

**This is a self-archived version of an original article. This version may differ from the original in pagination and typographic details.**

**Author(s):** Waselius, Tomi; Xu, Weiyong; Sparre, Julia Isabella; Penttonen, Markku; Nokia, Miriam S.

**Title:** -Cardiac cycle and respiration phase affect responses to the conditioned stimulus in young adults trained in trace eyeblink conditioning

**Year:** 2022

**Version:** Accepted version (Final draft)

**Copyright:** © 2022, Journal of Neurophysiology

**Rights:** In Copyright

**Rights url:** <http://rightsstatements.org/page/InC/1.0/?language=en>

**Please cite the original version:**

Waselius, T., Xu, W., Sparre, J. I., Penttonen, M., & Nokia, M. S. (2022). -Cardiac cycle and respiration phase affect responses to the conditioned stimulus in young adults trained in trace eyeblink conditioning. *Journal of Neurophysiology*, 127(3), 767-775.  
<https://doi.org/10.1152/jn.00298.2021>

1 **Cardiac cycle and respiration phase affect responses to the conditioned**  
2 **stimulus in young adults trained in trace eyeblink conditioning**

3 Authors: Tomi Waselius<sup>#</sup>, Weiyong Xu, Julia Sparre, Markku Penttonen & Miriam S. Nokia<sup>#</sup>

4 Affiliations for all authors: Department of Psychology, University of Jyväskylä, Finland and  
5 Centre for Interdisciplinary Brain Research, University of Jyväskylä, Finland

6 Running head: Bodily rhythms affect eyeblink classical conditioning

7 Address for corresponding author: Tomi Waselius, [tomi.waselius@jyu.fi](mailto:tomi.waselius@jyu.fi), Department of  
8 Psychology, P.O. Box 35, 40014 University of Jyväskylä, Finland

9

10 <sup>#</sup> T. W. and M. S. N. contributed equally to this work.

11

12

13 **Abstract**

14 Rhythms of breathing and heartbeat are linked to each other as well as to rhythms of the  
15 brain. Our recent studies suggest that presenting conditioned stimulus during expiration or  
16 during the diastolic phase of the cardiac cycle facilitates neural processing of that stimulus  
17 and improves learning in a conditioning task. To date, it has not been examined whether  
18 utilizing information from both respiration and cardiac cycle phases simultaneously allows  
19 even more efficient modulation of learning. Here we studied whether the timing of the  
20 conditioned stimulus to different cardiorespiratory rhythm phase combinations affects  
21 learning in a conditioning task in healthy young adults. The results were consistent with  
22 previous reports: Timing the conditioned stimulus to diastole during expiration was more  
23 beneficial for learning than timing it to systole during inspiration. Cardiac cycle phase  
24 seemed to explain most of this variation in learning at the behavioral level. Brain evoked  
25 potentials (N1) elicited by the conditioned stimulus and recorded using electroencephalogram  
26 were larger when the conditioned stimulus was presented to diastole during expiration than  
27 when it was presented to systole during inspiration. Breathing phase explained the variation  
28 in the N1 amplitude. To conclude, our findings suggest that non-invasive monitoring of  
29 bodily rhythms combined with closed-loop control of stimulation can be used to promote  
30 learning in humans. The next step will be to test if performance can also be improved in  
31 humans with compromised cognitive ability, such as in older people with memory  
32 impairments.

33 **Keywords**

34 breathing, heartbeat, event-related potential, learning

35

36 **New & Noteworthy**

37 We report for the first time that the rhythms of breathing and the beating of the heart have a  
38 phase combination that is indicative of a neural state beneficial for cognition. This suggests  
39 that bodily rhythms not only modulate cognition but that this phenomenon can be non-  
40 invasively harnessed to improve learning in humans.

41

## 42 **Introduction**

43 Bodily rhythms like cardiac cycle and respiration usually vary at their own pace without  
44 much conscious thought put into them. When we are in a relaxed psychophysiological state,  
45 breathing and heartbeats synchronize [1–3]. The beat-to-beat intervals of the heart are longest  
46 at the end of expiration [4] and shorter during inspiration [5]. This is called respiratory sinus  
47 arrhythmia (RSA). In addition, the last heartbeat of each expiration delays the onset of the  
48 following inspiration, the “working phase” of breathing, so that the cardiac “working phase”,  
49 systole, precedes inspiration onset by 150–500 ms [6,7]. This phenomenon is termed  
50 cardioventilatory coupling. Especially RSA is thought to increase efficiency and stability in  
51 pulmonary gas exchange [2] and to decrease energy use caused by heartbeats [8].  
52 Nevertheless, the root cause and outcome of cardiorespiratory synchrony is somewhat unclear  
53 [6].

54 Interestingly, cardiac cycle and respiration also synchronize with electrophysiological  
55 rhythms of the brain [9], which in turn affect neural processing of external information.  
56 Temporal correlations exist, for example, between cardiac cycle and the hippocampal theta  
57 oscillation (3–12 Hz) in rodents [10,11]. Theta oscillation is crucially involved in memory  
58 formation during spatial [12] and non-spatial tasks [13,14] as it, for example, regulates the  
59 firing of hippocampal principal cells. In addition to cardiac cycle, also the respiration rhythm  
60 couples with brain oscillations [15]. For example, electrophysiological oscillations in the  
61 limbic system are entrained by nasal breathing in humans at the delta (0.5–4 Hz), theta (4–8  
62 Hz) and beta (13–30 Hz) frequency bands [16]. In addition, hippocampal sharp-wave ripples  
63 (SWRs, 100–200 Hz), crucial for memory consolidation [17], are entrained by respiration in  
64 mice [18].

