Cerebellar theta oscillations are synchronized during hippocampal theta-contingent trace conditioning

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The hippocampus and cerebellum are critically involved in trace eyeblink classical conditioning (EBCC). The mechanisms underlying the hippocampal-cerebellar interaction during this task are not well-understood, although hippocampal theta (3–7 Hz) oscillations are known to reflect a favorable state for EBCC. Two groups of rabbits received trace EBCC in which a brain-computer interface administered trials in either the explicit presence or absence of naturally occurring hippocampal theta. A high percentage of robust theta led to a striking enhancement of learning accompanied by rhythmic theta-band (6–7 Hz) oscillations in the interpositus nucleus (IPN) and cerebellar cortex that were time-locked both to hippocampal rhythms and sensory stimuli during training. Rhythmic oscillations were absent in the cerebellum of the non-theta group. These data strongly suggest a beneficial impact of theta-based coordination of hippocampus and cerebellum and, importantly, demonstrate that hippocampal theta oscillations can be used to index, and perhaps modulate, the functional properties of the cerebellum.

Neurobiological oscillations act as timing signals in the brain, organizing and coordinating activity within and across different regions (1–5). Hippocampal theta oscillations, which are low frequency waves ranging from 3 to 7 Hz, serve as an index of hippocampal functional state and are within the frequency bandwidth that has been proposed to synchronize large areas or across long distances (3, 5). Eyeblink classical conditioning (EBCC), which is arguably one of the best-understood models of mammalian associative learning, relies on cerebellar circuitry for normal acquisition (6–11). It has been shown, for all EBCC paradigms examined, that lesions restricted to the anterior interpositus nucleus completely and permanently prevent acquisition of conditioned responses (CRs) in naïve animals and abolish CRs in well-trained animals without affecting the reflexive, unconditioned response (UR) performance (6, 8). Additionally, Larssell’s hemispheric lobule VI (HVI) is among the cerebellar cortical regions that are known to send direct projections to the IPN and play a modular role during trace EBCC by contributing information, related to the frequency and magnitude of CRs, that facilitates normal acquisition and expression of CRs (8, 10). The trace form of the EBCC paradigm, in which there is a stimulus-free period between the conditioned (CS) and unconditioned stimuli (US), also requires an intact hippocampus (12–15). Hippocampal neurons are known to increase unit firing rates in response to conditioning stimuli (15–17) and, perhaps critically, during the trace interval (12, 17). Questions concerning the timing, form, and location of possible hippocampal-related cerebellar responses during trace EBCC cannot be addressed solely by stimulation, lesion, or drug studies because those manipulations prevent the natural ebb and flow of theta states during a training session and often produce distorted oscillations (18) or abnormal cellular correlates of theta in hippocampus (19). Furthermore, complete removal of the hippocampus has been found to be less deleterious to delay EBCC than is disruption of theta by anticholinergic drugs or medial septal lesions (18, 20, 21). In the case of large hippocampal lesions, all hippocampal input to cerebellum is absent and the role of an intact hippocampus in trace EBCC can be inferred only indirectly (14, 20, 21). Our approach is to observe covariations of physiological responses in hippocampus and cerebellum under relatively intact conditions while pretrial hippocampal theta state is selected by a brain-computer interface (BCI). We have shown that theta in the hippocampus mediates hippocampal involvement in EBCC (17, 20, 22–24; see also ref. 25). Through the use of our BCI, we are able to limit EBCC training to two naturally occurring extremes of hippocampal theta such that each of two groups can be trained in either the explicit presence (T+) or absence (T−) of on-going theta. Theta-contingent trial presentations lead to: (i) significantly faster learning during both delay (23) and trace EBCC (17), (ii) a corresponding increase in hippocampal unit responses across days during trace EBCC (17), and (iii) a substantial reduction in age-related memory impairment (24). Here, we report that pretrial hippocampal theta state predicts the synchronization of hippocampal (CA1) and cerebellar local field potentials (LFPs) into a rhythmic theta oscillation that accompanies a striking cognitive/behavioral benefit over non-theta conditioning. This finding supports a hypothesized role for theta in coordinating a widely distributed memory system for trace EBCC and demonstrates the effective use of a hippocampal BCI to modulate cerebellar responses to conditioning stimuli.

