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Title: Hippocampal theta activity is selectively associated with contingency detection but not discrimination in rabbit discrimination-reversal eyeblink conditioning

Running title: Hippocampal theta activity and discrimination learning

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Abstract

The relative power of the hippocampal theta-band (~6 Hz) activity (theta ratio) is thought to reflect a distinct neural state, and has been shown to affect learning rate in classical eyeblink conditioning in rabbits. We sought to determine if the theta ratio is mostly related to the detection of the contingency between the stimuli used in conditioning or also to the learning of more complex inhibitory associations when a highly demanding delay discrimination-reversal eyeblink conditioning paradigm is used. A high hippocampal theta ratio was not only associated with a fast increase in conditioned responding in general, but also correlated with slow emergence of discriminative responding due to sustained responding to the conditioned stimulus not paired with an unconditioned stimulus. The results indicate that the neural state reflected by the hippocampal theta ratio is specifically linked to forming associations between stimuli rather than to the learning of inhibitory associations needed for successful discrimination. This is in line with the view that the hippocampus is responsible for contingency detection in the early phase of learning in eyeblink conditioning.

Classical eyeblink conditioning (Gormezano et al., 1962) in its basic form is a cerebellum-dependent form of learning (for a review, see Thompson, 2005); however, the hippocampal contribution becomes important when the relations between the conditioning stimuli are complex, as in the discrimination-reversal paradigm (Berger and Orr, 1983). During discrimination-reversal eyeblink conditioning, the subject is first trained to discriminate between one conditioned stimulus (CS) that leads to an unconditioned stimulus (US) and another CS, which is followed by nothing (CS+ and CS-, respectively). In the reversal phase, the pairing of the CSs with the US is reversed. Hippocampal multiple-unit activity (MUA) increases in relation to the acquisition of the CR per se during discrimination training, diminishes when reversal training is started, and increases again when the correct reversal response is acquired (Mauldin et al., 2008; Miller & Steinmetz, 1997). On the other hand, hippocampectomy does not affect the acquisition of the initial discrimination, but severely retards reversal learning (Berger and Orr, 1983; Weikart and Berger, 1986). The deficit is due to sustained conditioned responding to the new CS-, but since hippocampectomy does not disrupt extinction training (Schmaltz and Theios, 1972; Berger and Orr, 1983; Weikart and Berger, 1986), it cannot be explained by a general inability to inhibit responding to a CS previously paired with a US. Thus, hippocampal function might have a different role during the two training phases of discrimination-reversal conditioning.

Hippocampal electrophysiological activity in the awake animal is dominated by a ~6 Hz oscillation called theta activity (for a review, see Buzsáki, 2002). A high relative power of hippocampal theta activity (theta ratio) before training predicts fast learning during eyeblink conditioning in rabbits (Berry and Thompson, 1978; Nokia et al., 2008; 2009; for a review, see Seager et al., 2002). In addition, hippocampal theta -contingent training affects learning rate especially in the early phase of trace eyeblink conditioning (Griffin et al., 2004). It seems that the strength of the hippocampal theta activity reflects a neural state affecting the rate at

which the contingency between the CS and the US is acquired (see Prokasy, 1984).

Moreover, the hippocampal theta activity seems to be modified by the demands set by the experimental situation, such as the novelty of the external stimuli and whether there is an association between them to be learned or not (Nokia et al., 2008; 2009).

In the present study, we explored how the neural state reflected by the hippocampal theta ratio is 1) related to learning rate and 2) changes across delay discrimination-reversal eyeblink conditioning in rabbits. Hippocampal local field potentials (LFPs) were recorded within each trial. We hypothesized that the hippocampal theta ratio would correlate at least with the overall rate of CR acquisition and possibly also with the rate of discrimination learning. Since discrimination-reversal learning is a highly demanding task, we anticipated that the level of hippocampal theta activity would either rise or remain high throughout training.

The subjects were nine female New Zealand albino rabbits aged ~4 months and weighing ~3.7 kg at the time of surgery. All the experimental procedures were 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.

Three monopolar recording electrodes were chronically implanted into the right hippocampus (for details, see Korhonen, 1991; Nokia et al., 2008) 5 mm posterior, 4-7 mm lateral, and 6.7-7.2 mm below the bregma. A 10.42 kHz sampling rate was used for all recordings. The LFPs were band-pass-filtered between 0.1–200 Hz. Eyeblinks were recorded using an infrared recorder similar to that described by Ryan et al. (2006). All recordings were made in 2-s sweeps with a 1-s pre-stimulus period.

A delay paradigm with a 250 ms interstimulus interval was used in conditioning. The CSs were 2 and 8 kHz, 85 dB, 350 ms tones (counterbalanced) and the US was a 100 ms corneal airpuff (3 psi source pressure, sound pressure level 64 dB). Within each session, 60 paired (CS+) and 60 tone-alone (CS-) trials were presented pseudorandomly (inter-trial-interval 20–30 s). Ten to 20 discrimination sessions and 25 to 30 reversal sessions per animal were carried out. A criterion of >75% CRs to the CS+ and <25% CRs to the CS- was pursued in both phases.