65 Further and most importantly, brain responses to external stimuli and consequent behavior  
66 such as startle eyeblinks and premotor reaction times [19,20] and even associative learning  
67 [21] are modulated by the cardiac cycle phase. Regarding respiration, associative learning is  
68 enhanced when the significant stimuli are presented during expiration [22]. However, to our  
69 knowledge, there are no studies considering the combined effect of cardiac cycle and  
70 respiration phases on brain responses nor behavior. It is possible that utilizing combined  
71 information from these bodily rhythms might allow even more efficient modulation of  
72 behavior to the desired direction. Hence, we investigated the combined effect of cardiac cycle  
73 and respiration phase on learning in an associative task called trace eyeblink conditioning  
74 (TEBC). Participants were trained while watching a documentary film, using a tone as a  
75 conditioned stimulus (CS) and an air puff towards the corner of the right eye as an  
76 unconditioned stimulus. The presentation of the CS was fixed to a certain phase of the cardiac  
77 cycle (systole, SYS or diastole, DIA) and respiration (expiration, EXP or inspiration, INS) for  
78 each participant. In addition to conditioned responses also electroencephalogram (EEG) was  
79 recorded. Our previous data [21] showed mixed effects of cardiac cycle phase on CS-evoked  
80 brain responses in humans and in rabbits while we have not examined the effect of respiration  
81 phase. Based on our previous behavioral results [21, 22], we hypothesized that timing the CS  
82 to systole during inspiration would be less than optimal for learning TEBC whereas  
83 presentation of the CS to the diastole during expiration would be most favorable for learning  
84 (see Figure 1B).

85

## 86 **Materials and Methods**

### 87 *Participants*

88 Participants were recruited via student email lists. All participants gave informed written  
89 consent to this study and were free to discontinue participation in the experiment at any point.  
90 All participants received a reward (a movie ticket or a gift card) even if they discontinued the  
91 experiment at some point (however, no one did). The study was approved by the University  
92 of Jyväskylä Ethical Committee. A total of 59 young adults (12 males; aged 20–30 years;  
93 mean 23.4 years, standard error of mean 0.4 years) took part in the study. All participants  
94 were healthy with no history of psychiatric or neurological illnesses. They were not taking  
95 medication affecting the central nervous system, and they had no disabilities in hearing or  
96 vision.

### 97 *Physiological recordings*

98 Recording electrodes were attached after participants had signed the written consent.  
99 Respiration was recorded and monitored during the experiment with a reusable fabric belt  
100 (RESPA00000, Spes Medica, Italy), which was fastened on top of the clothes on the lower  
101 chest area. Heart rate was recorded using three electrocardiogram (ECG) electrodes (Kendall,  
102 H92SG); one electrode was placed on top of the right clavicle, one on the left lower ribs, and  
103 the grounding electrode on the back of the neck. Electromyography (EMG) to determine  
104 eyeblinks was recorded using two electrodes (70010-K/12, Ambu, Ballerup, Denmark) that  
105 were attached on top of the participant's right eye muscles (orbicularis oculi). EEG was  
106 recorded using a 128-channel EGI Sensor Net (Electrical Geodesics Inc., Hydrogel GSN 128,  
107 1.0). All signals were high-pass filtered (0.16 Hz) and low-pass filtered (250 Hz) online and

108 recorded with NeurOne Tesla (with Analog Out Option, Bittium Biosignals Ltd., Finland) at  
109 a 1-kHz sampling rate.

#### 110 *Experimental procedure*

111 We chose to use a between-subjects design with four groups (INS-SYS, INS-DIA, EXP-SYS,  
112 and EXP-DIA) to keep the paradigm simple and use just one CS and one US. We do  
113 acknowledge that a within-subjects design would be more powerful as there is quite a lot of  
114 inter-individual variability in TEBC, and this is something that should be addressed in  
115 follow-up studies.

116 The outline of the experimental procedure is presented in Figure 1A. The participants sat in a  
117 chair in front of a TV screen (Asus VG236 series H, 23"; distance: approximately 100 cm).

118 They were informed that the aim of the study was to record physiological responses to  
119 different types of stimuli while their attention was to be directed at a silent film depicting  
120 landscapes and animals. The participants were instructed to pay attention to the film and told  
121 that there would be questions considering the content of the footage after the recording  
122 session. They were also instructed to sit comfortably in the chair and not pay attention to the  
123 disturbing stimuli. In other words, the participants were led to believe that the idea was to  
124 study the disturbance caused by beeping sounds and air puffs on their attention towards the  
125 film.

#### 126 Trace eyeblink conditioning

127 The conditioned stimulus (CS) was a 200-ms, 440-Hz, 66-dB tone delivered via a  
128 loudspeaker situated in the lower right-hand corner of the room. The unconditioned stimulus  
129 (US) was an air puff (0.2 bar source pressure, 100 ms) targeted at the right eye and it was  
130 delivered via a plastic tube attached to modified safety goggles. Note that the air pressure was



131 low and none of the participants reported that the air puff hitting the eye was unbearable.  
132 During conditioning trials, a 600-ms trace interval separated the tone-CS offset and the  
133 airpuff-US onset. The presentation of the stimuli used for conditioning was controlled by  
134 custom software running on an Arduino-based device (ABD).