Results

Behavior. Behavioral results demonstrate that initiating acquisition trials based on CA1 theta-state enhances learning rate substantially. Groups differed significantly in percentage CRs on days 2, 3, and 4 [D2: M+ = 25.0%, M− = 3.3%, F (1, 9) = 6.02, P = 0.037; D3: M+ = 53.4%, M− = 3.1%, F (1, 9) = 15.43, P = 0.003; D4: M+ = 54.6%, M− = 9.6%, F (1, 9) = 12.03, P = 0.007]. Fig. 1A shows the percentage of CRs across days 1–4, illustrating the significant difference in group performance. Although group differences in percentage CRs were not significant on day 1 [M+ = 12.6%, M− = 0%, F (1, 9) = 3.69, P = 0.087], the number of trails to the third CR (classically thought to demarcate the end of learning phase 1; 26) was significantly different between groups [M+ = 41.8, M− = 174.2, F (1, 9) = 7.54, P = 0.023]. Trial 41 occurred on the first day of acquisition for T+ animals, while trial 174 typically occurred on day 4 for T− animals. The averaged cumulative CR plot shows the learning trajectories of T+ and T− groups diverging within the first 50 trials of acquisition day 1 (Fig. 1B), which is consistent with our prior theta-contingent trace EBCC findings (17).

Overview of Neural Recordings. Simultaneous LFPs from hippocampal field CA1, cerebellar IPN, and lobule HVI of cerebellar cortex were recorded from each subject. The T+ group averages of these LFPs displayed strong evoked potentials (EPs)

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followed by an immediate onset of robust, time-locked theta oscillations in response to stimulus presentations beginning on day 1 (Fig. 2; see Fig. S1 for electrode placements and Fig. S2 for LFPs on additional days). Specifically, for hippocampus, a regular theta rhythm was triggered by both CS and US in T+, while in T− group averages, a weaker theta was elicited by each stimulus onset (as has been reported for non-theta-triggered signals; ref. 27). The T+ oscillations were characterized by a longer latency onset, a less robust, faster, and less regular periodicity than those of the T− group (as shown in Figs. 3A and 4A), and were also less consistent across days (Figs. 2 and 4A and Fig. S2). In cerebellum, rhythmic theta in the averaged LFPs appeared at short latency in the T+ group, with the first positive peak of rhythmic theta at approximately 300 ms after CS and US onsets, superimposed upon a larger nontheta negative EP peak (Fig. 2). Conversely, in T− cerebellar LFPs, each EP ended with a clear, single negative peak showing little or no theta of regular periodicity superimposed on the negative EP following the CS or US (Fig. 2). This lack of rhythmicity, relative to the T+ LFPs, can also be seen in the cerebellar autocorrelations (Fig. 4A). Unlike the oscillations of CA1, theta in the IPN and HVI following each EP was combined with a low frequency (~0.5 Hz) positive baseline shift (Fig. 2), that can also be seen as the fundamental frequency of the cerebellar autocorrelations (Fig. 4A). In T+ autocorrelations, this slow shift is clearly modulated by theta (Fig. 4A). All LFPs of the T+ group exhibited a consistent 6–7 Hz theta frequency during the trial period, while the T− group displayed less rhythmicity and greater variability in frequency between structures (Fig. 3) as well as across days (Figs. S2 and S3). Crosscorrelations support a theta-based covariation of LFPs in hippocampus and cerebellum, but only in the T+ group (Fig. 4B). Thus, the state of the hippocampus reliably differentiated the T+ and T− groups in terms of cerebellar evoked and rhythmic responses to both conditioning stimuli.