After the experiments, the rabbits were anesthetized, perfused with saline and 10% formalin and the brains were removed. The brains were later sectioned and the slices stained with Prussian blue and cresyl violet. The electrode-tip locations were determined with the help of a stereotaxic atlas (Bures et al., 1967).

MATLAB (The MathWorks Inc., Natick, MA, USA) and SPSS (SPSS Inc., Chicago, IL, USA) were used for the data analysis. For behavioral analysis, 250 ms pre-CS and CR periods were determined. To meet the CR criterion, the CRmean had to exceed the PREmean + 4*SDpre. Trials during which the SDpre exceeded 4 times the session average of SDpre were excluded from the behavioral analyses. To assess the learning rate, the number of trials needed to reach the conditioning criterion (trials to criterion = TTC), a CR in 8 out of 9 consecutive trials, was determined. The rate of discrimination learning was quantified as the number of sessions needed to reach a 25% and a 50% difference in responding to the CSs. To determine the relative power of hippocampal theta activity [$\theta / (\delta + \theta)$] Fast Fourier Transform was run on the LFPs (see Nokia et al., 2008; 2009). The pre-trial theta ratio was determined from a 500-ms time period immediately preceding the CS onset. The post-trial theta ratio was determined from a 500-ms time period starting 400 ms after the CS onset, thus avoiding the effect of the event-related potentials.

To standardize the structure of the data, each animal's data was divided into ten equal-sized blocks of trials (i.e. "vincentized") per training phase. This was done by giving each trial within the discrimination/reversal phase a running number and then dividing the data into portions each representing 10% of the data. Repeated measures analysis of variance (ANOVA) was used in analyzing the changes across training (10 blocks x 2 CS types). Whenever an interaction between block and CS type was detected, separate ANOVAs were conducted for the CS- and the CS+ data. Due to the small sample size, the normality assumption required for the use of the Pearson correlation coefficient could not be met. Thus, Spearman's rho (r_s) was used.

Six of the rabbits had at least one correctly placed recording electrode in the pyramidal layer of the CA1, and were included in further analyses (see Fig. 1).

The behavioral results are summarized in Figure 2A. The mean (M) number of trials needed to reach the learning criterion was 407 trials (minimum = 266, maximum = 696, standard error of mean, SEM = 62) corresponding to the 3rd discrimination training block. On average, a 25% discrimination was acquired by the end of the 10th session (M = 9.83, minimum = 3, maximum = 18, SEM = 2.29) corresponding to the 6th discrimination training block. The criterion of 50% discrimination was obtained on average by the end of the 12th session (M = 11.17, minimum = 4, maximum = 20, SEM = 2.27) also corresponding to the 6th training block. In the reversal phase, all six animals reached a discrimination of 25%, but only four animals eventually reached a discrimination of 50%. The average number of sessions needed to reach the 25% discrimination was 18.5 (minimum = 15, maximum = 24, SEM = 1.48), corresponding to the 7th reversal training block. The average number of sessions needed to reach the 50% discrimination (here, n = 4) was 24.5 (minimum = 16, maximum = 28, SEM = 2.84), corresponding to the 9th block of reversal training.

The ANOVAs (block: $F [9, 90] = 19.23, p < .001$; CS type: $F [1, 10] = 13.91, p < .01$; block x CS type: $F [9, 90] = 6.03, p < .001$) showed that during discrimination training, the number of CRs increased in response to the CS+ (block: $F [9, 45] = 26.73, p < .001$), and first increased and then decreased in response to the CS- (block: $F [9, 45] = 6.53, p < .001$; Quadratic $F [1, 5] = 20.32, p < .01$). During reversal training, the difference in responding to the CS- and the CS+ was not as clear (block: $F [9, 90] = 5.65, p < .01$; CS type: ns.; block x CS type: $F [9, 90] = 12.23, p < .001$), although the number of CRs elicited by the CS- did decrease across training (block: $F [9, 45] = 11.14, p < .001$). Conditioned responding to the new CS+ was acquired rapidly within the first three reversal sessions (block 1, see Fig. 2A) and from then on remained at a fairly stable level throughout training (block: ns.).

Table 1 summarizes the correlations between the hippocampal theta ratio and learning rate. The hippocampal theta ratio recorded following the presentations of the training trial during the first session (1st se. post) correlated with the overall learning rate during the initial discrimination training (TTC), $r_s = -.94, p < .01$, and especially with the level of conditioned responding elicited by the CS- (CR % to CS-), $r_s = .94, p < .01$. Consequently, the first session post-trial theta ratio also correlated with the acquisition rate of the initial discrimination (25% diff.: $r_s = .81, p = .05$, and 50% diff.: $r_s = .97, p < .001$). In addition, also the average post-trial theta ratio recorded during discrimination training (Dis. post) correlated with discrimination rate (50% diff.): $r_s = .84, p < .05$. No correlation between the post-trial theta ratio and discrimination rate in the reversal phase was found, but the first session post-trial theta ratio (1st se. post), as well as the overall post-trial theta ratio recorded during discrimination training (Dis. post), both correlated with the level of behavioral responding (CR %) to both the CS+ and the CS- during the reversal phase, $r_s = .89, p < .05$ in all cases (see Table 1 rightmost column).