135 First, five US-alone trials with an inter-trial interval (ITI) of 5 s were presented to make sure  
136 that the participants felt comfortable enough to proceed with the experiment. After this, 5  
137 minutes of resting data were recorded, followed by five CS-alone trials to determine baseline  
138 eyeblink rate. Then, 50 CS+US classical conditioning trials were presented either at  
139 inspiration-systole, inspiration-diastole, expiration-systole or at expiration-diastole. Last, five  
140 CS-alone trials were presented as an extinction training block. A random ITI of 20-40 s was  
141 applied throughout the experiment.

142 To time the classical conditioning trials, the respiration, cardiac cycle, and EMG signals were  
143 conveyed to a custom script running in LabVIEW (National Instruments). Signals were  
144 sampled at 1 kHz. At each time point, the last second of respiration, ECG and EMG signals  
145 were analyzed. EMG was evaluated for spontaneous eyeblinks, that is, the signal had to stay  
146 below a set amplitude threshold to proceed with presenting the conditioning trial. The  
147 respiration signal was analyzed in two consecutive 500-ms windows. To trigger a trial, the  
148 signal amplitude during the latter 500-ms time window had to cross a set absolute threshold  
149 value (peak for inspiration, trough for expiration) and the signal had to either rise  
150 (inspiration) or fall (expiration) at a certain rate between the two consecutive time windows.  
151 In addition, R-peaks were detected from the ECG and used for timing the trial either at  
152 systole (immediately) or diastole (delayed from R-peak). Note that the threshold values for  
153 the EMG and for the respiration peak and rise (inspiration) and for the trough and fall  
154 (expiration) were set individually for each participant during the 5-minute baseline recording

155 prior to conditioning. As a result, when the participant was not spontaneously blinking, and  
156 respiration and cardiac cycle were at desired phases, LabVIEW sent a TTL pulse to the ABD,  
157 which then presented the actual conditioning stimuli. In addition, whenever voluntary  
158 movement of the participant was visible either in the monitoring video or in the breathing  
159 signal, the trial presentation was manually halted. Also, any changes in the overall breathing  
160 baseline were taken into account and ABD controlling LabView parameters were adjusted  
161 accordingly.

162 Two minutes of spontaneous breathing and ECG without any external stimuli were recorded  
163 after the conditioning session to visually confirm online that the experimental manipulation  
164 had not changed the respiration pattern overall and that the respiration belt signal quality had  
165 remained similar to that recorded before experimental manipulations. The whole procedure  
166 lasted about 40 minutes depending on the random ITI.

### 167 Questionnaire

168 After the experiment, participants answered background questions about age, sex, and  
169 handedness and five questions concerning the silent film (e.g., “What equipment did the man  
170 in the film use for travelling in the snow?”) and an open question about the disruptive stimuli.  
171 Questions about the film were asked to find out if participants had been concentrating on the  
172 film because attention has a serious impact on learning in classical conditioning [\[23\]](#).

173 Participants also answered seven true/false questions about the occurrence of the disruptive  
174 stimuli (e.g., air puffs occurred immediately after beeping sounds). These questions were  
175 asked to find out how conscious the participants became of the CS-US association. For the  
176 complete questionnaire (translated into English for reporting purposes), please see Appendix  
177 1.

178 *Data analysis*

179 Conditioned responses

180 The conditioned responses (CR) performed by each participant were analyzed offline using  
181 MATLAB (The MathWorks Inc.). First, the EMG signal was low pass filtered (40 Hz) and  
182 the absolute value of the signal was derived. Then the mean amplitude of the rectified EMG  
183 signal during a 500-ms pre-US period (MEAN<sub>pre</sub>) was calculated. In addition, the mean of  
184 the standard deviation of the signal amplitude during the 500-ms pre-CS period (SD<sub>pre</sub>) was  
185 determined. Learned responses were detected from a 200-ms time window immediately  
186 preceding the US. To qualify as a learned response, the rectified EMG signal amplitude had  
187 to exceed the following threshold:  $MEAN_{pre} + 2 * SD_{pre}$ . For statistical analysis, trials were  
188 grouped into blocks of five trials and the proportion (%) of conditioned responses per block  
189 was calculated. These measures were used as dependent variables when analyzing learning.  
190 Further, to create a simple measure of the outcome of TEBC, we determined the highest  
191 proportion (%) of conditioned responses during any given 10-trial block (50 trials, 5 blocks).  
192 This measure is referred to as the best performance in the TEBC task.

193 Event-related potentials (ERPs)

194 EEG data were analyzed using the MNE python [24]. First, EEG channels were visually  
195 inspected and bad channels were interpolated using spherical spline interpolation method  
196 [25]. Then fast independent component analysis (ICA) was applied to remove any eyeblink  
197 and cardiac artifacts related components [26]. Our previous study has shown that after  
198 applying ICA to remove cardiac related components, sometimes also referred to as heart-  
199 evoked potentials, the cardiac-related signal in EEG is virtually flat (see Figure 4 in Waselius  
200 et al. 2018). Then a band-pass filter of 0.1–30 Hz (zero phase finite impulse response filter  
201 with a Hamming-window) was applied to the continuous EEG recordings. After filtering, the

202 EEG signal was re-referenced to the common average. Then the EEG data were segmented  
203 into epochs spanning from -100 to 500 ms relative to the onset of the CS. The EEG epochs  
204 were manually checked to exclude any trials that were contaminated by movement-related  
205 artifacts or other high-amplitude noise. EEG epochs exceeding 100  $\mu$ V peak-to-peak  
206 amplitudes were excluded from further analysis. Finally, the event-related potentials (ERP)  
207 were obtained by averaging EEG epochs around the CS over all paired conditioning trials.

