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Hippocampal theta phase–contingent memory retrieval in delay and trace eyeblink conditioning

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ABSTRACT

Hippocampal theta oscillations (3–12 Hz) play a prominent role in learning. It has been suggested that encoding and retrieval of memories are supported by different phases of the theta cycle. Our previous study on trace eyeblink conditioning in rabbits suggests that the timing of the conditioned stimulus (CS) in relation to theta phase affects encoding but not retrieval of the memory trace. Here, we directly tested the effects of hippocampal theta phase on memory retrieval in two experiments conducted on adult female New Zealand White rabbits. In Experiment 1, animals were trained in trace eyeblink conditioning followed by extinction, and memory retrieval was tested by presenting the CS at troughs and peaks of the theta cycle during different stages of learning. In Experiment 2, animals were trained in delay conditioning either contingent on a high level of theta or at a random neural state. Conditioning was then followed by extinction conducted either at a random state, contingent on theta trough or contingent on theta peak. Our current results indicate that the phase of theta at CS onset has no effect on the performance of the behavioral learned response at any stage of classical eyeblink conditioning or extinction. In addition, theta-contingent trial presentation does not improve learning during delay eyeblink conditioning. The results are consistent with our earlier findings and suggest that the theta phase alone is not sufficient to affect learning at the behavioral level. It seems that the retrieval of recently acquired memories and consequently performing a learned response is moderated by neural mechanisms other than hippocampal theta.

1. Introduction

The hippocampus is a crucial brain structure in learning [1]. According to Buzsáki's [2] two-stage model of learning and memory, the hippocampus exhibits two states related to the encoding and strengthening of memory traces. When exploring its environment and focusing attention on external stimuli, hippocampal electrophysiological activity is dominated by rhythmic slow wave activity, the theta oscillations. Theta oscillations are most prominent in the hippocampal input region, the dentate gyrus (DG) and near the hippocampal fissure [3]; for a review, see Ref. [4] and are driven by GABAergic neurons of the medial septum [5]. When the level of attention lowers and hippocampal theta activity ceases, the output region of the hippocampus, the CA1, shows activation in the form of large amplitude sharp-wave ripples. Ripples are generated when input from the entorhinal cortex (EC) via the perforant path and the DG reaches the CA3. Within the CA3 autoassociative network, recently activated neuronal assemblies then reactivate [6], and further activate the CA1 pyramidal cells. Pyramidal cells of the CA1 project the signal back to the neocortex and complete the network loop. Thus, in Buzsáki's [2] model, the role of theta oscillations is suggested to be most crucial during the encoding of new information. However, others also suggest a role for theta in the retrieval of already encoded memories [7].

Lesion studies [8,9] and pharmacological manipulations [10] indicate that the hippocampus is needed at least in the early stages of learning in eyeblink conditioning [11]. Trace eyeblink conditioning is a hippocampus-dependent task [12,13] whereas delay eyeblink conditioning can be acquired even in the absence of the whole forebrain [14]. Yet studies suggest that hippocampal electrophysiological state, namely the level of theta, correlates with learning even during delay eyeblink conditioning [15–17]. According to Berry et al., when training is carried out during theta, animals learn delay eyeblink conditioning faster than do those trained in the absence of or during low levels of hippocampal theta activity [18]. This is a finding so far not reported elsewhere.

A computational model by Hasselmo et al. [7] proposes that not only the presence or absence of hippocampal theta might be important but that the phase of the theta cycle determines the optimal time

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windows for hippocampal encoding and retrieval of memories. First, the model suggests that encoding associations in memory is most effective when synaptic output from the EC to the hippocampus is strong. Second, the model postulates that retrieval of the learned associations is most effective when the EC input is weak. The fluctuation in the input from the EC to the hippocampus is congruent with the hippocampal fissure theta phase, the peak of theta corresponding to low EC input. Presenting the CS at the peak or trough also affected learning rate, since acquisition of conditioned response (CR) was slower when the CS onset coincided with the theta peak. However, in contradiction to the hypothesis, our results suggested that in well-learned animals the behavioral performance of the CR is not dependent on the theta phase regardless of a clear effect on neural responses [19]. However, a possibility remains that an effect might have been seen if memory retrieval had been probed at different stages of learning. Lesion studies suggest that the hippocampal contribution to eyeblink conditioning is strongest early in learning [11].

The studies reported here were conducted to further test if hippocampal theta phase has an effect on the retrieval of a recently acquired memory trace and the consequent performance of a learned response in various stages of the acquisition process as well as during extinction training. It might well be that although no behavioral effect was detected in our previous study on well-trained animals [19], there could be an effect when the memory trace is still in the labile state early in training [11]. This notion was addressed in Experiment 1. In line with the model of Hasselmo et al. [7], our hypothesis was that retrieval would be more efficient if the CS was presented at fissure theta peak. In addition to simple acquisition of a learned response, in Experiment 1 we also studied if the phase of theta moderates the conditioned responses during different stages of extinction training. Again, more efficient retrieval of the previously acquired memory trace manifested as a conditioned response was expected if the CS was presented at fissure theta peak. In Experiment 2, we first sought to confirm the results of the study by [18] on theta-contingent delay conditioning and then addressed the question of whether extinction of the learned response is also affected by theta phase. To do so, we first trained rabbits in delay eyeblink conditioning either during theta or regardless of neural state. We expected animals trained during theta to learn faster and/or better than those trained at random [18]. Then, in well-trained animals we conducted extinction either by presenting the CS alone randomly or always at the theta trough or at the theta peak. Compared to animals trained at a random state, we expected to see impeded extinction learning in animals trained contingent on fissure theta peak [19].

