Parallel acquisition of awareness and differential delay eyeblink conditioning

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There is considerable debate about whether differential delay eyeblink conditioning can be acquired without awareness of the stimulus contingencies. Previous investigations of the relationship between differential-delay eyeblink conditioning and awareness of the stimulus contingencies have assessed awareness after the conditioning session was finished using a post-experimental questionnaire. In two experiments, the point at which contingency awareness developed during the conditioning session was estimated from a button-press measure of expectancy of the unconditioned stimulus (US). In both experiments, knowledge of the stimulus contingencies and acquisition of differential delay eyeblink conditioning developed approximately in parallel. In Experiment 1 it was shown that predicting the US facilitated eyeblink conditioning compared with predicting the eyeblink response. In Experiment 2, a masking task was used that slowed down the emergence of awareness, and it was shown that differential conditioning only occurred in participants who were able to predict the US. The current findings challenge the hypothesis that differential delay eyeblink conditioning is entirely mediated by a functionally and neurally distinct nondeclarative learning system.

It is widely believed that memory is composed of several different abilities that depend on different brain systems (Squire 1992; Schacter and Tulving 1994; Eichenbaum and Cohen 2001). One popular distinction is between memory that can be consciously recalled (declarative memory) and nonconscious memory that supports skill and habit learning (nondeclarative memory). In the case of Pavlovian conditioning, which is experience dependent and expressed through performance rather than recollection, it is commonly assumed that there are two independent learning systems in operation (Reber and Squire 1994; Clark and Squire 1998). First, there is said to be a declarative learning system that is associated with conscious knowledge of the relationship between the conditioned stimulus (CS; e.g., a tone) and the unconditioned stimulus (US; e.g., an airpuff to the eye). Second, there is said to be a more primitive nondeclarative learning system that leads to the production of conditioned responses (CRs, e.g., an eyeblink) by the automatic formation of excitatory links between nodes representing the CS and the US. Because these two learning processes operate independently, it is postulated that CR production can occur without conscious awareness of the CS-US contingency. However, empirical reviews on human associative learning have shown that there is relatively little evidence for this dual-process model (Brewer 1974; Dawson and Schell 1985; Boakes 1989; Lovibond and Shanks 2002; Mitchell et al. 2009). By and large, conditioned responding is closely associated with awareness of the contingency, is sensitive to verbal and reasoning manipulations, and is impaired by cognitive load.

However, perhaps the strongest evidence in favor of a dual-process model of Pavlovian conditioning comes from a series of human eyeblink conditioning studies by Larry Squire, Robert Clark, and colleagues that use a delay procedure (in which the CS precedes, overlaps, and coterminates with the US). They have shown that contingency knowledge, that the CS predicts the US, appears to be unrelated to acquisition of differential delay conditioning in which one conditioned stimulus (CS+) is paired with the US, and a second conditioned stimulus (CS−) is presented alone (Clark and Squire 1998; Smith et al. 2005). In contrast, these same researchers have found that trace conditioning (in which a temporal gap separates the termination of the CS from the onset of the US) is only observed in those participants who are aware of the CS-US contingency (Clark and Squire 1998; Manns et al. 2000a,b). Clark and Squire have interpreted these results as supporting a dual-process model of eyeblink conditioning, in which trace conditioning relies on declarative knowledge that is mediated by the hippocampus and the neocortex and delay conditioning relies on procedural knowledge, which can be learned in an automatic and reflexive manner by cerebellar circuits (Clark and Squire 1998).

While the pattern of results reported by the Clark and Squire's group provides support for this dual process model, they are at odds with a number of other eyeblink conditioning studies, particularly with respect to the relationship between awareness and differential delay eyeblink conditioning. Previous studies of differential delay eyeblink conditioning have found that only contingency-aware participants are able to acquire differential conditioning (Nelson and Ross 1974; Perry et al. 1977; Benish and Grant 1980; Knuttila and Squire 1998). Manns et al. (2002) attempted to explain these differences by pointing out that there are some significant differences in the methodologies between their studies and the previous research, such as the complexity of the stimuli used, the way in which eyeblinks were measured, and whether voluntary responses were excluded from the analyses. However, subsequent research from our laboratory aimed at addressing these methodological differences has found differential delay conditioning only occurred in participants who were contingency aware, and not in participants who were unaware (Lovibond et al. 2011). It may seem that this failure to observe differential delay conditioning in unaware participants represents a null result and, hence, should be treated with caution. However, in fact, the reverse is true. The research is designed to assess whether there is a relationship between awareness and conditioning, and the null result is no association—the pattern that was
found by Clark and Squire (1998) and Smith et al. (2005). In principle, their null result could have been produced by low power, insensitive measures, or extraneous sources of variance.

One of the features of all of the aforementioned studies is their reliance on the post-experimental questionnaire as their measure of contingency awareness. Because a post-experimental questionnaire is temporally removed from the conditioning session, it is not the most precise measure of awareness. In fact, it may be the reliance on the post-experimental questionnaire to assess contingency awareness, which resulted in the contradictory findings between studies. In some studies it is possible that a number of the contingency-aware participants were misclassified as contingency unaware, thus resulting in the conclusion of unaware conditioning. Indeed, Dawson and Reardon (1973) found that using a long post-experimental questionnaire systematically underestimated contingency awareness. In addition to being a less precise measure of awareness, a post-experimental questionnaire does not allow one to determine the temporal relationship between the acquisition of awareness and the acquisition of differential responding.

To date, there have been only two studies in the eyeblink conditioning that have used an online measure of awareness, one with a delay arrangement of cues (Perry et al. 1979) and one with a trace arrangement of cues (Manns et al. 2000b). Both of these studies found a strong relationship between the emergence of contingency awareness and differential eyeblink responding. However, the study with a delay conditioning procedure used complex conceptual stimuli such as CSs, which Manns et al. (2002) argue would require awareness for participants to decipher what distinguished the CS+ and the CS−. Therefore, the aim of the present experiments was to assess the relationship between differential delay eyeblink conditioning, with simple auditory stimuli as CSs, and the emergence of contingency awareness using an online measure of contingency awareness. This will enable awareness to be classified more accurately, as well as determine approximately when during the conditioning session participants became aware, and whether differential conditioning appeared before or thereafter. Consequently, it should allow for better temporal resolution of the relationship between the acquisition of awareness and the acquisition of differential delay conditioning.

