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Acquisition of Differential Delay Eyeblink Classical Conditioning Is Independent of Awareness

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Abstract

There has been debate about whether differential delay eyeblink conditioning can be acquired without awareness of the stimulus contingencies. In 4 experiments, the authors reexamined this question. Older participants were tested with a tone and white noise (Experiment 1) or with 2 tones (Experiment 2). In addition, younger participants were tested with 2 tones (Experiment 3) or with 2 tones plus the parameters from an earlier study that had reported a relationship between conditioning and awareness (Experiment 4). Participants who were designated aware of the stimulus contingencies and participants who were designated unaware exhibited equivalent levels of differential eyeblink conditioning. Awareness of stimulus contingencies is not required for differential delay eyeblink conditioning when simple conditioned stimuli are used.

Memory is composed of different abilities that depend on different brain systems (Eichenbaum & Cohen, 2001; Schacter & Tulving, 1994; Squire, 1992). A fundamental distinction is between the capacity for conscious recollection (declarative memory) and a collection of nonconscious memory abilities (nondeclarative memory) that supports skill and habit learning, the phenomenon of priming, and other forms of experience-dependent behavior that are expressed through performance rather than recollection. The best studied example of nondeclarative memory in mammals is delay classical conditioning of the eyeblink response (Christian & Thompson, 2003; Clark, Manns, & Squire, 2002). In its simplest form (single-cue conditioning), a single neutral conditioned stimulus (CS), such as a tone, is presented just before an unconditioned stimulus (US), such as a mild puff of air to the eye. The CS and US overlap and coterminate. After repeated pairings of CS and US, the CS elicits a conditioned eyeblink response (CR) in advance of the US.

Only the cerebellum and related brainstem pathways are necessary for acquisition and retention of delay eyeblink conditioning. Accordingly, it has seemed reasonable to view delay eyeblink conditioning as relatively reflexive and automatic and to suppose that conditioning can occur in the absence of awareness about the stimulus contingencies. Indeed, for other forms of

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nondeclarative memory, researchers have found successful performance to be independent of awareness about what is learned (Bayley & Squire, 2002; Berns, Cohen, & Mintun, 1997; Cleeremans & McClelland, 1991; Reber & Squire, 1994; Willingham, Nissen, & Bullemer, 1989), and this independence of awareness and performance has been considered to reflect an important characteristic of nondeclarative memory (Eichenbaum & Cohen, 2001; Squire, 1992). Nevertheless, it has remained a matter of some debate whether delay classical eyeblink conditioning itself can proceed without awareness (Knuttinen, Power, Preston, & Disterhoft, 2001; Lovibond & Shanks, 2002; Manns, Clark, & Squire, 2002).

On the one hand, it is quite clear that delay classical conditioning is independent of awareness when a single CS is used (Manns, Clark, & Squire, 2001, 2002; Papka, Ivry, & Woodruff-Pak, 1997). On the other hand, there has been uncertainty about differential delay eyeblink conditioning and its relationship to awareness. In differential conditioning, one stimulus (the CS+) is followed by the US, whereas a second stimulus (the CS−) is not. Successful differential conditioning occurs when more CRs are elicited by the CS+ than by the CS−. There is no doubt that some differential conditioning paradigms do depend on participants becoming aware of the relationship of the CS+ and the CS− to the US. For example, in two early studies, the CS+ and CS− involved two-word phrases that were either grammatical or ungrammatical (Baer & Fuhrer, 1982; Perry, Grant, & Schwartz, 1977). In these cases, only participants who became aware of the grammaticality rule discriminated between the CS+ and CS−.

Yet the question of interest is not whether awareness might sometimes be required for successful differential delay conditioning, for example, when the distinction between the CS+ and CS− is conceptual or linguistic. The question is whether successful differential delay conditioning is ever independent of awareness about the stimulus contingencies. And, if so, how general is the phenomenon? What kinds of conditioned stimuli can support differential delay conditioning in the absence of awareness?

In 1998, Clark and Squire reported that differential delay conditioning to a tone and white noise succeeded as well for participants who became aware of the stimulus contingencies, according to a postconditioning true–false test, as for participants who did not become aware. Further, amnesic patients, including the severely amnesic patient E.P., conditioned as well as healthy volunteers. Moreover, in a subsequent study (Clark & Squire, 1999), differential delay conditioning succeeded as well for participants who were distracted by an attention-demanding secondary task as for participants who were not distracted.

These findings differ from the results of an often-cited study from the earlier literature. Nelson and Ross (1974) used two tones (800 Hz and 2100 Hz) as CS+ and CS− and found that participants designated as unaware failed to develop differential CRs across 100 training trials (also see Ross & Nelson, 1973, for an analysis of CRs from the final 40 trials of training in the same study). A possible reason why awareness was related to successful conditioning in this study (Nelson & Ross, 1974) but not when a tone and white noise served as the CSs (Clark & Squire, 1998) is that the two tones were not sufficiently salient or sufficiently easy to discriminate. Perhaps the CS+ and CS− need to be very distinct (e.g., a tone and white noise) if conditioning is to succeed without awareness. Alternatively, perhaps some other feature of this study made conditioning difficult unless participants became aware of the stimulus contingencies.

More recently, Knuttinen et al. (2001) used a tone and white noise as CS+ and CS− and closely followed the conditioning protocol used by Clark and Squire (1998). In this study, even with a tone and white noise as CS+ and CS−, participants who became aware of the stimulus contingencies conditioned much better than those who remained unaware. In fact, unaware participants exhibited little or no conditioning across 120 conditioning trials. These findings, together with the earlier study (Nelson & Ross, 1974), challenge the idea that differential delay conditioning can occur in the absence of awareness.

In four experiments, we have reexamined differential delay eyeblink conditioning and its relationship to awareness. Experiment 1 attempted to replicate the original study by Clark and Squire (1998), which tested older individuals and used a tone and white noise as CS+ and CS−. Additionally, the eyeblink responses from the earlier study (Clark & Squire, 1998) were rescored by an individual who was blind to whether a participant had been designated as aware or unaware. Experiment 2 used two tones as CS+ and CS− (800 Hz and 2100 Hz) and also tested older individuals. Experiment 3 used these same two tones as CS+ and CS− and tested younger individuals. Experiment 4 tested younger individuals and also followed as closely as possible the protocol used by Nelson and Ross (1974). In each of the four experiments, designation of participants as aware or unaware was determined in three different ways by applying different scoring criteria to our standard questionnaire. In addition, in Experiments 3 and 4, interviews were used together with the questionnaire to improve estimates of awareness and unawareness.

