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RAPID COMMUNICATION

CA3–CA1 long-term potentiation occurs regardless of respiration and cardiac cycle phases in urethane-anesthetized rats

Miriam S. Nokia | Tomi Waselius | Markku Penttonen

Department of Psychology and Centre for Interdisciplinary Brain Research, University of Jyvaskyla, Jyväskylä, Finland

Correspondence
Miriam S. Nokia, Department of Psychology, University of Jyvaskyla, P.O. Box 35, FI-40014, Jyvaskyla, Finland.
Email: miriam.nokia@jyu.fi

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Academy of Finland

Abstract

Breathing and heartbeat synchronize to each other and to brain function and affect cognition in humans. However, it is not clear how cardiorespiratory rhythms modulate such basic processes as synaptic plasticity thought to underlie learning. Thus, we studied if respiration and cardiac cycle phases at burst stimulation onset affect hippocampal long-term potentiation (LTP) in the CA3–CA1 synapse in urethane-anesthetized adult male Sprague–Dawley rats. In a between-subjects design, we timed burst stimulation of the ventral hippocampal commissure (vHC) to systole or diastole either during expiration or inspiration and recorded responses throughout the hippocampus with a linear probe. As classical conditioning in humans seems to be most efficient at expiration-diastole, we also expected LTP to be most efficient if burst stimulation was targeted to expiration-diastole. However, LTP was induced equally in all four groups and respiration and cardiac cycle phase did not modulate CA1 responses to vHC stimulation overall. This could be perhaps because we bypassed all natural routes of external influences on the CA1 by directly stimulating the vHC. In the future, the effect of cardiorespiratory rhythms on synaptic plasticity could also be studied in awake state and in other parts of the hippocampal trisynaptic loop.

KEYWORDS
breathing, heartbeat, hippocampus, learning, memory, plasticity

Long-term potentiation (LTP) (Bliss & Collingridge, 1993) of synaptic transmission is thought to support learning in the brain. Burst stimulation delivered at a frequency mimicking hippocampal theta (3–12 Hz) (Buzsaki, 2002) seems optimal for inducing LTP (Larson et al., 1986) while also lower frequencies (~2 Hz) have been reported effective (Grover et al., 2009). LTP is stronger in the hippocampal CA3–CA1 synapse if the stimulation bursts are delivered specifically during ongoing CA1 theta oscillation peaks (Holscher et al., 1997; Hyman et al., 2003; McCartney et al., 2004). Our previous studies suggest trace eyeblink conditioning (Clark et al., 2002) in rabbits is more efficient if trials are presented contingent on hippocampal sharp-wave ripples (SPW-Rs) (Nokia et al., 2010), an overall low hippocampal theta drive (Nokia & Wikgren, 2014) or during the CA1 theta peak rather than trough (Nokia et al., 2015). These effects are likely explained by the findings that theta regulates CA1 pyramidal cell excitability (Kamondi et al., 1998), the amplitude of hippocampal gamma oscillations (~25–140 Hz) as well as neuronal firing in the cortico-hippocampal loop (Bragin et al., 1995; Colgin, 2015; Lisman, 2005).

Theta-gamma coupling is commonly thought to support memory encoding (Colgin, 2015; Igarashi et al., 2014; Lega et al., 2014; Tort et al., 2009). Interestingly, theta and gamma oscillations link with respiration (Hammer et al., 2021; Macrides et al., 1982; Tort et al., 2018, 2021; Yanovsky et al., 2014). In fact, respiration synchronizes with the rhythmic electrophysiological activity of the brain at rest overall (Klimesch, 2018; Kluger & Gross, 2021; Parviainen et al., 2022) and specifically hippocampal SPW-Rs in the CA1 (Buzsaki, 2015; Girardeau et al., 2009; Maingret et al., 2016; Nokia et al., 2012) are time-locked to a certain phase of breathing in sleeping mice [(Karalis & Sirota, 2022), see also (Liu et al., 2017)]. According to our recent results in humans, trace eyeblink conditioning is more effective if the conditioned stimulus is presented to expiration rather than inspiration (Waselius et al., 2019) and diastole rather than systole (Waselius et al., 2018, 2022). Taken together, it seems that the ongoing bodily rhythm phase affects learning and memory. However, it is unclear if and how respiration and cardiac cycle might influence such a basic phenomenon as synaptic plasticity within the hippocampus. Thus, we studied the effects of respiration and cardiac cycle phase on LTP in the CA3–CA1 synapse in urethane-anesthetized rats. We expected that the optimal moment for inducing LTP in the CA3–CA1 synapse would be during expiration and diastole (Waselius et al., 2018, 2019, 2022).

