

Temporal Discrimination Learning in Severe Amnesic Patients Reveals an Alteration in the Timing of Eyeblink Conditioned Responses

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This study investigated whether the hippocampal system plays a modulatory role in the timing of conditioned responses (CRs) in eyeblink classical conditioning. Seven bitemporal amnesic patients and 7 controls were randomly presented 2 tone conditioned stimuli (CSs) that were individually paired with two different interstimulus intervals (ISIs) in a delay conditioning task. It was found that amnesic patients' CRs occurred significantly earlier than control participants' CRs at the longer ISI. Amnesic patients also produced significantly more nonadaptive CRs than did control participants, their level of acquisition was less than that of control participants after equating for ISI, and they did not show extinction with the longer ISI. These data suggest a role of the hippocampal system in controlling the precise timing of conditioned eyeblink responses and in acquiring and extinguishing responses within the context of a temporal discrimination task.

One of the more remarkable aspects of eyeblink classical conditioning is that the elicitation of the conditioned response (CR) is strictly dependent on the time interval between the conditioned stimulus (CS) and the unconditioned stimulus (US). The latency to CR onset and the rise time or peak latency vary in strict relation to the interstimulus interval (ISI) such that a CR reaches maximum amplitude just before the onset of the US (e.g., Coleman & Gormezano, 1977; Kehoe, Graham-Clarke, & Schreurs, 1989; Levey & Martin, 1968; Millenson, Kehoe, & Gormezano, 1977; Schneiderman, 1966; Schneiderman & Gormezano, 1964; Smith, 1968; Smith, Coleman, & Gormezano, 1969). An attempt to localize the brain systems that support this learning has been the goal of a great deal of research.

Neuropsychological studies conducted by Ivry and his colleagues have indicated that the cerebellum is critical for

the normal operation of an internal timing mechanism that could underlie the timing of classically conditioned eyeblink responses. Their studies have suggested that the cerebellum computes on-line intervals between successive stimuli that are important for the perception of time, production of response sequences, and computation of the intervals between stimuli and responses (Ivry & Keele, 1989; Ivry, Keele, & Diener, 1988). Additionally, Ivry and Diener (1991), in a series of three experiments, found that patients with cerebellar lesions were normal in making position judgments but were impaired in making judgments about the velocity of moving stimuli.

Woodruff-Pak and her colleagues have examined the relationship between eyeblink conditioning and a possible cerebellar timing mechanism. In one study, Woodruff-Pak, Papka, and Ivry (1996) reported that CRs in normal participants were negatively correlated with a measure of clock variability in the timed-interval tapping task (Ivry et al., 1988). Further, Woodruff-Pak and Jaeger (1997) found that the variability in this timing task was a significant predictor of conditioning performance across a wide age range.

The possible contribution of the cerebellar cortex in temporal learning using classical eyeblink conditioning was directly addressed in rabbits by Perrett, Ruiz, and Mauk (1993). In that study, rabbits were trained by using two discriminable tone CSs that were individually associated with two different ISIs (Mauk & Ruiz, 1992). Both CSs signaled the same US. After the rabbits had learned the temporal discrimination (i.e., their CRs were appropriately timed as a function of the CSs), they received aspiration lesions of the cerebellar cortex. After the lesions, both CSs elicited similarly timed CRs that peaked at very short latencies.

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The above studies suggest that the cerebellar cortex may be the locus of a timing mechanism that controls various aspects of learned behavior. There is some indication, however, that the hippocampal system may also play a role in processing temporal information. Support for this supposition was initially provided in a theoretical review by Solomon (1979). On the basis of evidence from rabbit eyeblink conditioning studies, he suggested that "one function that the hippocampus might participate in is a type of 'temporal mapping.' By this I simply mean registration of the temporal sequence of events" (p. 1277). This was a relatively radical position at the time, as most theories posited a spatial processing view of hippocampal function (e.g., O'Keefe & Nadel, 1978).

More recently, it has been demonstrated in both animals and humans that the hippocampal system is necessary for normal acquisition in the trace eyeblink conditioning task in which there is temporal separation between the CS and US. Specifically, Solomon, Vander Schaaf, Thompson, and Weisz (1986) found that lesions of the hippocampus disrupt acquisition of the trace, but not the delay, nictitating membrane CR (see also James, Hardiman, & Yeo, 1987; Port, Romano, Steinmetz, Mikhail, & Patterson, 1986). Moyer, Deyo, and Disterhoft (1990) found that acquisition could be totally eliminated with a 500-ms trace interval in rabbits that had undergone more complete hippocampectomies than in those studied by Solomon et al. (1986). Last, Kim, Clark, and Thompson (1995) reported that hippocampectomy eliminated retention and relearning of 500-ms trace eyeblink conditioning when done 24 hr after acquisition of the task.