**Experiment I: Predicting airpuff and predicting eyeblink**

One of the difficulties of using an online measure of contingency awareness during conditioning is that it influences the likelihood of participants becoming aware by drawing their attention toward the contingency. In order to adequately assess the relationship between awareness and conditioned responding, it is essential to assess conditioning as a function of a range of different time points for the emergence of awareness. To be able to see evidence for conditioning prior to the point of awareness, it is desirable to slow down the emergence of awareness to allow time for conditioned responding to emerge as a function of CS–US pairings. Conversely, if conditioned responding is related to awareness, then slowing down the emergence of awareness should also slow down the emergence of conditioned responding. Previous research has shown that asking participants to predict their blink response has the effect of slowing down the development of awareness, because participants tend not to become aware of the link between their eyeblink and the CS (Manns et al. 2000b). Consequently, Manns et al. (2000b) compared the development of conditioned responding in a differential trace conditioning procedure among participants instructed to press a button whenever they expected the airpuff (Predict Airpuff group) or whenever they believed they were about to blink (Predict Eyeblink group). They found that asking participants to predict the onset of US promoted awareness and facilitated conditioning compared with asking participants to predict their eyeblink. Experiment 1 was designed to replicate the essential procedure used by Manns et al. (2000b), but using a delay rather than a trace-conditioning procedure.

**Results**

To examine the development of the online measure of contingency knowledge across the entire conditioning session we compared the mean percentage of button presses made with the CS+ and CS− across the six blocks for both prediction groups (see Fig. 1). Both groups showed considerably greater button presses to the CS+ than to the CS−. This was confirmed by a significant main effect for CS type across both groups, \( F_{(1,36)} = 96.66, P < 0.05 \). This pattern of predictive button pressing increased in magnitude across successive blocks of trials, which was confirmed by a significant interaction for CS type and the linear trend across blocks, \( F_{(1,36)} = 18.16, P < 0.05 \). However, from Figure 1 it is apparent that there is greater differentiation in responding to CS+ and CS− for participants in the Predict Airpuff group compared with those in the Predict Eyeblink group. This is confirmed by a significant interaction between CS Type (CS+ vs. CS−) and Prediction group, \( F_{(1,36)} = 9.54, P < 0.05 \). Therefore, those participants asked to predict the airpuff quickly became aware of the differential signaling relationship between the CS+ and the CS− and the airpuff. Likewise, those participants asked to predict their eyeblink also largely became aware of a signaling relationship between the CS+ and CS− and their blink response, but not to the same degree as for those asked to predict the airpuff. This result is somewhat at odds with the findings of Manns et al. (2000b) who found that participants asked to predict their eyeblink did not become aware of the signaling relationship between the CS+ and CS− and their blink response.

To determine whether asking people to predict the airpuff or their eyeblink influenced CRs, we compared the percentage of CRs made with the CS+ and CS− over the six blocks of trials in the two prediction groups (see Fig. 2). From Figure 2 it can be seen that there was considerable differential responding in both groups. This was confirmed by a significant main effect for CS type, \( F_{(1,36)} = 68.55, P < 0.05 \). In addition, the CS Type interacted with linear trend over blocks, \( F_{(1,36)} = 7.18, P < 0.05 \), indicating that there was a progressive emergence of discrimination between

**Figure 1.** Mean percentage of button presses made to the CS+ and CS− on each of the six blocks of 20 trials in Experiment 1. Data for participants in the Predict Airpuff group are shown at left, and data for participants in the Predict Eyeblink group are shown at right. (CS+) Stimulus that was paired with the unconditional stimulus; (CS) stimulus that was presented alone.
CS+ and CS− over blocks. However, from Figure 2 it can also be seen that the degree of discrimination responding was much greater in the Predict Airpuff group than in the Predict Eyeblink group. Statistical analysis of the interaction between CS Type and Prediction group confirmed that this was a statistically significant result, $F_{1,360} = 6.09, P < 0.05$. Therefore, asking participants to predict the airpuff facilitated differential CRs to the CS+ and CS− compared with asking participants to predict their eyeblink. This result suggests that the emergence of differential eyeblink conditioning is not entirely independent of the cognitive processes occurring during conditioning, evidence that is at odds with a dual-system model of delay eyeblink conditioning, which would predict that focusing on the stimulus contingency would not affect conditioning.

To examine the relationship between awareness and conditioning at the participant level, we began by using Clark and Squire’s post-experimental questionnaire and awareness criterion to define awareness. Using this measure, there were a total of 32 aware participants and five unaware participants. Of the unaware participants, three were from the Predict Eyeblink group and two were from the Predict Airpuff group. A comparison of the awareness scores between groups revealed no difference between the groups on their scores on the post-experimental questionnaire, $t < 1$. This finding is in contrast to the findings of Manns et al. (2000b), who found that asking participants to predict their eyeblink substantially reduced their level of contingency knowledge compared with a group asked to predict the airpuff when assessed post-experimentally with the same questionnaire. The fact that the Predict Eyeblink participants in the present study largely became aware of the stimulus contingency may have influenced their propensity to become aware of the signaling relationship between the CS+ and CS− and their blink response, or vice versa. Regardless, asking people to predict their blink response did not appear to significantly affect their propensity to become aware. It may have slowed down the rate at which they became aware; however, as the online measure in the Predict Eyeblink group does not provide a pure measure of contingency, knowledge this is difficult to assess.

