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HIPPOCAMPO–CEREBELLAR THETA BAND PHASE SYNCHRONY IN RABBITS

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Abstract—Hippocampal functioning, in the form of theta band oscillation, has been shown to modulate and predict cerebellar learning of which rabbit eyeblink conditioning is perhaps the most well-known example. The contribution of hippocampal neural activity to cerebellar learning is only possible if there is a functional connection between the two structures. Here, in the context of trace eyeblink conditioning, we show (1) that, in addition to the hippocampus, prominent theta oscillation also occurs in the cerebellum, and (2) that cerebellar theta oscillation is synchronized with that in the hippocampus. Further, the degree of phase synchrony (PS) increased both as a response to the conditioning stimuli and as a function of the relative power of hippocampal theta oscillation. However, the degree of PS did not change as a function of either training or learning nor did it predict learning rate as the hippocampal theta ratio did. Nevertheless, theta band synchronization might reflect the formation of transient neural assemblies between the hippocampus and the cerebellum. These findings help us understand how hippocampal function can affect eyeblink conditioning, during which the critical plasticity occurs in the cerebellum. Future studies should examine cerebellar unit activity in relation to hippocampal theta oscillations in order to discover the detailed mechanisms of theta-paced neural activity.

Keywords: hippocampus, cerebellum, theta, oscillation, eyeblink conditioning, phase synchrony.

Common to all biologically meaningful learning is the engagement of multiple distinct phases and sub-processes that are governed by different, sometimes widely distributed, brain structures. Rabbit eyeblink conditioning (Gormezano et al., 1962), where a neutral conditioned stimulus (CS, e.g. a tone) is repeatedly paired with a reflex-eliciting unconditioned stimulus (US, e.g. a corneal airpuff) provides an example of a model system of learning which could be modified to tap into different aspects of the learning process. In this paradigm, the primary memory trace of the motor conditioned response (CR) is thought to be stored within the cerebellar deep nuclei and/or the cerebellar cortical area HVI (Thompson and Steinmetz, 2009; De Zeeuw and Yeo, 2005), whereas various cerebral areas can modulate the learning process. For instance, when the cognitive demands of the task increase, hippocampal contribution becomes critical (Beylin et al., 2001; Moyer et al., 1990; Woodruff-Pak and Disterhoft, 2008).

Hippocampal function, especially in the form of theta-band oscillation (~6 Hz; for a review see, e.g., Bland, 1986 or Buzsáki, 2002), has been linked to a host of cognitive processes, most notably to learning and memory (Buzsáki, 2005; Hasselmo, 2005; Kahana, 2006). The relative power of the hippocampal theta activity (theta ratio) recorded before learning correlates strongly with the learning rate during subsequent eyeblink conditioning in rabbits, as shown both using the simple delay paradigm and using the trace paradigm, during which learning is hippocampally mediated (Berry and Thompson, 1978; Berry and Seager, 2001; Griffin et al., 2004; Nokia et al., 2008, 2009; Nokia and Wikgren, 2009; Seager et al., 2002). Moreover, blocking hippocampal theta oscillation with, for example, scopolamine injections before eyeblink conditioning retards behavioral learning and virtually abolishes any learning-related unit responses in the hippocampus (Salvatierra and Berry, 1989).

Previous research begs the questions, how do the hippocampus and the cerebellum interact, and what is the special role of the theta oscillation? In order for the hippocampus to modulate memory trace formation in the cerebellum we have to assume interaction between these areas. One indication of interaction between two brain areas is synchronized oscillation (e.g. Fries, 2005; Singer, 1999). By synchronizing and desynchronizing their activity, brain structures can select which inputs arrive and are sent during a time window of maximal effect, i.e. cause a change in the desired direction in the excitability of the receiving group of neurons (Fries, 2005). We recorded LFPs simultaneously from the rabbit hippocampus, cerebellar cortex, and medial prefrontal cortex (mPFC) during trace eyeblink conditioning and assessed the degree of phase synchrony (PS) (Palva et al., 2005; Lachaux et al., 1998) between these structures. Since synchronous oscillatory activity supposedly indicates co-operation, and because hippocampal theta oscillation has been associated with the learning rate during cerebellum-dependent eyeblink conditioning, we expected to observe (1) theta oscillation also in the cerebellum and (2) PS between the hippocampus and the cerebellum at the theta band. In addition, we aimed to determine whether the degree of PS changes as a function of training and/or learning and