To date, three studies have been conducted to examine trace eyeblink conditioning in humans. Woodruff-Pak (1993) found that H.M. and a postencephalitic patient did acquire a mean percentage of CRs comparable to that of a control participant but did so when provided additional trials to reach criterion, suggesting that bitemporal amnesic patients can perform trace conditioning, albeit at an impaired rate. We have also examined the ability of amnesic patients with bilateral, medial temporal lobe damage and matched control participants to acquire and extinguish CRs in a trace eyeblink paradigm. McGlinchey-Berroth et al. (McGlinchey-Berroth, Carrillo, Gabrieli, Brawn, & Disterhoft, 1997) found that bitemporal amnesic patients produced significantly fewer CRs than control participants at three different trace intervals (500 ms, 750 ms, and 1,000 ms), although the same subjects did perform normally when previously tested in a delay conditioning task (Gabrieli et al., 1995). Patients did not show any evidence of CR acquisition in the 1,000-ms trace condition. Clark and Squire (1998) used a differential trace conditioning procedure in which one CS was consistently paired with the US (CS+) and a second CS was presented alone (CS-). Bitemporal patients were tested by using this procedure with a 1000-ms trace interval. They found that bitemporal amnesic patients were unable to show differential conditioning at this interval due to their failure to acquire CRs to the CS+. These patients, however, did show acquisition in a differential 700-ms delay task.

These data suggest that the hippocampal system may play

an important role in representing temporally distinct stimuli. Additional evidence suggests that the hippocampal system is not only necessary for processing temporally distinct information but that it may also be important for controlling or regulating the timing of responses that are initiated by the cerebellum (Christiansen & Schmajuk, 1992; James et al., 1987; McGlinchey-Berroth et al., 1997; Port & Patterson, 1984; Port et al., 1986). For example, Christiansen and Schmajuk (1992) found that hippocampal lesions in rats did not impair acquisition or extinction of CRs in a delay paradigm, but that the hippocampally lesioned rats showed significantly shorter CR onset latencies during acquisition and extinction and larger CR amplitude during acquisition. Similar findings have been reported with the trace conditioning paradigm (Moyer et al., 1990; Port et al., 1986; Solomon et al., 1986). In each of these studies, hippocampal lesions resulted in nonadaptive, short-latency CRs. In addition, we have recently reported a subtle timing deficit in humans, in which the peak latency of the CR in bitemporal amnesic patients occurred earlier than in nonamnesic control participants (McGlinchey-Berroth et al., 1997).

In the present study, we investigated the possibility that the hippocampal system is involved in modulating the timing of CRs directly. We used the discrimination paradigm developed by Mauk and Ruiz (1992), in which two tone CSs signal two different ISIs before US onset. Because the discrimination task used a delay paradigm, it was predicted that amnesic patients with bilateral, medial temporal lobe damage would display intact acquisition and extinction of CRs for each individual CS and ISI. However, because of the hippocampal system's hypothesized role in modulating the timing of CRs, comparisons across ISIs were expected to reveal alterations in the CR onset latency and CR peak latency. On the basis of the evidence regarding timing from the animal literature and our own previous study, the nature of this difference was predicted to be shorter onset and peak latencies and the occurrence of nonadaptive responses in the long ISI condition. Nonamnesic control participants were predicted to show CRs that were time-locked to the specific CS-ISI combination.

Method

Participants

The participants in this study were recruited from the Memory Disorders Research Center in Boston, MA. Each had participated in previous eyeblink conditioning studies (Gabrieli et al., 1995; McGlinchey-Berroth et al., 1997).

Amnesic participants. Seven amnesic patients were tested. Four became amnesic as the result of an anoxic episode, 2 from encephalitis, and 1 from status epilepticus. Bilateral damage to the hippocampal formation was confirmed by computerized tomography or magnetic resonance imaging in 5 of the 7 cases. Of the remaining 2 cases, 1 had enlarged ventricles and diffuse cortical atrophy, and 1 had moderate central and cortical atrophy (both were amnesic because of an anoxic episode).