Figure 3 shows the mean percentage of CRs that aware and unaware participants made to CS+ and CS− over the six blocks of trials. From Figure 3 it can be seen that there was considerable differential responding in both the aware and the unaware participants, which was confirmed by a main effect for CS type, $F_{1,131} = 26.90, P < 0.05$. Importantly, there was no interaction between CS type and awareness, $F < 1$. When follow-up analyses were conducted to evaluate eyeblink conditioning in the aware and unaware participants separately, both the aware as well as the unaware participant showed significantly more responding overall to the CS+ compared with the CS−, $F_{1,239} = 56.61, P < 0.05$, and $F_{1,41} = 11.49, P < 0.05$, respectively. This result could be interpreted as evidence for differential delay conditioning in the absence of awareness, but it may equally be indicative that the post-experimental questionnaire was not effective in differentiating aware from unaware participants. The button-press data provided some support for this possibility. For the Predict Airpuff group, the button press responses provide an alternative measure of awareness. While the very small number of participants classified as unaware in the Predict Airpuff group does not allow for any strong conclusions about the relationship between these different measures of awareness, one of the two participants who was classified as unaware according to the post-experimental questionnaire exhibited button-press responses indicative of awareness. Likewise, for the Predict Eyeblink group all three participants who were classified as unaware according to the post-experimental questionnaire exhibited button-press responses that indicated they were at least aware that they blinked more to the CS+ than to the CS−; whether this is evidence that they were also contingency aware is debatable.

For the Predict Airpuff group, the button presses also provide a trial by trial measure that can be used to estimate the point at which participants became aware that the CS+ predicted the occurrence of the airpuff and the CS− did not. To examine the temporal relationship between awareness and differential conditioning in this group we compared eyeblink CRs made to the CS+ and CS− before and after the point at which participants were considered to have acquired contingency awareness. We determined the point of awareness as the point at which differential button press responding to the CS+ and CS− first emerged. Figure 4 shows the mean percentage of button presses and the mean percentage of eyeblink CRs made to the 10 CS+ and 10 CS− preceding and following the acquisition of awareness. As the point of awareness is determined by the emergence of differential responding on the button-press measure, by definition there are comparable levels of button presses to the CS+ and CS− prior to the point of awareness and almost perfect differential after the point of awareness. The figure only includes participants for whom a distinct point of awareness could be determined, which was 20 of the 21 participants in the Predict Airpuff group. The number of participants who contribute to each data point is indicated below each data point. The majority of participants showed button-press responses indicative of awareness within a few trials of the beginning of the session, so relatively few participants contributed to the early trials preceding awareness. From the bottom panel of Figure 4 it can be seen that participants only appear to show evidence for differential conditioned responding following the acquisition of contingency awareness and delay eyeblink conditioning.

Figure 2. Mean percentage of eyeblink conditioned responses (CRs) made on each of the six blocks of 20 trials in Experiment 1. Data for participants in the Predict Airpuff group are shown at left, and data for participants in the Predict Eyeblink group are shown at right.

Figure 3. Mean percentage of eyeblink conditioned responses (CRs) made on each of the six blocks of 20 trials in Experiment 1. Data for Aware participants (defined by the Clark and Squire [1998] post-experimental questionnaire) are shown at left, and data for Unaware participants are shown at right.
Awareness and delay eyelink conditioning

A within-subject analysis of the average level of CRs to the CS+ and the CS− before and after the point of awareness yielded a main effect for CS type, $F_{1,17} = 7.69, p < 0.05$, no main effect for before and after awareness, $F < 1$, but an interaction between CS type and point of awareness, $F_{1,17} = 16.81, P < 0.001$. A follow-up comparison of average responding to the CS+ and the CS− before the point of awareness showed no evidence for differential responding $t_{17} = 1.31, P > 0.21$. However, a comparison of average responding to the CS+ and the CS− after the point of awareness showed that there was significantly more responding to the CS+ than the CS−, $t_{17} = 5.99, P < 0.001$. Although this analysis indicates that differential responding increased after the point that participants became aware, it does not necessarily indicate that the development of awareness is the critical factor in influencing conditioned responding. This pattern could merely be a function of the number of reinforced trials, of which there have always been more after the point of awareness than before the point of awareness.

To assess the degree to which the development of awareness and the number of reinforced trials that predicted differential eyelink responding, we used a multilevel model of differential eyelink responding on blocks of four trials (two CS+ and two CS−). Multilevel modeling was used because it can handle missing data on repeated measures, which was the case for participants who had high levels of voluntary CRs that were excluded from the analysis, and because it can model the variance at both levels within a nested data structure simultaneously (Snijders and Bosker 1999; Raudenbush and Bryk 2002). Differential eyelink CRs across 30 blocks of trials represented the lower level (Level 1), which was nested within participants (Level 2). Only the 20 participants for whom it was possible to determine the block during which they became aware were included in the analysis. To test whether awareness and/or trial block predicted differential eyelink CRs, the Level 1 model included trial blocks as a continuous variable and awareness as a dummy coded variable (0: not aware during block; 1: aware during block) and the Level 2 model included an estimate of random effects due to participant.

The simplified reduced-form model combining Levels 1 and 2 was:

$$\text{Differential CRs}_{ij} = B_{00} \text{(intercept)} + B_{10} \text{(Trial Block)} + B_{20} \text{(Awareness)} + e_i + \epsilon_{ij}$$

The equation specifies that differential eyelink CRs are a function of: a grand mean intercept ($B_{00}$) that varies randomly across all individuals ($e_i$); the main effect of trial block ($B_{10}$) and the main effect of awareness ($B_{20}$); $e_{ij}$ is deviation of each individual’s average from the overall average differential CRs. The model yielded a significant main effect of Awareness ($B_{20} = 0.286, SE = 0.075, t_{(530)} = 3.78, P < 0.001$), but not of Trial Block ($B_{10} = 0.003, SE = 0.003, t_{(538)} = 1.08, P = 0.282$). These results indicated that differential eyelink CRs were lower prior to awareness than after awareness, and that trial block did not have any predictive power when included in the model. To test the contribution that Awareness and Trial Block made to the overall model fit, we conducted two deviance tests. We compared the model fit of the model with Trial Block as the only predictor with the model that included both Trial Block and Awareness. The test of the difference in deviances between the two models was significant, $\chi^2(1) = 14.216, P = 0.0001$, which indicated that the model including Awareness provided a better fit to the data than the model without Awareness. Then we compared the model fit with Awareness as the only predictor with the model that included both Trial Block and Awareness. The test of difference in deviance between the two models was not significant $\chi^2(1) = 1.16, P = 0.282$, suggesting that Trial Block did not improve model fit. Thus, the results of these deviance tests in conjunction with the results of the multilevel modeling analyses suggest that Awareness is a better predictor of differential CRs than Trial Block.

