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Hippocampectomy Disrupts Trace Eye-Blink Conditioning in Rabbits

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The role of the hippocampus (HFC) in trace eye-blink conditioning was evaluated using a 100-ms tone conditioned stimulus (CS), a 300- or 500-ms trace interval, and a 150-ms air puff unconditioned stimulus (UCS). Rabbits received complete hippocampectomy (dorsal & ventral), sham lesions, or neocortical lesions. Hippocampectomy produced differential effects in relation to the trace interval used. With a 300-ms trace interval, HPC-lesioned Ss showed profound resistance to extinction after acquisition. With a 500-ms trace interval, HPC-lesioned Ss did not learn the task (only 22% conditioned responses (CRs) after 25 sessions, whereas controls showed >80% after 10 sessions), and on the few trials in which a CR occurred, most were "nonadaptive" short-latency CRs (i.e., they started during or just after the CS and always terminated prior to UCS onset). The authors conclude that the HPC encodes a temporal relationship between CS and UCS, and when the trace interval is long enough (e.g., 500 ms), that the HPC is necessary for associative learning of the conditioned eye-blink response.

There has been a lack of agreement regarding the role of the hippocampus in associative learning of the conditioned eye-blink or nictitating membrane (NM) response in rabbits. In delay conditioning, when the conditioned stimulus (CS) precedes and overlaps with the unconditioned stimulus (UCS), an intact hippocampus is not necessary for acquisition of the conditioned response(CR; Akase, Alkon, & Disterhoft, 1989; Schmaltz & Theios, 1972; Solomon & Moore, 1975). However, during trace conditioning, in which the CS and UCS do not overlap, the general consensus regarding the effects of hippocampus lesions has been less clear. Solomon, Vander Schaaf, Weisz, and Thompson (1986) first reported associative learning deficits in rabbits with dorsal hippocampus lesions during trace conditioning. Similar learning deficits on a bar-pressing task that incorporated a timing component were also reported in rats with fimbria-fornix lesions (Meck, Church, & Olton, 1984). In this study, rats learned to respond to a fixed-interval (20 s) schedule of reinforcement to avoid a footshock UCS. Initially, the tone CS was present throughout the fixed-time interval, but when the CS length was decreased, rats with fimbria-fornix lesions were unable to correctly time their responses to avoid the UCS. These studies suggested a role for the hippocampus in forming appropriate temporal associations. Associative learning deficits during trace eye-blink/NM conditioning were not corroborated by reports from other laboratories (James, Hardiman, & Yeo, 1987; Port, Romano, Steinmetz, Mikhail, & Patterson, 1986). However, these studies did report altered response-onset latencies during acquisition of the traceconditioning task. When a periorbital shock UCS was used, dorsal hippocampus lesions resulted in longer response latencies (James et al., 1987; Port et al., 1986), but when a corneal air puff UCS was used, hippocampal lesions produced shorter response-onset latencies (Port et al., 1986) relative to control rabbits.

Electrophysiological studies have continued to implicate involvement of the hippocampus in associative learning. For example, the fact that hippocampal pyramidal neurons alter their firing rates during classical conditioning has been shown in various species such as rat (Segal, 1973), rabbit (Akase, Deyo, & Disterhoft, 1988; Berger & Thompson, 1978a; Solomon et al., 1986), and cat (Patterson, Berger, & Thompson, 1979) when measured in vivo. These altered firing patterns were also shown to mimic the amplitude and time course of the conditioned behavioral response as the animal learned the task (Berger, Laham, & Thompson, 1980; Berger, Rinaldi, Weisz, & Thompson, 1983). This "neural modeling" of the behavioral response has been shown to be conditioning specific because these changes were not observed during unpaired pseudoconditioning trials (Berger & Thompson, 1978b).