Experiment 1

Method

Participants—The participants (9 men, 3 women) were volunteers or employees at the Veterans Affairs San Diego Healthcare System. They averaged 49.1 years of age (range: 32–71 years) and had an average of 15.9 years of education.

Procedure—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 noises and airpuffs. After giving informed consent, the participants were seated in a comfortable chair in a darkened room, approximately 0.7 m from a TV monitor. The CS+, CS−, and airpuff were first presented alone two or three times each to familiarize the participants with the stimuli. We administered 120 delay conditioning trials with an intertrial interval (ITI) of 10–15 s. During the conditioning session, participants watched a silent movie (*The Gold Rush*), which they were instructed to remember for a later memory test. Each 20-trial block consisted of 10 CS+ trials in which a tone or white noise presented through headphones was paired with the airpuff US (1,250-ms interstimulus interval) and 10 CS− trials in which a tone or white noise occurred in the absence of the airpuff US. The order of the CS+ and CS− trials was pseudorandom with neither trial type occurring more than twice in succession. The CSs were a 1,350-ms, 95-db 1000-Hz tone and a 1,350-ms, 95-db white noise. Each stimulus served equally often as CS+ and CS−. 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 & Squire, 2000).

Following the conditioning session, participants were given a true–false questionnaire that asked about aspects of the conditioning session. Ten questions concerned the silent movie, and 17 questions concerned the CS+, CS−, and US (Clark & Squire, 1999; Table 1). On the basis of their responses to these 17 questions (e.g., the CS+ predicted the US), participants were designated as aware or unaware of the stimulus contingencies. As described previously (Clark & Squire, 1998), participants who scored ≥ 13 correct on the 17-item questionnaire (binomial probability < .05) were designated as aware, and those who scored ≤ 12 correct were designated as unaware. The results were also analyzed using a more stringent criterion to designate participants as unaware (≤ 9 questions answered correctly, chance = 8.5; Manns et al., 2002). Lastly, designation of participants as aware or unaware was based only on the 8 questions (out of 17) that asked directly about the relationship between the CS+ and US or the CS− and the US (and excluded 9 questions that asked about the relationship between the CS+ and the CS

−). Participants were designated unaware in this case if they answered ≤ 4 questions correctly. These additional criteria for awareness were used to address the possibility raised by Lovibond and Shanks (2002) that our original method might have classified too many participants as aware or might have involved questions not directly relevant to awareness of the CS–US relationship.

Eyeblinks that occurred within a 500-ms window before the onset of the US were scored as CRs. In addition, for a response to the CS+ or CS− to be scored as a CR for a given participant, the eyeblink amplitude needed to be at least 20% of his or her mean eyeblink amplitude in response to the first 10 US presentations. In addition, discrete and unambiguous eyeblinks that failed to reach the 20% guideline were also scored as CRs. On average, this procedure resulted in an additional 6.7 trials per 120-trial session being scored as CRs. Finally, trials were eliminated from the analysis if they contained waveforms that appeared to be voluntary eyeblinks (large amplitude, early onset responses that were maintained until the termination of the US airpuff). Waveforms were also eliminated if they were associated with excessive blinking or if they were ambiguous and could not be classified. Each participant produced on average a total of 4.0 voluntary eyeblinks and 5.0 ambiguous eyeblinks during the 120-trial conditioning session, and these were eliminated from the analysis. Scoring of CRs was done without knowledge of whether participants were designated as aware or unaware.

Results

Figure 1 shows the percentage of differential CRs emitted by participants across 120 conditioning trials (percentage of CRs to the CS+ minus percentage of CRs to the CS−). Participants designated as aware ($n = 4$) obtained a mean score of 14.2 ± 0.6 on the 17-item questionnaire, and participants designated as unaware ($n = 8$) obtained a score of 10.0 ± 0.9, $t(10) = 3.3$, $p < .01$, which was no better than the chance score of 8.5, $t(7) = 1.8$, $p > .10$. Nevertheless, both groups performed similarly in the conditioning session. Across all 120 trials, the aware group emitted 15.8% ± 6.5% differential CRs, and the unaware group emitted 24.9% ± 4.1% differential CRs. An analysis of variance (ANOVA; Group × Block) revealed a linear effect of training block, $F(1, 10) = 25.8$, $p < .001$, but no effect of group and no interaction (Fs < 1.6). Additionally, both the aware and unaware groups exhibited an increase in differential CRs across training blocks as revealed by separate ANOVAs for each group (Fs > 13, ps > .05). Both aware and unaware groups did well at answering the 10 questions about the silent movie (8.8 vs. 9.1 items correct, respectively). Lastly, the correlation between awareness and average differential conditioning scores was weak and opposite in sign to what would be expected if awareness were related to conditioning ($r = −.48$, $p > .10$).

The results were quite similar to those reported previously for aware and unaware participants given differential delay eyeblink conditioning (Clark & Squire, 1998). In that study, the aware group emitted 19% differential CRs and the unaware group emitted 16% differential CRs. An ANOVA (Group × Block) of the data from that study revealed the same pattern of effects as was found in the present experiment, that is, a linear effect of training block, $F(1, 8) = 16.8$, $p < .01$, but no effect of group and no interaction (Fs < 1.0). In addition, as part of the present study, the original trial-by-trial eyeblink waveforms from Clark and Squire (1998) were rescored by an individual who was blind to each participant's designation as aware or unaware. This reanalysis yielded the same pattern of findings, that is, a linear effect of training block, $F(1, 8) = 7.7$, $p > .05$, but no effect of group and no interaction (Fs < 1.0). Thus, the earlier findings (Clark & Squire, 1998), the subsequent reanalysis of those findings, and the results of the present experiment all reached the same conclusion: Unaware participants acquired differential delay conditioning to the same extent as aware participants.

The findings from the present experiment were the same when different criteria were used to designate participants as aware or unaware. Thus, with a more stringent criterion (unaware being defined as ≤ 9 of 17 questions correct), 8 aware participants and 4 unaware participants emitted 19.4% ± 4.7% and 26.9% ± 11.7% differential CRs, respectively, $t(10) = 1.0$, $p > .30$. Both these scores were above zero ($p < .05$). When the designation of participants as aware or unaware was based on only the eight (out of 17) questions that asked directly about the CS–US relationship (unaware being defined as ≤ 4 questions correct), group membership remained the same, and the results were identical (19.4% vs. 26.9%) to the results just described for the more stringent criterion. In summary, knowledge of the stimulus contingencies was unrelated to differential delay conditioning.

