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The Role of the Hippocampus in Trace Conditioning: Temporal Discontinuity or Task Difficulty?

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It is well established that the hippocampal formation is critically involved in the acquisition of trace memories, a paradigm in which the conditioned (CS) and unconditioned stimuli (US) are separated by a temporal gap (Solomon et al., 1986). The structure is reportedly not critical for the acquisition of delay memories, where the CS and the US overlap in time (Berger & Orr, 1983; Schmaltz & Theios, 1972). Based on these results, it is often stated that the hippocampus is involved in "filling the gap" or otherwise associating the two stimuli in time. However, in addition to the presence of a temporal gap, there are other differences between trace and delay conditioning. The most apparent difference is that animals require many more trials to learn the trace task, and thus it is inherently more difficult than the delay task. Here, we tested whether the hippocampus was critically involved in delay conditioning, if it was rendered more difficult such that the rate of acquisition was shifted to be analogous to trace conditioning. Groups of rats received excitotoxic lesions to the hippocampus, sham lesions or were left intact. Using the same interstimulus intervals (ISI), control animals required more trials to acquire the trace than the delay task. As predicted, animals with hippocampal lesions were impaired during trace conditioning but not delay conditioning. However, when the delay task was rendered more difficult by extending the ISI (a long delay task), animals with hippocampal lesions were impaired. In addition, once the lesioned animal learned the association between the CS and the US during delay conditioning, it could learn and perform the trace CR. Thus, the role of the hippocampus in classical conditioning is not limited to learning about discontiguous events in time

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and space; rather the structure can become engaged simply as a function of task difficulty.

INTRODUCTION

Classical eyeblink conditioning is a form of associative learning that has been demonstrated in a range of animals, including humans. In a typical eyeblink conditioning procedure, a behaviorally "neutral" conditioned stimulus (CS), such as a burst of white noise, is repeatedly presented in close temporal proximity to an impending aversive unconditioned stimulus (US), such as a stream of air (i.e., "airpuff") directed at the eye or a periorbital stimulation of the eyelid. As a consequence, an association is formed between the CS and US such that the previously neutral CS acquires the capacity to elicit closure of the surrounding eyelid, i.e., a conditioned response (CR). Eyeblink conditioning exhibits a high degree of sensitivity to the temporal relationship between the CS and US. Characteristically, the acquisition of the conditioned response is most efficacious when the onset of the CS precedes the onset of the US by no more than several hundred ms and, under many circumstances, may be abolished entirely with interstimulus intervals (ISIs) as short as 1 s (Gormezano, 1966). Moreover, acquisition of the learned eyeblink response is typically impaired with "trace" intervals as short as 100 ms inserted between the offset of the CS and the onset of the US. Although all forms of associative learning are to varying degrees sensitive to each of these temporal manipulations, eyeblink conditioning is unique in its *degree* of sensitivity, i.e., its acquisition is disrupted by absolute temporal manipulations that would be inconsequential in most commonly studied forms of associative learning.

Procedures employing the temporal arrangements referred to as "delay" and "trace" conditioning are diagrammatically summarized in Fig. 1. In the case of delay conditioning, the CS coterminates with the onset or overlaps with the US, whereas in trace conditioning CS offset occurs prior to the onset of the US. Even in those instances where the interval between the offset of the CS and the onset of the US are the same, the insertion of a trace interval (i.e., a period void of the CS) between CS offset and US onset impedes the acquisition of the conditioned response. Thus, the insertion of a trace interval or temporal "gap" is believed to produce a unique adverse influence on associative learning (Kamin, 1961) (Shors et al., 2000).

Beyond its implications for understanding learning processes, trace conditioning is of interest to those concerned with the neurobiological basis for learning and memory because it is found to engage different anatomical substrates than the more elemental delay procedure. Richard F. Thompson and colleagues have determined that acquisition and expression of delay eyeblink conditioning are dependent on the cerebellum, as evidenced by the impairment of learning and abolition of established conditioned responding by lesions of the anterior interpositus nucleus (Clark et al., 1992; Krupa et al., 1993; Lavond et al., 1993; McCormick et al., 1982; Steinmetz et al., 1986; Yeo, 1999). However, with homologous training parameters, neither the acquisition nor the expression of the delay conditioned response is sensitive to hippocampal lesions (Berger & Orr, 1983; Schmaltz & Theios, 1972). While acquisition of trace conditioning is similarly dependent on the cerebellum (Woodruff-Pak et al., 1985), acquisition and a time-limited expression of trace conditioning are dramatically impaired following hippocampal lesions (Gabrieli et al., 1995; Kim et

