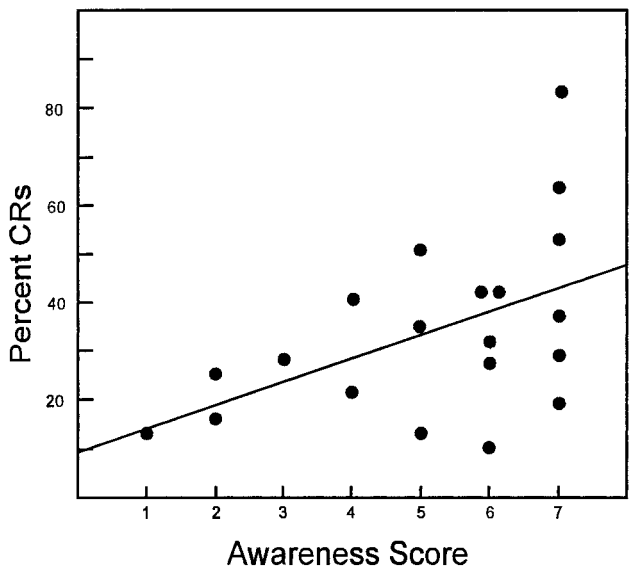

FIGURE 3. Relationship between awareness score obtained after the first 10 conditioning trials and strength of conditioning (percent CRs) across all 120 conditioning trials ($r = 0.49$, $P < 0.05$).

performed well on the 10 questions about the movie (mean number of correct answers = 9.4 ± 0.5).

DISCUSSION

In experiment 1, participants who were distracted by an attention-demanding, secondary task emitted fewer CRs than those who watched a silent movie. In experiment 2, participants who became aware of the relationship between the CS and the US early in the session conditioned to a greater extent than those who became aware of the relationship later in the session or who never became aware at all. Thus, awareness of the relationship between the CS and US early in the conditioning session predicted the magnitude of single-cue trace eyeblink conditioning.

Previous studies of elderly volunteers and amnesic patients (Clark and Squire, 1998, 1999) demonstrated the importance of awareness for differential trace conditioning. A recent preliminary report described the same finding for both young and aged individuals (Knuttinen et al., 1999). The present work establishes the generalizability of these findings to the much more widely studied case of single-cue conditioning. It had seemed possible that the differential conditioning procedure might introduce extra demands on information processing that do not apply to the single-cue paradigm, such that the importance of awareness would be limited to the differential conditioning procedure (LaBar and Disterhoft, 1998). However, the present findings for single-cue trace conditioning suggest that the importance of awareness for trace eyeblink conditioning is a general phenomenon that will be observed in many if not all conditioning protocols. Indeed, in a single-cue trace conditioning paradigm that used a 1,400-ms trace interval, Woodruff-Pak (1999) reported that volunteers (aged 15–30) who were aware that their blinking was related to the tone emitted more CRs during the first 10 conditioning trials than volunteers who were unaware.

What can the available data reveal about the nature of the relationship between awareness and trace eyeblink conditioning? First, distraction can reduce awareness and also attenuate trace conditioning (Clark and Squire, 1999; experiment 1, present study). Second, informing participants about the CS-US relationship increases awareness and improves trace conditioning (Clark and Squire, 1999). Third, awareness can develop early in conditioning (Woodruff-Pak, 1999; experiment 2, present study), and awareness early in conditioning predicts the magnitude of conditioning during the remainder of the session (experiment 2, present study). These findings indicate that awareness is not a result of successful conditioning. Rather, it appears to be the case either that awareness precedes and contributes to acquisition of trace CRs, or that awareness and trace conditioning develop concurrently.

It is possible that awareness ordinarily emerges during trace conditioning because awareness is a typical feature of hippocampus-dependent memory rather than because awareness is always a prerequisite for this form of memory (Eichenbaum, 1999). Although certain conditions may exist in which hippocampus-dependent memory can be acquired without awareness (Chun and Phelps, 1999), we suggest that most forms of declarative memory typically develop together with awareness. Thus, awareness emerges naturally when the hippocampus and related structures are carrying out their normal function of associating and binding together the elements of an episode for ultimate storage in the neocortex. Moreover, the emergence of awareness may be a reliable indicator that the system is operating effectively, because task conditions that reduce awareness also disrupt the system's function, and task conditions that promote awareness facilitate the system's function (Clark and Squire, 1999). By this view, awareness of what is being learned is indicative of a brain state (specifically, a state of interaction between the hippocampus and neocortex) that is optimal for forming and storing declarative memory. If so, the possibility must be considered that hippocampus-dependent learning in nonhuman animals may be accompanied by some form of awareness.