Finally, the data were nearly identical when responses were classified as CRs if they reached only 10% of the average UR amplitude rather than 20% (which was our standard method). Following this scoring procedure, the aware and unaware groups continued to perform similarly (12.0% vs. 23.6% differential CRs across 120 trials, $t(10) = 1.5$, $p > .1$).

Experiment 2

In Experiment 1 and in an earlier study (Clark & Squire, 1998), a tone and white noise were used as CS+ and CS−, and awareness of stimulus contingencies was unrelated to differential delay eyeblink conditioning. However, in another study (Nelson & Ross, 1974) when two tones (800 Hz and 2100 Hz) served as CS+ and CS−, participants designated as aware exhibited differential delay eyeblink conditioning but participants designated as unaware did not. This earlier finding raises the possibility that differential delay eyeblink conditioning is independent of awareness only under limited conditions. Perhaps two tones are more difficult to discriminate than a tone and white noise, such that successful conditioning requires attention and ultimately awareness of the stimulus contingencies. To explore this possibility, Experiment 2 involved the same parameters as Experiment 1 except that two tones (800 Hz and 2100 Hz) served as CS+ and CS−.

Method

Participants—The participants (14 men, 6 women) were volunteers or employees at the Veterans Affairs San Diego Healthcare System. They averaged 57.9 years of age (range: 33–79 years) and had an average of 14.4 years of education.

Procedure—The procedure was the same as for Experiment 1, except that two tones (800 Hz and 2100 Hz) were used as the CSs instead of a tone and white noise. The two tones served equally often as CS+ and CS−.

Results

Figure 2 shows the percentage of differential CRs emitted by participants across 120 conditioning trials. Participants designated as aware ($n = 6$) obtained a mean score of 14.3 ± 0.5 on the 17-item questionnaire, and participants designated as unaware ($n = 14$) obtained a score of 8.6 ± 0.4, $t(18) = 8.0$, $p < .001$, which was no better than the chance score of 8.5, $t(13) = 0.4$, $p > .70$. Nevertheless, both groups performed similarly in the conditioning session. Across all 120 trials, the aware group emitted 11.9% ± 3.9% differential CRs, and the unaware group emitted 12.4% ± 3.6% differential CRs. Both these scores were well above zero (ts > 3.0, ps < .05). An ANOVA (Group × Block) revealed a linear effect of training block, $F(1, 18) = 7.1$, $p < .05$, but no effect of group and no interaction (Fs < 1.0). Both aware and unaware groups did well at answering questions about the silent movie (9.8 vs. 9.4 items correct, respectively). Lastly, there was no correlation between awareness and average differential conditioning scores ($r = -.004$, $p > .90$).

The results were the same when different criteria were used to designate participants as aware or unaware. Thus, with the more stringent criterion (unaware being defined as ≤ 9 questions correct), 11 aware participants and 9 unaware participants emitted 11.0% ± 3.7% and 13.8% ± 4.2% differential CRs, respectively, $t(18) = 0.5$, $p > .60$. Both these scores were above zero (ps < .05). When only the 8 (out of 17) questions that directly assessed knowledge of the CS–US relationship were used to classify participants (unaware being defined as ≤ 4 questions correct), 10 aware and 10 unaware participants emitted 13.8% ± 3.0% and 10.7% ± 4.7% differential CRs, respectively, $t(18) = 0.6$, $p > .50$. Both these scores were also above zero (ps < .05).

Experiment 3

Experiment 2 showed that awareness of stimulus contingencies was unrelated to differential delay eyeblink conditioning even when two tones (800 Hz and 2100 Hz) served as CS+ and CS−. In this study and most of our earlier studies (Clark & Squire, 1998, 1999), the participants were middle-aged or older, often averaging more than 60 years of age. In contrast, in the earlier study that used two tones as CS+ and CS− (Nelson & Ross, 1974), the participants were undergraduate students. Perhaps younger participants readily become aware of the stimulus contingencies and, as a result of becoming aware, condition more successfully than participants who do not become aware. Experiment 3 explored this possibility by testing undergraduates.

Method

Participants—The participants (9 men, 10 women) were undergraduates at the University of California, San Diego, CA, who received class credit for participating. They averaged 20.4 years of age (range: 19–25 years).

Procedure—The procedure was the same as for Experiment 2, except that we interviewed the participants in some detail about the stimulus contingencies after they took the questionnaire. We had noted in Experiment 2 that a small number of participants reported that they knew that one tone (but not the other) predicted the airpuff but did not know which tone was which (this problem did not arise in Experiment 1 because one CS was a tone and the other was white noise). Depending on how such participants completed the questionnaire, they could be designated as either aware or unaware. Accordingly, on the basis of the postquestionnaire interview in Experiment 3, we analyzed the results both with and without the data from those participants who had knowledge of stimulus contingencies but were confused about which tone was the CS+.

Results

Figure 3A shows the percentage of differential CRs emitted by participants across 120 conditioning trials. Participants designated as aware ($n = 12$) obtained a mean score of 16.3 ± 0.5 on the 17-item questionnaire, and participants designated as unaware ($n = 7$) obtained a score of 8.5 ± 1.5, $t(17) = 9.2$, $p < .001$, which was no better than the chance score of 8.5, $t(6) = 0.4$, $p > .60$. Nevertheless, both groups performed similarly in the conditioning session. Across all 120 trials, the aware group emitted 13.0% ± 3.3% differential CRs, and the unaware group emitted 16.8% ± 3.2% differential CRs. Both these scores were well above zero (ts > 3.9, ps < .01). An ANOVA (Group × Block) revealed no effect of group and no interaction (Fs < 1.0). There was also no effect of training block for these young participants ($F < 1.0$) because rapid acquisition of differential responding occurred within the first block of training (see Figure 3B). Indeed, an ANOVA of the data from the first 20 trials (averaged in 4-trial blocks) did reveal a linear effect of training block, $F(1, 17) = 9.5$, $p < .01$. Both aware and unaware groups did well at answering questions about the silent movie (9.8 vs. 9.6 items correct, respectively). Lastly, there was no correlation between awareness and average differential conditioning scores (*r* = −.15, *p* > .90).