Results regarding changes in the hippocampal theta ratio (%) across training are presented in Figure 2B. The occurrence of the trial increased the hippocampal theta ratio (post-trial vs. pre-trial) throughout training (first session: $t(5) = 3.14, p < .05$; Discrimination: $t(5) = 3.18, p < .05$; Reversal: $t(5) = 3.55, p < .05$). The post-trial theta ratios showed no differences between the CS- and the CS+ trial types, but both showed some decrease towards the end of discrimination (block: $F(9, 90) = 3.99, p < .05$; block x CS type: ns.; CS type: ns.). No differences between the trial types or changes across training were found during reversal training. Furthermore, there was no change in the pre-trial theta ratios across discrimination or across reversal training. In addition to comparing the post-trial theta ratio on CS- and CS+ trials, comparisons were also made between CS- and CS+ trials divided based on CR performance (CR/non-CR). However, no significant differences were found.

We sought to determine how the neural state of the animal reflected by the hippocampal theta ratio affects the rate of CR acquisition as well as the rate of discrimination acquisition, and changes across delay discrimination-reversal eyeblink conditioning in rabbits. While a high hippocampal theta ratio predicted fast acquisition of the conditioned response, it also predicted high responding to the CS- and thus slow discrimination. No connection between the rate of discrimination and hippocampal theta ratio was found during reversal training, but a high theta ratio during discrimination training predicted high behavioral responding to both CSs during subsequent reversal training. The hippocampal theta ratio decreased towards the end of discrimination training and remained unchanged throughout reversal training, reflecting the demands set by the task at hand.

When combined with earlier findings (Berry and Thompson, 1978; Nokia et al. 2008; 2009), the results of the present study suggest that strong hippocampal theta activity reflects a state in which the subject is prone to making associations between external events – i.e. the

stimuli used in conditioning. In addition, in our present study, the higher the theta ratio was, the harder it was for the animal to cease responding to the non-reinforced stimulus. This would imply that a high hippocampal theta ratio leads to the formation of not only fast, but also strong and generalized associations between stimuli occurring in a short space of time. These findings might explain why removing the hippocampus, and thus possibly disrupting the neural state of the animal in terms of electrophysiological oscillatory activity in the theta-band, does not retard discrimination learning (Berger and Orr, 1983; Weikart and Berger, 1986) or simple extinction (Schmaltz and Theios, 1972). The absence of hippocampal contribution might, in fact, make it easier to respond appropriately during discrimination requiring inhibition of behavior, since the generalized association between the CS- and the US would be formed more slowly and would be possibly weaker.

With respect to the deficits in reversal learning induced by hippocampectomy shown in earlier studies (Berger and Orr, 1983; Weikart and Berger, 1986), the conclusions of our study are less straightforward. Our current results show only a predictive connection between the pre-training theta ratio and responding rate, but this does not exclude the possibility that there might also be a (possibly weaker) connection between the hippocampal theta ratio and the rate of discrimination learning during reversal training. If a connection was found in later studies, it would most probably be similar to that which we have found regarding initial discrimination training i.e. strong hippocampal theta activity predicts slow discrimination. Nevertheless, it seems that it is not the level of theta activity that explains why an intact hippocampus is needed for normal learning during reversal training.

Earlier studies (Mauldin et al., 2008; Miller & Steinmetz, 1997) have shown that hippocampal MUA and conditioned responding are differently related during the initial discrimination training and the following reversal training. Our results, showing a stronger connection between hippocampal theta activity and learning during discrimination training

than reversal training and a rapid acquisition of conditioned responding to the new CS+ in the beginning of reversal training are in line with the above mentioned results. Together these findings support a notion that some processes, like contingency detection (Prokasy, 1984), related to hippocampal function occur only, or at least are stronger, during the initial discrimination training compared to reversal training. In conclusion, our current study implies that the neural state reflected by the magnitude of the hippocampal theta activity affects primarily the rate and strength of association formation during simple motor learning also when a complex training paradigm is used.

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Figure Legends

Figure 1. Recording electrode locations.

Figure 2. Changes in conditioned responding and the hippocampal theta ratio as a function of training block. To standardize the structure of the data, each animal's data was divided into ten equal-sized blocks of trials per training phase (Discrimination/Reversal). The dotted vertical line shows when the initial discrimination (50% difference in response rates to the CS- and the CS+) was acquired. The solid vertical lines depict the standard error of mean. The text at the bottom of each panel refers to statistically significant results of repeated measures ANOVA conducted for the whole data (1.), and conducted separately for the CS types (2.). The asterisks refer to statistical significance: * $p < .05$, ** $p < .01$, *** $p < .001$.