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REFERENCES

Carrillo MC, Thompson LT, Gabrieli JDE, Disterhoft JF. 1997. Variation of the intertrial interval in human classical conditioning. Psychobiology 25:152–157.

Chun MM, Phelps EA. 1999. Memory deficits for implicit contextual information in amnesic subjects with hippocampal damage. Nat Neurosci 2:844–847.

Clark RE, Squire LR. 1998. Classical conditioning and brain systems: a key role for awareness. Science 280:77–81.

Clark RE, Squire LR. 1999. Human eyeblink classical conditioning: Effects of manipulating awareness of the stimulus contingencies. Psychol Sci 10:14–18.

Clark RE, Squire LR. In press. Awareness and the conditioned eyeblink response. In: Woodruff-Pak DS, Steinmetz JE, editors. Eyeblink classical conditioning: human. Norwell, MA: Kluwer Academic Publishers.

Daum I, Channon S, Canavan AG. 1989. Classical conditioning in patients with severe memory problems. J Neurol Neurosurg Psychiatry 52:47–51.

Eichenbaum H. 1999. Conscious awareness, memory and the hippocampus. Nat Neurosci 2:775–776.

Gabrieli JD, McGlinchey-Berroth R, Carrillo MC, Gluck MA, Cermak LS, Disterhoft JF. 1995. Intact delay-eyeblink classical conditioning in amnesia. Behav Neurosci 109:819–827.

Kim JJ, Clark RE, Thompson RF. 1995. Hippocampectomy impairs the memory of recently, but not remotely, acquired trace eyeblink conditioned responses. Behav Neurosci 109:195–203.

Knuttinen M-G, Power JM, Preston AR, Disterhoft JF. 1999. Age and awareness in eyeblink conditioning. Soc Neurosci Abstr 25:2069.

LaBar KS, Disterhoft JF. 1998. Conditioning, awareness, and the hippocampus. Hippocampus 8:620–626.

Mauk MD, Thompson RF. 1987. Retention of classically conditioned eyelid responses following acute decerebration. Brain Res 403:89–95.

McGlinchey-Berroth R, Carrillo MC, Gabrieli JDE, Brawn CM, Disterhoft JF. 1997. Impaired trace eyeblink conditioning in bilateral, medial-temporal lobe amnesia. Behav Neurosci 100:243–252.

Moyer JR, Deyo RA, Disterhoft JF. 1990. Hippocampectomy disrupts trace eye-blink conditioning in rabbits. Behav Neurosci 104:243–252.
Mulligan N, Hartman M. 1996. Divided attention and indirect memory tests. Memory Cogn 4:453–465.
Norman RJ, Buchwald JS, Villablanca JR. 1977. Classical conditioning with auditory discrimination of the eyeblink reflex in decerebrate cats. Science 196:551–553.
Schacter DL, Tulving E. 1994. Memory systems 1994. Cambridge, MA: MIT Press.
Solomon PR, Vander Schaaf RF, Thompson RF, Weisz DJ. 1986. Hippocampus and trace conditioning of the rabbit's classically conditioned nictitating membrane response. Behav Neurosci 100:729–744.
Squire L. 1992. Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. Psychol Rev 99:195–231.
Weiskrantz L, Warrington EK. 1979. Conditioning in amnesic patients. Neuropsychologia 17:187–194.
Woodruff-Pak DS. 1999. New directions for a classical paradigm: human eyeblink conditioning. Psychol Sci 10:1–3.
Woodruff-Pak DS, Papka M. 1996. Alzheimer's disease and eyeblink conditioning: 750 ms trace vs. 400 ms delay paradigm. Neurobiol Aging 17:397–404.

APPENDIX A

1) I believe the airpuff usually came immediately *before* the tone.	TF
2) I believe the airpuff usually came immediately *after* the tone.	TF
3) I believe the tone usually came immediately *before* the airpuff.	TF
4) I believe the tone usually came immediately *after* the airpuff.	TF
5) I believe the tone and airpuff were always closely related in time.	TF
6) I believe the tone and airpuff were only sometimes related in time.	TF
7) I believe the tone predicted when the airpuff would come.	TF