2. Materials and methods

2.1. Subjects

The subjects were 29 (Experiment 1, 13 rabbits; Experiment 2, 16 rabbits) adult female New Zealand White rabbits (Lidköpings kaninfarm, Sweden) weighing approximately 2.8 kg at the time of surgery. The rabbits were housed in individual cages at the Laboratory center of the University of Jyväskylä. Food and water were freely available, and room temperature and humidity were controlled. The rabbits were maintained on a 12/12-h light/dark cycle, with lights on at 8:00 a.m. All experiments were carried out during the light part of the cycle. All the experimental procedures, care and handling were executed in accordance with Directive 2010/63/EU of the European Parliament and of Council on the protection of animals used for scientific purposes. Animal handling was performed only by trained personnel and the rabbits were introduced to human contact and handling for a sufficient amount of time before the surgery.

2.2. Surgery

Before the surgery, rabbits were treated with subcutaneous injections (s.c.) of an anti-inflammatory drug (50 mg/mL carprofen [Rimadyl vet, Pfizer Inc. Animal Health], dose: 0.1 ml/kg) and with 2 ml of an analgesic drug (0.3 mg/ml buprenorphine [Temgesic, Schering-Plough Europe] diluted with 0.9 ml of 0.9% NaCl) to moderate acute pain after surgery. The rabbits were anesthetized with an intramuscular injection (i.m.) of ketamine–xylazine cocktail (7.8 ml of 50 mg/ml Ketaminol vet [Intervet International B.V.] mixed with 2.8 ml of 20 mg/ml Narcoxyl vet [Intervet International B.V.]). A dose of 0.8 ml/kg of the cocktail was injected i.m. before surgery. During surgery, additional doses of either the cocktail or ketamine alone were injected subcutaneously approximately every 20–30 min or as needed. Before the surgery, the rabbit's fur was shaved from the top of the head. Then, the rabbit was positioned in a stereotaxic instrument (Kopf Instruments) with the bregma 1.5 mm higher than the lambda. Eye gel was inserted into the rabbit's eyes. At this point, 2.0 ml of lidocaine (10 mg/ml Lidocain [Orion pharma]) was injected s.c. in the area of surgery before making the opening incision.

A longitudinal incision was made to the scalp and a local anesthetic (2 g of lidocaine-hydrochloride Xylocain [AstraZeneca]) was administered to the wound. Eight holes for electrodes were drilled into the skull along with four holes for the anchoring screws (5 mm anterior and 5 mm lateral to the bregma; 13 mm posterior and 5 mm lateral to the bregma). Two of the posterior and anterior screws were connected together and served as a reference and the ground, respectively, in the electrophysiological recordings. Eight monopolar recording electrodes (Teflon-insulated stainless steel wire; 0.125 mm uninsulated diameter [A-M Systems]) were chronically implanted into the left dorsal hippocampus, aiming four electrodes to the CA1 (4 mm posterior, 3.5–6.5 mm laterally from the bregma; electrode tip depth from the bregma 6–8 mm) and four above the hippocampal fissure (5 mm posterior, 4–7 mm laterally from the bregma; electrode tip depth 6.2–8.5 mm below the bregma). Wires, skull screws, preamplifier interface, one mounting screw for an air puff mount, and the incision area were cemented with dental acrylic. To prevent nausea after surgery, metoclopramide (0.1 ml/kg, concentration 5 mg/ml; Primperan [Sanofi Winthrop Industrie]) was administered s.c. and the rabbit was returned to her home cage wrapped in a towel. Recovery was monitored and the rabbits were medicated with analgesic (buprenorphine [Temgesic, Schering-Plough Europe] diluted with 0.9 ml of 0.9% NaCl) 4 h after surgery and then every 8 h for the next 44 h.

2.3. Experimental procedure

The experimental procedures are illustrated in Fig. 1. After one week of recovery from surgery, the animals were accustomed to a plexiglas restraining box without restraining and overall behavior was monitored. On the second day, restrained animals were habituated to the recording chamber and 30 min of spontaneous hippocampal local-field potentials (LFPs) were recorded. LFPs and electromyography (EMG, please see Section 2.4 below) from the right eye were recorded 5 min prior to, during and 1 min after each session. The inter-trial interval always varied randomly between 30 and 60 s. LabVIEW (National Instruments) was used to monitor neural activity and blinking online, to execute the experimental procedures and to present stimuli. The percentage of learned responses performed by each animal was analyzed after every session using MATLAB (The MathWorks Inc.). Please see Section 2.4 for further information.

2.3.1. Experiment 1: trace eyeblink conditioning, memory retrieval testing and extinction

During the first training session, 60 tone-alone (40-ms, 5-kHz, 75-dB tone) trials were presented regardless of neural state. In addition to hippocampal LFPs, EMG from the right eye was also recorded to determine the frequency of spontaneous eyeblinks elicited by the tone later used as a CS.

Trace eyeblink conditioning was carried out with the tone specified