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Abbreviations: ANOVA, analysis of variance; CS, conditioned stimulus; CR, conditioned response; EMG, electromyogram; FFT, fast fourier transform; HPC, hippocampus; HVI, cerebellar cortical area HVI; LFP, local-field potential; LTP/LTD, long-term potentiation/depression; mPFC, medial prefrontal cortex; PS, phase synchrony; US, unconditioned stimulus.

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whether, like the hippocampal theta ratio does, it predicts learning rate.

EXPERIMENTAL PROCEDURES

Subjects and surgery

The subjects were 27 adult male New Zealand white rabbits (Harlan, Netherlands, BV, Horst, Netherlands) aged ~4 months and weighing ~2.7 kg at the time of surgery. The rabbits were housed in individual metal cages on the premises of the animal research unit of the University of Jyväskylä. Food and water were freely available, and room temperature and humidity were controlled. All procedures were conducted during the light portion of the 12/12 hour light/dark cycle and implemented in accordance with the European Communities Council Directive (86/609/EEC, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do? uri=CELEX:31986L0609:EN:HTML) on the care and use of animals for research purposes. Every effort to minimize the number of animals and suffering caused to them was undertaken.

For a detailed description of the surgery see Nokia et al. (2009). Two or three monopolar recording electrodes were chronically implanted into the right hippocampus 5 mm posterior and 4–6 mm lateral to the bregma and one electrode into the right cerebellar cortex lobule HVI 1 mm anterior and 5 mm lateral to the lambda. In addition, two electrodes were implanted into the left mPFC 4–5 mm anterior and 0.8 mm lateral to the bregma. The electrodes were attached to a pin connector and the whole construction cemented in place with dental acrylic. At least 1 week was allowed for postsurgical recovery.

Stimuli and procedure

Prior to the experiments, the rabbits were placed (for approximately 20 min) in a Plexiglas restraining box located in a ventilated, electrically insulated, and sound-attenuated conditioning chamber to familiarize them with the experimental situation, and to ensure the functioning of the implanted electrodes. Thereafter, sessions were conducted once per day on consecutive days.

The CS was a 2 kHz, 85 dB, 200 ms tone and the US was a 100 ms corneal airpuff (0.35 bar source pressure, sound pressure level 64 dB) delivered through a nozzle (inner diameter 2 mm) placed approximately 1 cm away from the eye. A fan located inside the conditioning chamber behind the rabbit created a steady background noise of approximately 65 dB. E-Prime software (Psychology Software Tools Inc., Pittsburgh, PA, USA) was used to control the presentation of stimuli.

The rabbits were randomly assigned to unpaired ($n=10$) and paired ($n=17$) groups. Both received 10 daily sessions of either trace eyeblink conditioning (paired) or explicitly unpaired training (unpaired). The unpaired sessions consisted of 70 CS-alone and 70 US-alone trials presented in a random order with an intertrial interval averaging out at 20 s (range, 15–25 s). The conditioning sessions consisted of 80 trials: 60 conditioning, 10 CS-alone, and 10 US-alone trials were presented in a pseudorandom order with an average intertrial interval of 40 s (range, 30–50 s). During the conditioning trials, CS onset preceded US onset by 700 ms, thus creating a 500 ms trace period.