In the present experiment, the majority of participants became aware according to the post-experimental questionnaire, and of those participants who were classified as unaware according to the post-experimental questionnaire, their button-press data suggests that most of them may in fact have been aware. However, because some of these participants were asked to predict their eyelink rather than the airpuff, we cannot use the button-press data to definitively determine awareness of their contingency knowledge. Nonetheless, asking participants to predict the airpuff facilitated differential conditioned responding compared with asking participants to predict their eyelink. In the Predict Airpuff group, the majority of participants became aware of the stimulus contingency very early in the conditioning session. If differential conditioning in the absence of awareness emerges slowly, then it would not be possible to see the emergence of differential responding prior to the point of awareness under these conditions. Therefore, Experiment 2 was designed to slow down the emergence of contingency awareness, but using a procedure where all participants were asked to predict the airpuff with an online measure.

Experiment 2: Online measure of awareness with complex masking task

The aim of Experiment 2 was to apply a masking task with the intention of slowing down the rate of acquisition of contingency awareness, while at the same time tracking the trial-by-trial development of contingency knowledge with a button-press response predicting the airpuff. Such a technique has been used successfully in studies of electrodermal conditioning (Dawson and Biferno 1973). In their study, an “auditory perception task” served...
to mask CS–US contingency. In Experiment 2 we adapted the auditory masking task used by Dawson and Biferno (1973) to the eyeblink conditioning paradigm. The masking task we used involved asking participants to make loudness comparisons between spoken letters of the alphabet and a target noise (either a tone or white noise), which served as the CS+ and CS−. They were told that stimuli that were perceived as loud may induce an eyeblink startle response, and so we would be measuring their blink response using an infrared detector. They were also told that the eyeblink startle response tends to habituate across the session so we would be puffing them with air in the eye occasionally to counteract this habituation. Awareness was measured trial-by-trial by instructing participants to press a button just prior to when they believed the airpuff was about to occur.

Results
To examine the overall relationship between awareness and conditioning, we compared those participants who showed evidence of differential button-press responses indicative of contingency awareness with those who did not. The criterion used to determine awareness was higher average button-press responding during the CS+ than during the CS− across the entire session. Using this criterion, there were a total of 50 aware participants and 23 unaware participants. Figure 5 shows the mean percentage of CRs that all aware and unaware participants made to the CS+ and CS− over blocks. From Figure 5 it appears that discrimination between CS+ and CS− is restricted to aware participants. This was confirmed by a significant interaction between CS Type and Awareness, $F_{(1,71)} = 15.52, P < 0.05$. A separate analysis of the aware participants alone yielded a main effect for CS Type, $F_{(1,49)} = 55.01, P < 0.05$. In contrast, a similar analysis of the unaware participants alone showed no significant difference in responding to CS+ and CS− overall, $F_{(1,22)} = 1.96, P > 0.05$. Therefore, by using an online measure of contingency awareness, there was evidence for a strong relationship between contingency awareness and differential responding and no evidence of differential responding among participants who remained unaware during the entire session.

To examine the temporal relationship between awareness and conditioning, the same strategy was used as that in Experiment 1, whereby CRs made before and after the point of awareness were compared. Figure 6 shows the mean percentage of button presses and eyeblink CRs made to the 10 CS+ and 10 CS− preceding and following the point of awareness. The figure only includes participants for whom there could be determined a distinct point at which differential button presses emerged, of whom there were 41. The number of participants who contribute to each data point is indicated below each data point. From Figure 6 it can be seen that participants do not appear to show any evidence of differential conditioning prior to the point of awareness, whereas after the point of awareness participants begin to show evidence for differential conditioned responding. Notably, differential CRs did not emerge immediately after the point of awareness but began to emerge following four or five CS+ and CS− trials post awareness. This is different from the pattern of results in Experiment 1, where differential CRs appeared to emerge almost immediately after participants became aware. This difference between the results of Experiments 1 and 2 may be due to the difference in emphasis of task in the different experiments. In Experiment 1 the focus of the task was on predicting the occurrence of the airpuff, whereas in Experiment 2, predicting the airpuff was secondary to discriminating loudness. A focus on predicting the airpuff clearly facilitated the emergence of awareness, but it may have also influenced the emergence of differential CRs following awareness by focusing attention on the imminent onset of the airpuff.

A within-subject analysis of the average level of CRs to the CS+ and the CS− before and after the point of awareness yielded a main effect for CS type, $F_{(1,39)} = 35.30, P < 0.001$, a main effect for point of awareness, $F_{(1,39)} = 14.05, P < 0.001$, and an interaction between CS type and point of awareness, $F_{(1,39)} = 16.57, P < 0.001$. A follow-up comparison of average responding to the CS+ and the CS− before the point of awareness showed no evidence for differential responding, $t_{(39)} = 1.42, P > 0.10$. A comparison of average responding to the CS+ and the CS− after the point of awareness showed that there was significantly more responding to the CS+ than the CS−, $t_{(39)} = 7.53, P < 0.001$. Thus, as with the results from Experiment 1, there was no evidence for differential responding prior to the point of awareness, but differential responding emerged after the point of awareness. However, this pattern by itself does not necessarily indicate that the increase in differential responding is tied to the emergence of awareness.