In vitro studies using both biophysical and neurochemical analyses have also indicated that the hippocampus is involved in associative learning. A conditioning-specific reduction in the amplitude and du-

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ration of the afterhyperpolarization (AHP) that follows a burst of action potentials was demonstrated in rabbit hippocampal CA1 neurons after delay (Coulter et al., 1989; Disterhoft, Coulter, & Alkon, 1986) and trace (de Jonge, Black, Deyo, & Disterhoft, in press) eve-blink/NM conditioning when compared with the much larger and longer lasting AHPs from naive and pseudoconditioned rabbits. This AHP reduction has recently been shown to be highly correlated with behavioral acquisition of the conditioned response (Disterhoft, Golden, Read, Coulter, & Alkon, 1988). Also, LoTurco, Coulter, and Alkon (1988) noticed that high-frequency stimulation of the Schaffer collaterals yielded enhanced synaptic potentials in hippocampal CA1 neurons after classical conditioning. Alterations in the neurochemical properties of hippocampal neurons have also been found after classical conditioning. For example, Mamounas, Thompson, Lynch, and Baudry (1984) demonstrated enhanced glutamate binding in the dorsal hippocampi taken from eye-blink conditioned rabbits. More recently, Bank, DeWeer, Kuzirian, Rasmussen, and Alkon (1988) showed that classical conditioning resulted in an increased translocalion of protein kinase C from the cytosolic fraction to the membrane fraction in hippocampal CA1 neurons. A redistribution of membrane-associated protein kinase C (from soma to dendrites) has also been visualized in CA1 neurons after conditioning (Olds, Anderson, McPhie, Staten, & Alkon, 1989). These in vitro studies demonstrate learning-specific alterations that are intrinsic to the hippocampus because the hippocampal neurons were isolated from their normal afferent and efferent connections.

The existing discrepancies among the reported effects of hippocampus lesions during eye-blink conditioning may be due to variations in the lesion sizes reporterd in different studies. Recent studies from our laboratory have demonstrated, using a short-delay eye-blink conditioning paradigm, that large hippocampus lesions impaired consolidation of a partially learned response and retarded extinction when rabbits were overtrained (Akase et al., 1989). Thus far, in trace eye-blink/NM conditioning studies, lesions to only the dorsal hippocampus have been studied (James et al., 1987; Port et al., 1986; Solomon et al., 1986). In one of these studies, Solomon et al. (1986) reported both a learning deficit and the presence of "nonadaptive" shortlatency CRs in rabbits with dorsal hippocampus lesions. The other two studies failed to find acquisition deficits, but their lesions were less extensive. Consequently, sufficient hippocampus may have remained intact to mask any learning deficits that might otherwise have existed.

Given the discrepancies in the lesion literature coupled with the extensive electrophysiological and neurochemical data that suggest involvement of the hippocampus in classical conditioning, we reasoned that the role of the hippocampus in trace conditioning could be clarified through the use of more complete hippocampal lesions. Furthermore, because considerable evidence implicates the hippocampus in the temporal processing of information during associative learning (Akase et al., 1988; Berger, Alger, & Thompson, 1976; Meck et al., 1984; Port, Mikhail, & Patterson, 1985; Solomon, 1979, 1980; Solomon et al., 1986; Vinogradova, 1975), we assessed the effect of hippocampectomy on acquisition of the conditioned eye-blink response in rabbits using two different trace intervals. We wanted to determine whether alterations in the trace interval would yield alterations in the sensitivity of the task to removal of the hippocampus.

Experiment 1: 300-ms Trace Interval

Method

Subjects arid surgery. Experimentally naive young adult male New Zealand albino rabbits (Oryctolagus cuniculus) were obtained from a local breeder. They weighed 1.5-2.0 kg and were 3 months old. Subjects were assigned to one of three groups based on their surgical treatment: (a) sham-operated control rabbits (shams, n = 5), (b) neocortical-lesioned rabbits (neocorticals, n =5), and (c) hippocampus-lesioned rabbits (hippocampals, n = 5). All subjects were individually housed in a colony maintained on a 12:12-hr light-dark cycle with ad libitum access to food and water. Prior to surgery, rabbits were anesthetized with injections of xylazine (6 mg/kg im) and ketamine hydrochloride (45 mg/kg im). The subjects were then mounted in a stereotaxic instrument with bregma elevated about 2.0 mm above lambda. A midsagittal incision was made, and the skull was exposed. At this time, the incision for rabbits in the sham group was sutured closed, but for rabbits in the other two surgical groups, a 5×6 mm section of skull was removed bilaterally. This was done (beginning at a point 3 mm lateral from the midsagittal suture and 3 mm caudal from bregma) by first cutting a 5-mm lateral section followed by an additional 6-mm caudal section. The cutting was repeated to remove a rectangle of bone bilaterally. After removal of the overlying dura, either the neocortex overlying the dorsal hippocampus (neocorticals) or the neocortex plus the hippocampus (hippocampals) was removed by aspiration. The lesions were packed with Gelfoam and covered with bone wax, and the incision was closed. After surgery, all subjects were numerically coded so that the experimenter was unaware of any subject's surgical condition and allowed a 2-week recovery period prior to behavioral training.

