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Hippocampal lesions in rats differentially affect long- and short-trace eyeblink conditioning

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Abstract

Extensive previous research has implicated the hippocampus as an important structure for the acquisition of trace eyeblink conditioning. Evidence from multiple species and various lesioning methods shows that the disruption of conditioned responding (CR) may be partially dependent on the relative lengths of the conditioned stimulus (CS) period and the trace interval. The present study systematically manipulated the length of the CS and the trace interval while matching the interstimulus intervals (ISI) in rats with or without ibotenic acid hippocampal lesions. The long-trace interval condition had a CS duration of 50 ms and a trace interval of 500 ms. The short-trace interval condition had a 500 ms CS and a 50 ms trace interval. We found that control animals in the long-trace interval condition learned at a slower rate than the control animals in the short-trace interval condition. Lesioned animals in both the trace conditions showed deficits in acquisition. Lesioned animals in the short-trace interval condition acquired conditioned responses at a rate almost identical to that of the control animals in the long-trace interval condition. CR onset latencies were impaired for lesioned animals. Peak latencies were not different, indicating no difference in the adaptiveness of the CRs. These results suggest that while the hippocampus is important for acquisition of trace eyeblink conditioning, performance also depends on the parameters used for the task. In particular, the relative lengths of the CS period and the trace interval appear to be important.

Keywords: Eyeblink conditioning; Trace conditioning; Hippocampus; Ibotenic acid

1. Hippocampal lesions in rats differentially affect long- and short-trace eyeblink classical conditioning when the CS–US interval is matched

Trace eyeblink conditioning is characterized by a stimulus free temporal gap between the offset of the conditioned stimulus (CS) and the onset of the unconditioned stimulus (US). This is in contrast to the standard delay conditioning paradigm in which the CS overlaps the US. The basic brain circuitry required for acquisition and expression of conditioned responses (CRs) in the delay paradigm lies within the brainstem and the cerebellum [1]. While trace conditioning is also critically dependent upon the cerebellar circuitry [2], the role of the hippocampus in this task has been an area of interest and intense investigation for many years.

Early studies have produced contradictory results concerning the effects of hippocampal lesions on trace eyeblink conditioning in rabbits. Some groups reported that hippocampal lesions abolished CRs [3,4] while others reported that they did not [5,6]. These differences can be accounted for by varying definitions of the CR. Studies concluding that hippocampal lesions abolished trace CRs defined CRs as responses that were long latency and more coincident with the presentation of the US (i.e., responses that were considered "adaptive") [3]. In studies where the entire CS–US interval was examined for CRs, including short latency responses, hippocampal lesions did not appear to disrupt learning [3,5,6]. One conclusion was that the hippocampus may be involved in timing of the trace conditioning CRs, but not the acquisition [5,6]. Others concluded the hippocampus was necessary for acquisition of the trace CR [3].

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Other differences between the studies included use of an air puff [3,4,6] vs. a periorbital shock US [5,6]. Analysis of the differing US types found that periorbital shocks generally produce longer latency responses than air puffs in hippocampal lesioned animals [6], offering another explanation for the differing effects of hippocampal lesions. Tones [3,4,6] and white-noise [5] CS were also factors that differed when experiments were compared, although no formal analysis concerning differences in learning was reported.

These studies focused solely on the effects of lesions to the dorsal aspect of the hippocampus with variable amounts of damage [3,5,6]. Complete bilateral aspiration lesions of the dorsal and ventral hippocampus in rabbits differentially affected acquisition of CRs depending on the task parameters. Rabbits acquired CRs when a 100 ms tone CS and a 300 ms trace interval were used, but not when a 500 ms trace interval was used [7]. Most previous studies used a 250 ms tone CS and a 500 ms trace interval [3–6]. Additionally, hippocampal lesions did not alter the onset latencies or peak amplitudes when rabbits were trained with a 300 ms trace interval. This suggested that the hippocampus may only be necessary when the temporal demands of the task are sufficiently great (i.e., when the trace interval is relatively long) [7].

