Awareness in Classical Differential Eyeblink Conditioning in Young and Aging Humans

M.-G. Knuttinen, J. M. Power, A. R. Preston, and J. F. Disterhoft
Northwestern University Medical School

The role of awareness and its impact on learning the conditioned eyeblink response was investigated in both trace and delay discrimination eyeblink conditioning in young and aging participants, in 4 paradigms: delay 750, delay 1250, trace 500, and trace 1000. Participants concurrently watched a silent movie about which they were questioned afterward. Acquisition in both the trace and delay discrimination task was correlated with awareness of conditioning stimulus contingencies, regardless of age. Age-dependent deficits were observed in trace discrimination but not in delay discrimination, with more severe deficits appearing at the longer trace interval. The percentage of aware participants was also found to be greater in the young population than in the aging population. These results indicate that awareness or knowledge of stimulus contingencies may be an important contributor to successful acquisition in higher order discrimination tasks.

Considerable previous research has documented that both animals and humans can learn physical relationships and relational contingencies among sequenced stimuli (Hillner, 1979). For almost a century, psychologists have investigated the nature and importance of awareness and its role in the acquisition of the conditioned eyeblink response (Grant, 1973; Kimble, 1962; Spence, 1966). In the area of cognitive neuroscience, some of the questions central to the awareness debate are whether and in what manner knowledge can be used to modify classically conditioned eyeblink responding.

Classical conditioning serves as a powerful paradigm to assess the involvement of a simple psychological process, such as awareness, in associative learning. The eyeblink conditioning paradigm is a well-understood model of classical conditioning that is used to study the mechanisms of associative learning in animals (Disterhoft, Kwan, & Lo, 1977; R. F. Thompson et al., 1976) and in humans (Carrillo, Thompson, Gabrieli, & Disterhoft, 1997; Daum et al., 1993; Daum & Schugens, 1996; Gabrieli et al., 1995; McGlinchey-Berroth, Carrillo, Gabrieli, Brawn, & Disterhoft, 1997; Woodruff-Pak, 1988, 1993). Eyeblink conditioning requires that the subject associate a neutral stimulus (e.g., auditory conditioned stimulus [CS]) with a behaviorally significant stimulus (e.g., corneal airpuff unconditioned stimulus [US]). Different configurations of this paradigm allow for the analysis of simple and more complex learned associations. The delay conditioning version, used by Gormezano, Schneiderman, Deaux, and Fuentes

M.-G. Knuttinen, J. M. Power, A. R. Preston, and J. F. Disterhoft, Department of Cell and Molecular Biology and Institute for Neuroscience, Northwestern University Medical School.

Correspondence concerning this article should be addressed to J. F. Disterhoft, who is now at the Department of Physiology, Northwestern University Medical School, Searle 4-427, 303 East Chicago Avenue, Chicago, Illinois 60611. Electronic mail may be sent to jdisterhoft@northwestern.edu.

(1962), involves establishing an association between a tone CS that precedes, overlaps, and coterminates with a corneal airpuff US. Both lesion and recording studies have implicated the cerebellum and associated brainstem nuclei as necessary structures for the acquisition and retention of this task (Mauk & Thompson, 1987; McCormick, Clark, Lavond, & Thompson, 1984; McCormick, Guyer, & Thompson, 1982; McCormick, Lavond, & Thompson, 1983; McCormick & Thompson, 1984).

The hippocampus is known to play a crucial role in more complex conditioning paradigms, such as reversal learning (Berger & Orr, 1983) and latent inhibition (Solomon & Moore, 1975). In trace eyeblink conditioning, the subject is required to form a memory trace of the CS to bridge a stimulus-free time interval between the presentation of the CS and the US. This is a more memory-demanding task, and it has been hypothesized to require additional input to the cerebellar circuit that mediates delay conditioning (Weiss & Disterhoft, 1996). The temporal demand imposed by the silent trace interval engages the brainstem, cerebellum, and associated forebrain structures, including the hippocampus (Kim, Clark, & Thompson, 1995; McGlinchey-Berroth et al., 1997; Moyer, Deyo, & Disterhoft, 1990; Solomon, Vander Schaaf, Thompson, & Weisz, 1986). The eyeblink response task has been shown to be hippocampally dependent when the CS and US are separated by a 500-ms trace interval in rabbits (James, Hardiman, & Yeo, 1987; Kim et al., 1995; Moyer et al., 1990; Port, Mikhail, Romano, Steinmetz, & Patterson, 1986; Solomon et al., 1986) and a 250-ms trace interval in rats (Weiss et al., 1999). Studies in humans with temporal lobe amnesia have also shown trace eyeblink conditioning to be hippocampally dependent (Clark & Squire, 1998; Disterhoft, Carrillo, Hopkins, Gabrieli, & Kesner, 1996; McGlinchey-Berroth et al., 1997). Although hippocampal lesions do not affect performance in delay eyeblink conditioning (Gabrieli et al., 1995; Mauk & Thompson, 1987; Schmaltz & Theios, 1972), positron emission tomography studies have demonstrated increased hippocampal blood flow during and after acquisition of the task (Blaxton et al., 1996; Schreurs et al., 1997).

A more complex conditioning paradigm, discrimination and discrimination reversal, involves the presentation of two stimuli such that one CS is consistently paired with the US (CS+) and the other is always presented alone (CS−). Discrimination conditioning tasks can be in the form of delay or trace, depending on the duration and termination of the CS relative to the US. Results from studies in animals have shown that the acquisition of a simple two-tone discrimination is not disrupted by hippocampal lesions (Berger & Orr, 1983). Ross, Orr, Holland, and Berger (1984) assessed the effect of hippocampal lesions in the locomotor responses of rats and found no effect on a clicker-noise discrimination but found an impairment in acquisition of conditional discrimination, in which a serial light–tone combination was reinforced and a tone alone was not. The failure of conditional discrimination was due to very high response rates on unreinforced trials. Rabbits with hippocampal lesions are also able to acquire a new CS+ in discrimination reversal but are unable to inhibit responses to the new CS− (Berger & Orr, 1983; Orr & Berger, 1985).

In discrimination reversal, persons with medial temporal lobe amnesia are also able to acquire the new CS+ but are unable to inhibit responses to the new CS− (Carrillo et al., in press). Other investigations in unilateral temporal lobectomy patients have shown intact two-tone discrimination, whereas performance on a more complex conditional discrimination was found to be severely impaired compared with that of control participants (Daum, Channon, & Gray, 1992; Daum, Channon, Polkey, & Gray, 1991). In summary, the various classical conditioning paradigms have provided evidence for multiple memory systems (R. F. Thompson, 1986; R. F. Thompson et al., 1984). On a basic level of simple delay conditioning, the conditioned response (CR) develops primarily through cerebellum and brainstem mediation. However, with higher and more complex variations of conditioning, other brain regions become involved in addition to the brainstem and cerebellum.

On the Role of Awareness

Awareness of CS–US relationships has been well documented as important for verbal (Spielberger, 1962) and autonomic conditioning (Dawson & Biferno, 1973; Dawson & Furedy 1973; Grings, 1965), but addressing the role of awareness in human eyeblink conditioning has been a matter of considerable debate. As early as the 1930s, Hilgard and colleagues found that participants who reported awareness of which CS was reinforced and which was not reinforced showed better differential delay conditioning than those who did not note the stimulus contingencies (Hilgard, Campbell, & Sears, 1937). Subsequent studies, including Ross and Nelson (1973), demonstrated poor differential conditioning even after 300 trials when participants performed a concurrent masking task. This poor performance was attributed to similar acquisition for both the CS+ and the CS−. More recently, Carrillo, Gabrieli, and Disterhoft (2000) showed that performance of a masking–concurrent task yielded significant effects on two-tone delay discrimination eyeblink conditioning but not on single-cue delay or trace paradigms. Participants who were given a concurrent task, either watching a silent movie or performing a verbal shadowing task, did not exhibit significant discrimination when compared with controls not given a concurrent task. Discrimination condi-

tioning, therefore, may require an additional cognitive component that is not required in single-cue conditioning.