In brief, we recorded hippocampal local-field potentials (LFPs), electrocardiogram (ECG) and respiration and timed burst stimulation of the ventral hippocampal commissure (vHC) to systole or diastole during either inspiration or expiration in adult male Sprague–Dawley rats ($n = 51$). All experimental procedures were implemented in accordance with directive 2010/63/EU and the ARRIVE guidelines were followed. Rats were anesthetized with urethane (dose: 1.3 g/kg) and fixed to a stereotactic holder. A piezo sensor was placed under the rat's ribcage to record respiration and needles placed under the skin on each side of the animal were used to record ECG. A craniotomy was made over the left hippocampus and another over the contralateral vHC. A 32-electrode linear (65 μm separation) silicon probe (Atlas Neuroengineering) was implanted targeting the tip at the lower blade of the DG at ~3.6 mm below dura. A bipolar stainless-steel stimulation electrode was implanted to the vHC at ~4 mm below dura. LFPs (1–5000 Hz) and physiological signals were recorded at 20 kHz throughout the experiment.

The LTP protocol was conducted as previously described in (Lehtonen et al., 2022). Baseline responses were recorded during a 30-min session where a single weak pulse was presented every 30 s. Stimulation current (device max. 160 μA) was set so that it elicited a population spike and a reliable field excitatory postsynaptic potential (fEPSP) response at a delay of <10 ms in the pyramidal cell layer of the CA1 (CA1p). This was followed by burst stimulation (4 bipolar pulses at 100 Hz): Eight bursts were applied with an inter-burst interval of at least 200 ms and timed so that they took place either at inspiration-systole (IN-SYS), at expiration-systole (EX-SYS), at inspiration-diastole (IN-DIA) or at expiration-diastole (EX-DIA). Here the stimulation current was set so that the responses were near maximum in CA1p. This procedure was repeated 4 times at an interval of at least 20 s so that the total number of pulses was 128 ($4 \times 8 \times 4$). Then, to test whether LTP was induced, responses to the weak single-pulse stimulus were recorded for another 180 min. At last, rats were overdosed with sodium pentobarbital and decapitated, and brains were collected for histological analysis to confirm electrode placement as described in (Lehtonen et al., 2022).

We analyzed data only from rats in which the burst stimulation was accomplished so that the interval between bursts was on average less than 3 s (Grover et al., 2009) and the interval between burst trains was less than 30 s, and in which either a reliable stratum radiatum fEPSP ($n = 30$) or a CA1p population spike ($n = 28$) was present. Note that the groups were partially overlapping ($n = 22$) and hence the data comprised altogether 36 rats (IN-SYS, $n = 7$; EX-SYS, $n = 10$; IN-DIA, $n = 9$ and EX-DIA, $n = 10$). Figure 1a–d illustrates the timing of the vHC burst stimulation in relation to respiration and cardiac cycle in representative rats from each group. There were no group differences in the stimulation train interval (Mean, $M = 21.9$ (standard deviation, $SD = 1.5$) s, one-way ANOVA: F [3, 35] $= 1.19$, $p = .328$) or in the burst interval (1.9 [0.6] s; F [3, 35] $= 0.27$, $p = .846$).