Figure 5. Mean percentage of eyeblink conditioned responses (CRs) made on each of the six blocks of 20 trials in Experiment 2. Data for Aware participants (as defined by the online measure of contingency awareness) are shown at left, and data for Unaware participants are shown at right.

Figure 6. (Top) The mean percentage of button presses; (bottom) the mean percentage of CRs made to the 10 CS+ and 10 CS− trials preceding awareness, and the 10 CS+ and 10 CS− trials following awareness in Experiment 2. The numbers at bottom next to each pre-awareness and post-awareness trial indicate the number of participants who had awareness data that contributed to this trial.
As in Experiment 1, in order to test whether the point at which participants became aware predicted differential eyelblink CRs, we used a multilevel model of differential eyelblink responding on blocks of four trials. We constructed the model in the same way that we did for Experiment 1. The model yielded a significant main effect of Awareness ($B_{20} = 0.162, SE = 0.042, t_{(132)} = 3.84, P < 0.001$), but not for Trial Block ($B_{20} = 0.0007, SE = 0.0019, t_{(1114)} = 0.349, P = 0.73$). As with the results from Experiment 1, the model indicated that differential eyelblink CRs were lower prior to awareness than after awareness, and that trial block did not have any predictive power when included in the model with awareness. When we compared the model fit of the model with Trial Block as the only predictor with the model that included both Trial Block and Awareness, the difference in deviances between the two models was significant, $\chi^2(1) = 13.892, P = 0.0002$, which indicated that the model including Awareness provided a better fit to the data than the model without Awareness. However, when we compared the model fit of the model with Awareness as the only predictor with the model that included both Trial Block and Awareness, the difference in deviance between the two models was not significant, $\chi^2(1) = 0.121, P = 0.728$, suggesting that Trial Block did not improve model fit. Thus, as with the results from Experiment 1, both the results of these deviance tests and the results of the multilevel modeling analysis suggest that Awareness is a better predictor of differential CRs than Trial Block.

In summary, Experiment 2 indicated that there was a strong relationship between awareness and differential conditioning when using a delay procedure and an online measure of awareness. Participants who were unaware of the stimulus contingency showed no evidence for differential responding, and participants who became aware during the conditioning session showed no evidence of differential responding prior to the point of awareness, but showed evidence of differential responding after the point of awareness. Differential CRs were closely tied to the emergence of awareness and were not simply a function of the number of reinforced trials that a participant received. It is possible that the addition of the masking task in Experiment 2 may have made the task into something more “complex” than standard differential conditioning, and it is this increase in complexity that necessitates the involvement of contingency awareness in conditioning. However, while there were additional auditory stimuli presented in the same context as part of the masking task, the evidence for differential conditioning was always a comparison of differential responding between the tone and the white noise, one of which was consistently reinforced, while the other was not. Manns et al. (2002) argue that differential delay conditioning, where the positive and negative conditioned stimuli (CS+ and CS−) are a tone and white noise, can occur independently of awareness, so there is no a priori reason why the masking task would necessitate the involvement of awareness. Asking participants to make loudness judgments between the stimuli is likely to have added additional cognitive load; however, there is no a priori reason why this would have made the masking task a more “complex” task that necessitates awareness.

**Discussion**

Together, the results of Experiments 1 and 2 indicate a strong association between differential eyelblink conditioning and self-reported contingency knowledge when measured with an online measure of contingency awareness. Experiment 1 indicated that asking participants to predict the occurrence of the US facilitated differential responding compared with asking participants to predict their blink response. Experiment 2 indicated that only participants who were classified as aware according to the online measure of awareness showed evidence of differential responding. Furthermore, both experiments showed that among participants who became aware of the stimulus contingency, differential CRs did not emerge until shortly after the point that they became aware.

In Experiment 1 there was some evidence to suggest that participants who were classified as unaware according to the post-experimental questionnaire nonetheless showed evidence of differential CRs. However, many of these participants who were classified as unaware according to the post-experimental questionnaire showed differential button-press responses to the CS+ and the CS− during conditioning. This result suggests that, rather than being evidence for conditioning in the absence of awareness, it is perhaps evidence that the post-experimental questionnaire was not particularly sensitive in assessing awareness, and some of those classified as unaware may not have been unaware at all. Furthermore, the results from Experiment 2, which used an online measure of contingency awareness among all participants, found evidence for a strong relationship between awareness and conditioned responding, and no evidence for differential conditioning among those participants who were classified as unaware. These results may go some way toward explaining some of the discrepancies in the published literature with regard to the role of awareness in differential delay conditioning. The evidence for apparent unaware differential delay conditioning in the literature has been reported by Squire, Clark, and colleagues (Clark and Squire 1998; Smith et al. 2005). All of these experiments used the same post-experimental questionnaire that was used in Experiment 1 as the method for assessing awareness, which also resulted in apparent unaware differential conditioning. With a post-experimental questionnaire it is possible that some of the participants who were aware at the time of conditioning could respond to the questionnaire as though they were unaware due to forgetting or interference that occurred between conditioning and assessment (Dawson and Reardon 1973). Alternatively, some of the participants who were unaware at the time of conditioning could respond to the questionnaire as though they were aware, because information present in the questions could have triggered an awareness that was not present during conditioning. Perhaps, as has been suggested by Lovibond and Shanks (2002), some of the evidence for apparent unaware conditioning is due to the use of an insensitive measure of contingency awareness. Although determining awareness using a post-experimental questionnaire may be less than ideal, it is likely that there is some relationship between the post-experimental assessment of awareness and contingency knowledge at the time of conditioning that may be sufficiently sensitive to show a relationship between awareness and conditioning on some occasions, such as has been found by Nelson and Ross (1974), and more recently, Lovibond et al. (2011). Indeed, using the same post-experimental questionnaire as was used in the present study, Clark and Squire (1998) and Manns et al. (2000a) found evidence for a relationship between awareness and differential trace eyelblink conditioning. The fact that the same post-experimental questionnaire appears to discriminate aware and unaware individuals in the case of trace conditioning would seem to argue against the idea that unaware delay conditioning is simply an artefact of an insensitive measure of awareness. However, apart from the study of Clark and Squire (1998) this comparison in the sensitivity of the questionnaire between trace and delay conditioning is being made across different experiments, tested at different times by different experimenters. Thus, differences in the administration of the post-experimental questionnaire could be responsible for the apparent differences in its sensitivity between trace and delay conditioning and, indeed, between different instances of delay conditioning.
The use of an online measure of contingency awareness addresses some of the intrinsic limitations of the post-experimental questionnaire, such as determining whether participants were aware at the time of conditioning (Dawson and Biferno 1973). The evidence for a strong relationship between awareness and conditioned responding, found in Experiment 2 when awareness was assessed with an online measure of awareness, is perhaps due to the greater sensitivity of an online measure compared with a post-experimental measure of contingency awareness.