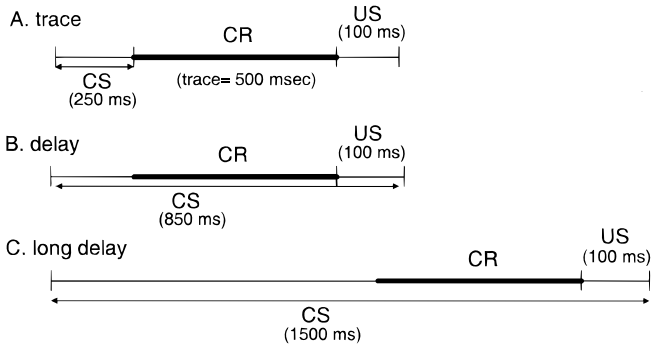

FIG. 1. Schematic diagram of (A) trace, (B) delay, and (C) long delay conditioning procedures.

al., 1995; Moyer et al., 1990; Solomon et al., 1986). Thus, while delay conditioning is dependent on an intact cerebellum, trace conditioning requires participation of both the cerebellum *and* the hippocampus. From these findings and similar ones, it has been suggested that the hippocampus plays a unique role in the formation of associations between stimuli that are discontiguous in time or space (Wallenstein et al., 1998). Alternatively, it has been suggested that a hippocampal–cerebellar network becomes necessary for timing conditioned responses when the CS–US interval is nonoptimal (LaBar & Disterhoft, 1998; Port et al., 1985) or that the hippocampus resolves stimulus relationships that are temporally ambiguous (Hoh et al., 1999; Murray & Ridley, 1999).

Despite the marked influence that observations of trace conditioning have had on the development of theories of hippocampal function, a closer consideration of relevant data led us to question whether a *unique* role for the hippocampus in trace conditioning has been demonstrated. Since trace conditioning is inherently more difficult than delay conditioning (Beylin & Shors, 1998; Clark & Squire, 1998; Gould et al., 1999a; Ivkovich et al., 2000), we questioned whether the hippocampus is involved in the processing of temporally discontiguous events or whether it is simply engaged as a function of task difficulty. For such a determination, it would be necessary to compare the effects of hippocampal lesion on trace conditioning *relative* to a delay conditioning arrangement that is comparably difficult for the animal to learn. To accomplish this, we extended the length of the ISI between CS and US onset during delay conditioning and established parameters whereby delay and trace conditioning were acquired at comparable rates. Basing task difficulty on learning rates, it was then possible to establish whether there is a *unique* role for the hippocampus in trace conditioning or whether it is simply engaged differentially as a function of increasing task difficulty.

In the first experiment, rats with hippocampal lesions were tested on delay versus trace paradigms in which the ISIs between the CS and US were equated (Fig. 1AB). In a second experiment, rats with hippocampal lesions were tested on trace conditioning, while others were tested on a long delay task with an extended ISI (i.e., longer than the standard delay (Fig. 1C)). As a second part of each experiment, we tested whether rats that first acquired the delay conditioning task could then perform the trace conditioning task and vice versa. This manipulation allowed us to determine whether the presence of the trace interval would interfere with learning despite the fact that the animal already associated the CS with the US. Also, the manipulation allowed us to assess the effects of hippocampal ablation on learning per se relative to the generation of CRs (i.e., performance variables).

EXPERIMENT 1

Acquisition of delay eyeblink conditioning is nonlinearly dependent on the ISI between the CS and US. Under most circumstances, optimal conditioning occurs with ISIs of 200–400 ms, while both shorter and longer ISIs can impair acquisition (Gormezano et al., 1983; Schniederman, 1966). In the first experiment, we questioned whether lesions of the hippocampus would prevent trace but not delay conditioning using the same ISI. In the delay condition, 750 ms intervened between the onset of a continuous CS (850 ms duration) and the onset of the US (Fig. 1A). For delay conditioning, the CS overlapped and coterminated with the US. In the companion trace procedure, the same 750-ms intervened between the CS and US onsets; however, the CS was only 250 ms in duration, resulting in a 500-ms trace interval in which the CS was absent prior to US onset (Fig. 1B). For both delay and trace tasks, the duration of the US was 100 ms. Acquisition and maintenance of the CR was assessed in intact, lesioned, and sham lesion animals. As discussed, we also reversed the learning paradigms for all groups of rats such that after trace conditioning they were exposed to delay conditioning and vice versa. This protocol allowed us to verify that hippocampal-lesioned animals trained with trace conditioning could indeed learn delay conditioning. Conversely, we could evaluate whether animals that had learned the delay CR could then perform the same response with the trace interval present.

Methods

Subjects. The subjects were 40 male Sprague–Dawley rats (Charles River) that were housed individually with unlimited access to Purina laboratory chow and water. Rats were maintained on a 12:12 light:dark cycle, with light onset at 0700 h. Testing occurred between 0900 and 1800 h.