The results were similar when different criteria were used to designate participants as aware or unaware. Thus, with the more stringent criterion (unaware being defined as ≤ 9 questions correct), 16 aware participants and 3 unaware participants emitted 13.7% ± 2.6% and 18.3% ± 6.0% differential CRs, respectively, *t*(17) = 0.7, *p* > .40. When only the 8 (out of 17) questions that directly assessed knowledge of the CS–US relationship were used to classify participants (unaware being defined as ≤ 4 questions correct), 15 aware and 4 unaware participants emitted 13.6% ± 2.8% and 17.4% ± 4.3% differential CRs, respectively, *t*(17) = 0.6, *p* > .50. These two scores were above zero (*p*s < .05).

Lastly, 2 participants who had been designated as unaware on the basis of the 17-item questionnaire reported that they were aware that one tone (but not the other) predicted the airpuff but were confused about which tone was which. When these 2 participants were eliminated from the data analysis, the results were unchanged. Across the different criteria for designating participants as aware or unaware, the average percentage of differential CRs across all trials did not change by more than 3.7%. Also, across all trials the average percentage of differential CRs emitted by the aware group and the average percentage of differential CRs emitted by the unaware group were always within 2.2% of each other.

Experiment 4

Experiment 3 demonstrated that awareness was unrelated to differential delay eyeblink conditioning even in young participants. Thus, neither the stimuli used as CS+ and CS− (Experiment 2) nor the age of the participants (Experiment 3) can explain the earlier finding (Nelson & Ross, 1974) that awareness was an important factor in differential delay eyeblink conditioning. Because there were other aspects of this earlier study that also distinguished it from our own studies (Clark & Squire, 1998, 1999) and from the present series of experiments (specifically, CS intensity and duration, US intensity, and ITI), Experiment 4 was designed to match the procedures of the earlier study (Nelson & Ross, 1974) as closely as possible.

Method

Participants—The participants (9 men, 16 women) were undergraduates at the University of California, San Diego, CA, who received class credit for participating. They averaged 20.1 years of age (range: 18–22 years).

Procedure—The procedure was the same as for Experiment 3, except that the CS intensity and duration, US intensity, and ITI were altered to match those in the earlier study (Nelson & Ross, 1974). The CSs were 900-ms, 85-db tones (800 Hz and 2100 Hz), and the US was a 100-ms, 0.75-psi airpuff (800-ms interstimulus interval). The ITI varied between 15, 20, and 25 s.

CRs were scored as in Experiments 1–3, except that in order to match the scoring procedures reported by Nelson and Ross (1974), we scored eyeblinks as CRs only if they occurred between 200 ms after the onset of the CS and before the onset of the US.

Results

Figure 4 shows the percentage of differential CRs emitted by participants across 120 conditioning trials. Participants designated as aware (*n* = 19) obtained a mean score of 15.1 ± 0.3 on the 17-item questionnaire, and participants designated as unaware (*n* = 6) obtained a score of 9.3 ± 0.7, *t*(23) = 8.3, *p* < .001, which was no better than the chance score of 8.5, *t*(5) = 1.3, *p* > .20. Conditioning was more modest in Experiment 4 compared to Experiments 1–3. Across all 120 trials, the aware group emitted 7.5% ± 3.7% differential CRs, and the unaware group emitted 3.3% ± 2.0% differential CRs. Differential CRs for aware participants were marginally greater than zero, $t(18) = 2.0$, $p = .06$, but differential CRs for unaware participants were not, $t(5) = 1.7$, $p = .16$. Lastly, an ANOVA (Group × Block) revealed a marginally significant linear effect of training block, $F(1, 23) = 3.4$, $p = .08$, but no effect of group and no interaction (*F*s < 1.0). Thus, there was no difference between the performance of the aware and unaware groups. Lastly, there was no correlation between awareness and average differential conditioning scores ($r = .23$, $p > .20$).

Because the unaware group performed poorly during the first block of 20 trials (−10.2% CRs), we also analyzed performance across Blocks 2–6. Across Blocks 2–6, the aware group emitted 8.8% ± 3.8% differential CRs, and the unaware group emitted 6.3% ± 2.7% differential CRs. Again, there was no difference between the aware and unaware groups ($F < 1.0$). Differential CRs for aware participants were greater than zero, $t(18) = 2.3$, $p < .05$, while differential CRs for unaware participants were marginally greater than zero, $t(5) = 2.3$, $p = .07$, perhaps because of the small number of participants in the latter group. Both aware and unaware groups did well at answering questions about the silent movie (9.3 vs. 9.3 items correct, respectively).

The results were similar when different criteria were used to designate participants as aware or unaware. Thus, with a more stringent criterion (unaware being defined as ≤ 9 questions correct), 22 aware participants and 3 unaware participants emitted 7.2% ± 3.2% and 1.0% ± 2.1% differential CRs, respectively, during Blocks 1–6, $t(23) = 0.7$, $p > .40$. When only the eight (out of 17) questions that directly assessed knowledge of the CS–US relationship were used to classify participants (unaware being defined as ≤ 4 questions correct), 20 aware and 5 unaware participants emitted 7.5% ± 3.6% and 2.4% ± 0.5% differential CRs, respectively, $t(23) = 0.7$, $p > .40$. These scores were above zero (*p*s < .05).

Lastly, 6 participants (5 designated as aware and 1 designated as unaware) were confused about which tone predicted the airpuff. When these participants were eliminated from the data analysis, the results were unchanged regardless of which method was used to designate participants as aware or unaware (averaged across all trials, the differential CRs were within 0.4% of the scores when these 6 participants were included).

Discussion

In four experiments, we found that differential delay conditioning was independent of awareness of the relationship between the CS+, CS−, and the US. Experiment 1 involved older participants and used a tone and white noise as CS+ and CS−. Those participants designated as aware and unaware exhibited equivalent levels of conditioning. In addition, a reanalysis of data from our earlier study (Clark & Squire, 1998) confirmed that aware and unaware participants exhibited equivalent levels of conditioning. Experiment 2, also with older participants, yielded the same result when two tones were used as CS+ and CS−. Experiment 3 tested younger participants and also found that awareness was not related to conditioning when two tones were used as CS+ and CS−. Experiment 4 obtained similar results in younger participants when the methods used by Nelson and Ross (1974) were followed as closely as possible.