**EP Peak Amplitudes.** For evoked responses to conditioning stimuli, peaks were denoted P1, P2, P3, and P4, where P1 and P2 corresponded to the first and second phase, respectively, of the CS-related EP, and P3 and P4 correspond to the first and second phase, respectively, of the US-related EP (see labeled peaks in Fig. 2). Taken individually, the amplitudes of the initial phase of each evoked response to the CS and US (P1 and P3), generally did not differ significantly between T+ and T− groups across days 1–4 in any structure (see SI Text for statistics). However, throughout T+ training, the IPN showed larger positive peak responses to US than CS, whereas the IPN in the T− group appeared to show the opposite relationship between CS and US EPs. Within-group difference scores, comparing P1 and P3 response amplitudes in IPN, revealed significant differences between theta groups on days 1–3 [D1: MT* = −73.81, Mrev* = −110.33, F (1, 9) = 6.20, P = 0.034; D2: Mrev* = −68.83, Mrev* = −208.10, F (1, 8) = 17.44, P = 0.003; D3: Mrev* = −90.97, Mrev* = −146.79, F (1, 9) = 6.14, P = 0.035], but not on day 4 [Mrev* = −75.27, Mrev* = 129.92, F (1, 9) = 5.07, P = 0.051]. Difference scores comparing amplitudes of P1 and P3 were not significant for CA1 or HVI (see SI Text for statistics).

The second phase of each EP (P2 and P4) showed distinct differences in cerebellar responses as a function of pretrial hippocampal state. For CS responses in IPN, amplitudes differed significantly between theta groups. Specifically, one-way ANOVAs on P2 amplitude (negative-going component of EPs to the CS) in IPN revealed that this EP peak was significantly larger in amplitude in the T− condition on days 1–3 [D1: Mrev* = −272.58, Mrev* = −508.61, F (1, 9) = 13.36, P = 0.005; D2: Mrev* = −216.33, Mrev* = −455.01, F (1, 8) = 13.84, P = 0.006; D3: Mrev* = −248.46, Mrev* = −505.75, F (1, 9) = 19.45, P = 0.002], but not D4 [Mrev* = −238.01, Mrev* = −352.85, F (1, 9) = 2.28, P = 0.165]. Similar results
occurred in HVI for P2, in that the negative-going EP peak was significantly larger in amplitude in the T+ group compared to T− group on days 1–3 

**Fig. 3.** Power analyses of average LFPs during whole trial period (from CS-onset to 1 s after US-offset) in CA1, IPN, and HVI on day 1. (A) Power spectra from 2 to 20 Hz show that, for rhythmic theta frequencies, power at approximately 6.5 Hz is apparent in CA1 and cerebellar structures of the T+ group (black trace; n = 6, in the T− group (gray trace; n = 5), power at approximately 7 Hz is present in CA1 (persisting across days), while power in cerebellum is much lower at the 6–7 Hz frequencies and is more variable across days (most commonly ~8 Hz). Area within dashed rectangle highlights 6–7 Hz bandwidth (see Fig. S3 for additional days). (B) Line graphs of percentage power at 6–7 Hz display significantly greater power in this frequency band in CA1 and IPN of the T+ group compared to T− on days 1–3. Error bars represent ± 1 SEM.

**Frequency.** Power spectral analyses of whole-trial (extending from CS-onset to 1 s after US-offset) average LFPs show that the large, slower waveforms of each EP produced frequency components with consistent power at 3 and 5 Hz (Fig. 3A). These spectral peaks are single, non-rhythmic evoked responses (positive-negative for cerebellar structures and the inverse for CA1). Specifically, the 5 Hz component reflects the first half of the EP, while the 3 Hz component reflects the second, opposite polarity part of the EP in responses to both CS and US. Group differences in percentage power at 3 and 5 Hz were not significant (Fig. 3B and C; see **SI Text** for statistics). As power in these EP frequency components does not vary between theta groups or structures, and are not significantly different between groups for each frequency, our analysis concentrated on the faster, rhythmic LFPs that differentiated our treatment groups and corresponded to differences in learning rate.