Apparatus and procedure. The apparatus and behavioral training were similar to those previously described (Disterhoft, Kwan, & Lo, 1977). During behavioral conditioning experiments, subjects were wrapped in cloth bags and placed in Plexiglas restraining boxes (Gormezano, 1966). Head movements were minimized by padded ear clamps attached to the restraining box. Subjects were trained in pairs in separate sound-attenuated chambers and habituated to the behavioral apparatus for three 30-min sessions prior to behavioral conditioning. Conditioning was to the right eye, and the right eyelid was held open nonaversively by stainless steel clips for detection of NM (third eyelid) extensions. Rabbits were then trained in a trace-conditioning paradigm consisting of a 100-ms, 90-dB (re 20 µN/m²) 6-kHz tone CS followed by a 300-ms trace interval (neither CS nor UCS presentation) in a sound-attenuated chamber. The UCS was a 150-ms, 2.5 pounds per square inch (17,236.892 N/m²) corneal air puff that was sufficient to elicit a reliable NM extension as the unconditioned response (UCR). Training sessions consisted of 80 CS-UCS paired trials presented at a random intertrial interval ranging from 40 to 80 s (M = 60 s). Rabbits were conditioned daily until a behavioral criterion of 80% CRs for two consecutive sessions was reached. Twenty-four hours after acquisition, extinction sessions were begun. Each subject received three consecutive extinction sessions consisting of 80 CS-only trials per session. All behavioral trial presentations, data acquisition, and data reduction were controlled by a microcomputer.



Figure 1. Reconstructions of largest and smallest lesions from six representative coronal sections from rabbits trained with a 300-ms trace interval. (Areas of tissue removal are solid black. Panel A: hippocampal lesions.)

Histology. After the 3rd day of extinction, rabbits in the neocortical and hippocampal groups were sacrificed with an overdose of sodium pentobarbital and perfused intracardially with normal saline followed by a 10% buffered Formalin solution. Brains were then removed and placed in a 10% Formalin solution for at least 1 week. Prior to cryostat sectioning, brains were placed in a sucrose solution overnight. Coronal sections of 50- μ m thickness were made through the length of the hippocampus, and every 10th section was saved and stained with cresyl violet.

Results

Histology. A summary of lesion sizes for subjects in the hippocampal and neocortical lesion groups is presented in Figure 1. Hippocampectomized rabbits were found to have had complete removal of the dorsal hippocampus, the fimbria-fornix, and most of the ventral hippocampus. One subject (J623) received a total bilateral hippocampectomy. No damage to the thalamus was observed, and the cingulate/retrosplenial cortex was generally intact.

Neocortical rabbits were lesioned to control for the unavoidable extrahippocampal damage that occurred during hippocampectomy. The neocortical subjects received lesions of the neocortex overlying the dorsal hippocampus, and these lesions did not damage any hippocampus (Figure 1 B). These lesions also spared the cingulate/ retrosplenial cortex and represented analogous regions of the neocortex initially removed during hippocampal aspirations.

Acquisition. Hippocampectomy had no significant effect on the number of trials required to reach a behavioral criterion of 80% CRs for 2 consecutive sessions when a 300-ms trace interval was used. All rabbits acquired the task to criterion within 960 trials or 12 training sessions (range: shams, 400–800; neocorticals, 240–960; hippocampals, 400–960). prior to beginning extinction. On average, hippocampectomized rabbits acquired the task approximately 1 session faster, but this difference was not significant, *F*(2, 12) = 0.28, *ns*. Figure 2 shows a comparison of the percentage of CRs during the last 2 days of training (criterion sessions). A response was classified as a CR if extension of the NM occurred during the interval between the CS onset and UCS onset. An analysis of variance (ANOVA) of the percentage of CRs during the last 2 criterion sessions revealed no significant difference between groups, *F*(2, 12) = 0.515, *ns*.