The results from Experiment 1 are entirely consistent with our initial report that differential delay conditioning is unrelated to awareness (Clark & Squire, 1998). Yet they differ from the results of a similar study (Knuttinen et al., 2001). That study found that neither older nor younger participants who were trained with a tone and white noise successfully acquired differential CRs if they remained unaware of stimulus contingencies. In fact, neither the older nor younger unaware groups exceeded 10% differential CRs across 120 conditioning trials. The discrepancy between these findings and the findings from Experiment 1 (as well as Clark

& Squire, 1998) is a puzzle because the two studies employed the same methods for the most part.

One difference between the studies was that the CSs were presented at 75 db in Knuttinen et al. (2001) and at 95 db in Experiment 1. Another difference is that Knuttinen et al. administered 30 pseudoconditioning trials prior to the conditioning session, whereas we administered only 6–9 of such trials in Experiment 1. Lastly, Knuttinen et al. used two devices to measure eyeblinks. In addition to the infrared reflective sensor that they used to measure eyeblinks (as in Experiment 1 of the present study), Knuttinen et al. also attached electromyography (EMG) electrodes above each participant's right eye. The infrared reflective sensor was attached to the airpuff delivery device and therefore would not have directed participants' attention to their eyeblink responses. In contrast, perhaps the EMG electrodes near the eye directed attention toward the eyeblink responses themselves and interfered with the automatic development of differential CRs.

It is interesting to note that Knuttinen et al. (2001) reported that the failure of their unaware participants to discriminate between the CS+ and CS− was due to a failure to respond to either CS. Yet according to previous studies, failure to exhibit differential conditioning typically occurs because CRs develop to both the CS+ and the CS− rather than only to the CS+ (Carrillo, Gabrieli, & Disterhoft, 2000; Nelson & Ross, 1974). In these cases, distraction reduced differential conditioning but did not prevent the acquisition of CRs. These observations appear consistent with the idea that some factor in the study by Knuttinen et al. (e.g., the presence of EMG electrodes) might have interfered with the production of CRs. One way to interpret the data is to suppose that conditioning was poor in all participants and that aware participants appeared to produce differential CRs because they emitted voluntary eyeblinks that were scored as true CRs.

True CRs are reflexive, nonvolitional responses. Nonetheless, an eyeblink response can be brought under voluntary control. If individuals become aware of the stimulus contingencies, they are in a position to blink their eyes voluntarily in order to avoid the airpuff. This type of response could be disproportionately represented in aware participants. Voluntary eye closures have been described as short-latency responses involving complete eye closures that are maintained until the onset of the US (Spence & Ross, 1959; Spence & Taylor, 1951). We have endeavored to identify and exclude voluntary eye closures by visually examining every eyeblink waveform and excluding those responses that began earlier than 500 ms before the US and that persisted until the US was presented. Knuttinen et al. (2001) did not discuss voluntary responses, and one cannot determine if such responses were a factor in their study. We note only that the average CRs illustrated in their Figure 3 began more than 700 ms before the US and persisted until the US. By our criteria, individual responses with these characteristics would have been excluded from analysis. If voluntary eye closures were frequent and were sometimes scored as CRs, then aware individuals (who would be capable of voluntary eye closures) would likely perform better than unaware individuals (who would not exhibit voluntary eye closures).

In any case, other studies from some of these same investigators suggest that awareness is not important for differential delay eyeblink conditioning. First, amnesic patients, who would be expected to have difficulty developing and maintaining awareness, nevertheless acquired normal levels of CRs during single-cue delay conditioning (Gabrieli et al., 1995). Second, Carrillo et al. (2001) reported that amnesic patients exhibited normal differential delay conditioning and that their performance was unrelated to awareness. It is also of interest that rabbits with hippocampal lesions were entirely normal at acquiring two-tone differential delay eyeblink conditioning (Berger & Orr, 1983).

In the present study, differential conditioning was unrelated to awareness when two tones were used as CS+ and CS− (Experiments 2 and 3). It is interesting to note that the level of conditioning achieved in Experiments 2 and 3 was lower than in Experiment 1 when a tone and white noise served as CS+ and CS−. Thus, differential responding in Experiment 1 was 21.9% across 120 trials, but differential responding in Experiment 2 (older participants) and in Experiment 3 (younger participants) was only 12.2% and 14.1%, respectively: Experiment 1 vs. Experiments 2 and 3, $t(49) = 2.26$, $p < .05$. This difference was driven by the greater number of CRs to the CS+ in Experiment 1 than in Experiments 2 and 3 (the number of CRs to the CS− was comparable across experiments). Apparently, it is more difficult to discriminate between two tones than to discriminate between a tone and white noise.

The results of Experiment 4 were similar to the results of Experiments 1–3 in that there was no difference in the average number of differential CRs emitted by participants designated as aware and participants designated as unaware. Nevertheless, only modest levels of conditioning were achieved by aware and unaware participants, and it remains possible that a difference between aware and unaware groups could have been detected if more robust conditioning had been achieved. Thus, the percentage of differential CRs for the 19 aware participants (7.5%) was greater than zero ($p < .06$), whereas the percentage of differential CRs for the 6 unaware participants (3.3%) was not. Note, though, that when differential CRs were averaged across Blocks 2–6 (instead of Blocks 1–6), the percentage of differential CRs for unaware participants (6.3%) was marginally greater than zero ($p = .07$; for aware participants, 8.8%, $p < .05$). In any case, even in Experiment 4, performance of the aware and unaware groups was not significantly different in any comparison.

In the study by Nelson and Ross (1974) (on which Experiment 4 was based) there were also many more aware than unaware participants (17 vs. 7), and the percentage of differential CRs emitted by unaware participants (12.5%) was not significantly greater than zero (as in our Experiment 4). It is also true that Nelson and Ross did not report whether their aware participants exhibited significantly more conditioning than their unaware participants (21.6% vs. 12.5% differential CRs). Accordingly, some question remains about how much better their aware participants performed in comparison with their unaware participants and, correspondingly, how different their results were in comparison with ours.