Surgery. Bilateral excitotoxic lesions to the hippocampus were adapted from the methods of Jarrard (1989). Rats were anesthetized with an intraperitoneal injection of Nembutal (60 mg/kg), which was supplemented as necessary during surgery with additional doses of 5–15 mg. The skull over the hippocampus was removed and a Hamilton syringe was used to infuse *N*-methyl-D-asparate (NMDA; 15 mg/ml) into 26 sites throughout the hippocampus. NMDA was infused 0.05 μl/min through a pulled glass micropipette that provided minimal damage to the overlying cortex. Following injection, pipettes were left in place for 3 min to limit NMDA flow within the injection pathway. Coordinates and volumes for the 26 infusion sites are presented in Table 1 and were as suggested by Jarrard (1989), using the Paxinos and Watson atlas (1986). Sham surgeries consisted of lowering the pipette, filled with 0.9% saline, to the same coordinates and leaving them in place for 1 min without infusion. A control group was left surgically intact. All rats were implanted with electrodes to record the electromyographic (EMG) activity for determination of the eyeblink and to deliver the periorbital stimulation to elicit the eyeblink reflex. The rats were fitted with headstages attached to four electrodes as previously described (Servatius & Shors, 1996; Skelton, 1988). Electrodes consisted of silver wire (0.005 in. without insulation; 0.007 in. with insulation) which were implanted subcutaneously to emerge through and around the eyelid. The wires were deinsulated on one end and the other was attached through gold pins to a strip connector that served as a headstage.

TABLE 1
Stereotaxic Coordinates Indicating the Position of Each Injection Site along the Entire Medial–Temporal Extent of the Hippocampus

Anterior–posterior	Medial–lateral	Dorsal–ventral
−2.4	±1.0	−3.4
−3.0	±1.4	−3.4*, −2.6*
	±3.0	−3.0
−4.0	±2.6	−3.3*, −2.3*
	±3.7	−3.0
−4.9	±3.9	−7.0, 3.5*
	±4.1	−3.8
−5.7	±5.1	−5.8, 4.9, 4.0

Note. Anterior–posterior and medial–lateral coordinates are presented in millimeters in relation to bregma, while dorsal–ventral injection sites are in reference to the surface of dura mater. A volume of 0.1 μl of NMDA was infused at each location, except where an asterisk denotes 0.05 μl. These parameters were as suggested by Jarrard (1989).

The headstage was attached to the skull with acrylic and surrounded by a plastic cap for security. The scalp was closed with stainless steel wound clips. Postoperatively, rats were injected intramuscularly with 300,000 units of penicillin (Butler). Rats were given at least 4 weeks to recover prior to behavioral testing.

Conditioning apparatus and procedure. Headstages were connected to a cable that allowed free movement within the conditioning chamber. Of four implanted electrodes, two delivered periorbital shock. The other two electrodes transmitted EMG activity that was filtered to pass 0.3–1.0 kHz and amplified (10K) with a differential AC amplifier and passed to a 16-bit A/D card (DAS, 1600, Keithley-Metrabyte, Tauton, MA).

Rats were acclimated to the conditioning apparatus for 1 h with the ventilating fans and house lights operating. During this time, rats were not exposed to conditioning stimuli and spontaneous blink rate was recorded. Twenty-four hours later, rats were returned to the conditioning apparatus and eyeblinks were recorded. A total of 30 samples were collected for 550 ms with an intertrial interval (ITI) of 20 $\pm$ 10 s. A blink during the sampling period was considered a response. To determine whether hippocampal lesions altered responding to the white noise stimulus prior to training, rats were exposed to 10 white noise CSs (320 ms; 82–83 dB; ITI, 20 $\pm$ 10 s) prior to training. If an eyeblink occurred during the first 100 ms of the white noise stimulus, it was considered a sensitized response to the CS.

Half of the animals in each surgical group (hippocampal, lesion, sham, and intact) were exposed to 600 trials (300/day) of trace eyeblink conditioning with a 500-ms trace interval between a 250-ms, 82- to 83-dB burst of white noise CS and a 100-ms, 0.7-mA periorbital shock US (Fig. 1). The same animals were subsequently exposed to 600 trials (300 trials/day) of delay conditioning with an 850-ms CS overlapping and coterminating with a 100-ms US. The ISI (CS onset to US onset) for both tasks was 750 ms. The remaining half of the rats in each of the three surgical groups were trained with the same conditioning procedures, but in the opposite order, i.e., 600 trials of delay conditioning followed by 600 trials of trace conditioning. Every 10-trial sequence consisted of 1 CS-alone presentation, 4

paired presentations of the CS and US, a US-alone presentation, and 4 paired presentations of the CS and US. The ITI was randomized with a mean of 20 ± 10 s.

To detect the occurrence of an eyeblink, the maximum EMG response that occurred during a 250-ms prestimulus baseline recording period was added to four times its standard deviation. Responses that exceeded that value and were longer than 3 ms were considered eyeblinks. Eyeblinks were considered CRs if they began 500 ms prior to US onset. Eyeblink performance was computed as a percentage of CRs to the CS.

Histology. Rats were anesthetized with an overdose of Nembutal and perfused through the heart with 10% buffered Formalin. Brains were removed and placed in 10% Formalin with 30% sucrose for at least 5 days. Brains were frozen, sectioned (50 μm), mounted on gelatinized slides, and stained with cresyl violet (Fig. 2). Lesions were considered complete if cells in the entire dorsal hippocampus were destroyed and more than 85% of those in the ventral hippocampus were absent. Inclusion was also dependent on the absence of any thinning of the cortical mantle, especially the entorhinal cortex.