Demographic and neuropsychological characteristics of the amnesic participants are presented in Table 1. It is clear from these data that all of the patients were severely memory impaired as shown by their poor recall performance ($M = 59.71$, $SE = 3.52$) on

Table 1
Patient Demographic and Neuropsychological Characteristics

Patient	Age	Ed.	WAIS-R		WMS-R		Warrington	
			Verbal IQ	General memory	Attention	Delay	Faces	Words
Amnesic Group								
1	57	16	105	76	92	51	41	35
2	60	20	109	65	89	61	34	30
3	47	16	111	81	107	69	32	31
4	68	18	103	68	93	66	33	35
5	38	14	95	90	115	<50	29	33
6	38	12	104	88	108	71	41	40
7	69	18	126	102	114	<50	32	35
Control Group								
8	72	12	93					
9	59	16	128					
10	55	16	115					
11	43	14	94					
12	36	13	97					
13	47	16	97					
14	59	14	97					

Note. The control participants were not tested on the Wechsler Memory Scale-Revised (WMS-R) or the Warrington Recognition Test. The Wechsler Adult Intelligence Scale-Revised (WAIS-R) and the WMS-R scaled scores yield a normalized, age adjusted mean of 100. The WMS-R does not provide scores below 50. On the Warrington Recognition Test, 1 point is scored for each of 50 items. Age and education (Ed.) are expressed in years.

the Wechsler Memory Scale-Revised (WMS-R; Wechsler, 1987) and their poor recognition performance for verbal ($M = 34.14$, $SE = 1.24$) and nonverbal ($M = 34.57$, $SE = 1.76$) material on the Warrington Recognition Test (Warrington, 1984). They had preserved intellectual and attentional function, however, as indicated by their verbal score performance ($M = 107.57$, $SE = 3.62$) on the Wechsler Adult Intelligence Scale-Revised (WAIS-R; Wechsler, 1981) and their WMS-R attention score ($M = 102.57$, $SE = 4.15$).

Nonamnesic control participants. Seven control participants were recruited from a pool of volunteers and screened to be free of any neurological disease or illness. The control group was matched to the amnesic patients with regard to age ($M = 54$, $SE = 4.85$), education ($M = 14$, $SE = 0.63$), and verbal intelligence as measured by the WAIS-R ($M = 99.00$, $SE = 2.80$). Results of *t*-tests indicated that the amnesic patients and control participants were equivalent on each of these measures ($ps > .14$).

Apparatus

The apparatus used was a modified version of that used for eyeblink conditioning in the rabbit (Akase, Thompson, & Disterhoft, 1994; Thompson, Moyer, Akase, & Disterhoft, 1994), and one that we have used in previous eyeblink conditioning studies with humans (Gabrieli et al., 1995; McGlinchey-Berroth, Carrillo et al., 1997; McGlinchey-Berroth, Cermak, et al., 1995). Eyeblink movements were monitored with an infrared diode phototransistor aimed at the right eye. This device monitors and amplifies light reflectance from the cornea in a 0–5 VDC range, which is then digitized and stored by the computer. In this system, eyeblink amplitude is an inverse function of the amount of reflected light contacting the phototransistor aimed at the cornea. The detector was adjusted so that the baseline or null setting was near 1 V when the eye was open

and at the highest amplitude possible (less than 5 V) when the eye was fully closed. The detector and the airpuff delivery nozzle were attached to an adjustable arm that was mounted on a headpiece worn by the participants.

Stimuli

There were two CSs. The first (CS1) was an 85-dB, 1-kHz tone, and the second (CS2) was an 85-dB, 5-kHz tone. The tones were delivered binaurally over earphones and were presented for a total of either 450 or 850 ms. The US was a 100-ms, corneal airpuff delivered to the right eye that coterminated with the CS. The magnitude of the airpuff averaged 3 psi. The assignment of CS1 and CS2 to ISI1 (350 ms) and ISI2 (750 ms) was counterbalanced across participants.

Procedure

Participants were individually brought into the laboratory, and the examiner went over the informed consent form with them. After providing consent, they were seated in an upright chair and fitted with the eyeblink apparatus. Throughout the session, the experimenter sat in the same room out of the direct view of the participant and answered questions as they arose. The experimenter read the following instructions:

Please listen carefully to the following instructions. Remain seated comfortably and look straight ahead, avoiding all eye movements such as looking around the room. Please do not touch the headband or earphones at any time during the experiment yet if you feel uncomfortable or feel you need to adjust anything please let me know and I will stop the experiment to make any adjustments.