In addition to providing a superior assessment of contingency awareness, the use of an online measure of awareness allows an assessment of the temporal relationship between the emergence of awareness and differential responding. The present experiments show that awareness and differential delay responding emerge in parallel. Manns et al. (2000b) have shown a similar pattern of parallel emergence of awareness and differential conditioned responding in a trace-conditioning procedure, but this is the first time that this has been shown in a delay-conditioning procedure. For both Experiment 1, where awareness occurred very early in the conditioning session, and Experiment 2, where awareness was somewhat delayed by the imposition of a masking task, it was not until after the point of awareness that there were reliably more CRs to the CS+. Indeed, once awareness was acquired, the emergence of differential CRs, at least on a group basis, was relatively rapid.

If it is the case that awareness is sufficient, but not necessary for differential delay conditioning to occur, then any task that promotes the development of awareness should facilitate the emergence of differential responding. Thus, the facilitation of differential responding in the Predict Air puff compared with Predict Eyeblink group in Experiment 1 may be due to the task instructions in Group Air puff facilitating the emergence of awareness of which stimulus predicted the airpuff. However, if awareness is sufficient, but not necessary, then any task that inhibits the development of awareness should not ultimately prevent the emergence of conditioning. Thus, the failure to see any evidence of differential delay conditioning in Experiment 2 among individuals who never became aware of which stimulus predicted the airpuff, suggests that either 120 conditioning trials is insufficient for conditioning to emerge in the absence of awareness, or that awareness is both necessary and sufficient for differential delay conditioning.

The fact that the memory trace for delay eyeblink conditioning is formed and stored in the cerebellum and that forebrain structures do not appear to be necessary for the acquisition and retention of the CR using a delay procedure is often cited as supporting the notion that awareness is not necessary for delay eyeblink conditioning, either single cue or differential (Clark and Squire 1998; Manns et al. 2002). This evidence indicates that damage to the cerebellum and related brain-stem structures interfere with both the learning and the expression of eyeblink conditioning in both humans and nonhuman animals (McCormick and Thompson 1984a,b; Gerwig et al. 2006). In contrast, lesions of forebrain structures including the hippocampus have been shown to have little or no effect on delay eyeblink conditioning, both in single-cue and differential conditioning paradigms (Berger and Orr 1983; Solomon et al. 1986; Mauk and Thompson 1987). Because forebrain structures are not necessary for the acquisition or retention of delay eyeblink conditioning, it is assumed that conditioning can occur in the absence of awareness.

There are two possible ways in which the present data might be reconciled with the evidence concerning the brain systems underlying conditioning. First, they could be seen as challenging the assumption that conscious awareness of the stimulus relationship is entirely mediated by forebrain structures. For example, it has been argued by Merker (2007) that the limited-capacity sequential mode of operation of certain midbrain structures are better candidates for certain aspects of conscious processing than the thalamocortical complex alone. Second, if you reconsider the neural data, particularly the data concerning the involvement of the hippocampus in delay conditioning in the intact organism, then there is considerable evidence that the hippocampus is important in modulating delay eyeblink conditioning, particularly in the case of differential conditioning.

Although hippocampal lesions do not prevent delay eyeblink conditioning, there is considerable evidence to suggest that activity in the hippocampus significantly modulates delay conditioned responding, both differential and single-cue conditioning. Studies of firing rates in hippocampal pyramidal cells have shown learning-related neural plasticity over the course of delay eyeblink conditioning, which are strongly predictive of the subsequent emergence of CRs (Berger et al. 1983; Green and Arenos 2007). Neuroimaging studies in humans show similar learning-related activity in the hippocampus during delay eyeblink conditioning (Blaxton et al. 1996). Moreover, hippocampal activity seems to be causally related to the rate of acquisition of delay eyeblink conditioning. For example, the amount of spontaneous hippocampal theta activity before training predicts the subsequent rate of learning during delay conditioning (Berry and Thompson 1978). In particular, the presence of hippocampal theta activity during the early stage of learning is correlated with increases in CS-associated firing rates in the hippocampus, as well as subsequent behavioral CRs (Nokia et al. 2008). Moreover, administration of CS–US pairing in the presence of hippocampal theta activity, especially in the early phase of learning, facilitates the acquisition of delay eyeblink conditioning (Seager et al. 2002). Consequently, if contingency knowledge is associated with the hippocampus being effectively engaged in modulating cerebellar activity, then the pharmacological and electrophysiological data suggest that appropriate contingency knowledge should facilitate delay eyeblink conditioning.