Results

Hippocampal lesions did not impair acquisition of delay conditioning using the same ISI as used during trace conditioning [$F(2, 17) = 0.03$; $p = .97$] (Fig. 3A). All groups (intact, sham, and lesion) exposed to delay conditioning increased the number of CRs over 600 trials [$F(5, 85) = 5.47$; $p < .001$]. Hippocampal lesions did not affect subsequent trace conditioning after exposure to delay conditioning [$F(2, 17) = 1.94$; $p = .17$].

Hippocampal lesions did impair trace conditioning [$F(10, 80) = 2.21$; $p = < .05$] (Fig. 3B). Groups of rats with hippocampal lesions were impaired relative to sham-operated rats [$F(1, 16) = 6.45$; $p < .05$] and intact rats [$F(1, 16) = 11.68$; $p < .005$], whereas sham-operated and intact rats were not different from each other [$F(1, 16) = 0.72$; $p = .41$]. Groups of sham and intact rats increased their CRs over the course of 600 trials of trace conditioning, whereas the group with the hippocampal lesions did not. Performance of rats with hippocampal lesions did not differ from that of sham and intact rats when they were subsequently trained on the delay paradigm [$F(2, 12) = 0.16$; $p = .85$].

Hippocampal lesions did not affect spontaneous blink rate [$F(2, 37) = 1.27$; $p = .29$] or responding to the CS prior to training [$F(2, 37) = 0.59$; $p = .56$]. During phase one of conditioning (the first 600 trials), sham and intact rats emitted more CRs during delay conditioning than sham and intact rats did during trace conditioning [$F(1, 33) = 11.48$; $p < .005$, and $F(1, 33) = 4.78$; $p < .05$, respectively]. This effect was evident early in training (within 100 trials), as depicted in Fig. 3.

EXPERIMENT 2

In the prior experiment, we replicated the common observation that hippocampal lesions impair the acquisition of trace but not delay conditioning (Solomon et al., 1986). This selective deficit in trace conditioning occurred despite the fact that the ISI between the CS and US was identical between conditions. Nonetheless, intact and sham-operated animals acquired the trace CR more slowly than did animals trained with the delay procedure, a result consistent with our assertion that trace conditioning is an inherently

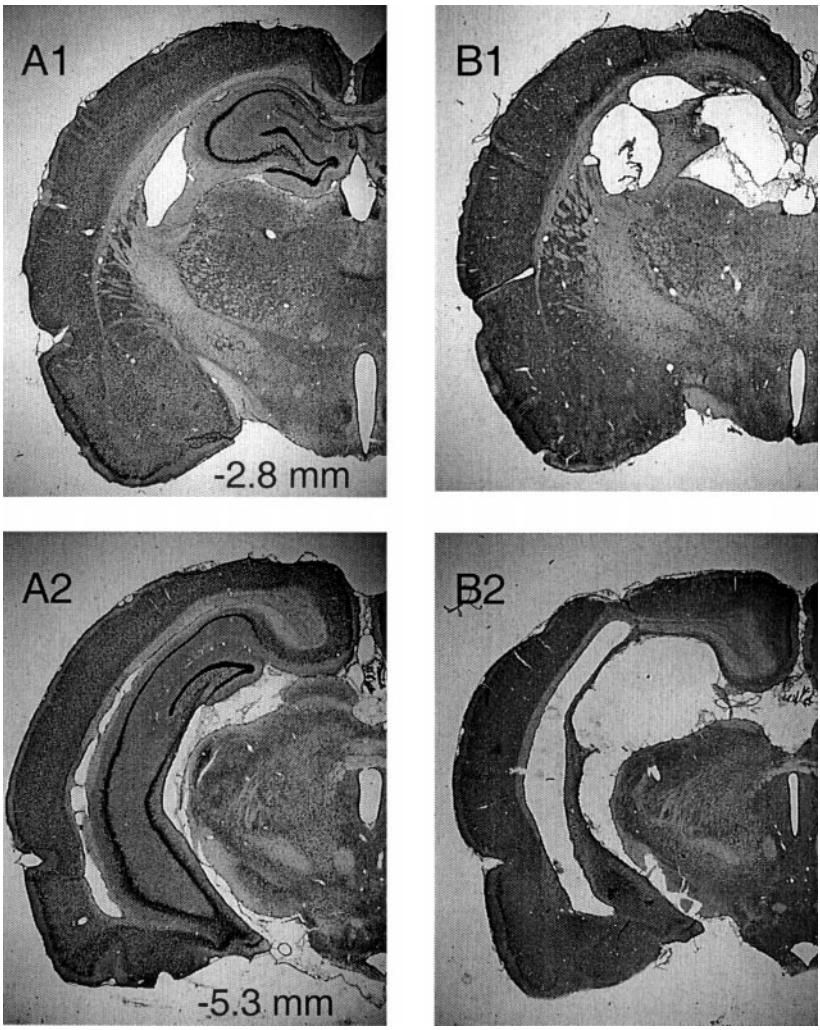

FIG. 2. Photomicrographs of representative cresyl violet-stained rat brain slices through the dorsal (−2.8 mm in relation to bregma) and ventral (−5.3 mm) hippocampus, in matched coronal sections for control (A1, A2) and lesioned (B1, B2) subjects (Paxinos & Watson, 1986).