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Figure 2. Response rates expressed as percentage of CRs over the last two acquisition sessions (criterion sessions) and three consecutive test sessions (extinction sessions) using a 300-ms trace interval. (Error bars represent the SE for the 5 subjects in each group. Post hoc comparisons were made using Tukey's HSD test. An asterisk denotes significant difference when compared to hippocampals, p < .05).

1

2

CRITERION SESSIONS

2

EXTINCTION SESSIONS

Comparisons between CR and UCR amplitudes were also performed. Neither the CR amplitude nor the UCR amplitude was significantly different between the three groups of rabbits during the last 2 days of training, F(2, 12) = 0.372, ns, and F(2, 12) =0.206, ns, for CR and UCR amplitudes, respectively. Also, there were no significant group differences in mean CR onset latencies, F(2, 12) = 0.237, ns, during the last two criterion sessions.

Extinction. In contrast to acquisition, a number of differences between the hippocampectomized and control subjects were seen during extinction. First, hippocampectomized rabbits showed no evidence of extinction within 240 test trials (three sessions). Figure 2 shows the mean percentage of CRs during three consecutive extinction sessions. A CR was defined as an extension of the NM occurring within 550 ms (CS + trace interval + 150 ms) after CS onset. This deliberately included the previous UCS period because we wanted to look at shifts in response latency that might occur during the extinction process. ANOVA of the percentage of CRs during the three test sessions revealed a significant lesion effect, F(2, 12) = 4.196, p < .05, and a significant extinction effect, F(2, 12) = 4.196, p < .05, and a significant extinction effect, F(2, 12) = 4.196, p < .05, and a significant extinction effect. 24) = 8.794, p < .0025. Hippocampectomized rabbits showed virtually no extinction (84% CRs on Extinction Day 3), whereas neocorticals and shams dropped below 55% CRs by the 3rd day of extinction (see Figure 2). Hippocampectomized rabbits also showed considerably larger response amplitudes during extinction, F(2, 12) = 8.576, p < .005. Figure 3A illustrates the mean response amplitudes during the three extinction sessions. Response amplitudes were averaged for all extinction trials within a particular session. An ANOVA for average response latency also revealed a significant lesion effect, F(2, 12) = 4.816, p < .05, as well as a significant extinction effect, F(2, 24) = 9.047, p < .0025. This reflected the fact that the response latencies of subjects in the hippocampal group remained constant, whereas the latencies in the other two groups increased as extinction occurred (Figure 3B). Thus, by the 3rd day of extinction, the average response latency for the hippocampectomized rabbits was nearly half that of either control group.

Discussion

The results from Experiment 1 indicate that hippocampectomy had no significant effect on acquisition of the traceconditioned eye-blink response using a 300-ms trace interval. However, the extinction data strongly support the hypothesis that the hippocampus modulates this simple associative learning task. Previous studies have demonstrated differences in expression of the CR in delay conditioning after dorsal hippocampus lesions (Akase et al., 1989; Orr & Berger, 1985; Port et al., 1985), even though the hippocampus appears not to be part of the minimal essential circuitry required for acquisition of classical delay conditioning (Desmond & Moore, 1982; Haley, Thompson, & Madden, 1988; Mauk & Thompson, 1987; Mc-Cormick & Thompson, 1984; Yeo, Hardiman, & Glickstein, 1985, 1986). This modulation may be in the form of a perseveration (Kimble & Kimble, 1965) or an inability to give up previously learned responses, a deficit also reported during discrimination reversal learning (Berger & Orr, 1983; Orr & Berger, 1985). The present findings are in agreement with previous reports of slower rates of extinction in well-trained rabbits with hippocampal lesions using a short-delay paradigm (Akase et al., 1989; Schmaltz & Theios, 1972). These findings, coupled with the impairment seen in hippocampus-lesioned rabbits during discrimination reversal learning (Orr & Berger, 1985), suggest that a generalized effect of hippocampus removal may be an inability to modify previously learned responses.