These issues notwithstanding, our results do seem to differ from what was reported by Nelson and Ross (1974) in one respect. We observed relatively low levels of conditioning in both aware and unaware participants (7.5% vs. 3.3%; or for Blocks 2–6, 8.8% vs. 6.3%). Nelson and Ross observed higher levels of conditioning (21.6% for aware participants and 12.5% for unaware participants). Note too that the methods used in our Experiment 4 did not match the methods used by Nelson and Ross in every respect. Their participants were told that the purpose of the study was to “measure their *reactions* [italics added] to certain events” (p. 3), whereas in our Experiment 4, participants were told that the purpose of the study was to test the effects of distraction on memory. Also, Nelson and Ross used a potentiometer to measure eyeblinks, which involved adhering a thread to each participant's eyelid, whereas in our Experiment 4 an infrared reflective sensor was attached to the airpuff delivery device and did not make contact with the participant. Perhaps these differences were important. Again, however, it remains unclear how different the results of their study and our study actually are with respect to the importance of awareness for conditioning.

Our main finding across four experiments was that aware and unaware participants achieved similar levels of conditioning. This finding did not depend on the method used to classify participants as aware or unaware. Lovibond and Shanks (2002) suggested that too lenient a criterion for classifying participants as aware or unaware can lead to a misclassification of some aware participants as unaware and might thereby conceal differences in conditioning between aware and unaware participants. In particular, they suggested that classifying participants as unaware who score < 13 correct on a 17-item test is too lenient. They also questioned whether items on the 17-item test that did not directly assess knowledge of the relationships between the CS+ and the US and the CS− and the US were relevant to the kind of awareness that needed to be assessed.

In the present studies, we classified participants as aware or unaware in three different ways. First, we classified participants as unaware in the traditional way, that is, if they scored < 13 correct on the 17-item test (a score of ≥ 13 correct is significantly above chance, $p < .05$). In this way, we could compare our results with what we found in previous studies (Clark & Squire, 1998, 1999). Second, following the suggestion of Lovibond and Shanks (2002), we classified participants as unaware only if they scored ≤ 9 correct on the 17-item test (chance = 8.5). Third, again following Lovibond and Shanks, we classified participants as unaware according to their responses to the eight questions that asked directly about the relationship between the CS+ and US or the CS− and the US (participants were classified as unaware if they scored ≤ 4 correct). In all four experiments, regardless of which method was used to classify participants as aware or unaware, the findings were the same. There were no differences in the average differential CRs produced by aware and unaware groups. Finally, in Experiments 3 and 4, a supplemental structured interview was given in addition to the 17-item questionnaire in order to improve our estimate of awareness and unawareness. When we excluded from the analysis those participants who in fact had knowledge of the stimulus contingencies but were confused about which tone was the CS+ and which was the CS−, the results were the same as when those participants were included. Lastly, no correlations were found between awareness scores and differential conditioning scores.

In summary, the results from Experiments 1–4 indicate that the acquisition of differential delay eyeblink conditioning is unrelated to awareness. Thus, the findings for differential delay eyeblink conditioning and awareness are the same as the findings for single-cue delay eyeblink conditioning and awareness (Manns et al., 2001, 2002; Papka et al., 1997). This conclusion is restricted to standard differential delay conditioning with simple stimuli such as a tone and white noise or two different tones. The results are different when discrimination between the CS+ and CS− is more difficult (such as when the CSs differ in grammaticality; Baer & Fuhrer, 1982; Perry et al., 1977), when the stimulus contingencies undergo reversal (Carrillo et al., 2001), or in more complex conditioning paradigms such as conditional discrimination (Bellebaum & Daum, 2004; Daum, Channon, & Canavan, 1989; Daum, Channon, Polkey, & Gray, 1991; Fortier et al., 2003; for further discussion, see Clark & Squire, 2004). In these cases, awareness appears to be important for successful conditioning. In addition, as demonstrated repeatedly, awareness of stimulus contingencies is also important in both single-cue and differential trace-conditioning paradigms (Clark & Squire, 1998, 1999; Knuttinen et al., 2001; Manns, Clark, & Squire, 2000a, 2000b).

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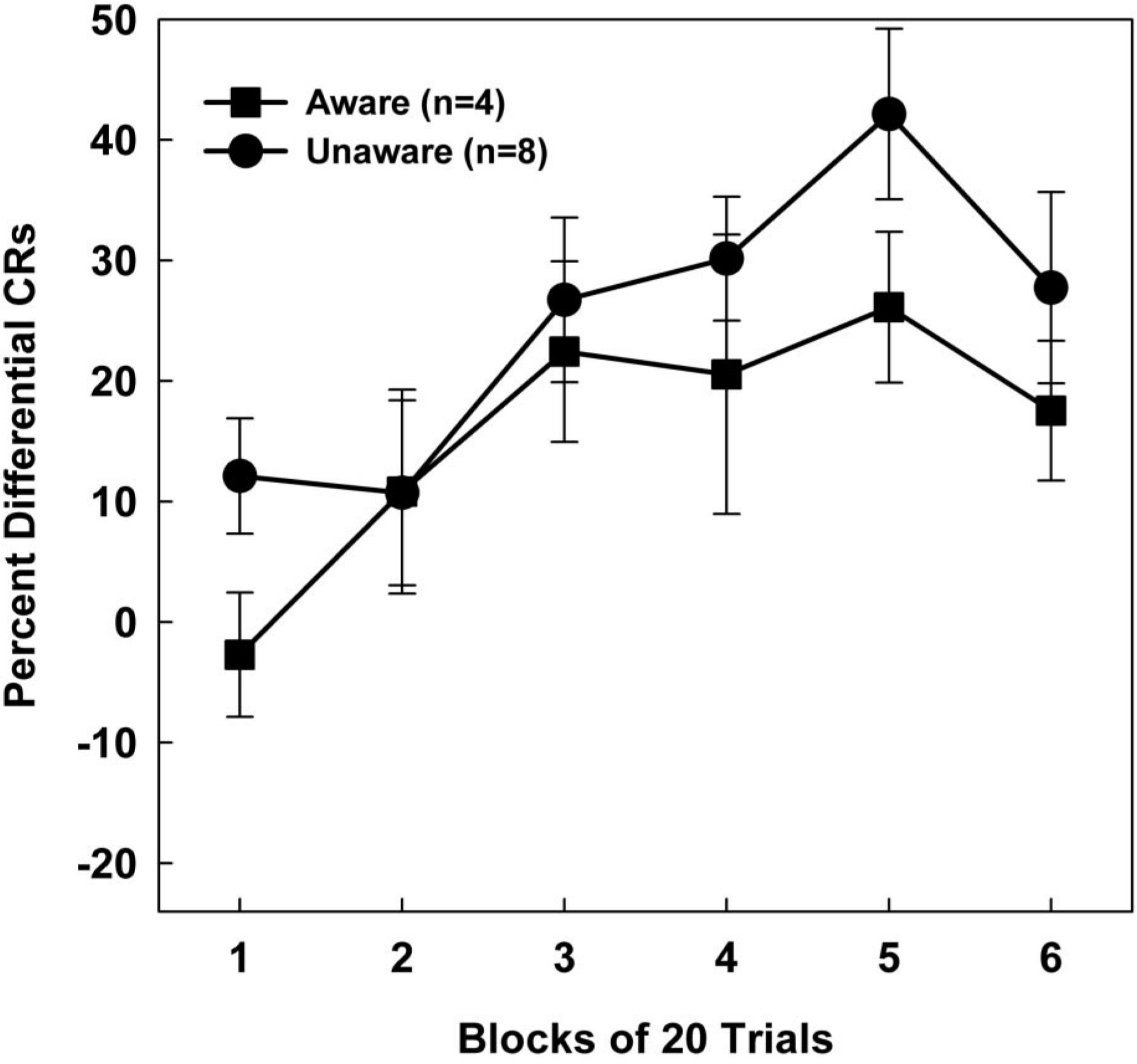