more "difficult" task than delay conditioning. In the next experiment, we increased the difficulty of the delay task by increasing the ISI. In pilot studies, we determined that delay conditioning with a 1400-ms ISI (hereafter designated "long delay," Fig. 1c) supported a rate of acquisition that was significantly lower than the shorter delay conditioning procedure (750-ms ISI) and comparable to the trace conditioning procedure used in Experiment 1. Here we tested the effects of hippocampal lesions on learning the long delay versus the trace task. One group of rats received excitotoxic lesions to the hippocampus (as in Experiment 1) and another received sham surgeries. Eyeblinks that occurred within 500 ms of US onset were considered CRs.

Methods

Subjects. Adult male Sprague–Dawley rats (n = 27; Zivic-Miller) were housed as described in Experiment 1. Because the sham-operated animals did not differ from the

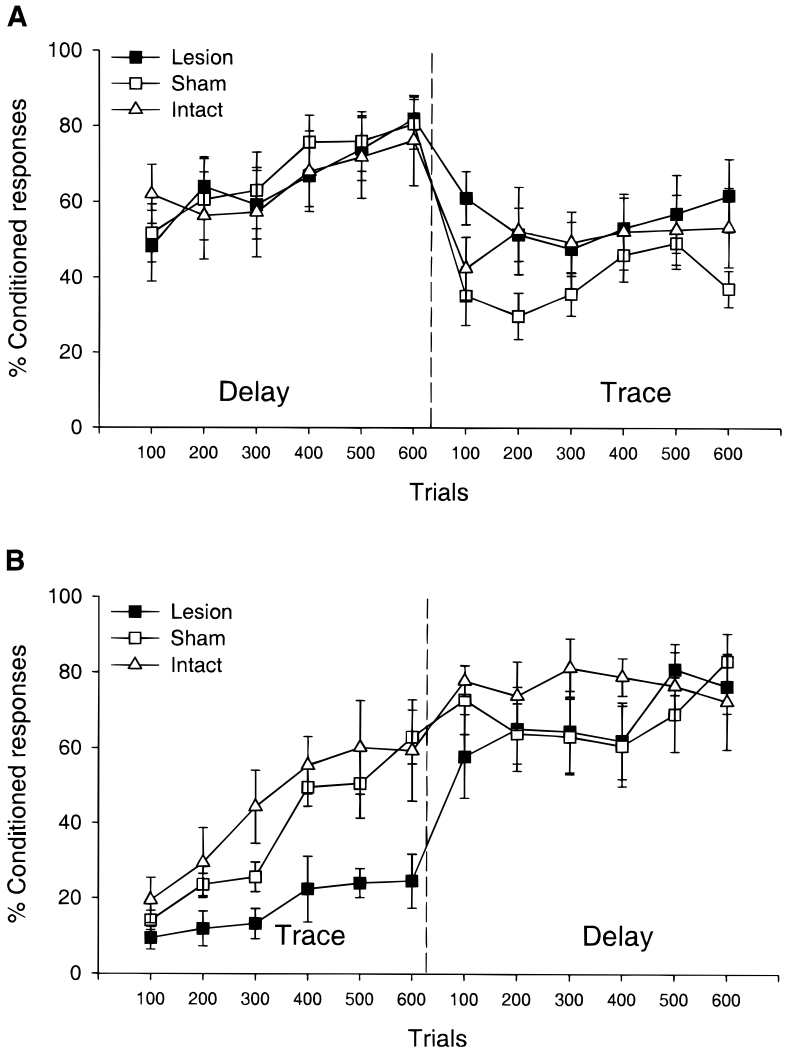

FIG. 3. (A) Effect of hippocampal lesions on the percentage of CR (±SE) over the course of 600 trials of delay conditioning and 600 trials of trace conditioning. Hippocampal lesions did not impair acquisition of delay conditioning nor subsequent performance of the trace CR. (B) Effect of lesions on the percentage of CR (±SE) over the course of 600 trials of trace conditioning and 600 trials of delay conditioning. Hippocampal lesions impaired the acquisition of trace conditioning but not the performance of subsequent delay conditioning.

intact animals in performance in Experiment 1, we only compared lesioned rats to those exposed to a sham surgery (i.e., no intact rats were examined in this study). All surgical procedures and apparatus were as used in Experiment 1.