Stratum radiatum fEPSP slope and CA1p population spike amplitude were analyzed from 6/6 rats belonging to IN-SYS, 10/8 rats belonging to EX-SYS, 7/8 rats belonging to IN-DIA and 7/6 rats belonging to the EX-DIA group, respectively. To quantify LTP, the steepest slope of the stratum radiatum fEPSP within a 1-ms window at 4–6 ms after stimulation onset was derived from both the baseline and the post-burst recordings. Then the values were divided by the mean of the values during the baseline period and multiplied by 100. This relative measure was averaged per rat and per 30-min epoch (7) and used for statistical analyses. Additionally, the amplitude of the population spike at CA1p was derived and treated like the stratum radiatum fEPSP. Further, to determine the effect of respiration and cardiac cycle phase on response properties overall ($n = 28/30$ rats), each single-pulse vHC stimulation was assigned to one of four categories: EX-SYS, EX-DIA, IN-SYS, and IN-DIA. Response properties were normalized to the mean calculated separately for pre- and post-burst stimulation. Note that while the number of stimulations per category (pre: $M = 15$ ($SD = 6$), post: 90 (34)) was determined randomly due to the steady 30-s inter-stimulus interval, the very good signal-to-noise ratio ensured by our technical set-up enabled reliable analysis of the response properties even with fewer repetitions.

Results of our experiment indicate that respiration and cardiac cycle phase at burst stimulation delivery did not affect subsequent LTP in the CA3-CA1 synapse (Figure 1g–j): Levene's test indicated no differences between groups in variances of either dependent measure at any time point. Stratum radiatum fEPSP slope ($n = 30$ rats) during the first 30-min period after burst stimulation was on average 141 ($SD = 42$) % and during the last 30-min period of the recording 96 (31) % of that evoked during the baseline recording (cardiac cycle phase (2) × respiration phase (2) repeated measures (7) ANOVA, main effect of time: F [6, 156] $= 21.44$, $p < .001$, large effect size according to partial eta squared: $\eta^2 = 0.452$). There was no significant main effect of respiration phase (F [1, 26] $= 2.09$, $p = .161$) or cardiac cycle phase (F [1, 26] $= 1.52$, $p = .229$) and no significant interactions