You will hear and feel a series of stimuli during the session. These stimuli will consist of some tones or beeps and a light puff of air. Please feel free to blink whenever you want. All you are asked to do is to concentrate on what is going on and just let your natural reactions take over.

Each conditioning session consisted of 90 conditioning trials. For approximately half of the participants, CS1 was followed by the 350-ms ISI before US onset, and CS2 was followed by the 750-ms ISI before US onset. For the remaining half of the participants, the reverse was true. There were 45 trials of each trial type, presented in random order. There were 30 extinction trials after the conditioning trials. During extinction, the two CSs were presented alone, also in random order. A schematic diagram of the two types of conditioning trials is presented in Figure 1. Before the onset of each trial, there was a 650-ms baseline recording period. The intertrial interval during conditioning and extinction averaged 10 s but varied randomly from 8 to 12 s (Carrillo, Thompson, Gabrieli, & Disterhoft, 1997).

Definitions

An eyeblink was only scored as a CR if it was three standard deviations greater than the mean baseline response amplitude. Eyeblinks with a latency of less than 100 ms after CS onset were recorded as alpha responses and were not considered CRs (Gormezano, 1966). Additionally, the number of nonadaptive CRs was tabulated. These were responses that met the criteria for a CR but for which there was a partial opening of the eyelid before the onset of the US.

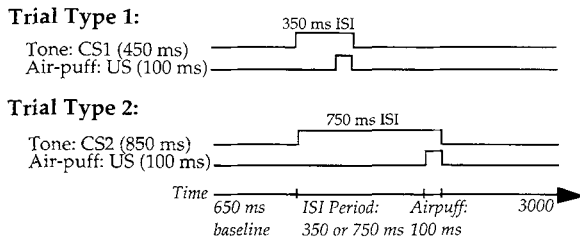


Figure 1. Time course of the temporal discrimination task. CS = conditioned stimulus; US = unconditioned stimulus; ISI = inter-stimulus interval.

Results

The two primary measures that are informative with regard to response timing are CR onset latency and CR peak latency. Each of these measures was analyzed in separate analyses of variance (ANOVAs) examining the effect of group (amnesic or control) and ISI (350 ms or 750 ms). We conducted planned comparisons on the interaction term to examine whether a shift in either of the latency measures occurred from the 350-ms condition to the 750-ms condition and whether group differences were present at either of the two ISIs. A secondary measure of timing was also examined in a one-way ANOVA that compared the number of nonadaptive CRs across the 2 groups.

Acquisition was evaluated by examining the percentage of trials on which a CR occurred and measuring the CR amplitude in a Group \times ISI ANOVA. To equate the two ISI conditions, only the final 250 ms before US onset was considered in the 750-ms ISI condition. Extinction was evaluated in two ANOVAs that examined the percentage of CRs as a function of group and condition (conditioning vs. extinction) for each ISI condition.

Temporal Learning

For CR onset latency, there was a main effect of ISI, $F(1, 12) = 16.59, p < .001$, but no significant effect of group or Group \times ISI interaction ($ps > .12$). Onset latencies in the 750-ms ISI condition ($M = 340.08, SE = 19.29$) were significantly longer than the onset latencies in the 350-ms ISI condition ($M = 257.86, SE = 9.87$). Planned comparisons indicated that this shift was significant for both the amnesics, $F(1, 12) = 4.96, p < .05$, and control participants, $F(1, 12) = 12.48, p < .01$. However, there was a tendency for the onset latency to differ between the two groups in the 750-ms condition, $F(1, 12) = 3.65, p < .08$, suggesting a possible alteration in the onset latency of the amnesics' CRs. There was no group difference in the 350-ms condition ($p > .5$).

A significant alteration in timing was observed in amnesic patients in the peak latency measure (see Figure 2). Although there was a main effect of ISI, $F(1, 12) = 168.97, p < .0001$, that followed a similar pattern as the onset latency measure (350 ms: $M = 322.32, SE = 6.38$; 750 ms: $M = 557.60, SE = 21.03$), the significant group effect indicated that amnesics' peak latency was shorter than that of control participants, $F(1, 12) = 4.55, p = .05$. Control

participants' latency averaged 476.43 ms ($SE = 42.34$) from CS onset, whereas amnesic patients' latency averaged only 437.07 ms ($SE = 36.95$). Planned comparisons revealed that the tendency for amnesics' CRs to peak earlier than normal was, in fact, significant only in the longer, 750-ms ISI condition, $F(1, 12) = 4.72, p = .05$, in which the amnesic patients' peak latency was approximately 60 ms shorter than that of the control participants. The peak latency did not differ between groups in the 350-ms condition ($p > 0.5$). Importantly, though, both groups did demonstrate a significant shift in peak latency from the 350-ms to the 750-ms ISI, $F(1, 12) = 69.98, p < .0001$, and $F(1, 12) = 100.35, p < .0001$, for amnesics, and controls, respectively. The fact that the patients performed similarly in the 350-ms condition and that both groups evidenced a significant shift in peak latency likely explains why the interaction term failed to reach significance, $F(1, 12) = 1.36, p < .3$, even though the amnesics did show a significant alteration in peak latency in the 750-ms ISI condition.