The trace interval used in this experiment was relatively short (300 ms) when compared with a previous study that used a 500-ms trace interval to demonstrate a learning deficit after dorsal hippocampus lesions (Solomon et al., 1986). We hypothesized that, if the hippocampus is involved in bridging the "temporal gap" between the CS and the UCS, the 300-ms trace interval used in this first experiment may have been short enough to mask this role. Consequently, we increased the temporal demands of the task by extending the trace interval to 500 ms and examined the effects of hippocampectomy on trace conditioning in Experiment 2.

Experiment 2: 500-ms Trace Interval

Method

Subjects and surgery. Subjects and treatment groups were the same as in Experiment 1, except that there were 6 subjects in each group. Prior to surgery, rabbits were anesthetized and mounted in a stereotaxic instrument as in Experiment 1. A midsagittal incision was made and two screw holes, located 3 mm and 8 mm rostral to bregma (approximately 4 mm lateral to the midsagittal suture), were drilled bilaterally. Skull screws (No. 2, 7 mm long, stainless steel) were then inserted, and dental cement was molded around the four screws to provide an attachment point for a skull cap (consisting of four 2-cm-long plastic bolts centered in a disk of dental cement, 3 cm in diameter and 5 mm deep). At this time, the incision for rabbits in the sham group was sutured closed, and the skull cap was attached with dental cement. For rabbits in the other two surgical groups, aspiration lesions and wound closure were performed as in Experiment 1, and the skull cap was attached.



100

85

HIPPOCAMPAL ▲ NEOCORTICAL

□ SHAM

1

PERCENT CRS

40

25



Figure 3. Summary of effects of hippocampectomy on response amplitude and response latency during three consecutive extinction sessions using a 300-ms trace interval. (Panel A: mean response amplitude. Panel B: mean response latency. Error bars represent the *SE* for the 5 subjects in each group. Post hoc comparisons were made using Tukey's HSD test. An asterisk denotes significant difference when compared with hippocampals, p < .05.)

All subjects were allowed a 2-week recovery period prior to behavioral training.

Apparatus and procedure. The apparatus used for the behavioral studies was similar to that used in Experiment 1, with some minor exceptions. Pilot studies suggested that an increased training time would be required for behavioral acqusition. Therefore, all rabbits were fixed in a stereotaxic device by attaching the skull cap to prevent excessive head movements and thus greatly decrease the need for human intervention during a conditioning session. We found that the rabbits adapted quite well to this procedure and showed no obvious signs of discomfort (Disterhoft et al., 1977). In fact, rabbits fitted with a skull cap and restrained during conditioning required fewer training sessions to reach behavioral criterion (unpublished observations). Consequently, padded ear restraints (to prevent excessive head movements) were unnecessary and not used. Subjects were conditioned as in Experiment 1 except that a 500-ms trace interval was used. Behavioral training and data reduction were controlled by a Standard 80286 computer using a custom-designed hardware interface and software package (Akase & Disterhoft, 1990). Prior to behavioral conditioning experiments, rabbits received one 90-min habituation session. Subjects were conditioned for a maximum of 25 sessions or until a criterion of 80% CRs in a single session was reached. Removal of brains, sectioning, and staining of rabbit coronal sections were performed as in Experiment 1.

Results

Histology. Six subjects received bilateral hippocampal lesions, the largest and smallest of which are depicted in Figure 4A. In all 6 subjects, the lesions involved the dorsal hippocampus, the fimbria-fornix, and the ventral hippocampus. In all cases the thalamus was completely intact, and the cingulate/retrosplenial cortex was virtually without damage. Two subjects (J712 and J727) received complete bilateral hippocampectomies, whereas in the other 4 subjects a small amount of the ventral hippocampus still remained unilaterally.

An additional 6 subjects received bilateral lesions restricted to the neocortex overlying the dorsal hippocampus (Figure 4B). As in Experiment 1, these subjects received neocortical lesions analogous to the neocortex initially removed during hippocampal aspirations. In all cases, the cingulate/retrosplenial cortex and the fimbria-fornix were virtually without damage.