Furthermore, manipulations that disrupt hippocampal activity significantly impair delay eyeblink conditioning (Solomon et al. 1983). Sub-seizure electrical stimulation of the hippocampus during delay eyeblink conditioning disrupts the acquisition, but not the expression of eyeblink CRs (Salaia et al. 1979). Systemic injection of the cholinergic antagonist scopolamine, which alters hippocampal neuronal activity, severely disrupts delay eyeblink conditioning in rabbits (Moore et al. 1976) and in humans (Solomon et al. 1993). Lesions of medial septum, which modulates hippocampal activity through cholinergic projections, disrupt the acquisition delay eyeblink conditioning in rabbits (Allen et al. 2002). Humans with damage to the basal forebrain structures including the medial septum, as a result of anterior communicating artery aneurism rupture, also show impaired delay eyeblink conditioning compared with matched controls (Myers et al. 2001). In fact, patients with medial septum damage showed no appreciable learning across 70 training trials. It is presumed that medial septum damage in humans disrupts delay eyeblink conditioning through disruption of septohippocampal projects that disrupt hippocampal activity. Indeed, it has been repeatedly shown in animal models of eyeblink conditioning that altering hippocampal activity is much more detrimental to delay eyeblink conditioning than removing the hippocampus (Solomon et al. 1983). Consequently, if the absence of contingency knowledge is associated with a disruption of hippocampal activity, then the pharmacological and electrophysiological data suggest that eyeblink conditioning should be impaired in the absence of contingency knowledge. However, this would tend to suggest that with extended conditioning, even participants who are unaware of the stimulus contingency would eventually acquire CRs, because although disrupted hippocampal activity appears
to impair delay conditioning, it usually does not entirely prevent it. It remains an open question as to whether differential delay eyeblink conditioning would eventually develop with an increased number of CS–US pairings. In the present experiment, 60 CS+ and 60 CS− presentations was not sufficient to see evidence for differential delay eyeblink conditioning in participants who were unaware of the contingency. This is the same number of trials that has been used in previous experiments, which claim to see evidence of differential delay conditioning in the absence of awareness.

How is it that awareness of the stimulus contingency contributes to the acquisition of the conditioned response? One mechanism that has been suggested by Bolles is that the CS activates an expectation for the US, and thereby elicits an appropriate CR. However, there is reliable evidence to show that under certain circumstances it is possible to dissociate eyeblink CRs from expectancy for the airpuff. Specifically, Perruchet (1985) showed in a single-cue partial reinforcement design that when responding is assessed as a function of recent reinforcement history, participants show a double dissociation between their pattern of expectancy and their pattern of eyeblink CRs. Specifically, participants expected the airpuff to occur if they had received a number of CS-alone trials, and did not expect the airpuff to occur if they had received a number of CS–US trials, that is, they showed a classic gambler’s fallacy for when they expected the airpuff to occur (Burns and Corpus 2004). However, participants’ CRs showed the opposite pattern; they decreased CRs following CS-alone trials and increased CRs following CS–US trials. This dissociation of expectancy and conditioned responding has been shown to be reliable (Weidemann et al. 2009) and to be present with a trace as well as a delay-conditioning procedure (Weidemann et al. in press). Therefore, contingency awareness does not appear to influence conditioned responding directly through expectancy for the unconditioned response. Indeed, in the present findings the level of differential eyeblinks was well below the level of differential button presses. This suggests that eyeblink CRs are being generated, at least in part, independent of expectancy for the airpuff. Instead of eyeblink CRs being directly influenced by expectancy for the airpuff, declarative learning about the CS–US contingency appears to facilitate the emergence of the differential eyeblink CRs.

Materials and Methods

Participants

Participants were undergraduate students from the University of New South Wales who received course credit for participation. In Experiment 1 there were 38 participants (15 males and 23 females) and they were randomly allocated to the two prediction groups (Predict Airpuff: n = 21; Predict Eyeblink: n = 17). In Experiment 2 there were 73 participants (30 males and 43 females).

Apparatus

Experiment 1

The two CS stimuli were an 85-dB, 1000-Hz tone and an 85-dB white noise signal. Both stimuli were of 3000 ms duration and were delivered via Sennheiser HD515 headphones. The US was a 100-msec, 15-psi puff of air (measured at the point of generation by an eyeblink airpuff unit [San Diego Instruments]). It was delivered to the left eye via 2 m of flexible plastic tubing, terminating in a 1-mm nozzle. This was attached to the left frame of a pair of spectacles worn by the participant. The nozzle had an infrared emitter and sensor (San Diego Instruments) attached for recording eyeblinks. Conditioning and recording was carried out by a Med Associates, Inc. SG-500 experimental interface connected to an Intel Pentium computer. Med-PC experimental control software (Med Associates, Inc.) was used to program the conditioning session and to record the button-press and eyeblink data.

Following Clark and Squire (1998) and Smith et al. (2005), the silent movie The Gold Rush (Chaplin 1925) was used. The movie was screened on a 30 cm × 36 cm computer monitor, ~50 cm in front of the participant.

Experiment 2

The CS stimuli were an 80-dB 1000-Hz tone and an 80-dB white noise signal. The masking task consisted of three different recorded audio files followed by either the CS+ or CS−. In total, there were five different audio files used in the experiment, which consisted of five different spoken letters of the alphabet, each of which were ~1 sec in duration. However, on any given trial only three audio files were used, which were randomized from trial to trial. The loudness of the audio files were varied from ~40 to 85 dB. However, on each trial one of the audio files was ~80 dB in volume and the other two files were either louder or softer. Following each trial, participants were presented with an instruction screen that read “Which spoken letter was most similar in volume to the final auditory stimulus?” This was displayed ~50 cm in front of the participant on a 30 cm × 36 cm computer monitor. Participants were instructed to make their response by speaking into a microphone. The stimulus presentation and the recording of eyeblink and button-press responses were controlled by LabView software (National Instruments).