Last, the percentage of trials in which a CR occurred but was nonadaptive (as depicted in Figure 3) was significantly different between the two groups, $F(1, 12) = 8.87, p = .01$. On average, 29% ($SE = 0.071\%$) of the CRs that amnesics produced were nonadaptive, compared with only 6% ($SE = 0.033\%$) for the control participants. As depicted by the standard deviations represented in Figure 4, there was some variability in this measure: Two of the patients' CRs were nonadaptive approximately half of the time, and 2 additional patients produced nonadaptive CRs on over one third of the trials. The control group produced very few nonadaptive CRs overall, with only 1 participant's nonadaptive CRs approaching 20% of total responses.

Conditioning

The mean percentage of CRs differed as a function of both group, $F(1, 12) = 6.12, p < .05$, and ISI, $F(1, 12) = 19.28$,

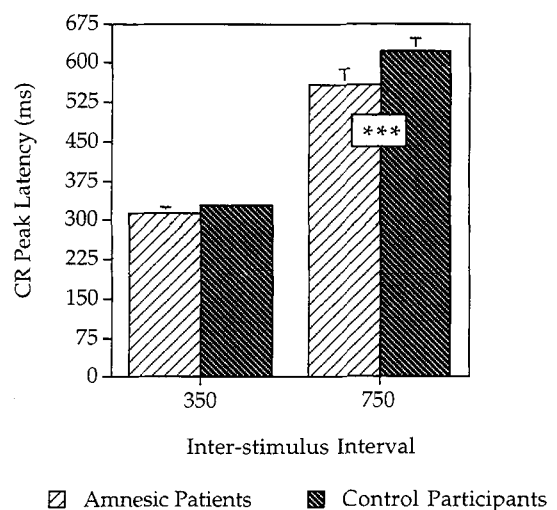


Figure 2. Mean peak latency ($\pm SEM$) for conditioned responses (CRs) in the 350-ms and 750-ms interstimulus interval conditions, displaying the significant alteration in timing in the amnesic patients. *** $p = .05$.

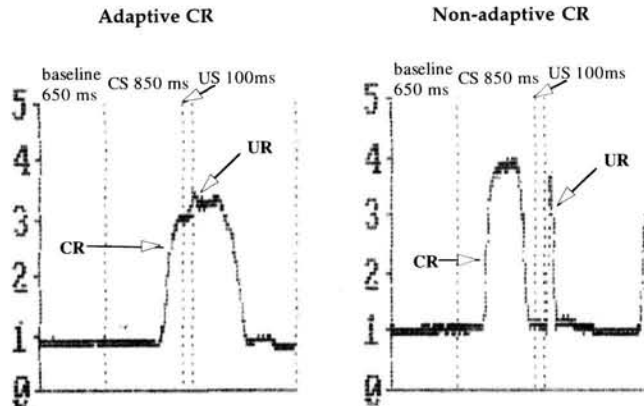


Figure 3. Conditioning trials illustrating adaptive and nonadaptive conditioned responses (CRs). Adaptive responses are ones in which the eyelid closure continues into the unconditioned response (UR) onset, protecting the eye from the airpuff. Nonadaptive responses are ones in which the eyeblink closure ends prematurely, leaving the eye exposed to the unconditioned stimulus.

$p < .001$. Overall, amnesics averaged 45.71 CRs ($SE = 4.55$), whereas control participants averaged 59.84 CRs ($SE = 4.66$). These data point to an overall impairment in acquisition by the amnesic patients on this discrimination task. In addition, there were significantly more CRs in the 750-ms condition ($M = 63.17$, $SE = 4.71$) than in the 350-ms condition ($M = 42.38$, $SE = 3.35$), even though the two intervals had been equated for the purposes of this analysis. Mean CR amplitude differed only as a function of ISI, $F(1, 12) = 10.13$, $p < .01$. Amplitudes were higher in the 750-ms ISI condition ($M = 2753.17$, $SE = 131.92$) than in the 350-ms condition ($M = 2403.28$, $SE = 128.51$).