208 Next, the ERP data were grand averaged across all participants. Two major ERP components  
209 were evident: An auditory N1, which peaked around 112 ms, and auditory P2, which peaked  
210 around 189 ms after the onset of the CS. In addition, the center of activities for both N1 and  
211 P2 peaks were around the channels number 6, 7 and 106 (128-channel EGI Sensor Net),  
212 which are located around the center of the head (see Figure 3A). This pattern (vertex  
213 negative-positive potentials) is consistent with our previous study [21] and other studies [27,  
214 28]. Based on this, auditory N1 and P2 mean amplitudes were extracted from each participant  
215 for further statistical analysis from a 30-ms time window around the grand average N1 (112  
216 ms) and P2 (189 ms) peaks from channels number 6, 7 and 106.

## 217 Statistics

218 One way analysis of variance (ANOVA) and independent samples t-test were used to  
219 examine differences between groups in single variables. Repeated-measures (rm) ANOVA  
220 was used to analyze changes across training and differences between the groups in  
221 conditioned responding: Five-trial averages (blocks, 10) were used as a within-subjects factor  
222 and respiration phase (2) and cardiac cycle phase (2) as between-subjects factors. Univariate  
223 ANOVA was used to examine the effects of respiration phase (2) and cardiac cycle phase (2)  
224 on single variables: best performance, N1 and P2 amplitude. Cohen's d or partial eta squared  
225 ( $\eta^2$ ) are reported for statistically significant differences.

226 **Results**

227 *Participants concentrated on watching the documentary film*

228 Of the 59 participants, 56 answered correctly to all the questions about the film content  
229 (questions 1–5 of Appendix 1) and the rest of them had only one missing answer.  
230 Respectively, only 27 of 59 participants answered correctly to the questions about how the  
231 disruptive stimuli were presented (questions 7–13 of Appendix 1). This indicates that the  
232 participants were generally well concentrated on watching the film and not on the  
233 conditioning stimuli.

234 *Participants trained during expiration-diastole made more conditioned responses than those*  
235 *trained during inspiration-systole*

236 Fifty-one out of 52 participants made conditioned responses at some point during the TEBC  
237 and were included in the analyses (EXP-DIA:  $n = 13$ , EXP-SYS:  $n = 13$ , INS-DIA:  $n = 12$ ,  
238 INS-SYS:  $n = 13$ ) (see Figure 2). Participants in all groups responded (i.e., blinked their eye)  
239 at an equal rate (mean  $\pm$  standard error of mean: 10 %  $\pm$  2 percentage units) to the CS during  
240 the CS-alone trials (one way ANOVA:  $F [3, 47] = 1.146$ ,  $p = 0.340$ ). To test the effects of  
241 breathing (EXP:  $n = 26$  vs. INS:  $n = 25$ ) and cardiac cycle phase (DIA:  $n = 25$  vs. SYS:  $n =$   
242 26) on TEBC, we analyzed the conditioned response data with rm ANOVA using respiration  
243 phase (2) and cardiac cycle phase (2) as between-subjects factors and block (10) as the  
244 within-subjects factor. In addition to the statistically significant main effect of block ( $F [9,$   
245 423] = 8.051,  $p < 0.001$ ,  $\eta^2_p = 0.146$ ) a statistically significant main effect of cardiac cycle  
246 phase ( $F [1, 47] = 6.109$ ,  $p = 0.017$ ,  $\eta^2_p = 0.115$ ) was detected. Interactions were not  
247 statistically significant (within-subjects:  $F [9, 423] = 0.434$ – $0.963$ ,  $p = 0.461$ – $0.889$ ; between  
248 subjects:  $F [1, 47] = 0.471$ ,  $p = 0.496$ ) nor was the main effect of breathing phase ( $F [1, 47] =$

249 0.852,  $p = 0.361$ ). Next, to test our hypothesis directly, an independent samples t-test was  
250 used to analyze the difference in conditioned responses to CS during all blocks (10, average)  
251 between EXP-DIA and INS-SYS. Conditioned responding was higher in the EXP-DIA ( $63 \% \pm 18$   
252 percentage units) than in the INS-SYS group ( $44 \% \pm 19$  percentage units),  $t(24) =$   
253  $2.588$ ,  $p = 0.016$ , Cohen's  $d = 1.015$ .

254 To further analyze the outcome of TEBC we determined a measure of best performance for  
255 each participant as the highest proportion (%) of conditioned responses during any given 10-  
256 trial block. Univariate ANOVA indicated a significant difference in best performance  
257 between participants trained at systole vs. diastole (cardiac cycle phase:  $F[1, 47] = 7.667$ ,  $p =$   
258  $0.008$ ,  $\eta^2_p = 0.140$ ; respiration phase,  $F[1, 47] = 0.357$ ,  $p = 0.553$ ; interaction,  $F[1, 47] =$   
259  $0.008$ ,  $p = 0.930$ ). To directly test our hypothesis, we performed a comparison between just  
260 the EXP-DIA ( $82 \% \pm 17$  percentage units) and the INS-SYS ( $62 \% \pm 22$  percentage units)  
261 groups using independent samples t-test which indicated a significant difference:  $t(24) =$   
262  $2.734$ ,  $p = 0.012$ , Cohen's  $d = 1.073$ .