Conditioning procedure. Rats were acclimated to the conditioning apparatus as described and 24 h later returned to measure spontaneous blink rate and responding to the CS prior to training. Half of the animals in each surgical group were exposed to 1200 trials of trace eyeblink conditioning with a 750-ms ISI and a 500-ms trace interval between the CS and the US. They were then exposed to 300 trials of long delay conditioning with a CS of 1500 ms that overlapped and coterminated with a 100-ms US. The remaining

half of the rats in each surgical group were first exposed to 1200 trials of long delay conditioning, followed by 300 trials of trace conditioning. As in Experiment 1, rats received 300 trials/session, and sessions were conducted on 5 consecutive days. Eyeblink performance was computed as a percentage of CRs to the CS.

Results

Hippocampal lesions impaired acquisition of trace conditioning [$F(1, 10) = 27.63$; $p < .001$] as well as long delay conditioning [$F(1, 11) = 8.46$; $p < .01$] (Fig. 4). However, with extensive training (>900 trials) on the long delay paradigm, rats with hippocampal lesions increased their number of CRs and many acquired the CR by 1200 trials (≥60% responding to the CS). Moreover, during the last 200 trials of long delay training, hippocampal-lesioned and sham-operated control groups did not differ [$F(1, 10) = 1.26$; $p = .29$, and $F(1, 10) = 1.69$; $p = .22$, respectively]. Thus, hippocampal lesions impaired early acquisition of both trace and long delay conditioning, but the deficit was more severe and persistent during trace conditioning [$F(1, 20) = 20.74$; $p < .001$]. As in Experiment 1, hippocampal-lesioned rats were not impaired during trace conditioning after having acquired the CR using the long delay procedure [$F(1, 8) = 3.00$; $p = .12$].

DISCUSSION

Richard Thompson and colleagues first demonstrated that the hippocampus was critically involved in trace conditioning in 1986 (Solomon et al., 1986). Since then, numerous studies have concurred with these findings (e.g., Kim et al., 1995; Moyer et al., 1990; Schmaltz & Theios, 1972; Solomon et al., 1986; Weiss et al., 1999). Because the animal must maintain a "trace" of the CS in order to associate it with the US later in time, it has been suggested that the hippocampus is involved in "bridging the gap" between the two stimuli, by either maintaining or resurrecting the representation of the CS. The purpose of the present study was to determine what aspect of trace conditioning necessarily engages the hippocampus—is it the unique temporal characteristic of the task (i.e., the trace interval) or is it because trace conditioning is a more difficult task to acquire than delay? In Experiment 1, hippocampal-lesioned, sham-lesioned, and intact rats were trained either on a trace task with a 500-ms trace interval or a delay task using the same interstimulus interval. Rats with lesions to the hippocampus did not acquire the trace conditioned response, whereas they did acquire the delay conditioned response. These data are consistent with those of studies mentioned indicating that the hippocampus is critically involved in trace, but not delay conditioning (Kim et al., 1995; Schmaltz & Theios, 1972; Solomon et al., 1986; Weiss et al., 1999).

Also consistent with past studies (Clark & Squire, 1998; Gould et al., 1999a; Ivkovich et al., 2000), intact animals acquired the delay conditioning task at a faster rate than they did trace conditioning, raising the possibility that task difficulty may be an important factor. To test this idea, we extended the ISI of a delay task to 1400 ms (long delay) and obtained similar rates of learning in control animals to those observed during trace conditioning (Fig. 1). Using this modified procedure we found that lesions to the hippocampus impaired acquisition of delay conditioning. These data represent a significant departure from current theories about the role of the hippocampus in delay versus trace conditioning