Acquisition of conditioned responses. None of the hippocampectomized rabbits acquired the task within the allotted 25 training sessions, whereas all of the sham and neocortical rabbits reached criterion within 16 training sessions, F(2, 15) = 48.997, p < .0001. A summary of the mean number of trials required to reach behavioral criterion is presented in Figure 5. Analyses of the percentage of CRs during the last day of training revealed that the hippocampectomized rabbits showed significantly fewer CRs than both control groups, F(2, 15) = 122.416, p < .0001. A CR was defined as an extension of the NM that occurred after CS onset but prior to UCS onset and included both "adaptive" long-latency CRs and nonadaptive short-latency CRs. Mean values for the percentage of CRs on the last training session were as follows: shams 82.7 ± 0.99 (*SE*), neocorticals 82.7 ± 1.04 , and hippocampals 22.3 ± 5.27 .

Topography of conditioned responses. Although all of the hippocampectomized rabbits failed to learn the task within the maximum allowable training sessions, they did show some CRs (responses after CS onset but before UCS onset). A large percentage of their CRs, however, were of the non-adaptive short-latency type (Solomon et al., 1986). A short-latency CR was defined as a CR that returned to baseline level prior to onset of the UCS (and thus did not overlap with the UCR). Figure 6 shows a typical example of a short-latency CR expressed by a hippocampectomized rabbit as compared with the normal adaptive long-latency CRs typically expressed by the sham and neocortical control rabbits. Although sham and neocortical rabbits showed shortlatency CRs during the 1st day of training, only the hippocampectomized rabbits still expressed a large percentage of these nonadaptive short-latency CRs on the last day of training, F(2, 15) =204.937, p < .0001 (see Figure 7). Finally, the associative learning deficits seen in the hippocampectomized subjects were present in the absence of significant differences in UCR amplitudes during the last training session, F(2, 15) = 3.395, ns; that is, these were learning deficits rather than nonspecific performance deficits.



Figure 4. Reconstructions of largest and smallest lesions from six representative coronal sections taken from rabbits conditioned with a 500-ms trace interval. (Areas of tissue removal are solid black. Panel A: hippocampal lesions. Panel B: neocortical lesions.)

Discussion

The results from Experiment 2 demonstrate that removal of the hippocampus disrupted acquisition of the trace eye-blink conditioning task when a 500-ms trace interval was used. This effect was so dramatic that hippocampectomized rabbits failed to learn this task even after 25 days of training (maximum allotted sessions). This learning deficit was not due to a generalized sensorimotor deficit because there was no difference in UCR amplitude between the hippocampectomized rabbits and the controls. The sham and neocortical control subjects were capable of bridging the temporal gap between the CS and the UCS when this longer trace interval was used, whereas the hippocampectomized subjects were incapable of bridging this temporal gap. This is evidenced by two observations. First, the hippocampectomized rabbits showed only 22.3% CRs (including nonadaptive short-latency CRs) after 2000 conditioning trials or 25 training sessions, suggesting that these rabbits would not have reached the behavioral criterion (80% CRs) regardless of the number of training sessions they received. Second, the hippocampectomized rabbits showed a significantly greater percentage of "inappropriately timed" short-latency CRs (Solomon et al., 1986) throughout behavioral conditioning. Sham and neocortical control rabbits typically expressed short-latency CRs only during the first training session (see Figure 7). This suggests that removal of the hippocampus severely impaired the rabbit's ability to make correct associations between temporally discontiguous stimuli (Rawlins, 1985; Solomon et al., 1986). Our observations are also consistent with the results from studies in which rats with fimbria-fornix lesions demonstrated an inability to appropriately time their responses when a temporal gap was introduced after they had learned a fixed-interval schedule of reinforcement training (Meck et al., 1984). These results from our second experiment suggest that the hippocampus is indeed essential for rabbits to formulate the appropriate temporal association between the CS and the UCS in trace conditioning when longer trace intervals are used.



Figure 5. Summary of mean number of trials required to reach a behavioral criterion of 80% conditioned responses in a single session. (Subjects were trained, using a 500-ms trace interval, for a maximum of 25 sessions [2000 trials]. No hippocampectomized subject reached criterion. Error bars represent *SE* for the 6 subjects in each group. Post hoc comparisons were made using Tukey's HSD test. Double asterisks denote significant difference when compared with hippocampals, p < .01.)