Spectral analysis of whole-trial LFPs on days 1–4 show that, for rhythmic theta frequencies, power at approximately 6.5 Hz was apparent in all three structures of the T+ group, while the T− group displayed significantly lower power at this frequency (as determined by percentage power analysis below; see Fig. 3B). When peaks did occur in the T− group they were higher frequency (7–9 Hz) and consistently smaller than the 6.5 Hz peaks of the T+ group (see Fig. S4 for day 1 and Fig. S3 for additional days). Specifically, T− CA1 showed an expected theta response to the conditioning stimuli but at higher frequency than T+. (see also Fig. 3A), T− IPN displayed small and variable peaks, and T− HVI had no salient frequency peaks greater than 5 Hz. These trends continued across the first 4 days of acquisition. Analyses comparing percentage power at 6–7 Hz during the first 4 days of acquisition revealed significant differences between groups in CA1 and IPN on days 1–3 [CA1: D1: F (1, 9) = 8.48, P = 0.017; D2: F (1, 9) = 8.40, P = 0.02; D3: F (1, 9) = 9.43, P = 0.015; IPN: D1: F (1, 9) = 6.50, P = 0.031; D2: F (1, 8) = 6.13, P = 0.038; D3: F (1, 9) = 37.29, P = 0.000] but not on D4 [CA1: D4: F (1, 9) = 4.52, P = 0.062; IPN: D4: F (1, 9) = 3.84, P = 0.082]. In HVI, group differences were not significant [D1: F (1, 9) = 5.23, P = 0.051; D2: F (1, 9) = 3.85, P = 0.085; D3: F (1, 9) = 5.17, P = 0.053; D4: F (1, 9) = 2.80, P = 0.129]. Together, these frequency analyses provide significant quantitative support for group differences in the visual appearance of rhythmicity in the LFPs (Fig. 2).

**Rhythmicity.** Time-series analyses (auto- and cross-correlations) of oscillations concentrated on the US-post period (a 1-s period beginning at 1,500 ms) because the estimates of theta phase and rhythmicity in whole-trial correlations were significantly distorted by the large, nonrhythmic stimulus driven EPs at the 600-ms interstimulus interval in all structures in both groups (as shown in above power analysis, Fig. 3A). Autocorrelograms of averaged post-US LFPs showed clear rhythmicity in CA1 and both cerebellar structures of the T+ group. The T− group displayed less robust rhythmicity and a faster, more variable frequency in CA1, and, importantly, no rhythmicity in either cerebellar structure (Fig. 4A). Similar periodicities were observed in trace period autocorrelations (**SI Text**). It is clear from the LFP plots in Fig. 2 that there was time-locked theta during the trace period, exhibiting more cycles of regular periodicity with a shorter latency onset in every brain structure of the T+ group in contrast to T−.

Crosscorrelograms of CA1 and cerebellar LFPs revealed rhythmic coordination of CA1 with IPN and lobule HVI at theta frequency only in the T+ condition (Fig. 4B). A negative correlation at lag time 0 indicates that CA1 and cerebellar waveforms are essentially 180° out of phase (although some hippocampal laminae, e.g., adjacent to the hippocampal fissure, would likely be out of phase with CA1 theta (3) and therefore in phase with cerebellum). The dominant frequency of phase synchronization is approximately 6–7 Hz between CA1 and both cerebellar structures in the T+ condition. Conversely, in the T−
due to different frequencies in the two structures as revealed by power spectra (see Fig. 4 in the T system). The systematic covariation of theta in hippocampus and cerebellum. Phase synchronization repeats at 6–7 Hz in the cerebellum at 6–7 Hz. The T leads to strong, rhythmic coordination across regions of the T group (Right) may be due to different frequencies in the two structures as revealed by power spectra (see Fig. 4A). Crosscorrelations between IPN and HVI reveal that cerebellar areas in phase in both T conditions, but that only the T+ group is modulated at theta periodicity.

**Discussion**

The above findings demonstrate a significant impact of hippocampal theta-triggered training in regulating response properties of cerebellar circuits or, at the very least, serving as a useful covariate of significant differences in cerebellar and behavioral responsiveness. Prior work had shown that, for trace EBCC, the hippocampus was part of the necessary circuit but its role was not clear (12–17). The major findings of the current study are that the presence of pretrial hippocampal theta occurs in conjunction with a substantial increase in acquisition rate, accompanied by (i) amplitude modulation of cerebellar evoked responses to conditioning stimuli, (ii) cerebellar theta oscillations that are time-locked to the sensory stimuli in awake, behaving animals, and (iii) synchronization of hippocampal and cerebellar IPN and HVI LFPs at 6–7 Hz theta frequency. The demonstration of time-locked hippocampal and cerebellar LFPs as well as a theta-based covariation between them begins to characterize the possible interactions between these structures during trace EBCC.