However, there are a number of other studies, including Grant (1973) and Frcka, Beyts, Levey, and Martin (1983), that have failed to relate awareness to conditioning performance. Recent work on the role of awareness by Clark and Squire (1998) suggested a novel approach for the study of the neural mechanisms of eyeblink conditioning. Clark and Squire proposed that trace eyeblink conditioning may differ from delay conditioning by requiring that the knowledge of the stimulus relationship be built up and remembered across many trials during an experimental session. They explored this possibility by testing amnesic patients and control volunteers in both delay and trace discrimination eyeblink conditioning while also assessing the knowledge the participants developed about the CS–US association. Their recent findings support the idea that trace discrimination conditioning occurs only in those individuals who exhibit awareness of the CS–US contingency, whereas performance in delay discrimination is independent of such awareness.

Although Clark and Squire's (1998) study suggested a possible separation between delay and trace discrimination, the participants used in their study were amnesic patients and their age-matched controls (mean age = 67.5 years and 66.9 years, respectively). These participants represent an aging population. It is known that aging humans are impaired in their ability to do single-tone delay (Solomon, Pomerleau, Bennett, James, & Morse, 1989; Woodruff-Pak & Thompson, 1988) and trace eyeblink conditioning (Carrillo, Fitzpatrick, & Disterhoft, 1995). The first goal of the present study was to address whether the learning deficits seen in Clark and Squire's study could be attributable to an age-dependent phenomenon. Second, given the inconsistency between previous reports on the role of awareness, this study also explored whether awareness in both young and aging subjects was related to successful acquisition of trace and/or delay discrimination eyeblink conditioning.

Method

Participants

Participants for this study included healthy individuals in two different age ranges: young adults (20–35 years of age) and older adults (60–75 years of age). A total of 134 participants, 69 young and 65 aging, took part in the study and were randomly assigned to one of four conditioning paradigms (see Figure 1).

Participants in each of the four paradigms were divided into two groups; one received white noise as the CS+ and the other group received a pure tone as the CS+. Young subjects were recruited from the Northwestern University community through posted notices. Older adults were recruited from a cohort of nominated controls who participate in normal aging studies through the Buehler Center on Aging of Northwestern University Medical School. These participants were selected from a database for the following criteria: no history of stroke, cancer, heart problems, Parkinson's disease, substance abuse, kidney or liver problems, and no hearing or vision loss. All participants received a small payment for taking part in the study.

Apparatus

The apparatus used was a modified version of that used for eyeblink conditioning in the rabbit (Akase, Thompson, & Disterhoft, 1994; L. T. Thompson, Moyer, Akase, & Disterhoft, 1994). Eyeblink responses were
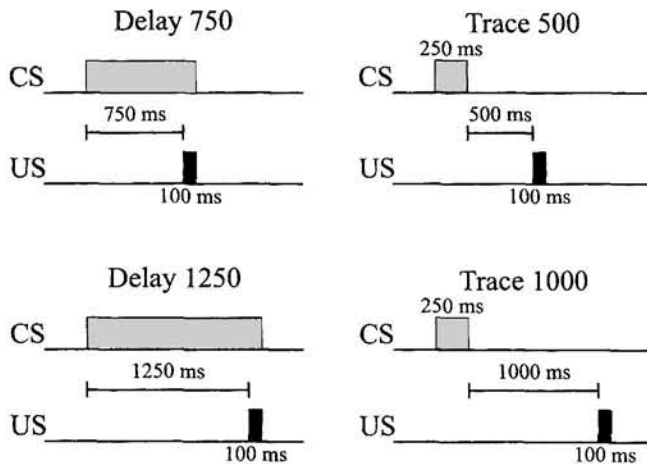
Figure 1. Participants were divided equally among four paradigms. Left: Delay 750 and delay 1250 paradigms in which the conditioned stimulus (CS), either tone or static/white noise, overlapped and coterminated with the airpuff unconditioned stimulus (US). Right: The trace paradigms, in which either a 500- or 1,000-ms silent trace interval occurred between the offset of the CS and the onset of the US. Both delay and trace parameters consisted of identical interstimulus onset intervals (i.e., 750 ms for the delay 750 and trace 500 tasks, 1,250 ms for the delay 1250 and trace 1000 tasks). All of these paradigms were used in a discrimination task in which one CS (CS+) was consistently paired with the US (as shown) and the other CS (CS−) was always presented alone.

measured with electromyograph (EMG) electrodes (Nicolet Biomedical, Madison, WI) placed over the orbicularis oculi muscle of the right eye. This approach to recording CRs also serves as a useful tool when using functional magnetic resonance imaging to monitor eyeblink responses during eyeblink conditioning (Knuttinen et al., 2000; Preston et al., 2000). The EMG responses were subsequently filtered, rectified, and integrated. An adjustable headband was worn to support the airpuff delivery nozzle. In addition to recording eyeblink responses with EMG, eyeblink movements were also monitored by an infrared diode–phototransistor aimed at the right eye. This device monitors and amplifies light reflectance from the cornea in a 0–5-V range, which is digitized and stored by computer. Figure 2 shows an example indicating that the standardized reflectance and EMG measurements are equivalent in their ability to detect appropriate eyeblink responses.

Volunteers were told they would be participating in a study of the effects of distraction on learning and memory, in a manner similar to Clark and Squire's study (1998). After signing an informed consent form, participants were seated and were administered the Folstein Mini-Mental Examination (Folstein, Folstein, & McHugh, 1975). One person (73 years of age) who received a score less than the normal range of 28–30 was excluded from the study. In addition to this behavioral assessment, each participant underwent audiometric testing to determine the auditory threshold at 1000 Hz, the frequency of the CS tone. For subjects whose auditory threshold in either ear was between 5 and 15 dB above normal (25 dB), the amplitude of the CS was adjusted accordingly (Solomon et al., 1989). Participants whose auditory threshold was greater than 40 dB were deleted from the study. Only 1 participant (68 years of age) failed to meet this criterion and had to be excluded from the study.

Participants were then fitted with the eyeblink apparatus and were told to make themselves comfortable. They were seated in a dimly lit room and told to fixate on the television screen situated in front of each of them throughout the session. A silent movie, Charlie Chaplin's *"The Gold Rush,"* was played for the participants to watch (Woodruff-Pak & Thomp-

son, 1988). The following instructions were given to each of the participants:

> Please listen carefully to the following instructions: We are studying the effects that distracting tones, static or white noise, and airpuffs have on learning and memory. Please watch the silent movie on the television screen, after which I will be asking you questions about it following this session. During the movie you will be receiving a variety of distractions. Please stay alert and watch the movie. If you feel uncomfortable and feel you need to adjust something, let me know and I'll stop the experiment to make any adjustments you need. Do you have any questions?

After giving the instructions to the participants, the experimenter sat in the same room out of the participants' view.

Behavioral Parameters

Participants initially received a sequence of 30 randomly presented pseudoconditioning (unpaired stimuli) trials (i.e., tone alone, white noise alone, and airpuff alone) before receiving 120 randomly presented discrimination conditioning trials—60 CS+ (paired with US) and 60 CS−. These initial pseudoconditioning control trials were used to determine unconditioned response (UR) amplitudes and basal rates for eyeblinks to CS-alone presentations. Airpuff-alone trials also serve to decrease sensitization responses that may otherwise have occurred during subsequent conditioning trials. Although presenting the unpaired pseudoconditioning trials before paired conditioning trials may slow response acquisition somewhat, parametric analysis of behavioral studies has demonstrated that relatively fast and robust learning curves do occur after the conditioning trials begin (Carrillo et al., 1997).

The discrimination conditioning parameters were similar to those used by Clark and Squire (1998). For the delay versions of the task, the CS was either a 75-dB, 1-kHz tone or white noise; either 850 ms or 1,350 ms in duration; with a 5-ms rise–fall time; delivered binaurally through earphones. This tone has been effective in previous conditioning studies in humans (Carrillo et al., 1995). This CS overlapped and coterminated with a 100-ms, 3-psi corneal airpuff US delivered to the right eye. For half of the subjects, the CS+ was the tone and the CS− was the white (static) noise. The other half of the subjects received the reverse. The trace conditioning parameters consisted of the same CS parameters, except the CS+ had a 250-ms duration and was followed by a silent, stimulus-free period of either 500 or 1,000 ms, which was subsequently followed by a 100-ms, 3-psi airpuff to the right eye. The intervals between the onset of the CS and the US were comparable between the delay 850 and trace 500 paradigms and between the delay 1350 and trace 1000 paradigms. No airpuff was paired with the CS−. A baseline period of 750 ms was monitored before the onset of each trial, and eyeblink responses were monitored for a period of 2,500 ms. After pseudoconditioning, a total of 120 conditioning trials (in 20-trial blocks) were presented, with an intertrial interval ranging from 10–15 s. The order of conditioning trials was random, except that neither trial type occurred more than twice consecutively. Our behavioral parameters were identical to those used by Clark and Squire (1998), with the exception that we used a short block of initial pseudoconditioning trials and a 75-dB tone as opposed to an 85-dB tone.