General Discussion

There were three major findings from these experiments, (a) Removal of the hippocampus resulted in a resistance to extinction after acquisition of a short (300-ms) trace-conditioning task. Even after 3 testing sessions, hippocampectomized rabbits exhibited almost no extinction, (b) When a longer (500-ms) trace interval



Figure 6. Summary of conditioned response (CR) types: an example of a typical nonadaptive short-latency CR from a hippocampectomized rabbit (top panel) as compared with the adaptive long-latency CR typically expressed by neocortical (middle panel) and sham (bottom panel) controls.



Figure 7. Summary of conditioned response (CR) types: mean percentage of short-latency CRs compared between surgical groups on the first and the last day of behavioral training. (Short-latency CRs are expressed as percentage of total CRs. Values indicate M \pm *SE* for the 6 subjects in each group. Post hoc comparisons were made using Tukey's HSD test. An asterisk denotes significant difference when compared with hippocampals, p < .05.)

was used, all of the hippocampectomized subjects failed to learn the association between the CS and the UCS after 25 training sessions, (c) Many of the small number of CRs exhibited by the hippocampectomized rabbits (when trained using a 500-ms trace interval) were of a nonadaptive short-latency type. These shortlatency CRs started either during or shortly after the CS and always returned to baseline level prior to UCS onset. These CRs were not effective in minimizing the impact of the UCS presentation and were of a different temporal configuration from the CRs shown by subjects in both control groups, which peaked at UCS onset.

Comparisons Between Trace and Delay Conditioning Lesion Studies

The data from our present experiments suggest a temporal processing role of the hippocampus in associative learning (Berger et al., 1976; Hoehler & Thompson, 1979; Meck et al., 1984; Port et al., 1985; Solomon, 1979, 1980). This becomes evident by comparing the results from lesion studies using delay conditioning and trace conditioning at short and long trace intervals. Previous studies (using delay conditioning) have shown that hippocampal lesions result in alterations in CR latencies (Port et al., 1985) and CR amplitudes (Akase et al., 1989; Orr & Berger, 1985) without noticeable effects on rates of acquisition. The most prominent and consistent effect has been slower rates of extinction of the conditioned response in well-trained rabbits with hippocampal lesions (Akase et al., 1989; Schmaltz & Theios, 1972). In Experiment 1, we used trace conditioning with a short (300-ms) trace interval and found slower rates of extinction (see Figure 2). In fact, the hippocampectomized rabbits demonstrated essentially no extinction throughout three testing sessions. This behavioral finding is strongly reminiscent of the data reported by Berger and Orr (1983) in their discrimination reversal experiments. They found that rabbits with hippocampal lesions could not extinguish their responses to the former CS^+ (now CS^-), although acquisition of responding to the new CS^+ was unimpaired.

When we used a longer (500-ms) trace interval, we found that the hippocampectomized rabbits failed to learn the task (see Figure 5). Even after 25 sessions, the hippocampal rabbits expressed only 22.3% CRs, whereas all controls reached that level before the 6th session (data not shown). Given this level of responding after 2000 conditioning trials, it is unlikely that the hippocampectomized rabbits would have learned the task regardless of the number of training sessions they received. We also noted significantly more nonadaptive short-latency CRs in hippocampectomized subjects (see Figures 6 and 7). These short-latency CRs were unique, because we only observed them in large percentages among the hippocampectomized rabbits. As a matter of fact, all control rabbits virtually stopped showing these short-latency CRs after the 1st training session-well before they learned the association. This suggests that although we only trained the hippocampal rabbits for 25 days, they were not learning the association, because nearly 50% of their small percentage of CRs (22.3%) were short-latency CRs (see Figures 6 and 7). Our observations convincingly corroborate those reported in a previous study by Solomon et al. (1986) in which they used a 500-ms trace interval to demonstrate learning deficits after dorsal hippocampus lesions.