The behavioral learning rates yielded in the present study for T+ and T− are consistent with those in previous reports, which also showed T+ and T− animals differing from yoked controls (17, 23). Thus, our current data replicate earlier behavioral effects and extend the neural correlates to include differential LFP responses in hippocampus and in cerebellar areas that are required for normal association and expression of CRs in trace EBCC.

Most interpretations of the essential role of hippocampus in trace EBCC center around its participation in sustaining CS-related activity through the trace period until US onset (11, 12, 17, 28). Our results show a strong, coordinated rhythmicity at theta within and between structures during this period in the T+ group but not the T− group. These data suggest that robust oscillations might help facilitate neural plasticity, known to be associated with theta frequencies in both hippocampus (3, 29–32) and cerebellum (33–36). The coordination of hippocampal and cerebellar theta in the T+ group may phase-lock their excitability at a periodicity that favors long-distance communication (3, 5).

In our EP analyses, the smaller negative EP in response to the CS in the T+ condition may, in part, be attributed to the rapid emergence of theta oscillations with a positive peak at theta periodicity during the negative phase of the EP (Fig. 2). This result demonstrates a significant relationship of hippocampal theta state with the CS response in the cerebellum (both IPN and HVI) and may imply that the behavioral benefit of theta-triggering involves a short-latency activation of theta rhythmicity in the trace interval that superimposes on the negative cerebellar EP in T+ only. Further, the relative CS versus US positive peak amplitudes for IPN differed as a function of hippocampal theta states, showing an effect across the CS-US interval. This could be critically important in trace conditioning in which the CS terminates hundreds of milliseconds before the US arrives.

The circuitry of the cerebellum has been shown to support resonant frequencies within the theta bandwidth (34–36). Moreover, there is evidence that such oscillations synchronize activity...
within cerebellar hemispheres as well as between cerebellar and cortical regions (37, 38). Those studies on cerebellar oscillations at theta frequency suggest that our methods may be engaging resonant frequencies of cerebellar circuits that favor plasticity and optimize timing. Our finding extends this by showing stimulus time-locked cerebellar oscillations that appear coordinated with hippocampal rhythmicity at consistent 6–7 Hz periodicity, and accompanied by a substantial cognitive enhancement.

These observations have important implications for the locus and mechanisms of essential participation of the hippocampus in trace conditioning, such as the modulation of neural pathways by which CS and US information may be transmitted to the cerebellum. Prominent models of EBCC suggest that the US activates the inferior olive (IO), which provides strong synaptic input to cortical Purkinje cells via climbing fibers, with collaterals to IPN (34, 35, 39, 40). These models also implicate pontine nuclei (PN), activated by the CS, as providing mossy fiber input to granule and Golgi cells, also with collateral input to IPN, and ultimately to the parallel fiber system, which synapses onto Purkinje cells. While the IO/climbing fiber system can generate oscillatory potentials in the theta range (41), our results suggest the need for studies on the latter (CS) pathway for two major reasons.

First, earlier research had demonstrated that hippocampal and prefrontal lesions prevent trace, but not delay, EBCC, suggesting essential contributions from forebrain to cerebellum during the trace paradigm (12–14, 28, 42). If, as hypothesized by the models, the role of hippocampus in trace EBCC is a continuation of the excitatory CS response into and through the trace interval, our results would predict major differences in amplitude and/or duration of PN responses covarying with hippocampal theta state. There is significant evidence of theta periodicity in unit activity of cerebellar granule and Golgi cells in the PN to parallel fiber pathway (35, 36), but their dependence on, or relation to, hippocampal theta is unknown. One recent proposal by Kalmbach et al. (28) for trace EBCC is that mossy fiber input, controlled by forebrain structures such as hippocampus and prefrontal cortex, serves to sustain CS responses through the trace interval to overlap with the US so that cerebellar circuits can form CS-US associations. Our results suggest that cerebellar theta (which occurred here only in the T+ condition) may create rhythmic patterns of mossy/parallel fiber excitability through the trace interval that allow time-locked US responses from the IO to arrive under optimal conditions for plasticity. Furthermore, to the extent that hippocampal theta is known to be the basis of synchronization of forebrain areas during learning- and memory-related tasks (43, 44), the mechanisms put forth by Kalmbach et al. (28) for post-CS forebrain activation of PN mossy fibers would very likely be modulated at the periodicity of hippocampal theta.