Once the session was terminated, the eyeblink apparatus was removed, and participants were then given a true–false postexperimental questionnaire identical to that used by Clark and Squire (1998). The questionnaire asked participants to describe aspects of the conditioning session (e.g., "I felt a puff of air to my eye"), how well they remembered the movie (e.g., "A bear was shot and killed"), and how well they remembered the relationships between the CS+, CS−, and US. The 17 critical questions addressed the temporal relationships between the CS+, the CS−, and the US (e.g., "I believe the tone predicted when the airpuff would come"). Participants responded to the questions in a fixed presentation and were
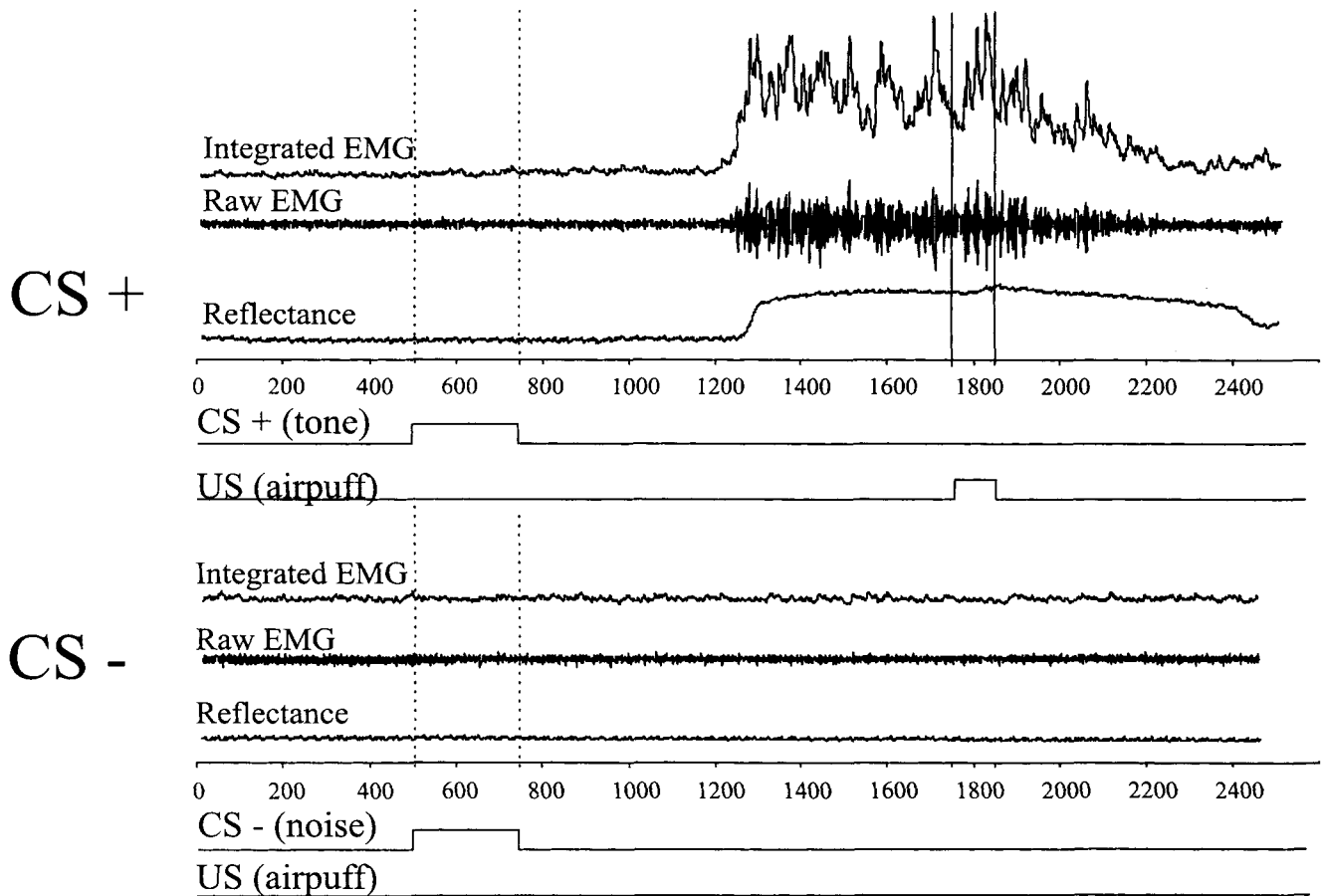
Figure 2. Sample electromyograph (EMG) and reflectance recording from single trials of an individual in the trace 1000 paradigm, illustrating the discrimination of a response to the conditioned stimulus (CS+, always presented with the unconditioned stimulus [US]) and no response to the CS− (always presented alone). Top: Raw and integrated EMG and reflectance measures of the observed eyeblink response (conditioned response) elicited to the CS+. Bottom: The lack of response elicited to the CS−. The bottom half of each graph outlines the presentation of the 250-ms tone or static/white noise relative to the 100-ms corneal airpuff. Eyeblink responses were monitored for a period of 2,000 ms.

told not to change their answers. In all groups, participants were classified as "aware" if they scored significantly above the level of chance (more than 13 of the 17 critical questions answered correctly). "Unaware" subjects were those who answered less than 13 of the 17 critical questions correctly. It has been shown that recognition memory tests reveal knowledge about stimulus relationships during classical conditioning in instances where recall tests do not reveal such knowledge (Dawson & Reardon, 1973).

Data Analysis

Eyeblink responses that reached 4 SD above the mean baseline amplitude for a minimum duration of 10 ms were classified as CRs if they occurred more than 100 ms after CS onset (to correct for voluntary responses; Gormezano, 1966). Alpha, or short latency, responses were classified as those eyeblink responses occurring during the first 100 ms of the CS (Gormezano, 1966) and were not counted as CRs. The UR amplitude was used to confirm that participants were adequately stimulated to permit conditioning to occur and that the unconditioned reflex was intact. The magnitude of the UR blink was equated between young and aging participants to compensate for the fact that aging participants tend to be

somewhat less responsive to the airpuff. Analyses in humans and rabbits have indicated that age-related conditioning deficits are not attributable to altered sensorimotor responsivity in aging subjects (Solomon et al., 1989; L. T. Thompson, Moyer, & Disterhoft, 1996; Woodruff-Pak & Thompson, 1988).