Figure 1.

Experiment 1. Percentage of differential CRs for each block of 20 trials (percentage of CRs to the CS+ minus percentage of CRs to the CS−). Participants were designated as aware or unaware on the basis of a questionnaire that asked about the relationship between the CS+, CS −, and unconditioned stimulus (mean age = 49 years). The conditioned stimuli were a tone and white noise. Error bars show the standard error of the mean. CR = conditioned response; CS+ = conditioned stimulus followed by the unconditioned stimulus; CS− = conditioned stimulus not followed by the unconditioned stimulus.

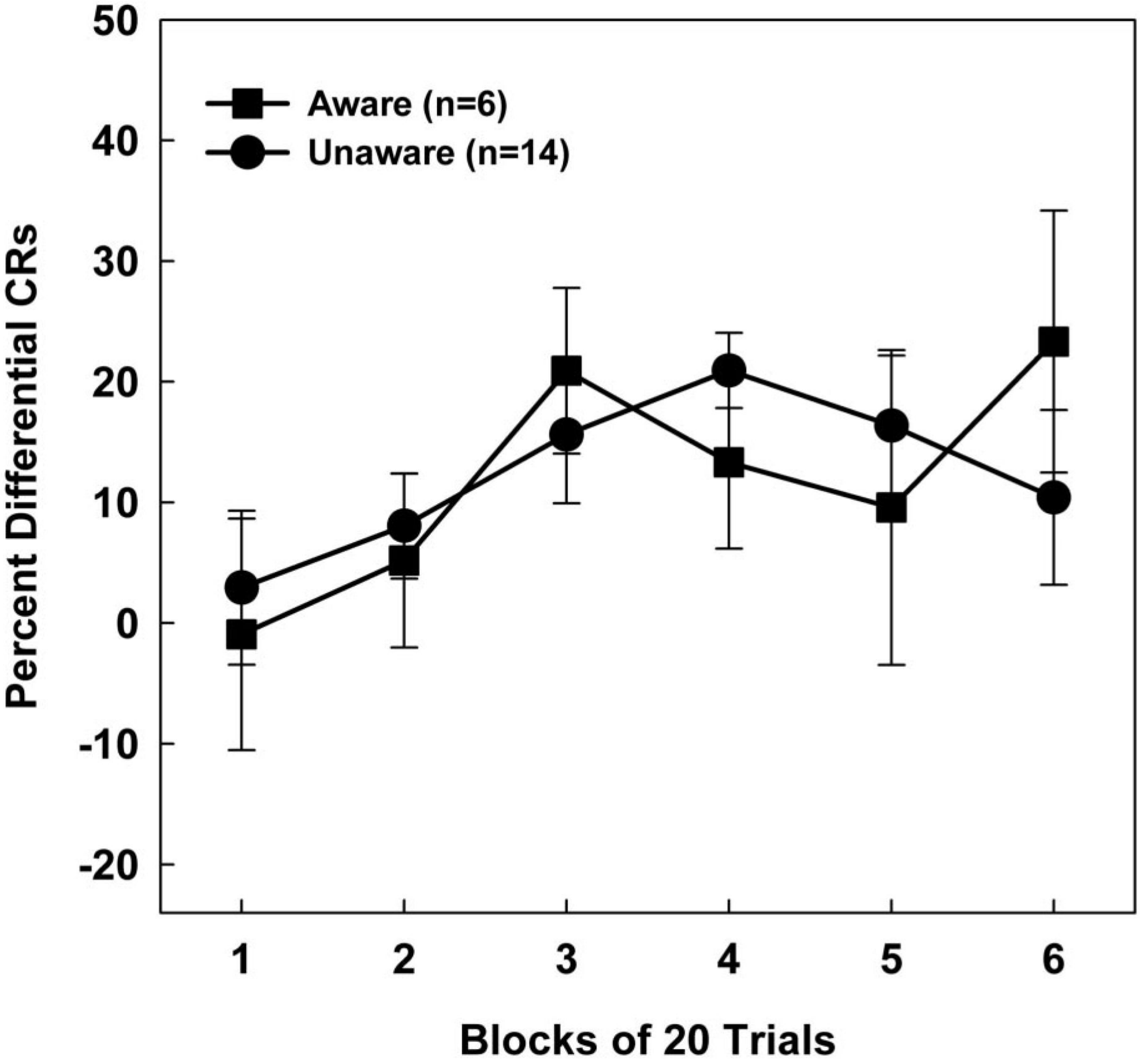

Figure 2.

Experiment 2. Percentage of differential conditioned responses (CRs) across six blocks of 20 trials for participants designated as aware or unaware (mean age = 58 years). The conditioned stimuli were an 800-Hz and a 2100-Hz tone. Error bars show the standard error of the mean.