Other studies also used a 500-ms trace interval but failed to find a learning deficit, although effects on conditioned response latencies during acquisition were noted (James et al., 1987; Port et al., 1986). An important consideration regarding the apparent controversy over the effects of hippocampal lesions on trace conditioning at a 500-ms trace interval is the extent of hippocampal damage. Our studies involved the removal of essentially all of the hippocampus, whereas previous studies used lesions to only the dorsal aspect of the hippocampus. The study reported by Solomon et al. (1986) involved larger lesions to the dorsal hippocampus than the lesion used in the studies that failed to replicate them (James et al., 1987; Port et al., 1986). Because our lesions removed nearly all of the hippocampus, it is possible that Solomon et al. (1986) removed enough dorsal hippocampal tissue to induce a learning deficit, although not as dramatic as the deficit we report in this article. The other two groups (James et ah, 1987; Port et al., 1986) apparently removed just enough hippocampus to reveal differences in response onset latencies but not enough to notice any learning deficit. This interpretation is consistent with previous studies on humans in which severe learning and memory deficits were noted only in those cases in which extensive bilateral damage to the hippocampus was sustained (Scoville & Milner, 1957). Although these patients also suffered damage to other brain regions, a more recent study by Zola-Morgan, Squire, and Amaral (1986) suggests that severe learning and memory impairments can be obtained in humans with damage limited to the entire rostral-caudal length of hippocampal subfield CA1. Thus, it would appear that our use of nearly complete hippocampal lesions contributed to the profound learning deficit reported in Experiment 2, in which a 500-ms tree interval was used.

Role of Hippocampus in Trace Eye-Blink Conditioning

A large body of literature suggests that the hippocampus is involved in the association of temporal events (Akase et al., 1989; Berger & Thompson, 1978b; Disterhoft et al., 1986; Meck et al., 1984; Port et al., 1986; Scoville & Milner, 1957; Solomon, 1980: Solomon et al., 1986; Zola-Morgan et al., 1986). Most of the results from lesion studies using delay eye-blink/NM conditioning suggested some form of modulation of expression of the CR, either in latency to onset (Port et al., 1985) or CR amplitude (Akase et al., 1989). Hippocampal neurons show modeling of the behavioral response during delay (Berger et al., 1980, 1983; Berger & Thompson, 1978a, 1978b) and trace (Akase et al., 1988; Solomon, et al., 1986) conditioning, when measured in vivo. Intrinsic ionic alterations have been seen in vitro after both delay (Coulter et al., 1989; Disterhoft et al., 1986) and trace (de Jonge et al., in press) eye-blink/NM conditioning. Finally, enhanced glutamate binding (Mamounas et al., 1984), enhanced synaptic potentials (LoTurco et al., 1988), and translocation of protein kinase C from the cytosolic fraction to the membrane fraction (Bank et al., 1988; Olds et al., 1989) have been observed in hippocampal CA1 pyramidal neurons of eye-blink conditioned rabbits but not control rabbits. These studies suggest that the hippocampus is involved in the mechanism by which animals learn the association between the CS and the UCS.

Our studies used the trace conditioning paradigm and clearly elucidated the additional role of the hippocampus in bridging the temporal gap between the CS and UCS as required in the traceconditioning paradigm. During acquisition training, the lack of a hippocampus can be compensated for if the temporal requirements are insufficient (300-ms trace interval, Experiment 1). This is not true, however, when the temporal component is increased (500-ms trace interval, Experiment 2). When the trace interval was extended to either 750 or 1000 ms, the temporal requirements were too great for even normal rabbits to learn the association (unpublished observations; 2 subjects used for each trace interval). These data are consistent with a temporal processing view of hippocampal function previously suggested by other researchers using a variety of learning paradigms (Berger et al., 1976; Meck et al., 1984; Port & Patterson, 1984; Port et al., 1986; Solomon, 1980; Solomon et al., 1986).

Also note that we used a CS duration (100 ms) that was shorter than those used in previously reported studies (James et al., 1987; Port et al., 1986; Solomon et al., 1986). This could have maximized the need for the rabbit to formulate an appropriate shortterm "memory trace" necessary for the correct association between the CS and the UCS. Thus, through the use of a short CS duration coupled to a relatively long (500-ms) trace interval, the need for an intact hippocampus emerged; the hippocampectomized subjects simply did not learn the task.

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