263 To summarize, participants in all groups readily acquired the conditioned response and those  
264 trained during diastole made more conditioned responses than those trained at systole.  
265 Specifically, and in accordance with our hypothesis, participants trained during expiration-  
266 diastole made more conditioned responses than those trained during inspiration-systole.

267 *The conditioned stimulus evoked a larger NI response in participants trained during*  
268 *expiration than in those trained during inspiration*

269 High-quality EEG data were recorded from 40 participants with valid behavioral data (10 in  
270 each group, see Figure 3). In analyzing the EEG data, we followed the same logic as for the  
271 conditioned responses: Univariate ANOVA revealed a significant effect of respiration phase

272 on the N1 amplitude ( $F [3, 36] = 12.219, p = 0.001, \eta^2_p = 0.253$ ; cardiac cycle phase:  $F [3,$   
273  $36] = 0.632, p = 0.432$ ; interaction:  $F [3, 36] = 0.737, p = 0.396$ ) but not on the P2 amplitude  
274 ( $F [3, 36] = 0.567, p = 0.456$ ; cardiac cycle phase:  $F [3, 36] = 3.144, p = 0.085$ ; interaction:  $F$   
275  $[3, 36] = 0.698, p = 0.409$ ). To follow up on our direct hypothesis of better learning in the  
276 expiration-diastole group compared to inspiration-diastole group, we performed independent  
277 samples t-test on the N1 and P2 amplitudes. The N1 amplitude was larger in the EXP-DIA  
278 compared to the INS-SYS group ( $t [18] = 2.766, p = 0.013, \text{Cohen's } d 0.135$ ) but there was  
279 no difference in P2 amplitude ( $t [18] = 1.626, p = 0.121$ ). To conclude, N1 responses were  
280 largest in the EXP-DIA group and overall larger N1 responses were evoked when the CS was  
281 presented during expiration rather than during inspiration.

282

283 **Discussion**

284 Respiration rhythm and cardiac cycle are known to synchronize to each other [6], to modulate  
285 brain activity [11, 15] and to affect, for example, perception and learning [29, 30]. However,  
286 it is unknown whether combinations of respiration and cardiac cycle phases modulate  
287 learning. Here, healthy young adults were trained in trace eyeblink classical conditioning,  
288 timing the conditioned stimulus based on four combinations of respiration and cardiac cycle  
289 phases (inspiration-systole, inspiration-diastole, expiration-systole, expiration-diastole; see  
290 Figure 1). Based on results of our previous studies [21, 22], we assumed that the diastolic  
291 phase during expiration would be a beneficial phase for stimulus presentation when learning,  
292 whereas systolic phase during inspiration would be less beneficial for learning.

293 As expected, timing the CS onset to diastole during expiration resulted in more frequent  
294 conditioned responding compared to timing the CS onset to systole during inspiration.  
295 Further, conditioned responding was overall more frequent if the CS was timed to diastole  
296 than to systole. Parallel differences were also observed in electrophysiological brain  
297 responses evoked by the CS: The N1 response was larger in amplitude when the CS occurred  
298 during expiration, and especially when it occurred during expiration and diastole. Together  
299 these results support our main assumption and our previous findings [21, 22] that bodily  
300 rhythms can be used to facilitate learning in humans.

301 Most importantly, our current study indicates that learned behavior can be modulated by the  
302 combinatory phases of breathing and the cardiac cycle. Overall, participants in our study  
303 acquired the conditioned eyeblink very fast, within the first few training blocks. As  
304 hypothesized, in our participants trained exclusively during the “resting states” of the heart  
305 and respiratory muscles (expiration-diastole), performance of a learned motor response was  
306 more likely compared to that in participants trained in the “working phase” of these organs



307 (inspiration-systole). Further, the phase of the cardiac cycle was the main factor explaining  
308 this difference. This result is in contrast with our earlier finding indicating no effect of  
309 cardiac cycle phase on learning in humans [21]. However, this could be explained by the  
310 further development of the conditioning paradigm in terms of triggering the trials to systole  
311 or diastole, which was more accurate in the current experiment. Namely, the delay from the  
312 R-peak was individually adjusted to suit each participant's heart rate instead of using a set  
313 delay for all participants. We also did not detect a main effect of breathing phase on  
314 conditioned responding, again in contrast with our earlier finding [22]. However, it could be  
315 that as in the current experiment the timing of the CS hinged on the R-peak, the phases of the  
316 respiration (EXP vs. INS) are not directly comparable to those in our earlier study. Namely,  
317 in our current study, the onset of the CS was delayed until the next heartbeat within the  
318 expiration or the inspiration phase of breathing while in the Waselius et al. 2019 study a CS  
319 was triggered immediately as the desired breathing phase was detected. In any case, putting  
320 all evidence together, it seems that the neural state during diastole and expiration might be  
321 most favorable for acquiring an auditory CS–somatosensory US association and then  
322 performing a learned motor response. This conclusion is in line with all our findings, current  
323 and previous [21, 22]. It is also in line with a report of faster reactions to and higher saliency  
324 evaluations of auditory startle stimuli when presented during expiration rather than during  
325 inspiration [31]. Further support comes from studies reporting greater startle eyeblink  
326 responses to auditory stimuli presented at diastole than systole [19, 20].