The mean unconditioned response amplitude was evaluated to determine if there was a difference between the groups. Unconditioned response amplitude averaged 2130 mV ($SE = 129.00$) for the amnesics and 2484.12 mV

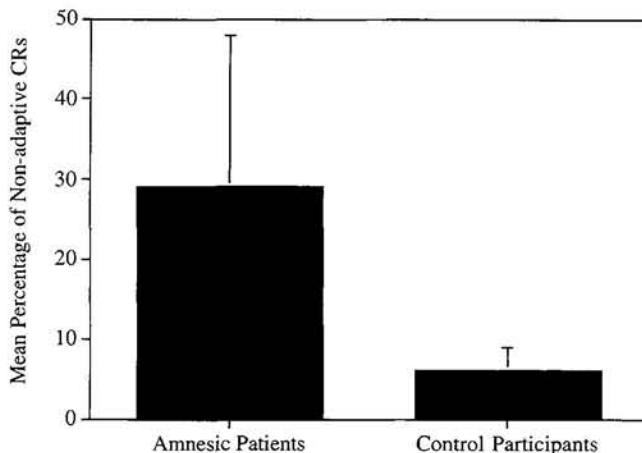


Figure 4. Mean percentage and standard deviation of nonadaptive conditioned responses (CRs) produced by amnesic patients and control participants.

($SE = 141.36$) for the control participants. This difference was not significant ($p > .12$).

Extinction

Both groups showed successful extinction of CRs given a 350-ms ISI. A Group \times Condition (conditioning vs. extinction) ANOVA revealed a main effect of condition, $F(1, 12) = 92.97$, $p < .001$, and a Group \times Condition interaction, $F(1, 12) = 5.95$, $p < .05$. As expected, the overall effect of condition was due to a significantly greater percentage of CRs during conditioning trials ($M = 42.38$, $SE = 3.34$) than during extinction trials ($M = 14.76$, $SE = 3.36$). Analysis of the interaction indicated that both the amnesics, $F(1, 12) = 25.95$, $p < .001$, and the control participants, $F(1, 12) = 72.96$, $p < .001$, showed a significant difference between conditioning and extinction (see Figure 5a). Control participants, however, did produce a greater percentage of trials with a CR during conditioning than did the amnesics, $F(1, 12) = 7.52$, $p < .05$. The groups did not differ in the percentage of CRs during extinction trials ($p > .4$).

A significant impairment in extinction was found in the amnesics in the 750-ms ISI condition (see Figure 5b). A Group \times Condition ANOVA revealed a main effect of condition, $F(1, 12) = 24.08$, $p < 0.001$, and a Group \times Condition interaction. Analysis of the interaction indicated

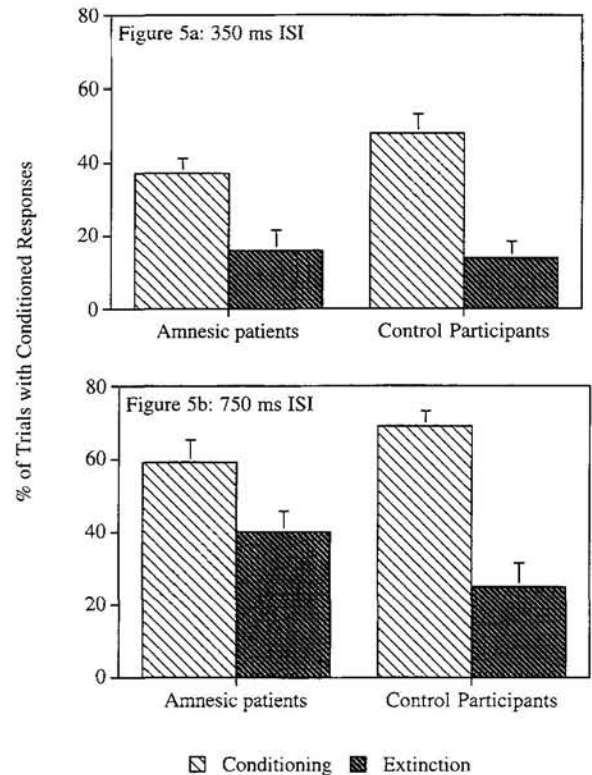


Figure 5. Comparison of the mean percentage ($\pm SEM$) of trials on which a conditioned response was produced during conditioning trials and extinction trials after equating the two interstimulus interval (ISI) conditions. Patients extinguished normally with a 350-ms ISI (a), but showed a deficit in extinction with a 750-ms ISI (b).

that there was not a significant difference between conditioning and extinction for the amnesic patients ($p > 0.14$) but that there was a significant difference for the control participants, $F(1, 12) = 28.56, p < 0.001$.