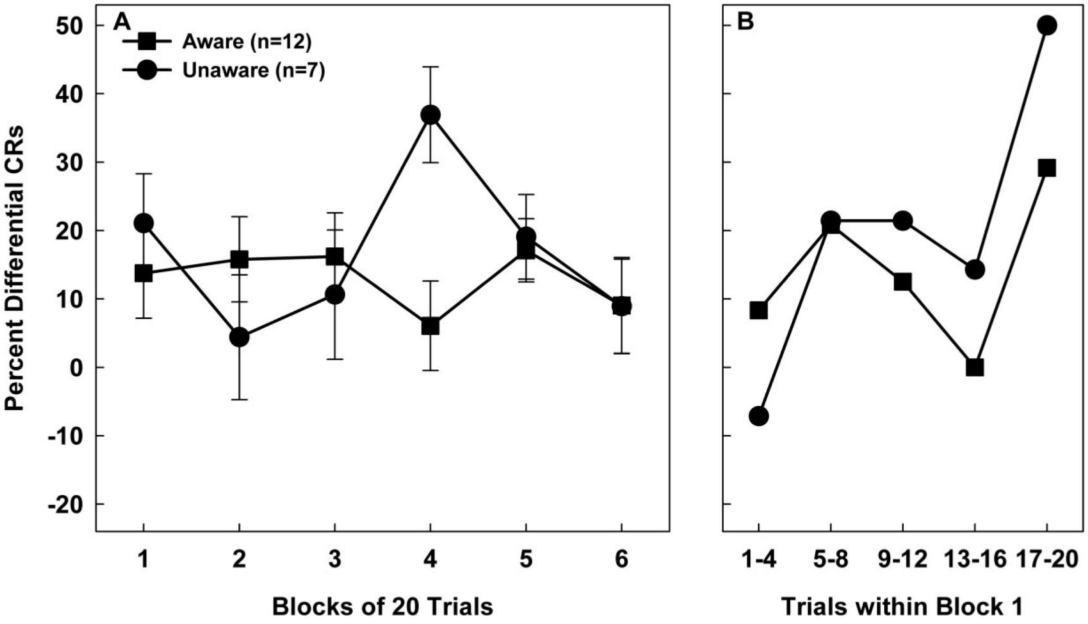

Figure 3.

Experiment 3. A: Percentage of differential conditioned responses (CRs) across six blocks of 20 trials for participants designated as aware or unaware (mean age = 20 years). The conditioned stimuli were an 800-Hz and a 2100-Hz tone. Error bars show the standard error of the mean. B: Percentage of differential CRs during the first 20 trials.

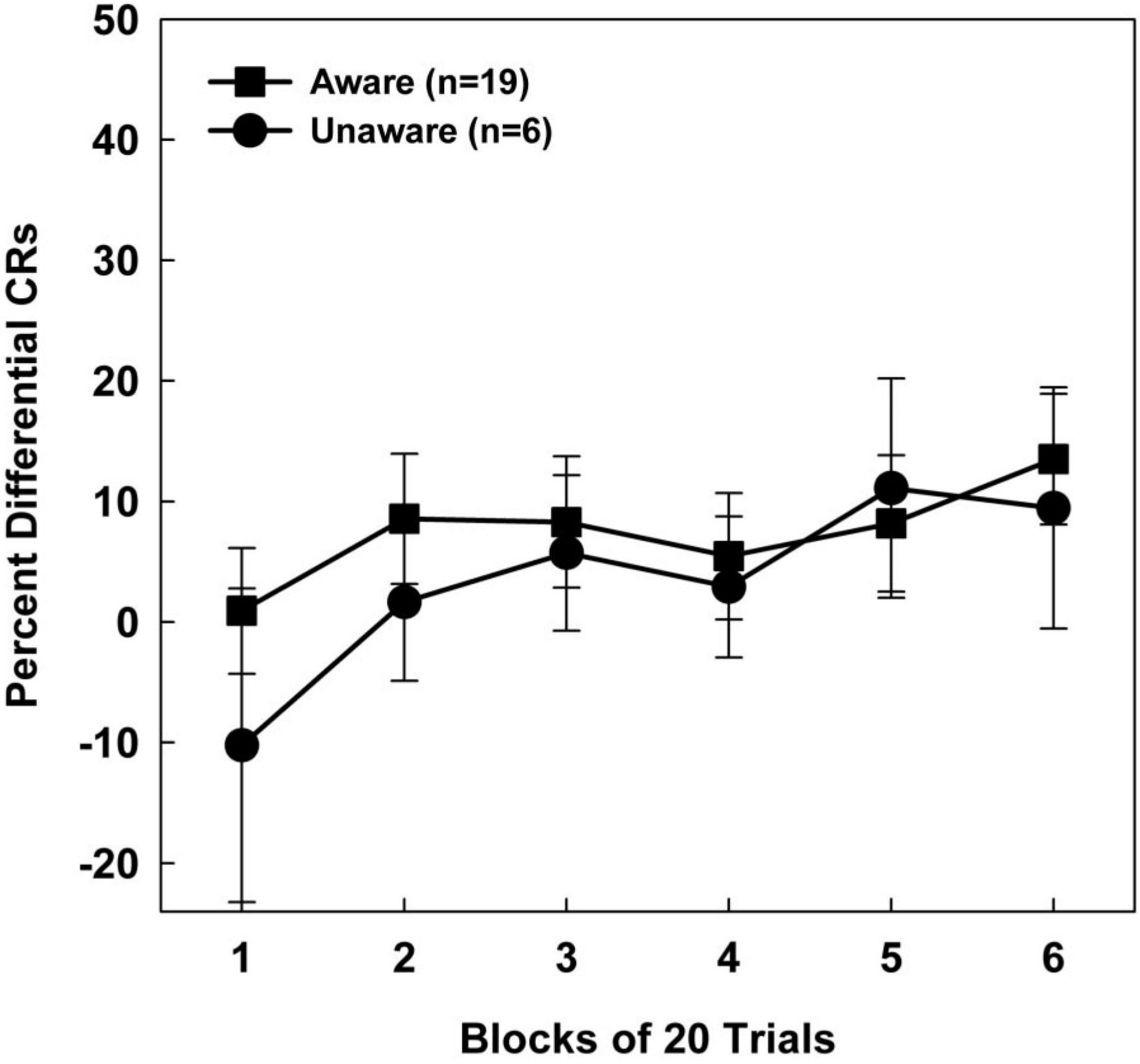

Figure 4.

Experiment 4. Percentage of differential conditioned responses (CRs) across six blocks of 20 trials for participants designated as aware or unaware (mean age = 20 years). Conditioned stimulus (CS) duration and intensity, unconditioned stimulus (US) intensity, and intertrial interval were as described by Nelson and Ross (1974). The CSs were an 800-Hz and a 2100-Hz tone. Error bars show the standard error of the mean.