Results

Awareness and Discrimination

Young and aging participants were divided into four discrimination paradigms: trace 500 ($n = 17$ aging, 19 young); trace 1000 ($n = 16$ aging, 16 young); delay 750 ($n = 16$ aging, 17 young); and delay 1250 ($n = 16$ aging, 17 young). A repeated measures analysis of variance (ANOVA) showed an overall increase in the differential CR percentage (defined as percentage of CRs to CS+ minus percentage of CRs to CS−) across six blocks of 20 conditioning trials in trace 500, $F(5, 160) = 5.83, p < .0001$; trace 1000, $F(5, 160) = 2.66, p = .0246$; delay 750, $F(5, 150) = 15.53, p < .0001$; and delay 1250, $F(5, 150) = 7.49, p < .0001$. Figure 3
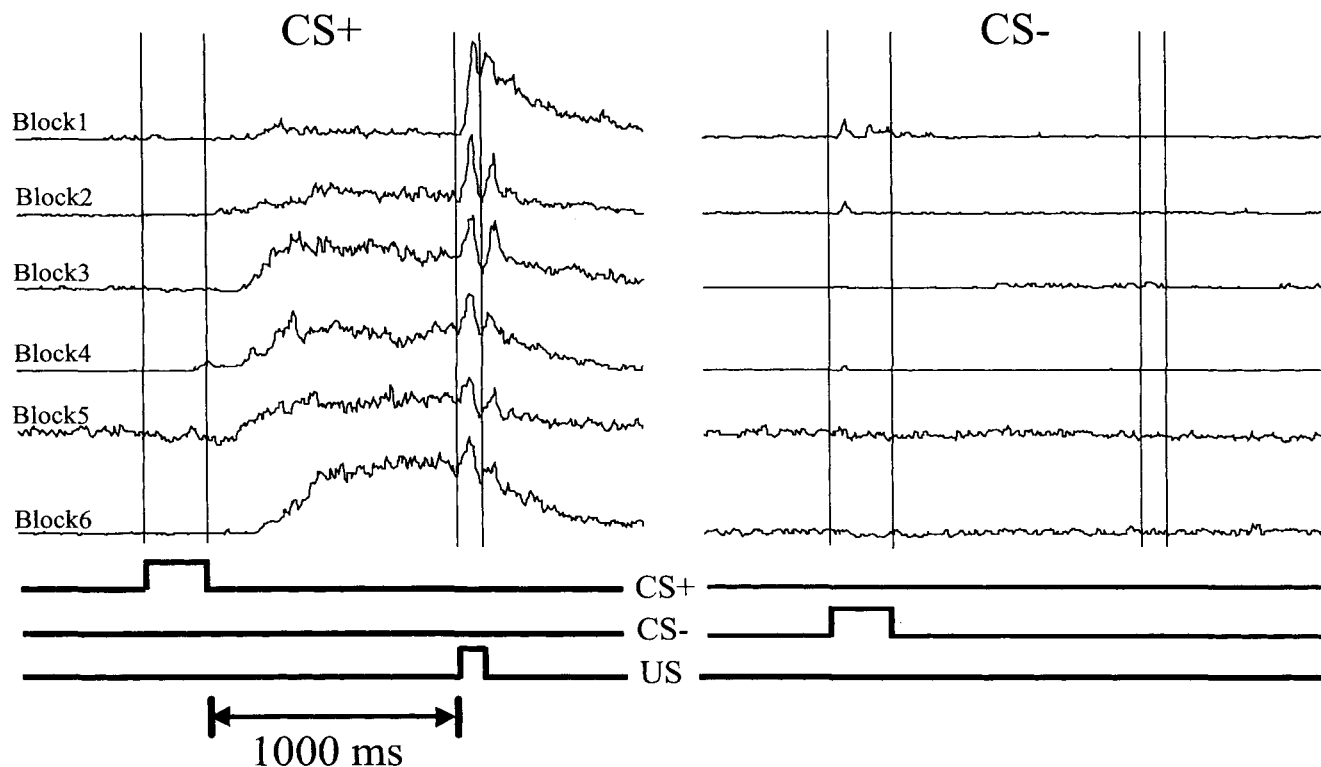
Figure 3. Development of the conditioned response (CR) to the conditioned stimulus (CS+, always presented with the unconditioned stimulus [US]) over the course of training within a single conditioning session in the trace 1000 paradigm. Each conditioning block consisted of 20 trials. Depicted here are the averaged responses of these 10 CS+ and 10 CS− (always presented alone) trials. Left: The increasingly developing CR over the course of six trial blocks. Early in training, a small response was given to the CS+ that subsequently increased in amplitude and latency until reaching the peak response. Right: In a similar yet opposite fashion, initial responses were made to the CS− that slowly disappeared as the training session continued.

exemplifies this increase in the development of the CR elicited to the CS+, as well as the lack of CRs to the CS−, during the course of a trace 1000 conditioning session for a single participant.

A significant interaction between amount of discrimination and awareness was also present across all paradigms: trace 500, $F(5, 160) = 5.05, p = .0003$; trace 1000, $F(5, 160) = 3.29, p = .0074$; delay 750, $F(5, 150) = 10.24, p < .0001$; and delay 1250, $F(5, 150) = 2.42, p = .0381$. Figure 4 shows the differential responding percentages obtained in both the young and aging aware individuals. Both young and aging aware participants were able to discriminate at a level of at least 50% in both the delay 750 and delay 1250 tasks. In the trace 500 task, the aware subjects were able to increase their percentage of differential CRs from less than 10% in the first block of conditioning to approximately 40% at the end of the session, regardless of age. The unaware subjects, however, were not able to discriminate at better than 10% even after 120 conditioning trials (60 CS+ and 60 CS−). In a similar manner, the aware young participants in the trace 1000 paradigm showed an increase in their discrimination values from 10% in Block 1 to approximately 40% at the end of the conditioning session; the aware aging participants reached asymptote at about 25% in this paradigm. The unaware participants were severely impaired and did not attain more than 0% discriminative responding across the entire conditioning session. This lack of discrimination observed in

the unaware population was attributed to the lack of CRs elicited to either the CS+ or the CS−.

Age and Discrimination

We next examined age as a factor in discriminative learning ability in an analysis that combined both the aware and unaware participants for each age group. A repeated measures ANOVA indicated no significant effect of age on performance in either the delay 750 or delay 1250 paradigms (see Figure 5). All young and all aging participants were performing equivalently in their ability to discriminate between the CS+ and the CS− by the sixth block of conditioning. However, there was a trend for the aging participants to be impaired in delay conditioning, as illustrated by their lower percentage of differential responding, especially within the first four blocks of conditioning as compared with the young participants (Figure 5). A significant effect of age on performance was noted in the trace 500, $F(5, 170) = 2.36, p = .0411$, and trace 1000, $F(5, 170) = 2.44, p = .0368$, discrimination tasks. Both young and aging participants were able to reach a level of approximately 35% discriminative responding in both delay paradigms by the end of the conditioning session (Block 6). However, compared with their younger counterparts, aging participants were impaired in their discriminative performance in both the trace 500 and (more
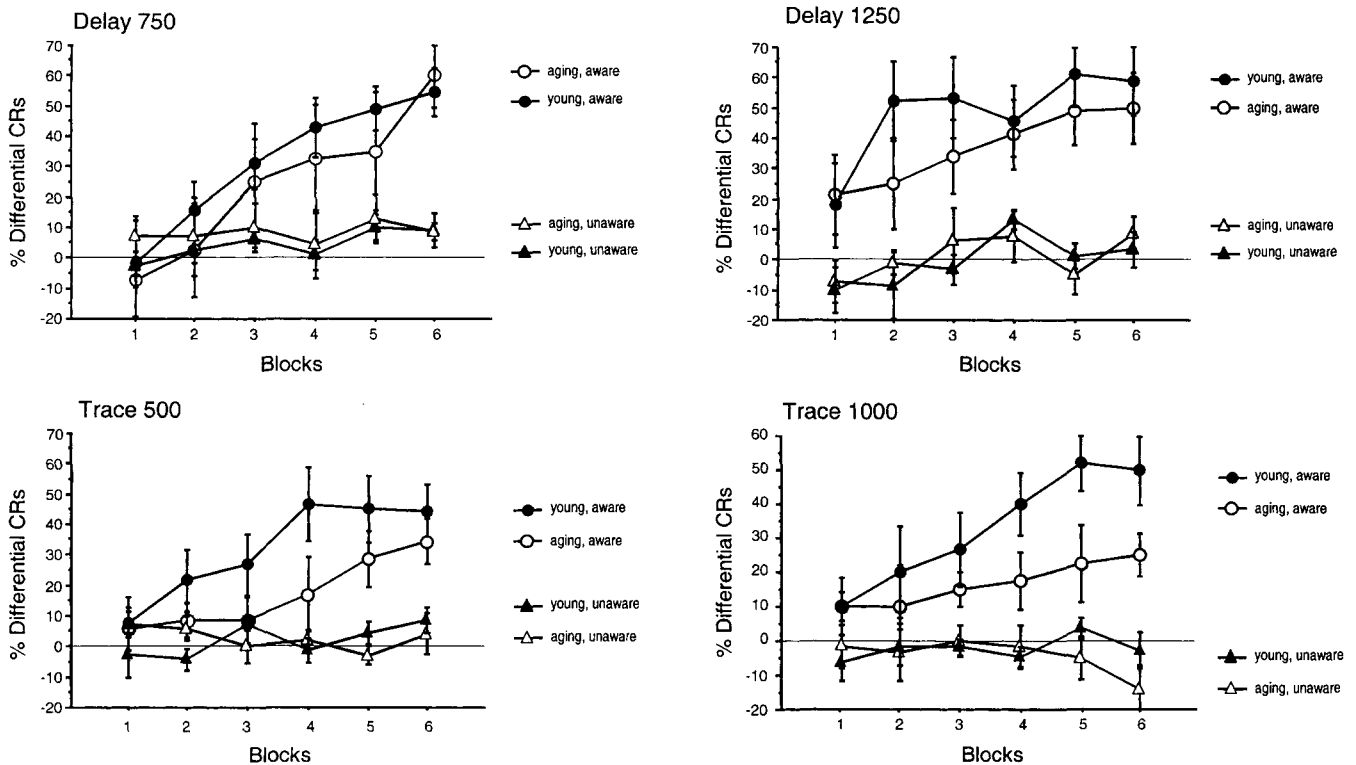
Figure 4. Percentage of differential eyeblink conditioned responses (CRs) for each block of 20 trials (% CRs to the conditioned stimulus [CS+, always presented with the unconditioned stimulus] minus the percentage of CRs to the CS− [always presented alone]). Top: Both young aware and aging aware participants attained a level of at least 50% differential CRs in the delay 750 and delay 1250 paradigms, compared with only 10% differential CRs in the unaware groups. Bottom: Young and aging aware subjects increased their percentage of differential CRs across the conditioning session in the trace 500 and trace 1000 paradigms. The young participants learned better than the aging participants in the trace 500 paradigm and (even more so) in the trace 1000 paradigm.