Bilateral aspiration lesions of the dorsal and ventral hippocampus in rats prevented the acquisition of CRs in a paradigm with a 250 ms tone CS, 250 ms trace interval, and a 100 ms air puff US [8]. The hippocampal rats also had fewer adaptive CRs (those occurring within 200 ms of the US), reduced area under the curve in the CRs, shorter CR durations, and shorter peak latencies. These results replicated previous studies with rabbits [3,4,7]. Additionally, the trained rats were switched to a paradigm with a 250 ms CS and a 100 ms US that was presented immediately after tone offset. The lesions produced no effect when this training procedure was used and the authors concluded that the procedure was similar to delay conditioning [8].

Another study questioned whether trace conditioning engaged the hippocampus because of the temporal relationship between the CS and the US or if it was fundamentally a more difficult task. Excitotoxic lesions of the hippocampus impaired learning in a trace conditioning task with a 250 ms tone CS and a 500 ms trace interval. Lesions did not impair a delay task that was matched for interstimulus interval (ISI). Hippocampal lesions also partially impaired acquisition of a more difficult delay conditioning paradigm with a 1400 ms ISI. Results of this study indicated that the hippocampus was engaged when the task was both sufficiently difficult and the temporal demands of the task exceeded the capacity of the cerebellar and brainstem circuitry alone [9].

While the hippocampus appears to be important for trace eyeblink conditioning, there are instances when the hippocampus appears not to be necessary for trace learning. For example, when the trace interval is sufficiently short relative to the CS, animals seem to be able to acquire the task [7,8]. The present study aimed to systematically study the role of the hippocampus in trace eyeblink conditioning by comparing animals with lesions of the hippocampus to control animals trained on a long- or short-trace interval eyeblink conditioning task. Unlike previous studies [7,8], the CS–US intervals were matched so that relative to the CS onset, the presentation of the US was consistent for both groups. This design allowed us to make direct comparison between the timing of the responses between the two conditions and also control for effects of different CS–US interval lengths. Additionally, we used ibotenic acid to selectively destroy hippocampal cell bodies, thus limiting damage to the overlying cerebral cortex and fibers of passage [10].

2. Methods

2.1. Subjects

The subjects for this study were thirty-nine Long–Evans Blue Spruce male ($n=19$) and female ($n=20$) rats obtained from Harlan (Indianapolis, IN, USA). The rats were pseudorandomly divided into the three groups: intact controls ($n=16$), hippocampal lesions with ibotenic acid ($n=17$), and sham lesions ($n=6$).

All animals were housed in a vivarium in the Department of Psychological and Brain Sciences on the Indiana University-Bloomington campus. Animals were group housed with no more than 4 rats per cage (48 cm×20 cm×26 cm) until surgery. Following surgery, animals were individually housed for the remainder of the study. All animals had ad-lib access to food and water. The vivarium was on a 12 hour light–dark cycle with lights on at 0700. All experiments and surgical procedures took place during the light phase. All experimental and surgical procedures were carried out in accordance with the guidelines published in the National Institutes of Heath *Guide for the Care and Use of Laboratory Animals* and were approved by the Bloomington Institutional Animal Care and Use Committee.

2.2. Surgery

2.2.1. Ibotenic acid and sham hippocampal lesions

Ibotenic acid (IBO) lesions of the hippocampus were performed using the methods previously described [10]. Rats were anesthetized with a single intramuscular (im) injection (2.0 ml/kg) of an anesthetic cocktail consisting of sterile physiological saline (9.0 mg/kg), Ketamine (74.0 mg/kg), Xylazine (3.7 mg/kg), and Acepromazine (0.74 mg/kg). Supplemental boosters of Ketamine were given as needed during surgery at a standard dose of 0.1 ml per im injection. The head was shaved and scrubbed with a betadine scrub, rubbing alcohol, and betadine solution. The rat was then placed in the stereotaxic apparatus, the eyes covered with ointment, and the skull was exposed. After the skull dried, it was adjusted so lambda was level to bregma. The skull overlying the hippocampus was removed with a dental drill and a sharp drill bit. Injections of IBO (1 mg/μl) were made at the coordinates shown in Table 1 with a 1 μl syringe (Hamilton; Reno, NV, USA). After all injections were completed, the hole in the skull was packed with gel foam and the animal was prepared for eyeblink conditioning as described below.

Sham lesion animals underwent a similar surgery as those with IBO lesions. The cortex was penetrated and the needle was lowered to the proper dorsal–ventral coordinates and left in place for 1 min without injecting any solution into the brain.