Second, the PN have long been implicated in the initiation of hippocampal theta (45, 46) so that, if there is a common generator for the oscillations observed in both hippocampus and cerebellum, patterns of activity in PN should interact with hippocampal theta states controlled by our BCI and address questions about the direction of influence between the structures. It should be noted that the precise phase locking over the distance between hippocampus and cerebellum (indicated by the peak at zero lag in the crosscorrelations) would be surprising if one region were directly driving the other, but would be quite consistent with a common pacemaker for both structures. The idea of a common pacemaker must be qualified by anecdotal observations during nontrial periods that show variability in the occurrence and frequency of theta between structures. Specifically, in the uncontrolled ITI, theta could be observed in one or both regions or neither at a given point in time. Moreover, during trials, our T− group clearly shows theta in CA1 while it is absent in both cerebellar structures. Thus a simple, unitary pacemaker appears unlikely unless it becomes activated during the trial period in T+ only.

Evidence for the direction of influences from cerebellum to forebrain exists, primarily the disruption of hippocampal learning-related neural responses after cerebellar lesions (47). Additionally, as mentioned above, hippocampal and prefrontal lesion studies demonstrate an essential forebrain to cerebellum connection during trace EBCC (12–14, 28, 42). These directional influences are not mutually exclusive, especially when trial duration is measured in hundreds of milliseconds. Our findings, based on a hippocampal BCI, cannot be used to conclude either direction of influence but do suggest physiological theta states under which interactions between hippocampus and cerebellum might be optimized.

Generalizing across species, studies have shown theta oscillations to be implicated in human cognitive processing, with beneficial effects in acquisition, retrieval, and spatial navigation (e.g. 48). If tasks could be acquired and performed when there are periods of maximal theta, cognitive processes might be enhanced. This raises the possibility that our methods could be used to ameliorate human cognitive deficits, such as age-related memory impairment, which we have already demonstrated using EBCC in animals (24). This technology provides a useful means of observing and manipulating clearly different functional brain states, without lesions or drugs, that could be adapted in the future to other species, brain structures, or oscillatory frequencies.

Methods

Subjects. Subjects were 11 New Zealand White rabbits (Oryctolagus cuniculus) supplied by Myrtle’s Rabbity. All rabbits were maintained on a 12-h light–dark cycle, with training conducted during the light phase. The rabbits were allowed free access to food and water in their home cages. All procedures involving animals were approved by the Miami University Institutional Animal Care and Use Committee.

Surgery. After each rabbit was preanesthetized with a ketamine (50 mg/kg, i.m.) and xylazine (10 mg/kg, i.m.) mixture or triopental sodium (Pentothal); 5% solution; 0.3 mL, i.v.), a small nylon suture loop was placed in the nictitating membrane for later attachment to a potentiometer for movement transduction. Each animal was secured in a stereotaxic frame and maintained on 2% isoflurane anesthesia for the duration of surgery. The head was oriented so that lambda was 1.5 mm ventral to bregma. Coordinates for placement of bilateral hippocampal electrodes were 4.5 mm posterior to bregma, 5.5 mm lateral to the midline suture and approximately 3.0 mm ventral to dura (17) (Fig. S1A). Cerebellar electrodes in IPN and Larsell’s lobule HVI were implanted ipsilaterally to the trained eye. Based on previous recording studies, IPN coordinates were: 0.0–1.5 mm anterior, 2.0–6.0 mm lateral, 12.5–14.5 mm ventral to lambda (7), and anterior cerebellar cortex coordinates were: 3.0 mm anterior, 5.0 mm lateral, 10.0–15.0 mm ventral to lambda (49) (Fig. S1 B and C). The dorsal-ventral placement of all electrodes was verified by simultaneous electrophysiological monitoring. All recordings were then monopolar with a skull screw as reference. Electrodes were insulated except for 50 to 70 μm at the tip, and impedances ranged from 200 to 500 kΩ.