severely) the trace 1000 task. In fact, aging participants were not able to reach over a level of 20% differential conditional responding in trace 500, compared with a level of 30% reached by the young participants. This aging deficit was even more pronounced in the trace 1000 task, in which aging participants could not attain a level greater than 10% differential CRs, compared with the 25% discriminative responding attained by the young participants. Note that the deficit in the trace paradigms was not attributable to age-related differences in awareness, given that the aging aware participants were still performing at a lower level of discriminative responding compared with the young aware participants (Figure 4).

Postexperimental Questionnaire

All aware and unaware participants in both age groups performed equivalently in answering questions regarding movie content (see Figure 6). Aware and unaware individuals in both age groups and across all four paradigms answered no less than 9 of the 10 questions addressing movie content correctly. Furthermore, aware individuals were able to answer no less than 13 of the 17 critical questions that specifically addressed the relationships between the stimuli presented (e.g., "I believe the airpuff usually came immediately after the tone" or "I believe the static noise

predicted when the airpuff would come"). Unaware individuals were those who answered less than 13 of the 17 critical questions (Figure 6).

A chi-square analysis showed that the percentage of aware subjects across all paradigms was significantly less in the aging population than in the young population: delay 750, $\chi^2(1, N = 33)$ = 52.16, $p < .0001$; delay 1250, $\chi^2(1, N = 33)$ = 10.18, $p = .0014$; trace 500, $\chi^2(1, N = 36)$ = 20.87, $p < .0001$; trace 1000, $\chi^2(1, N = 32)$ = 16.63, $p < .0001$. At least 60% of the young population was classified as aware across all paradigms. However, less than 50% of the aging population was classified as aware in each of the paradigms.

Discussion

Much of the previous literature on trace eyeblink conditioning in both animals and humans has focused on single-cue conditioning (Manns et al., 2000a; McGlinchey-Berroth et al., 1997; Port et al., 1986; Solomon et al., 1986; Woodruff-Pak, 1993). However, trace discrimination was hypothesized to be more difficult than delay discrimination because even single-cue trace conditioning is more difficult than single-cue delay conditioning (Solomon & Groccia-Ellison, 1996; L. T. Thompson et al., 1996). In the present study, awareness of stimulus contingencies was correlated with success-
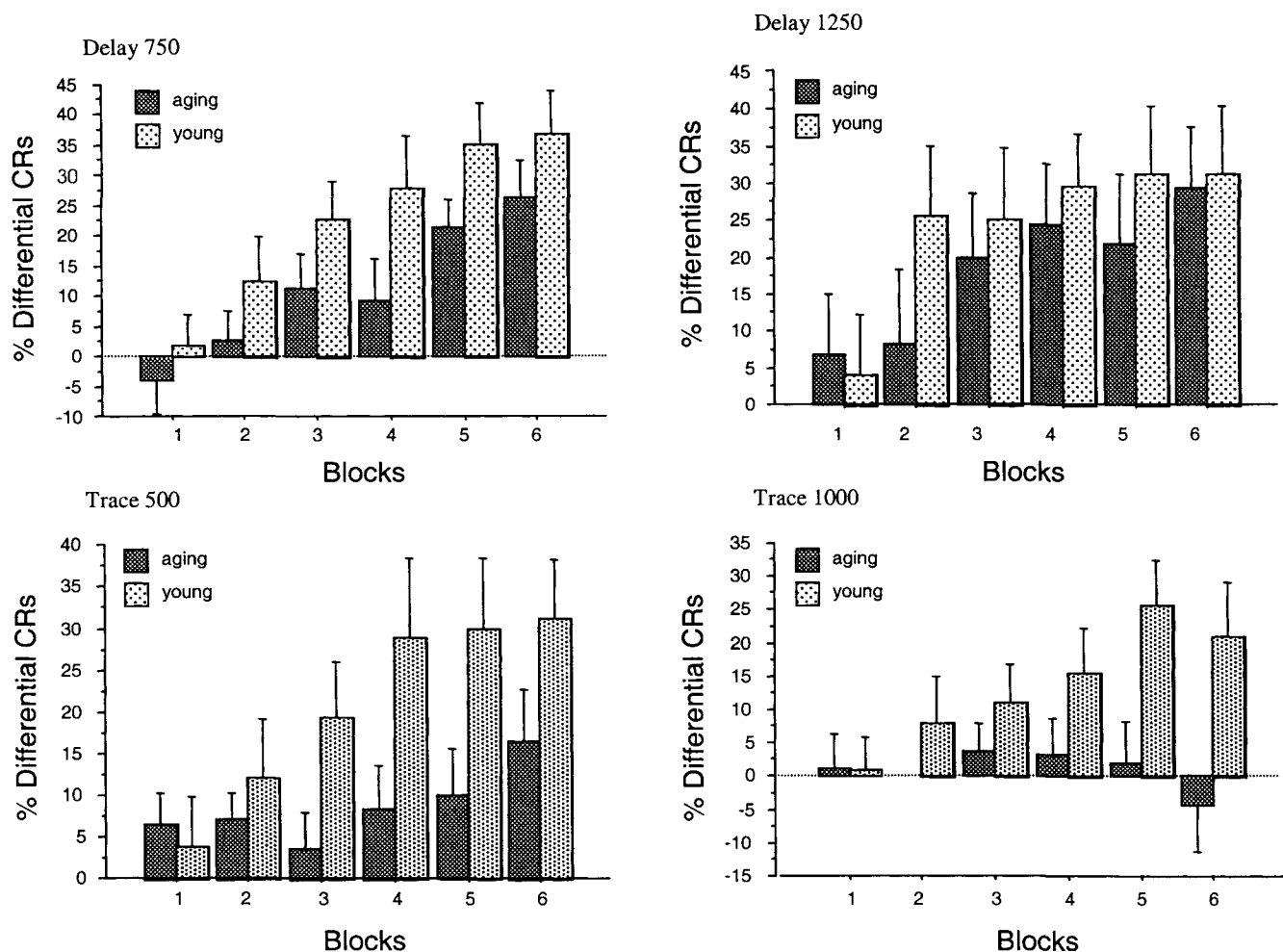
Figure 5. Data presented as the percentage of differential conditioned responses (CRs) for each block of 20 trials. Top: Aging and young participants increased their differential responding across the delay training sessions. Young participants learned at a slightly increased rate, but both young and aging participants were performing equivalently at Block 6 of training. Bottom: Young participants had an increased rate of learning in both the trace 500 and trace 1000 discrimination paradigms. Aging participants were severely impaired in conditioning in the trace 500 paradigm and (especially) in the trace 1000 paradigm.

ful acquisition of both trace 500 and trace 1000 discrimination eyeblink conditioning. Individuals were able to successfully discriminate in trace conditioning only when they had access to the conscious knowledge about the temporal relationships between the stimuli during the conditioning session. These findings are consistent with the results observed by Clark and Squire (1998, 1999; also, Manns et al., 2000a, 2000b). Furthermore, the ability to discriminate successfully in these conditions was found regardless of participant age.