327 Our current results suggest that respiration and cardiac cycle phases affect learning itself and  
328 not just the performance of the conditioned response, as 1) there is no difference between  
329 groups during the CS-alone treatment or the very first conditioning trials and 2) there is a  
330 clear distinction in the probability of a conditioned eyeblink once it reaches a plateau (see

331 Figure 2). According to Prokasy's theory (1984), during eyeblink conditioning the  
332 participants first learn an association between the conditioned stimulus and the unconditioned  
333 stimulus. Then they learn to shut their eye before the irritating air puff, that is, they learn to  
334 perform the motor conditioned response. Over time, with extended training, the conditioned  
335 eyeblink is adjusted temporally so that it optimally protects the eye from the flow of air [32].  
336 Considering this, it seems that in our current experiment the effects of the neural state  
337 indicated by the phases of the bodily rhythms center on the acquisition of the CS-US  
338 contingency and the motor conditioned response taking place early in training and not so  
339 much on the later phases of the process when the CR is further adjusted. Learning the CS-US  
340 association during trace eyeblink conditioning is considered to be hippocampus-dependent  
341 [33–35] because of the gap between the CS-offset and the US onset while the simpler version  
342 of the task where the two stimuli partially overlap relies solely on the cerebellum responsible  
343 for motor learning [36, 37]. Thus, it is possible that the neural state indicated by diastole  
344 during expiration is related to a more efficient acquisition of the CS-US association, perhaps  
345 involving the hippocampus, and to a more reliable execution of the conditioned motor  
346 response governed by the cerebellum.

347 As anticipated based on the behavioral results, electrophysiological brain responses evoked  
348 by the conditioned stimulus also differed between the experimental groups in our study.  
349 Specifically, the N1 component of the ERP responses was largest in the participants trained  
350 in diastole during expiration and an overall larger N1 was evoked when the CS was presented  
351 during expiration rather than during inspiration (Figure 3). This suggests that the CS evoked  
352 more synchronous neural activity and was possibly perceived as more surprising or salient  
353 [38] during expiration (and diastole). It should be noted that earlier studies have not  
354 addressed how respiration might modulate auditory ERPs, but it has been suggested that

355 auditory startle stimuli are subjectively rated more intense if presented to mid-expiration [31].  
356 Regarding the effects of cardiac cycle phase, in our earlier study [21] the N1 of the ERP was  
357 in fact higher in amplitude during systole than diastole. However, Schulz and colleagues  
358 (2020) found the N1 to be higher in amplitude during diastole than systole when studying the  
359 effects of the cardiac cycle phase on responses to auditory startle stimuli [20]. Clearly, the  
360 modulation of neural responses by bodily rhythms should be explored in more detail and  
361 considered in data analysis. One might claim, for example, that a fixed inter-stimulus interval  
362 could result in the stimulus being presented repeatedly in the same phase of respiration and/or  
363 cardiac cycle, depending on the rate. This might then affect the amplitude of the different  
364 ERP components.

365 When it comes to the mechanism behind the link between bodily rhythm phases, brain  
366 function and behavior, not a lot is known. It does seem clear that the spontaneous rhythms of  
367 the brain relate to the rhythms of breathing and heartbeat [9], but a deeper mechanistic  
368 explanation of the anatomical and functional connections is missing. One of the most studied  
369 phenomena is the link between respiration, olfaction, and related brain activity. Several  
370 studies report that respiration rhythm and phase are connected to brain oscillatory activity  
371 [15,16,18,39–41]. In most studies, the connection seems to be limited to nasal respiration [16]  
372 and to crucially depend on the function of the olfactory bulb neurons [18]. Some of the  
373 respiration-driven brain rhythms might even be separate from the traditional brain rhythms,  
374 such as theta paced by subcortical structures [14]. Specifically, sniffing rodents are reported  
375 to display hippocampal oscillations that occur at the theta-frequency and couple with  
376 respiration but are not theta [39]. Since oscillations are the foundation of information transfer  
377 in the brain [42–44], it is obvious that rodents largely dependent on their olfactory senses  
378 could benefit from this coupling of respiration and brain oscillations [45]. As an

379 evolutionarily close relative to rodents, humans might also possess this characteristic.  
380 Interestingly, olfaction and spatial memory seem to be congruent in humans: A good sense of  
381 smell is linked to good navigating ability [46]. Curiously, here and in our previous studies the  
382 “sniffing phase”, meaning inspiration, has emerged as the *less* optimal phase for associative  
383 learning of a causal connection between an auditory cue and an aversive somatosensory  
384 stimulation [22]. This might be related to the observations that hippocampal sharp-wave  
385 ripples (SPW-Rs) known to be crucial for memory consolidation [47, 48] and reflect a state in  
386 which rabbits acquire eyeblink conditioning better [47, 48] are more likely to occur during  
387 expiration than inspiration in mice [18]. Whether hippocampal SPW-Rs are more likely to  
388 occur during expiration in the diastolic phase of the cardiac cycle in humans is not known and  
389 should be studied in the future. Further, it should be tested whether limiting breathing to the  
390 oral route would possibly abolish the link between cardioventilatory rhythms and conditioned  
391 responding [16]. In sum, we suggest that the findings of our current experiment could be in  
392 part explained by the link between bodily rhythms and brain oscillatory activity, especially by  
393 that between expiration and SPW-Rs.