Recordings

Eyeblinks were measured using stainless steel wire hooks placed around the upper and lower eyelids for the duration of the training session. To acquire neural measures, a low-noise pre-amplifier (MPA8I, Multi Channel Systems, Reutlingen, Germany) was directly attached to the electrode coupler anchored with dental acrylic to the rabbit's head. A flexible, insulated cable was used to connect the animal to the amplifier (Axon Cyberamp 380, Molecular Devices Corporation, Union City, CA, USA). Both the neural data and the bipolar electromyogram (EMG) were recorded with AxoScope (Molecular Devices Corporation) software and digitized (Digidata 1322A, Molecular Devices Corporation) using a 10.26 kHz sampling rate. Before digitization, the LFPs were band-pass-filtered between 0.1 and 4000 Hz, and the EMG was filtered between 30 and 300 Hz.

Data analyses

Clampfit (Molecular Devices Corporation), MATLAB (The MathWorks Inc., Natick, MA, USA) and SPSS (SPSS Inc., Chicago, IL, USA) were used for the data analyses.

Eyeblinks. The EMG signal was further high-pass filtered over 100 Hz and Hilbert-transformed. Following this, an envelope curve following the peaks of the signal was calculated using the real and imaginary parts of the Hilbert transformation. Baseline EMG activity was calculated for each animal and session as the mean of the maximum EMG amplitude during a 500 ms pre-stimulus period (MAXpre). In addition, the mean of the standard deviation of the EMG activity during the 500 ms pre-stimulus period (SDpre) was determined. Eyeblinks were defined as EMG activity exceeding a threshold of [MAXpre+4×SDpre]. Blinks occurring during the 500-ms period following the offset of the tone (trace period) were counted as CRs. The learning rate was quantified as the number of trials needed to reach the 5th CR and the learning criterion.

Phase synchrony calculations. To obtain comparable data from the control and experimental groups, only CS-alone and US-alone trials were included in the analyses. Phase synchrony (PS) between two signals was calculated as described by Palva et al. (2005). First, the LFP signals were band-pass filtered (delta ~2 Hz, theta ~6 Hz, alpha ~12 Hz, beta ~20 Hz, and gamma ~60 Hz). Next, the filtered signals were transformed into a complex form using the Hilbert transform. Following this, the amplitudes of the signals were normalized to 1 (one) by dividing each data point by its absolute value. Then, the phase difference of the two signals in comparison was calculated by multiplying the first signal with the complex conjugate of the second signal (each data point of each trial). Finally, the PS was derived by averaging the phase difference matrix over trials, taking the absolute value.

In order to standardize the PS values, 100 surrogate datasets per each real PS were created by shuffling the real trials. The PS was then determined for each surrogate dataset and the mean and the 95th percentile of the surrogate PSs derived. The mean of the surrogate PS values was subtracted from the real PS and the outcome divided point-by-point by the difference between the 95th percentile and the mean of the surrogate PSs ([realPS–surrPS]/[surr95th percentile–surrPS]) yielding a standardized PS. Thus standardized PS values exceeding 1 (one) represent statistically significant synchrony at the level of $P<.05$. The group level statistical significance for the PS values was calculated using binomial statistics.

Theta ratio. The FFT was calculated from the 1-s pre-stimulus period data, with a resolution of 0.5 Hz. The theta ratio was then calculated using (theta/[delta+theta]). The delta and theta frequencies were used as the sole reference for theta, firstly, because in absolute power, delta and theta frequencies are fairly comparable and, secondly, because the absolute power of the higher frequencies (8 Hz<) is considerably lower than that of theta.

To compare PS during periods of a high versus low hippocampal theta ratio, the tone-alone (10) and airpuff-alone (10) trials from each session (10) and each animal were sorted according to the hippocampal theta ratio calculated from the pre-stimulus period. Then, the trials with the lowest and highest theta ratio were selected to form sets of 10 trials per animal, pre-stimulus theta ratio level and stimulus type.