Awareness was also shown to be a factor in successful performance in both delay 750 and delay 1250 discrimination eyeblink conditioning, regardless of age. These findings are consistent with those of previous studies (Carrillo et al., 2000; Mayer & Ross, 1969; Ross, 1971; Ross & Nelson, 1973), which have demonstrated that reducing attention and, presumably, awareness (such as by divided attention or performance in masking or concurrent tasks) significantly decreased and sometimes eliminated differen-

tial responding. Given that all of the participants in this study had perfect or near perfect scores on questions regarding the content of the movie, it is clear that they perceived watching the movie as the primary goal of the testing session. Participants who were unaware of the conditioning stimulus contingencies apparently had their full attention directed at the movie, whereas aware participants were able to both watch the movie and determine the temporal relationship between the conditioning stimuli. Our findings support Ross and Nelson's (1973) proposal that an additional cognitive or awareness process, sensitive to several variables, that may not affect single-cue conditioning may be involved in differential conditioning. It is also possible, however, that unconscious cerebellum-dependent learning may appear should the unaware participants receive several hundred more trials of non-hippocampally dependent delay eyeblink conditioning. Although this is a possibility, it seems somewhat unlikely given that the unaware subjects in all four paradigms did not reach a level
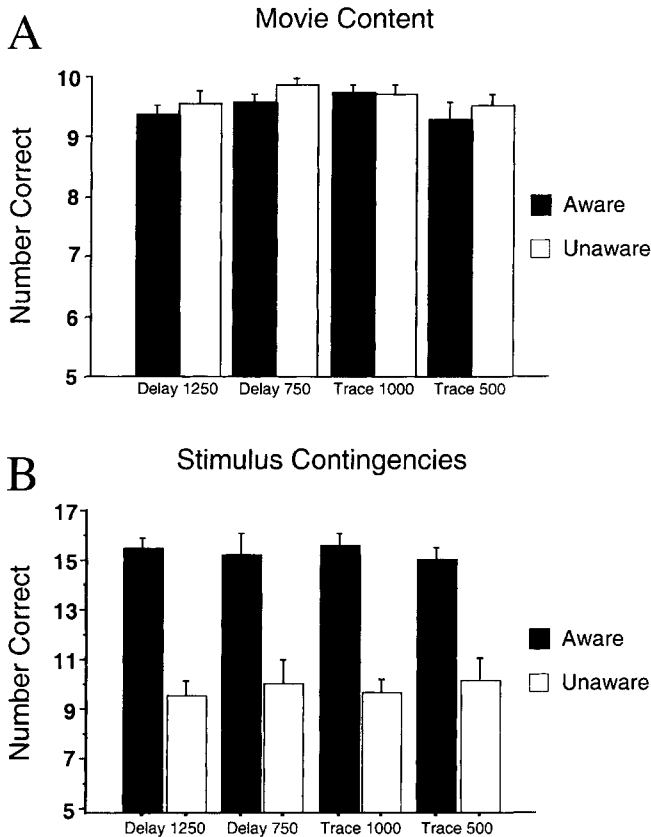
Figure 6. A: Aware and unaware young and aging participants performed equivalently in their ability to answer questions about the content of the silent movie. B: Number of correct responses to the 17 critical questions addressing the temporal relationships between the conditioned stimulus (CS+ [always presented with the unconditioned stimulus], CS− [always presented alone]), and the unconditioned stimulus. Both young and aging aware participants answered approximately 15 of 17 questions correctly, whereas unaware participants obtained no more than 10 of 17 correct responses.

beyond 10% differential CRs by the end of the training session. It would be interesting to see, however, whether presenting more delay discrimination trials may trigger the onset of cerebellum-dependent learning.

It is unclear why our results on the effects of awareness on delay discrimination tasks differ somewhat from the results observed by Clark and Squire (1998). The methods we used closely followed those of their study. The differences between the procedures are that we used a block of pseudoconditioning trials before conditioning trials to decrease sensitization responses and that we used a 75-dB tone rather than an 85-dB tone. An additional 6 aging participants were trained in exactly the same manner as Clark and Squire (1998) in the delay discrimination tasks to determine whether these differences may have altered the correlation of awareness with acquisition of delay discrimination conditioning that we observed. However, our results with this procedure were exactly the same as those obtained with the pseudoconditioning trials in place and the slightly softer tone. Furthermore, every one of the young and aging participants tested in the delay discrimi-

nation tasks who was able to successfully discriminate was classified as aware of the relationships between the CS and the US. That is, the correlation between acquisition of delay discrimination eyeblink conditioning and awareness of the stimulus contingencies was perfect in our sample. Manns, Clark, and Squire (2001) suggest that the difference between our study and theirs, which showed no correlation between awareness and acquisition in single-cue delay conditioning, may lie in our respective definition of CRs. They excluded as voluntary responses any eyeblinks that began before 500 ms prior to US onset. This would mean, for example, that the averaged response shown to develop in the successive panels of Figure 3 would not have been counted as a CR by their scoring criterion. We might note that this particular CR shows no evidence of a short-latency alpha response, increased in amplitude across trial blocks, and is an example of a trace discriminative CR for which our findings on awareness converge with those of Squire and colleagues, even though we used different scoring criteria (Clark & Squire, 1998; Manns et al., 2000a, 2000b). Future studies relating awareness to acquisition of delay eyeblink conditioning and using our alternative scoring criteria will be required to determine whether CR definition criteria may explain the divergence of our findings from those of Squire and colleagues on this one point.

Our results support the hypothesis that single-cue conditioning involves a detection process, whereas more complex conditioning paradigms, such as discrimination, may involve a detection as well as a recognition component. Because masking or concurrent tasks seem to have a profound effect in differential conditioning, this additional recognition component may be linked to an individual's awareness or other cognitive involvement. Persons with moderate amnesia who exhibited normal performance in a measure of attention (Wechsler Memory Scale—Revised) were able to successfully discriminate; however, those with severe amnesia who performed poorly on behavioral indices of attention were impaired in delay discrimination (Carrillo et al., in press). The poor performance illustrated by participants with severe amnesia suggests that an additional cognitive component may be necessary for successful acquisition of delay discrimination.

Memory has been broadly classified into nondeclarative (implicit) procedural learning and declarative (explicit) learning. Nondeclarative memory is hippocampally independent and represents a skill or process that is acquired through training and is limited to the context in which it was acquired. This category includes simple forms of associative conditioning such as delay eyeblink conditioning, which is hippocampally independent in animals and humans (Gabrieli et al., 1995; Schmaltz & Theios, 1972; Woodruff-Pak, 1993). Declarative memory, however, requires conscious recollection and the integrity of the hippocampus. As the processing demands of conditioning paradigms increase in complexity, the hippocampus and temporal lobe become essential contributors (Gluck & Myers, 1997; LaBar & Disterhoft, 1998; Moyer et al., 1990; Phillips & LeDoux, 1992; Port et al., 1986; Ross et al., 1984; Solomon et al., 1986). Understanding the conditions in which the hippocampus becomes a crucial element to conditioned behavior has intrigued scientists interested in classical conditioning models. Clark and Squire (1998) proposed that the declarative knowledge in trace conditioning paradigms is established by means of interactions between the hippocampus and the neocortex. This interaction was hypothesized to influence the circuitry underlying the CR.

Declarative memory may be a cognitive by-product or correlate of successful hippocampally dependent behavioral conditioning and not a prerequisite for it (LaBar & Disterhoft, 1998). An important issue in probing the interactions of awareness with behavior is that postexperimental questionnaires do not address whether the declarative or neurobiological aspects of conditioning are established first. An on-line method of assessing awareness on a trial-to-trial basis as conditioning occurs has begun to address this question (Manns et al., 2000a, 2000b).

The present results may shed a different perspective on the role of hippocampal learning. LaBar and Disterhoft (1998) addressed the many facets of conditioned learning that require hippocampal input, including spatiotemporal binding, the enabling of response-interval timing, and the ability to filter out irrelevant stimulation. The degree to which these processes are taxed may determine when the hippocampus makes an essential contribution to conditioned learning. A declarative account of conditioning, therefore, still leaves open multiple possibilities of the underlying processes. Our results suggest that declarative and behavioral aspects of learning may be activated in a parallel fashion in different neural systems (LaBar & Disterhoft, 1998; Manns et al., 2000a, 2000b).