Conditioning procedure

Experiment 1

Participants were tested individually in a dimly lit room separate from the apparatus room. They were told that they were taking part in a study on how distraction affects their memory for a movie, and the distracting stimuli would be noises and airpuffs. They were instructed to try to remember as much of the movie as possible despite the distracting stimuli, as they would be asked questions on it later. Participants in the Predict Airpuff group were instructed to press a button just prior to when they believed the airpuff was about to occur. Conversely, participants in the Predict Eyeblink group were instructed to press a button just prior to when they believed they were about to blink. To allow familiarization and calibration of the recording equipment, each participant received two presentations of both the tone and white noise and a single airpuff. The blink response to this initial airpuff presentation was recorded and was assessed to determine whether it was of sufficient magnitude to be reliably measured. If the eyeblink reflectance measure in response to the airpuff was not present or was very small, then the airpuff nozzle and the infrared sensor was adjusted to point more directly into the subject’s eye, and another airpuff was presented. This process was repeated until a reliable eyeblink response could be measured. No participant required more than five initial airpuff trials in order to position the airpuff nozzle and infrared sensor appropriately to obtain a reliable eyeblink measure. Following the conditioning session, all participants completed the post-experimental questionnaire, which was the same questionnaire used by Clark and Squire (1998) and Smith et al. (2005).

The conditioning session consisted of 120 conditioning trials, half of which were CS− alone trials and the other half were CS+ trials. The allocation of CS+ and CS− was counterbalanced across participants. On CS+ trials, the US was presented 1250 msec after the onset of the CS. The CS and US then overlapped for 100 msec and terminated concurrently. On CS− trials, the CS was presented alone for 1350 msec. Each trial was separated from the next with an intertrial interval (ITI) of 10–15 sec. Each trial was presented in random order, with the restriction that there could be no more than five trials of the same type in a row and that each block of 20 trials included 10 CS+ and 10 CS− trials.

Experiment 2

Participants were told that they would be taking part in an auditory perception task in which they would listen to a series of
different sounds that all varied in loudness. Their task was to identify which of the first three sounds was the same volume as the last sound on each trial. In addition, they were informed as to why the puff of air was going to be administered (i.e., to prevent habituation of the eyeblink response) and why they were required to press the button when they expected the airpuff (i.e., as their expectation for the airpuff was likely to make them blink more). To allow familiarization and calibration of the recording equipment, each participant received one presentation of each auditory stimulus (i.e., tone, white noise, and one of the wave files) and a single airpuff.

The conditioning session consisted of 120 conditioning trials, half of which were CS+– alone trials, and the other half were CS+– trials. On all conditioning trials participants were serially presented with three different auditory files of spoken letters of varying volumes that were followed by either the CS+ or the CS−. Each of the auditory files was separated by a 1-sec interval and the last auditory file and the CS was separated by a 1-sec interval. The allocation of CS+− and CS− was counterbalanced across participants. On CS+− trials, the US was presented 1250 msec after the onset of the CS. The CS and US then overlapped for 100 msec and terminated concurrently. On CS− trials, the CS was presented alone for 1350 msec. On both CS+− and CS− trials, 350 msec after the CS-terminated, participants were presented with an instruction screen that read, “Which spoken letter was most similar in volume to the final auditory stimulus?” to which they were required to respond by speaking into the microphone. Each trial was separated from the next with an interval interval (ITI) of 10–15 sec. Each trial was presented in random order, with the restriction that there could be no more than five trials of the same type in a row, and that each block of 20 trials included 10 CS+− and 10 CS−− trials.

**Scoring and analysis**

An eyeblink was defined as a CR if it occurred between 750 and 1250 msec after the onset of the CS (i.e., in the 500-msec time interval before the onset of the US on CS–US trials). In addition, to be a CR, the maximal amplitude of a blink in response to both the CS+− and CS− had to be at least 20% of the same participant’s maximum blink amplitude in response to the first five US presentations of the conditioning session. For every participant, a percent score of CRs was calculated for CS+− and CS−− on each block of 20 trials, based on the proportion of total trials for which a CR was recorded.

In addition, some researchers have argued that raw eyeblink recordings are contaminated with voluntary blinks, and that such blinks can be distinguished from true CRs by their early onset and high magnitude (Spence and Ross 1959). Although this method has been questioned (Gormezano 1965), it was used by Clark and Squire (1998) and by Smith et al. (2005), so we followed a similar procedure. We used a computer program to identify trials in which an eyeblink commenced before a 300-msec pre-US window, persisted until the end of the US presentation, and showed a magnitude of >70% of the mean UR magnitude recorded on the first five trials of the experiment. These trials were eliminated from the analysis.

In order to determine the point of awareness, we ran a change point analysis (Taylor 2000) on differential button-press responding on successive blocks of two CS+− and two CS−− trials, where average responding on CS−− trials was subtracted from average responding on CS+− trials to give a possible value of $-1$, $-0.5$, 0, 0.5, or 1. The first block during which there was a significant increase in differential responding according to the change point analysis (Taylor 2000), which uses a combination of cumulative sum charts and bootstrapping to detect change, was determined to be the block when participants became aware. For participants who did not have a change point because their differential button-press responding was consistent throughout, if they showed evidence of high levels of positive differential button-press responding, that is, >80% more button presses on CS+− trials than CS−− trials, then the earliest trial block that positive differential responding occurred in was determined to be the block when they became aware, otherwise they were excluded from the analysis. The particular trial in which participants became aware was determined to be the first trial in which they made a button-press response to the CS+ in the trial block where they became aware as determined by the change point analysis.

Both the eyeblink and button press data were analyzed with a set of planned contrasts using a multivariate, repeated measures model (O’Brien and Kaiser 1985). These analyses included Prediction group in Experiment 1 (Predict Airpuff vs. Predict Eyeblink) and Awareness (Aware vs. Unaware) as group variables in Experiment 1 and Experiment 2 and Stimulus Type (CS+− vs. CS−−) and Blocks as within-subject variables. Following Clark and Squire (1998), participants in Experiment 1 were classified as aware with the post-experimental questionnaire if they gave correct responses on at least 13 of the 17 critical questions that refer to the relationship between the CS+−, CS−−, and the US. In Experiment 2 participants were classified as aware according to the button-press responding if they showed higher average button-press responding during the CS+− than during the CS−−.

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Awareness and delay eyelink conditioning


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