#### 394 *Limitations*

395 There are some limitations in this study that we want to point out. Most obvious is the  
396 somewhat small sample size and small effect sizes. Nevertheless, the effect sizes in the ERP  
397 and behavioral results were large in our previous studies with similar group sizes and  
398 experimental set-up. Thus, we were confident that a similar group size should be large  
399 enough to detect possible effects in our current study as well. In addition, one must remember  
400 that associative learning in TEBC is by no means representative of all learning. In fact, in our  
401 current follow-up studies we have employed a different type of associative memory task,  
402 more closely resembling real-life situations. Moreover, here bodily rhythms modulated

403 auditory stimulus processing in a way that might or [\[22\]](#) might not be similar to how these  
404 rhythms modulate processing of visual or somatosensory stimuli in different settings. Last,  
405 we have previously reported [\[22\]](#) that participants have high RSA during this kind of an  
406 experimental setup but we did not take into account if the cardioventilatory coupling was  
407 strong or not and whether it showed fluctuations during the experiment [\[4\]](#). In future studies,  
408 it might be worth investigating if the level of cardioventilatory coupling has an additional  
409 effect on learning.

#### 410 *Conclusion*

411 To summarize, this study is the first to demonstrate that both breathing, and heartbeat  
412 rhythms influence the brain processing of external stimuli and learning about those stimuli.  
413 Learned responding is more likely when an auditory conditioned stimulus is presented during  
414 the resting phase of the heartbeat when breathing out instead of during the working state of  
415 the heartbeat when breathing in. In addition, the N1 component of the auditory-evoked  
416 potential is larger when the stimulus is presented during expiration (and diastole) than when it  
417 occurs during inspiration (and systole). These findings suggest that non-invasive  
418 measurement of cardiorespiratory rhythms combined with closed-loop control of stimulation  
419 can be utilized to promote learning in humans. The next step will be to test if performance  
420 can also be improved in humans with compromised cognitive ability [\[49, 50\]](#). Further, it will  
421 be interesting to test the effects of bodily rhythm phases on learning tasks more closely  
422 resembling real-life situations.

## 423 References

- 424 1. **Brecher GA, Hubay CA.** Pulmonary blood flow and venous return during spontaneous  
425 respiration. *Circ Res* 3: 210–214, 1955.
- 426 2. **Yasuma F, Hayano J-I.** Respiratory sinus arrhythmia: why does the heartbeat synchronize with  
427 respiratory rhythm? *Chest* 125: 683–690, 2004.
- 428 3. **Ludwig C.** Beitrage zur Kenntniss des Einflusses der Respirationsbewegungen auf den Blutlauf im  
429 Aortensysteme. *Arch Anat Physiol Leipzig* 13: 242–302, 1847.
- 430 4. **Galletly DC, Larsen PD.** Relationship between cardioventilatory coupling and respiratory sinus  
431 arrhythmia. *Br J Anaesth* 80: 164–168, 1998.
- 432 5. **Hirsch JA, Bishop B.** Respiratory sinus arrhythmia in humans: how breathing pattern modulates  
433 heart rate. *Am J Physiol* 241: H620–9, 1981.
- 434 6. **Elstad M, O’Callaghan EL, Smith AJ, Ben-Tal A, Ramchandra R.** Cardiorespiratory  
435 interactions in humans and animals: rhythms for life. *Am J Physiol Heart Circ Physiol* 315: H6–  
436 H17, 2018.
- 437 7. **Coleman WM.** The psychological significance of bodily rhythms. *Journal of Comparative*  
438 *Psychology* 1: 213–220, 1921. <https://doi.org/10.1037/h0067228>.
- 439 8. **Grossman P, Taylor EW.** Toward understanding respiratory sinus arrhythmia: relations to cardiac  
440 vagal tone, evolution and biobehavioral functions. *Biol Psychol* 74: 263–285, 2007.
- 441 9. **Klimesch W.** The frequency architecture of brain and brain body oscillations: an analysis. *Eur J*  
442 *Neurosci* 48: 2431–2453, 2018.
- 443 10. **Komisaruk BR.** Synchrony between limbic system theta activity and rhythmical behavior in rats. *J*  
444 *Comp Physiol Psychol* 70: 482–492, 1970.
- 445 11. **Pedemonte M, Goldstein-Daruech N, Velluti RA.** Temporal correlations between heart rate,  
446 medullary units and hippocampal theta rhythm in anesthetized, sleeping and awake guinea pigs.  
447 *Autonomic Neuroscience* 107: 99–104, 2003. [https://doi.org/10.1016/s1566-0702\(03\)00132-2](https://doi.org/10.1016/s1566-0702(03)00132-2).
- 448 12. **O’Keefe J, Recce ML.** Phase relationship between hippocampal place units and the EEG theta  
449 rhythm. *Hippocampus* 3: 317–330, 1993.
- 450 13. **Nokia MS, Waselius T, Mikkonen JE, Wikgren J, Penttonen M.** Phase matters: responding to  
451 and learning about peripheral stimuli depends on hippocampal  $\theta$  phase at stimulus onset. *Learning*  
452 *& Memory* 22: 307–317, 2015. <https://doi.org/10.1101/lm.038166.115>.
- 453 14. **Buzsáki G.** Theta oscillations in the hippocampus. *Neuron* 33: 325–340, 2002.
- 454 15. **Tort ABL, Brankačk J, Draguhn A.** Respiration-Entrained Brain Rhythms Are Global but Often  
455 Overlooked. *Trends in Neurosciences* 41: 186–197, 2018.  
456 <https://doi.org/10.1016/j.tins.2018.01.007>.
- 457 16. **Zelano C, Jiang H, Zhou G, Arora N, Schuele S, Rosenow J, Gottfried JA.** Nasal Respiration  
458 Entrain Human Limbic Oscillations and Modulates Cognitive Function. *J Neurosci* 36: 12448–  
459 12467, 2016.