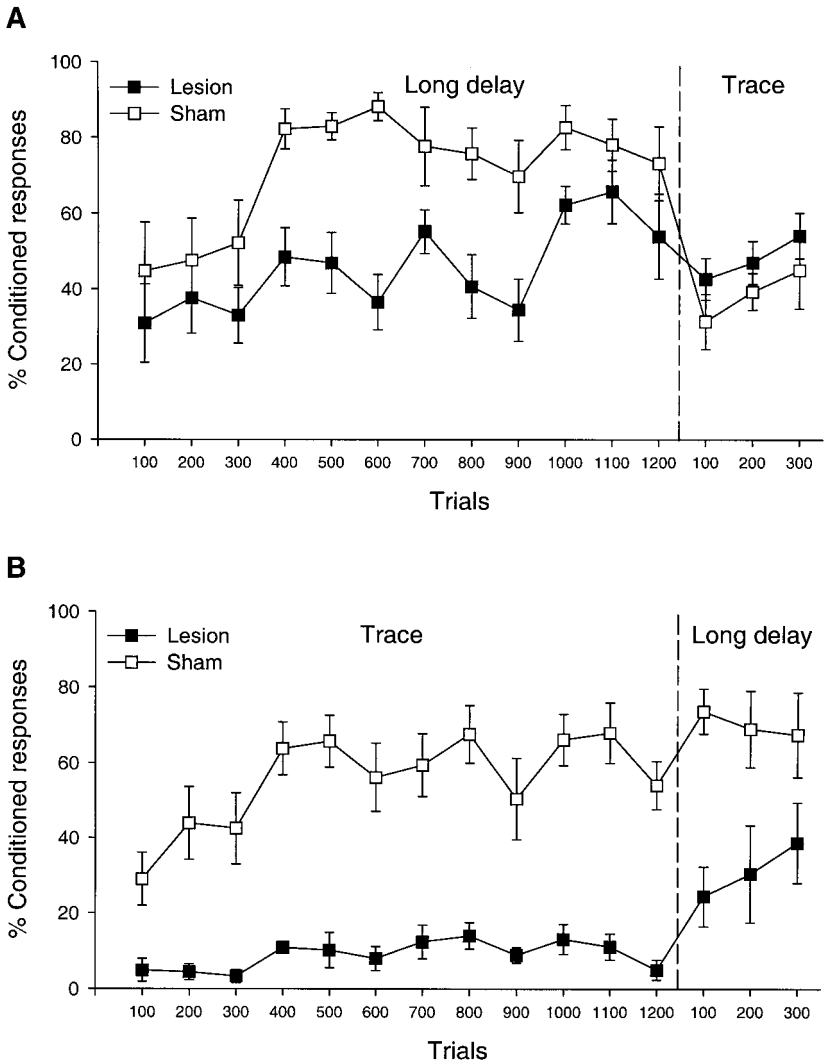

FIG. 4. (A) Effect of hippocampal lesions on the percentage of CR (±SE) over the course of 1200 trials of long delay and 300 trials of trace conditioning. Hippocampal lesions impaired acquisition of long delay, although lesioned rats acquired the CR after 1100 trials. Hippocampal lesions did not impair subsequent trace conditioning. (B). Effect of lesions on the percentage of CR (±SE) over the course of 1200 trials of trace conditioning and 300 trials of long delay conditioning. Hippocampal lesions impaired the acquisition of trace conditioning and the subsequent performance of the long delay CR.

by indicating that hippocampal lesions can impair classical delay conditioning if it is sufficiently difficult to acquire. It is noted that rats with hippocampal lesions were able to overcome the impairment with extensive training (>1000 trials). Thus, the hippocampus is involved in acquiring the more difficult task of long delay but the structure is not essential for learning to occur. In contrast, rats with hippocampal lesions did not acquire the CR in the trace task even after 1200 trials of training. In summary, these data confirm early claims by Thompson and colleagues indicating that the hippocampus is necessary for trace conditioning and extend those claims to include a temporary yet important role for the hippocampus in delay conditioning when the task is rendered more difficult.

In addition to evaluating the effects of hippocampal lesions on trace versus delay and long delay conditioning, we tested whether animals that learned the association between the CS and the US during delay conditioning could then perform the trace conditioned response. In the first experiment, animals trained with the delay paradigm were able to perform the trace task at a later time (Fig. 3A). Thus, the presence of the trace per se does not dictate that an intact hippocampus is necessary to perform the CR. However, since the ISIs are the same for delay and trace in Experiment 1, and therefore the animals learn to blink at essentially the same time, it could be argued that they already have acquired the appropriate blink latency and thus are not impaired when the trace is introduced. This potential confound was resolved in Experiment 2; animals were first trained with long delay and acquired adaptive responses occurring about 1000 ms after CS onset. Then they were exposed to the trace paradigm where the adaptive response occurred much sooner, at about 500 ms after CS onset. Our prior result was confirmed, as there was also no deficit in trace conditioning in rats with hippocampal lesions (Fig. 4). These results are important for at least two reasons. First, they suggest that once the animal has learned the association, it can continue to perform the task even when a trace interval is present. Second, since the lesioned animal can perform the trace response after delay conditioning, the lesion-induced deficit observed when rats are initially exposed to trace conditioning is not a performance or expression deficit.

The fact that rats can learn trace after delay conditioning is potentially enlightening with regard to learning in H.M. and other patients with anterograde amnesia. After undergoing hippocampectomy for uncontrollable seizures, H.M. was unable to learn many new tasks, but was surprisingly able to perform the trace conditioned eyeblink response. Since the hippocampus is considered necessary for trace conditioning, these results were inconsistent with those of the existing literature. However, H.M. was trained on delay conditioning before being trace conditioned (Woodruff-Pak, 1993). Thus, similar to rats in the present study, he had already learned the association and was not impaired simply by the insertion of a trace interval. Early as opposed to late involvement of the hippocampus in conditioning is consistent with temporal properties of neurons during acquisition of the eyeblink CR. It is well established that activity of hippocampal pyramidal cells correlates positively with the learned response (Berger et al., 1983; Orr and Berger, 1985). Early during trace conditioning, unit activity in CA1 pyramidal cells is increased first to the US and then (shortly before expression of the CR) to the CS as well (McEchron & Disterhoft, 1999). But once the CR has been acquired, unit activity decreases. Similarly, functional imaging studies indicate that metabolic activity in the hippocampus increases during early phases of learning but decreases with continued training (Buchel et al., 1999).