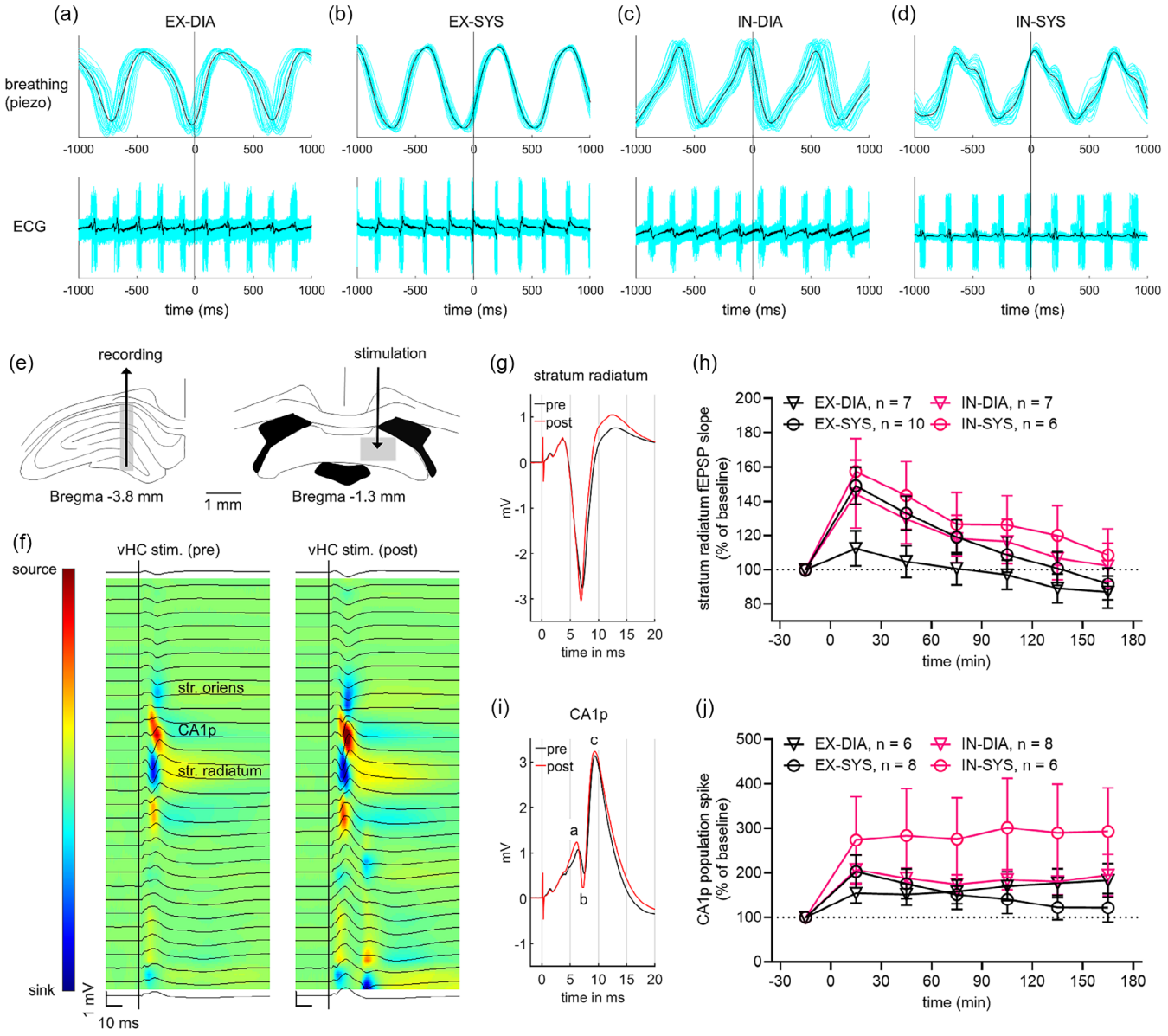

FIGURE 1 Burst stimulation timed to either expiration (EX) or inspiration (IN) and either to the diastole (DIA) or systole (SYS) was equally effective in inducing LTP at the hippocampal CA3-CA1 synapse in adult male Sprague–Dawley rats under urethane anesthesia. (a–d) Ventral hippocampal commissure (vHC) stimulation timing relative to the piezo signal indicating respiration phase (top) and the ECG signal indicative of cardiac cycle phase (bottom) in representative rats. All 32 individual signal traces illustrating the variation in the timing of stimulation bursts are plotted in cyan. The average of the signal is plotted on top in black. (e) Stimulations were delivered to the right vHC and LFPs were recorded from the left dorsal hippocampus. (f) Representative examples of hippocampal responses before (pre) and after (post) vHC burst stimulation in one rat. Current source density (CSD) is expressed in color and LFP average traces are plotted on top in black. (g–j) Burst stimulation at all cardiorespiratory phase combinations was equally effective in producing LTP, regardless of the measure used. The stratum radiatum fEPSP is illustrated in panel G and the slope was calculated as the change in signal amplitude at ~4–6 ms after stimulus onset. As illustrated in panel I, the CA1p population spike amplitude was calculated as $[(a - b) + (c - b)]/2$. In both (g) and (i), lines represent average of 60 stimulations, immediately prior to and following the burst stimulation in one representative rat. In panels (h) and (j), error bars indicate standard error of mean.

(within-subjects: F [6, 156] = 0.50–1.59, p = .212–.623; between-subjects: F [1, 26] = 0.11, p = .746). The results of repeated measures ANOVA conducted on CA1p population spike amplitude were similar to those reported for the fEPSP slope: LTP was induced (main effect of time: F [6, 144] = 9.68, p < .001, large effect size according to partial eta squared: $\eta^2 = 0.287$) but there was no effect of respiration phase (F [1, 24] = 2.46, p = .130) or cardiac cycle phase (F [1, 24] = 0.72, p = .404) and no interactions were detected (within-subjects: F [6, 144] = 0.77–1.79, p = .176–.474; between-subjects: F [1, 24] = 1.25, p = .275). In the overlapping data (n = 22) the average post-burst stratum radiatum fEPSP slope correlated statistically significantly and positively with the average post-burst CA1p population