Age-dependent effects were also observed in the trace, but not delay, discrimination paradigms. The most severe aging deficits on conditioning performance were observed in the trace 1000 task. Our results coincide with those of Finkbiner and Woodruff-Pak (1991), in that longer interstimulus intervals offer a higher level of difficulty for aging humans in the trace conditioning paradigm. Aging rabbits have also been shown to be impaired in single-cue trace, but not delay, eyeblink conditioning (Graves & Solomon 1985; Solomon & Groccia-Ellison 1996; L. T. Thompson et al., 1996). Both Woodruff-Pak and Thompson (1988) and Solomon, Blanchard, Levine, Velazquez, and Groccia-Ellison (1991) have shown that age-related deficits in humans are more pronounced at shorter delay intervals in single-cue delay eyeblink conditioning. For example, Solomon et al. (1991) compared young and aged adults in a 400-, 650-, and 900-ms delay eyeblink task and found significant age-related differences, primarily in the delay 400-ms task. In a similar fashion, Woodruff-Pak and Thompson (1988) showed that aging effects in humans, especially those in the 60–83 age range, are largest at short interstimulus intervals (i.e., 400 ms). It is possible that the delay intervals used in the present study fell within the longer delay intervals and were not sufficiently short to detect age-associated deficits. Furthermore, even taking into account the young–aging difference in awareness, there is still an aging deficit in trace conditioning (exhibited in Figure 4), in which the aging aware subjects performed at a lower level of discriminative responding as compared with the young aware subjects. It should also be stressed that most of the experiments with aging humans have used single-cue conditioning paradigms, and age-associated deficits may be quantitatively different in discrimination paradigms.

The present results also demonstrate that, across all paradigms, the percentage of young participants classified as aware was higher than that of aging participants. This is the first study we know of that has evaluated the effect of age on awareness in associative learning. Our findings are consistent with the common observation that older individuals are less adept at multitasking and must be able to maintain their attentive cognitive influences on one task at a time to successfully perform that task. All of the aging partici-

pants in this study who were classified as aware were performing equivalently to their younger counterparts by the end of the conditioning session. In all of the paradigms, however, the aging population was learning at a much slower acquisition rate, predominantly observed in the trace discrimination variations of the paradigm. Additional investigations using imaging techniques such as functional magnetic resonance imaging may further elucidate how the aging process may interact with awareness and, furthermore, how awareness may mediate performance in more complex forms of learning.

References

Akase, E., Thompson, L. T., & Disterhoft, J. F. (1994). A system for quantitative analysis of associative learning: Real-time software for MS-DOS microcomputers. *Journal of Neuroscience Methods, 54*, 119–130.

Berger, T. W., & Orr, W. B. (1983). Hippocampectomy selectively disrupts discrimination reversal conditioning of the rabbit nictitating membrane response. *Behavioural Brain Research, 8*, 49–68.

Blaxton, T. A., Zeffiro, T., Gabrieli, J. D. E., Bookheimer, S. Y., Carrillo, M. C., Theodore, W., & Disterhoft, J. F. (1996). Functional mapping of human learning: A positron emission tomography study of eyeblink conditioning. *Journal of Neuroscience, 16*, 4032–4040.

Carrillo, M. C., Fitzpatrick, C. T., & Disterhoft, J. F. (1995). Varying the trace interval in eyeblink conditioning in young and aging humans. *Society for Neuroscience Abstracts, 21*, 1708.

Carrillo, M. C., Gabrieli, J. D. E., & Disterhoft, J. F. (2000). Selective effects of division of attention upon discrimination conditioning. *Psychobiology, 28*(3), 293–302.

Carrillo, M. C., Gabrieli, J. D. E., Hopkins, R. O., McGlinchey-Berroth, R., Fortier, C. B., Kesner, R. P. & Disterhoft, J. F. (in press). Spared discrimination and impaired reversal eyeblink conditioning in patients with temporal lobe amnesia. *Behavioral Neuroscience*.

Carrillo, M. C., Thompson, L. T., Gabrieli, J. D. E., & Disterhoft, J. F. (1997). Variation of the intertrial interval in human classical conditioning. *Psychobiology, 25*, 152–157.

Clark, R. E., & Squire, L. R. (1998, April 3). Classical conditioning and brain systems: The role of awareness. *Science, 280*, 77–81.

Clark, R. E., & Squire, L. R. (1999). Human eyeblink classical conditioning: Effects of manipulating awareness of the stimulus contingencies. *Psychological Science, 10*, 14–18.

Daum, I., Channon, S., & Gray, J. A. (1992). Classical conditioning after temporal lobe lesions in man: Sparing of simple discrimination and extinction. *Behavioural Brain Research, 52*, 159–165.

Daum, I., Channon, S., Polkey, C. E., & Gray, J. A. (1991). Classical conditioning after temporal lobe lesions in man: Impairment in conditional discrimination. *Behavioral Neuroscience, 95*, 396–408.

Daum, I., & Schugens, M. M. (1996). On the cerebellum and classical conditioning. *Current Directions in Psychological Science, 5*, 58–61.

Daum, I., Schugens, M. M., Ackermann, H., Lutzenberger, W., Dichgans, J., & Birbaumer, N. (1993). Classical conditioning after cerebellar lesions in humans. *Behavioral Neuroscience, 107*, 748–756.

Dawson, M. E., & Biferno, M. A. (1973). Concurrent measurement of awareness and electrodermal classical conditioning. *Journal of Experimental Psychology, 101*, 82–86.

Dawson, M. E., & Furedy, J. F. (1973). The role of awareness in human differential autonomic classical conditioning: The necessary gate hypothesis. *Journal of Experimental Psychology, 101*, 55–62.

Dawson, M. E., & Reardon, P. (1973). Construct validity of recall and recognition post-conditioning measures of awareness. *Journal of Experimental Psychology, 98*, 308–315.

Disterhoft, J. F., Carrillo, M. C., Hopkins, R. O., Gabrieli, J. D. E., &

Kesner, R. P. (1996). Impaired trace eyeblink conditioning in severe medial temporal lobe amnesics. *Society for Neuroscience Abstracts, 732,* 1866.

Disterhoft, J. F., Kwan, H. H., & Lo, W. D. (1977). Nictitating membrane conditioning to tone in the immobilized albino rabbit. *Brain Research, 137,* 127–143.

Finkbiner, R. G., & Woodruff-Pak, D. S. (1991). Classical eyeblink conditioning in adulthood: Effects of age and interstimulus interval on acquisition in the trace paradigm. *Psychology and Aging, 6,* 109–117.

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). "Mini-Mental State": A practical method for grading cognitive state of patients for the clinician. *Journal of Psychiatric Research, 12,* 189–198.

Frcka, G., Beyts, J., Levey, A. B., & Martin, I. (1983). The role of awareness in human conditioning. *Pavlovian Journal of Biological Science, 18,* 69–76.

Gabrieli, J. D. E., McGlinchey-Berroth, R., Carrillo, M. C., Gluck, M. A., Cermak, L. S., & Disterhoft, J. F. (1995). Intact delay-eyeblink classical conditioning in amnesics. *Behavioral Neuroscience, 109,* 819–827.

Gluck, M., & Myers, C. (1997). Psychobiological models of hippocampal function in learning and memory. *Annual Review of Psychology, 48,* 481–514.

Gormezano, I. (1966). Classical conditioning. In J. B. Sidowski (Ed.), *Experimental methods and instrumentation in psychology* (pp. 385–420). New York: McGraw-Hill.

Gormezano, I., Schneiderman, N., Deaux, E., & Fuentes, I. (1962). Nictitating membrane: Classical conditioning and extinction in the albino rabbit. *Science, 13,* 33–34.

Grant, D. A. (1973). Cognitive factors in eyelid conditioning. *Psychophysiology, 10,* 75–81.

Graves, C. A., & Solomon, P. R. (1985). Age-related disruption of trace but not delay classical conditioning of the rabbit's nictitating membrane response. *Behavioral Neuroscience, 99,* 88–96.

Grings, W. W. (1965). Verbal-perceptual factors in the conditioning of autonomic responses. In W. F. Prokasy (Ed.), *Classical conditioning: A symposium* (pp. 71–89). New York: Appleton-Century-Crofts.

Hilgard, E. R., Campbell, A. A., & Sears, W. N. (1937). Conditioned discrimination: Development of discrimination with and without verbal report. *American Journal of Psychology, 49,* 564–580.

Hillner, K. P. (1979). *Conditioning in contemporary perspective.* New York: Springer Publishing.

James, G. O., Hardiman, M. S., & Yeo, C. H. (1987). Hippocampal lesions and trace conditioning in the rabbit. *Behavioural Brain Research, 23,* 109–116.

Kim, J. J., Clark, R. E., & Thompson, R. F. (1995). Hippocampectomy impairs the memory of recently, but not remotely, acquired trace eyeblink conditioned responses. *Behavioral Neuroscience, 109,* 195–203.

Kimble, G. A. (1962). Classical conditioning and the problem of awareness. *Journal of Personality, 3,* 27–45.

Knuttinen, M.-G., Weiss, C., Parrish, T. B. LaBar, K. S., Gitelman, D. R., Power, J. M., Mesulam, M.-M., & Disterhoft, J. F. (2000). Event-related fMRI of delay eyeblink conditioning, *Neuroimage, 11,* 416.