It was first reported in 1972 that the hippocampus is not required for delay conditioning (Schmaltz & Theios, 1972). Later, Thompson and colleagues found that trace conditioning was dependent on integrity of the structure (Solomon et al., 1986). Since then, interest in trace conditioning in the area of neurobiology has been increasing, especially with reports that learned responses other than eyeblinks require the hippocampus when a trace interval is present. For example, classical fear conditioning is dependent on the amygdala during delay conditioning, but also engages the hippocampus during trace conditioning (McEchron et al., 1998). Similarly, conditioned heart rate responding becomes hippocampal-dependent in the presence of a trace interval (McEchron et al., 2000). In humans, it has been demonstrated that amnesics with hippocampal damage cannot acquire

a trace eyeblink response but can acquire the delay response (Clark & Squire, 1998). Moreover, some studies suggest that awareness is involved in acquisition of trace memories. Intact humans that were aware of the contingency acquired the trace CR, whereas those that were unaware did not (Clark & Squire, 1998; Manns et al., 2000). Awareness did not impinge on acquisition of the delay conditioned response. It should be noted that awareness is not a *necessary* feature of hippocampal-dependent learning. In a contextually implicit memory task, human amnesics with medial temporal lobe damage were impaired relative to normal controls, although neither exhibited awareness for the contextual memories (Chun & Phelps, 1999).

Why is trace conditioning more "difficult" to learn than delay conditioning? Usually, it is assumed that maintaining or resurrecting a memory trace of the CS in order to associate it later with the US requires a processing capability for which the hippocampus is uniquely suited. But there are additional aspects of trace conditioning that may contribute to its difficulty (Desmond & Moore, 1991). For one thing, the animal must distinguish between the CS–US association and the US–CS association, since the stimuli do not co-occur and the context between the stimuli is similar. Indeed, trace conditioning is more rapidly acquired and similar in rate to delay conditioning if a distinguishing feature is placed within the temporal gap (Kamin, 1965; Kaplan, 1984). Given the present data, one would predict that such manipulation would reduce if not eliminate the deficit observed after hippocampal lesions. An additional reason asserted for the difficulty of trace conditioning is timing of the CR. Previous studies have reported that lesions of the hippocampus result in shorter, sometimes "nonadaptive" CR latencies (Port et al., 1986). If the hippocampus is involved in timing of the response, one might expect that hippocampal lesions would impair trace conditioning even after acquiring a delay CR. Such a deficit would be expected especially in Experiment 2, in which the animal learns to blink at a different time during trace than long delay conditioning. There was no deficit, supporting the notion that once the CS and US are associated, the hippocampus is no longer necessary for performance or timing of the trace CR.

Finally, we address the definition of task difficulty and how it relates to other studies of hippocampal lesions and learning. Specifically, are other learning deficits after hippocampal lesions attributable to task difficulty? As an example, place learning in the Morris water maze is dependent on the hippocampus but cue-based learning is not, and the former is more difficult, at least to the extent that rats acquire the cue version faster than the place version. Moreover, rats that are previously familiarized with the maze procedure learn faster (Perrot-Sinal et al., 1996) and those trained with a nonspatial task (Saucier & Cain, 1995) or another spatial task (Bannerman et al., 1995) are not impaired later on the spatial task even in the absence of NMDA receptor-dependent LTP in the hippocampus. Thus, as the task becomes easier to learn, the deficits in hippocampal plasticity are inconsequential. From a different perspective, it has been reported that the ability to acquire a delay eyeblink task develops earlier than a trace task (about postnatal day 23 for delay versus 30 for trace), unless the delay task is rendered more difficult using a very long ISI (Ivkovich et al., 2000). Thus, it appears that task difficulty requires some aspects of neural processing that are not yet functional in the young animal and engage the hippocampus of the adult.

The dentate gyrus continues to produce new neurons throughout life (Altman & Das, 1965; Gould et al., 1997). Although most of these cells die within weeks, their survival

is greatly enhanced by hippocampal-dependent but not hippocampal-independent learning, such as trace but not delay conditioning (Gould et al., 1999a; Gould et al., 1999b; Shors et al., 2000). More importantly, we have recently found that the newly generated neurons are involved in the formation of trace but not delay memories (Shors et al., 2001). Given the difference in acquisition rates between trace and delay conditioning, it also may be the case that the new neurons become engaged as task demands increase.

Do the present results prove that the dependence of trace conditioning on the hippocampus is due to task difficulty and not the presence of a trace interval? They do not. Rather they suggest that both aspects of the task can engage the hippocampus and to varying degrees. It is apparent that the hippocampus is especially critical to trace conditioning since none of the lesioned animals acquired the trace CR even after more than 1000 trials, whereas as a number of lesioned animals eventually acquired the long delay task. It could be argued that the long delay task used here remained less difficult. Regardless, the present data illustrate that hippocampal lesions, can adversely affect delay conditioning provided that the conditions are sufficiently challenging to the animal.

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