spike amplitude ($r = 0.69$, $p < .001$). Finally, to examine a possible connection of the burst interval (s) on LTP (Grover et al., 2009), we calculated correlations but these were not significant (stratum radiatum fEPSP slope: $n = 30$, $r = -0.25$, $p = .181$; CA1p population spike: $n = 28$, $r = -0.30$, $p = .105$). To summarize, LTP was induced in the CA3-CA1 synapse of urethane-anesthetized male Sprague–Dawley rats by burst stimulation (4 pulses at 100 Hz) of the vHC delivered 8 times at intervals of ~2 s repeated 4 times every ~22 s. Cardiac cycle or respiration phase at the time of burst stimulation did not affect subsequent measures of LTP. There was also no connection between the burst stimulation interval and the subsequent LTP.

An example of the hippocampal response profile to vHC stimulation prior to and after LTP induction is illustrated in Figure 1f. The randomly delivered vHC weak single-pulse stimulations were divided into categories based on stimulation timing: EX-DIA, EX-SYS, IN-DIA, and IN-SYS. Repeated measures ANOVA indicated that respiration and cardiac cycle phase at stimulation onset did not modulate CA1 responses to vHC stimulation either pre or post burst stimulation (slope of the stratum radiatum fEPSP, pre: F [3, 87] = 0.31, $p = .695$; post: F [3, 87] = 1.23, $p = .297$; CA1p population spike amplitude, pre: F [3, 81] = 0.95, $p = .421$; post: F [3, 81] = 1.04, $p = .345$) (data not shown). That is, vHC stimulation elicited a similar response in the CA1 irrespective of the phase of the cardiac cycle or respiration at stimulation onset.

To summarize, according to our results, respiration and cardiac cycle phase do not directly govern synaptic plasticity or response properties in the CA3–CA1 synapse. This is in contrast to our expectations based on the previously reported links between respiration rhythm and hippocampal theta (Macrides et al., 1982; Tort et al., 2018, 2021; Yanovsky et al., 2014) and between hippocampal theta phase and CA1 LTP (Holscher et al., 1997; Hyman et al., 2003; McCartney et al., 2004). It could be that while respiration seems to affect neuronal excitability in the CA1 (Karalis & Sirota, 2022), it does not affect it as systematically or as directly as for example the theta oscillation governed by cholinergic projections from the medial septum to the hippocampus (Buzsaki, 2002). It could also be that for respiration to impose an effect on hippocampal synaptic plasticity, the stimulation should involve sensory processing, such as activation of the olfactory bulb (Liu et al., 2017; Zelano et al., 2016). In our current study, we bypassed all sensory influences on the CA1 by directly stimulating the vHC under anesthesia. As breathing and heart beat under urethane anesthesia do not markedly differ from those recorded in natural sleep (Silver et al., 2021), it is unlikely that the use of urethane would explain our results. It would be interesting to also study the effects of bodily rhythms on LTP in awake animals in the future. Further, it might be that rather than the specific phase combination of respiration and cardiac cycle, the key for modulating hippocampal LTP would be to monitor the changes in cardiovascular coupling or respiratory sinus arrhythmia (RSA) (Elstad et al., 2018), or the level and nature of coupling between respiration and hippocampal theta (Tort et al., 2021). Furthermore, it is possible that although respiration and cardiac cycle phase did not modulate CA3-CA1 synaptic plasticity or responses to vHC stimulation, they might do so when stimulation is targeted to the perforant path conveying information from the entirhinal cortex (EC) to the dentate gyrus (DG). In fact, we recently found that the dentate spike affects DG responses to EC inputs but poses no effect at the intra-hippocampal synapses, that is, DG-CA3 or CA3–CA1 (Lehtonen et al., 2022). These considerations could be addressed in future studies.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Miriam S. Nokia https://orcid.org/0000-0003-2222-6118

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