LaBar, K. S., & Disterhoft, J. F. (1998). Conditioning, awareness, and the hippocampus. *Hippocampus, 8,* 620–626.

Manns, J. R., Clark, R. E., & Squire, L. R. (2000a). Awareness predicts the magnitude of single-cue trace eyeblink conditioning. *Hippocampus, 10,* 181–186.

Manns, J. R., Clark, R. E., & Squire, L. R. (2000b). Parallel acquisition of awareness and trace eyeblink classical conditioning. *Learning and Memory, 7,* 267–272.

Manns, J. R., Clark, R. E., & Squire, L. R. (2001). *Single-cue delay eyeblink conditioning is unrelated to awareness.* Manuscript submitted for publication.

Mauk, M. D., & Thompson, R. F. (1987). Retention of classically condi-

tioned eyelid responses following acute decerebration. *Brain Research, 403,* 89–95.

Mayer, M. J., & Ross, L. E. (1969). Effects of stimulus complexity, interstimulus interval, and masking task conditions in differential eyeblink conditioning. *Journal of Experimental Psychology, 81,* 469–474.

McCormick, D. A., Clark, G. A., Lavond, D. G., & Thompson, R. F. (1982). Initial localization of the memory trace for a basic form of learning. *Proceedings of the National Academy of Sciences, USA, 79,* 2731–2735.

McCormick, D. A., Guyer, P. E., & Thompson, R. F. (1982). Superior cerebellar peduncle lesions selectively abolish the ipsilateral classically conditioned nictitating membrane/eyelid response of the rabbit. *Brain Research, 244,* 347–350.

McCormick, D. A. Lavond, D. G., & Thompson, R. F. (1983). Neuronal responses of the rabbit brainstem during performance of the classically conditioned nictitating membrane/eyelid responses. *Brain Research, 271,* 73–88.

McCormick, D. A., & Thompson, R. F. (1984). Neuronal responses of the rabbit cerebellum during acquisition and performance of a classically conditioned nictitating membrane/eyelid response. *Journal of Neuroscience, 4,* 2811–2822.

McGlinchey-Berroth, R., Carrillo, M., Gabrieli, J. D. E., Brawn, C. M., & Disterhoft, J. F. (1997). Impaired trace eyeblink conditioning in bilateral medial temporal lobe amnesia. *Behavioral Neuroscience, 111,* 873–882.

Moyer, J. R., Jr., Deyo, R. A., & Disterhoft, J. F. (1990). Hippocampectomy disrupts trace eyeblink conditioning in rabbits. *Behavioral Neuroscience, 104,* 243–252.

Orr, W. B., & Berger, T. W. (1985). Hippocampectomy disrupts the topography of conditioned nictitating membrane responses during reversal learning. *Behavioral Neuroscience, 99,* 35–45.

Phillips, R. G., & LeDoux, J. E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behavioral Neuroscience, 106,* 274–285.

Port, R. L., Mikhail, A. A., Romano, A. G., Steinmetz, J. E., & Patterson, M. M. (1986). Retention and acquisition of classical trace conditioned responses by rabbits with hippocampal lesions. *Behavioral Neuroscience, 100,* 745–752.

Preston, A. R., Knuttinen, M.-G., Christoff, K., Glover, G. H., Gabrieli, J. D. E., & Disterhoft, J. F. (2000). The neural basis of classical eyeblink conditioning: An event-related fMRI study. *Society for Neuroscience Abstracts, 26,* 709.

Ross, L. E. (1971). Cognitive factors in conditioning: the use of masking tasks in eyelid conditioning. In H. H. Kendler & J. T. Spence (Eds.), *Essays in neobehaviorism: A memorial volume to Kenneth W. Spence* (pp. 161–185). New York: Appleton-Century-Crofts.

Ross, L. E., & Nelson, M. N. (1973). The role of awareness in differential conditioning. *Psychophysiology, 10,* 91–94.

Ross, R. T., Orr, W. B., Holland, P. C., & Berger, T. W. (1984). Hippocampectomy disrupts acquisition and retention of learned conditioned responding. *Behavioral Neuroscience, 98,* 211–225.

Schmaltz, L. W., & Theios, J. (1972). Acquisition and extinction of a classically conditioned reflex in hippocampectomized rabbits (*Oryctolagus cuniculus*). *Journal of Comparative and Physiological Psychology, 79,* 328–333.

Schreurs, B. G., McIntosh, A. R., Bahro, M., Herscovitch, P., Sunderland, T., & Molchan, S. E., (1997). Lateralization and behavioral correlation of changes in regional cerebral blood flow with classical conditioning of the human eyeblink response. *Journal of Neurophysiology, 77,* 2153–2163.

Solomon, P. R., Blanchard, S., Levine, E., Velazquez, E., & Groccia-Ellison, M. E. (1991). Attenuation of age-related conditioning deficits in humans by extension of the interstimulus interval. *Psychology and Aging, 6,* 36–42.

Solomon, P. R., & Groccia-Ellison, M. (1996). Classic conditioning in

aged rabbits: Delay, trace, and long-delay conditioning. *Behavioral Neuroscience, 110*, 427–435.

Solomon, P. R., & Moore, J. W. (1975). Latent inhibition and stimulus generalization of the classically conditioned nictitating membrane response in rabbits (*Oryctolagus cuniculus*) following dorsal hippocampal ablations. *Journal of Comparative and Physiological Psychology, 89*, 1192–1203.

Solomon, P. R., Pomerleau, D., Bennett, L., James, J., & Morse, D. L. (1989). Acquisition of the classically conditioned eyeblink response in humans over the life span. *Psychology and Aging, 4*, 34–41.

Solomon, P. R., Vander Schaaf, E. R., Thompson, R. F., & Weisz, D. J. (1986). Hippocampus and trace conditioning of the rabbit's classically conditioned nictitating membrane response. *Behavioral Neuroscience, 100*, 729–744.

Spence, K. W., (1966). Cognitive and drive factors in the extinction of the conditional eye blink in human subjects. *Psychology Review, 73*, 445–458.

Spielberger, C. (1962). The role of awareness in verbal conditioning. *Journal of Personality, 30*, 73–101.

Thompson, L. T., Moyer, J. R., Jr., Akase, E., & Disterhoft, J. F. (1994). A system for quantitative analysis of associative learning: I. Hardware interfaces with cross-species applications. *Journal of Neuroscience Methods, 54*, 109–117.

Thompson, L. T., Moyer, J. R., Jr., & Disterhoft, J. F., (1996). Trace eyeblink conditioning in rabbits demonstrates heterogeneity of learning ability both between and within age groups. *Neurobiology of Aging, 17*, 619–629.

Thompson, R. F. (1986, August 29). The neurobiology of learning and memory. *Science, 233*, 941–947.

Thompson, R. F., Berger, T. W., Cegavske, C. F., Patterson, M. M., Roemer, R. A., Teyler, T. J., & Young, R. A. (1976). A search for the engram. *American Psychologist, 31*, 209–227.

Thompson, R. F., Clark, G. A., Donegan, N. H., Lavond, D. G., Madden, J., Mamounas, L. A., Mauk, M. D., & McCormick, D. A. (1984). Neuronal substrates of basic associative learning. In L. R. Squire & N. Butters (Eds.), *Neuropsychology of memory* (pp. 424–442). New York: Guilford.

Weiss, C., & Disterhoft, J. F. (1996). Eyeblink conditioning, motor control, and the analysis of limbic-cerebellar interactions. *Behavioral and Brain Sciences, 19*, 479–481.

Weiss, C., Knuttinen, M.-G., Power, J. M., Patel, R. I., O'Connor, M. S., & Disterhoft, J. F. (1999). Trace eyeblink conditioning in the freely moving rat: Optimizing the conditioning parameters. *Behavioral Neuroscience, 113*, 1100–1105.

Woodruff-Pak, D. S. (1988). Aging and classical conditioning: Parallel studies in rabbits and humans. *Neurobiology of Aging, 9*, 511–522.

Woodruff-Pak, D. S. (1993). Eyeblink classical conditioning in H.M.: Delay and trace paradigms. *Behavioral Neuroscience, 107*, 911–925.

Woodruff-Pak, D. S., & Thompson, R. F. (1988). Classical conditioning of the eyeblink response in the delay paradigm in adults aged 18–83 years. *Psychology and Aging, 3*, 219–229.