Spontaneous Blinks and Alpha Responses

Neither the number of spontaneous blinks nor the number of short latency alpha responses differed between the groups ($ps > .4$). The mean number of spontaneous blinks was 30.71 ($SE = 4.61$) for the amnesics and 25.43 ($SE = 4.28$) for the control participants. The mean number of alpha responses for the amnesics was 2.79 ($SE = 0.38$) and 3.50 ($SE = 0.96$) for the control participants.

Discussion

The precise timing of classical conditioning eyeblink responses in a temporal discrimination task was altered by medial temporal lobe lesions in humans. The peak latency of bitemporal amnesics' CRs occurred significantly earlier than that of control participants for trials in which the ISI was relatively long (i.e., 750 ms). A similar trend was also noted in the onset latency measure. Additionally, a considerable number of CRs produced by hippocampally damaged amnesics were classified as nonadaptive; that is, there was at least a partial opening of the eyelid before the onset of the US. In fact, nearly half of the CRs produced by 2 patients met our criteria as nonadaptive. Notably, however, the amnesic patients showed peak and onset latencies equivalent to those of control participants when the ISI was relatively short (i.e., 350 ms), as well as a significant shift in peak and onset latencies for trials with a 350-ms ISI compared with trials with a 750-ms ISI. Thus, it appears that medial temporal lobe damage disrupts the ability to precisely time the elicitation of the CR and to maximize its adaptive and protective benefit, but it does not eliminate the ability to time the CR entirely.

These findings may be somewhat surprising in light of the evidence indicating that the cerebellum, not the hippocampal system, is critical for controlling the timing aspects of a number of cognitive processes (e.g., Ivry, 1993), including eyeblink classical conditioning (Perrett et al., 1993). However, the amnesic patients did show evidence of learning the temporal discrimination, evidenced by significantly longer onset and peak latencies in the 750-ms ISI condition than in the 350-ms ISI condition. In contrast, lesions to the cerebellar cortex produced CRs to both CSs that peaked at very short latencies in rabbits that had previously displayed differentially timed responses (Perrett, Ruiz, & Mauk, 1993). Thus, lesions to the cerebellar cortex may eliminate the appropriate expression of a learned temporal discrimination, whereas lesions to the hippocampal system may produce a more subtle alteration in the timing of CRs.

Perrett, Ruiz, and Mauk (1993) suggested that the dissociation that they observed between generating a CR and the timing of the CR might be due to the existence of at least two sites of neural plasticity. One site, located within the cerebellar deep nuclei, is essential for the learning and

expression of the CR (e.g., Daum, Ackermann, Lutzenberger, Dichgan, & Dirbaumer, 1993; Lavond, Lincoln, McCormick, & Thompson, 1984; Lye, O'Boyle, Ramsden, & Schady, 1988; Solomon, Stowe, & Pendlebury, 1989; Topka, Valls-Sole, Massaquoi, & Hallett, 1992; Woodruff-Pak, Lavond, & Thompson, 1985; Yeo, Hardiman, & Glickstein, 1985), whereas the second site, located within the cerebellar cortex, is responsible for the timing aspects of the CR. These two sites together support the generation of CRs in a generally appropriate time frame. The current data suggest a possible third site of plasticity involved in expression of associative learned responses, located within the medial temporal lobe memory system. Although not essential in a delay paradigm, this site may represent temporal information on a more detailed level than in the cerebellar cortex. This representation may be used to modulate the activity of the cerebellar cortex and thus ensure that the CR is elicited at a time that maximizes its protective value.

Theoretical support for the notion that the hippocampus may play a modulatory role in conditioning was provided by Grossberg and Merrill (1996), who have recently developed a neural computational model of the role of the hippocampus and cerebellum in adaptively timed learning. According to their model, hippocampal timing circuits help to maintain attention toward goal objects during variable-task-related delays, whereas cerebellar timing circuits control the release of CRs. In this way, the hippocampus modulates the activity of the cerebellum. Without the hippocampus, they argue, the cerebellum generates CRs prematurely.