Training Groups. Rabbits were randomly assigned to one of two groups, a theta-triggered (T+) group (n = 6) which received trace EBCC trial presentation in the explicit presence of each animal’s naturally occurring hippocampal theta, or a non-theta-triggered (T−) group (n = 5), which received trace EBCC in the explicit absence of theta. Procedures for theta-contingent trial presentation have been described in ref. 23 (for details, see SI Text). An earlier study from our lab has compared theta-contingent trial presentation during trace EBCC relative to yoked controls that received the same intertrial interval (ITI) and number of trials per day as its partner (either T+ or T−) irrespective of pretrial theta activity (17). The T− group learned faster, and T+ more slowly, than their respective controls. The two yoked control groups did not differ significantly in learning rates, ruling out possible systematic differences in ITI or number of trials per session as confounds. Thus, no yoked control groups were used in the present study.

Behavioral Training. After 5–7 days of postsurgical recovery and 2 days of adaptation to the conditioning chamber (where the rabbit was restrained in a standard Plexiglas box inside a Faraday cage and no stimuli were presented for ~45 min), each animal underwent theta-contingent trace EBCC. This hippocampal theta-contingent form of EBCC is characterized by a 1000-ms intertrial interval (ITI) followed by a 500-ms-stimulus-free trace period and a subsequent 100-ms corneal air puff US (3 p.s). For both groups, a daily, paired session was approximately 90 min in duration with a minimum ITI of 60 s. The number of trials was dependent...
upon the brain-computer interface, approximately 50 per day for each group. Animals were trained to a behavioral criterion of eight CRs in any nine consecutive trials and a maximum of 4 days. Histology. At the conclusion of training, each rabbit was anesthetized and small marking lesions were made at the tip of each recording electrode by passing a 200 μA, 10-s direct current. Animals were then killed with an overdose of sodium pentobarbital (Euthol, 0.2205 mg/kg i.v.) and perfused transcardially with physiological saline (0.9%) and formalin (10%) solutions. Serial frozen sections (40 μm) were mounted and stained with Prussian blue to mark electrode tips and a safranin counterstain. Only animals with electrodes properly placed in CA1, IPN, and HVI were included in the study. Hippocampal LFP recordings for animals with more ventral hippocampal placements in locations toward the hippocampal fisure (e.g., lacunsum moleculare) were inverted for field potential analyses (3). No systematic differences in electrode locations were found between groups.

Data Analysis. All neural and behavioral data were gathered with a DataWave interface (DT304) using the 16-channel, 12-bit, 400-kHz aggregate analog to digital converter. Behavioral data were hand-scored by using traditional learning criteria such as the number of trials to the third CR (indicative of initial acquisition of the CS-US contingency; 26), percentage CRs by day, and cumulative CRs, which gives an accurate overview of learning trajectories. A CR was defined as nictitating membrane movement (>0.5 mm) between 80 and 500 ms after tone offset. Electrophysiological activity from all electrodes was band-pass filtered 1–200 Hz and sampled at 500 Hz. Activity from each electrode tip was averaged across trials for individual subjects and across animals to produce daily group LFPs (Fig. 2 and Fig. 52). Frequency analyses were computed on the whole trial period, which extended from CS-onset to 1 s after US-offset. Spectral analyses were computed on group average LFPs (Fig. 3A and Fig. 53), and percentage power was computed individually by subject and then averaged (Fig. 3B). To exclude the impact of temporal regularities induced by the interstimulus interval and the waveform of the non-regularity EPs, auto- and cross-correlations of LFPs were performed on a 1-s sample of rhythmic activity after the end of the large amplitude response evoked by the US (beginning at 1,550 ms; see Fig. 4). MatLab and Microsoft Excel were used for all offline analyses and creation of data plots.

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