These data may also help to define the nature of the hippocampal activity that has been found during eyeblink conditioning in both animal and human studies. Electrophysiological studies have shown conditioning-specific changes in the firing rates of pyramidal cells in the CA1 region of the rabbit hippocampus that correlate with the development of CRs (Akase, Deyo, & Disterhoft, 1988; Berger & Thompson, 1978a, 1978b; McEchron & Disterhoft, 1997; Miller & Steinmetz, 1997; Weiss, Kronforst-Collins, & Disterhoft, 1996). Conditioning-specific changes in the amplitude and duration of the afterhyperpolarization have also been demonstrated in rabbit hippocampal CA1 and CA3 neurons after conditioning (Coulter et al., 1989; Disterhoft, Coulter, & Alkon, 1986; Moyer, Thompson, & Disterhoft, 1996; Thompson, Moyer, & Disterhoft, 1996), and these changes have been shown to correlate highly with behavioral acquisition of CRs (Disterhoft, Golden, Read, Coulter, & Alkon, 1988; Moyer et al., 1996; Thompson et al., 1996). In humans, positron emission tomography studies have imaged hippocampal activation in conjunction with acquisition during delay conditioning (Blaxton et al., 1996; Logan & Grafton, 1995). All of these studies suggest that the hippocampus must play some role in the acquisition of associative learning, even though that role may be nonessential, as appears to be the case in delay conditioning (because delay conditioning can be acquired after hippocampal lesions). The findings from the current study suggest that one such role may be in the modulation of the temporal aspects of conditioned responses acquired by the cerebellum.

The amnesic patients in this temporal discrimination task

were mildly impaired in their ability to acquire conditioned responses, as evidenced by a significant main effect of group in the Group \times ISI ANOVA examining the mean percentage of CRs. This finding was unexpected given that some studies have shown intact discrimination learning in amnesics. For example, it has been reported that amnesic patients can acquire differential conditioning during a two-tone discrimination task (Carrillo, Hopkins, et al., 1997; Clark & Squire, 1998). Similarly, Daum also found that amnesic patients showed significant discrimination learning that was similar to that of control participants (Daum, Breitenstein, Ackermann, & Schugens, 1997; see also Daum, Channon, & Gray, 1992). Perhaps the impairment observed in the present study is attributable to the fact that the discrimination itself was temporal in nature. Given that we now believe that the hippocampal system is involved in the processing of temporal motor behavior, it may not be so surprising that lesions to this system would interfere with acquisition within the context of a temporal discrimination task.

A final point of discussion is the lack of a significant difference between conditioning trials and extinction trials for the amnesic patients in the 750-ms ISI condition. This finding suggests a possible impairment in the amnesic patients' ability to extinguish the CR in the 750-ms ISI condition. This impairment could not simply be due to the fact that the amnesic's acquisition rate was lower in this condition because they also generated more CRs during the extinction trials than did control participants. Consequently, as far as we are aware, this is the first demonstration of impaired extinction following hippocampal system damage in humans. This finding is puzzling in light of the fact that these same amnesic patients showed normal extinction in previous eyeblink conditioning studies (Gabrieli et al., 1995; McGlinchey-Berroth et al., 1997). Daum, Channon, and Gray (1992) also reported normal extinction in a group of temporal lobectomy patients, although in another study with severely amnesic patients the findings were ambiguous (Daum, Channon, & Canavan, 1989). Impaired extinction after hippocampal system damage is not without precedent, however. Moyer, Deyo, and Disterhoft (1990) reported a profound impairment in extinction in hippocampectomized rabbits in a 300-ms trace conditioning study. Thus it appears that under certain conditions, hippocampal damage (or removal) may interfere with the extinction of CRs. It is interesting to note that the current study and the earlier rabbit study by Moyer et al. (1990) both required temporal processing, whereas the Daum et al. (1989, 1992) studies used a delay paradigm that did not have a temporal component. This cannot be the whole story, however, because we found intact extinction in our amnesic patients during trace conditioning. Perhaps the combined requirements of discriminating a sensory characteristic such as tone frequency and temporal processing demands make the task cognitively complex enough to require hippocampal system involvement for extinction to occur. Future studies are needed to directly address the role of the hippocampal system during extinction.

In conclusion, the current data support the idea that the hippocampus may play an important role in modulating the

temporal aspects of learned conditioned behavior that is most likely generated and localized within the cerebellum. It appears that the removal of this important modulatory input for cerebellar activity can cause an impairment in the timing of CRs that mimics to a lesser extent the impairment seen after cerebellar cortical damage.

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