- 460 17. **Buzsáki G.** Hippocampal sharp wave-ripple: A cognitive biomarker for episodic memory and  
461 planning. *Hippocampus* 25: 1073–1188, 2015. <https://doi.org/10.1002/hipo.22488>.
- 462 18. **Liu Y, McAfee SS, Heck DH.** Hippocampal sharp-wave ripples in awake mice are entrained by  
463 respiration. *Sci Rep* 7: 8950, 2017.
- 464 19. **Schulz A, Reichert CF, Richter S, Lass-Hennemann J, Blumenthal TD, Schächinger H.**  
465 Cardiac modulation of startle: effects on eye blink and higher cognitive processing. *Brain Cogn* 71:  
466 265–271, 2009.
- 467 20. **Schulz A, Vögele C, Bertsch K, Bernard S, Münch EE, Hansen G, Naumann E, Schächinger**  
468 **H.** Cardiac cycle phases affect auditory-evoked potentials, startle eye blink and pre-motor reaction  
469 times in response to acoustic startle stimuli. *Int J Psychophysiol* 157: 70–81, 2020.
- 470 21. **Waselius T, Wikgren J, Halkola H, Penttonen M, Nokia MS.** Learning by heart: cardiac cycle  
471 reveals an effective time window for learning. *J Neurophysiol* 120: 830–838, 2018.
- 472 22. **Waselius T, Wikgren J, Penttonen M, Nokia MS.** Breathe out and learn: Expiration-contingent  
473 stimulus presentation facilitates associative learning in trace eyeblink conditioning.  
474 *Psychophysiology*: e13387, 2019. <https://doi.org/10.1111/psyp.13387>.
- 475 23. **Clark RE, Manns JR, Squire LR.** Classical conditioning, awareness, and brain systems. *Trends*  
476 *Cogn Sci* 6: 524–531, 2002.
- 477 24. **Gramfort A, Luessi M, Larson E, Engemann DA, Strohmeier D, Brodbeck C, Goj R, Jas M,**  
478 **Brooks T, Parkkonen L, Hämäläinen M.** MEG and EEG data analysis with MNE-Python. *Front*  
479 *Neurosci* 7: 267, 2013.
- 480 25. **Perrin F, Pernier J, Bertrand O, Echallier JF.** Spherical splines for scalp potential and current  
481 density mapping. *Electroencephalogr Clin Neurophysiol* 72: 184–187, 1989.
- 482 26. **Hyvärinen A.** Fast and robust fixed-point algorithms for independent component analysis. *IEEE*  
483 *Trans Neural Netw* 10: 626–634, 1999.
- 484 27. **Carpenter AL, Shahin AJ.** Development of the N1–P2 auditory evoked response to amplitude rise  
485 time and rate of formant transition of speech sounds. *Neurosci Lett* 544: 56–61, 2013.
- 486 28. **Näätänen R, Sams M, Alho K, Paavilainen P, Reinikainen K, Sokolov EN.** Frequency and  
487 location specificity of the human vertex N1 wave. *Electroencephalogr Clin Neurophysiol* 69: 523–  
488 531, 1988.
- 489 29. **Park H-D, Correia S, Ducorps A, Tallon-Baudry C.** Spontaneous fluctuations in neural  
490 responses to heartbeats predict visual detection. *Nat Neurosci* 17: 612–618, 2014.
- 491 30. **Perl O, Ravia A, Rubinson M, Eisen A, Soroka T, Mor N, Secundo L, Sobel N.** Human non-  
492 olfactory cognition phase-locked with inhalation. *Nat Hum Behav* 3: 501–512, 2019.
- 493 31. **Münch EE, Vögele C, Van Diest I, Schulz A.** Respiratory modulation of intensity ratings and  
494 psychomotor response times to acoustic startle stimuli. *Neurosci Lett* 711: 134388, 2019.
- 495 32. **Prokasy WF.** Acquisition of Skeletal Conditioned Responses in Pavlovian Conditioning.  
496 *Psychophysiology* 21: 1–13, 1984.
- 497 33. **Kim JJ, Clark RE, Thompson RF.** Hippocampectomy impairs the memory of recently, but not  
498 remotely, acquired trace eyeblink conditioned responses. *Behavioral Neuroscience* 109: 195–203,  
499 1995.

- 500 34. **Moyer JR Jr, Deyo RA, Disterhoft JF.** Hippocampectomy disrupts trace eye-blink conditioning  
501 in rabbits. *Behav Neurosci* 104: 243–252, 1990.
- 502 35. **Solomon PR, Vander Schaaf ER, Thompson RF, Weisz DJ.** Hippocampus and trace  
503 conditioning of the rabbit's classically conditioned nictitating membrane response. *Behav Neurosci*  
504 100: 729–744, 1986.
- 505 36. **Medina JF, Nores WL, Ohyama T, Mauk MD.** Mechanisms of cerebellar learning suggested by  
506 eyelid conditioning. *Curr Opin Neurobiol* 10: 717–724, 2000.
- 507 37. **Thompson RF, Steinmetz JE.** The role of the cerebellum in classical conditioning of discrete  
508 behavioral responses. *Neuroscience* 162: 732–755, 2009.
- 509 38. **Friston K.** The free-energy principle: a rough guide to the brain? *Trends Cogn Sci* 13: 293–301,  
510 2009.
- 511 39. **Nguyen Chi V, Müller C, Wolfenstetter T, Yanovsky Y, Draguhn A, Tort ABL, Brankač J.**  
512 Hippocampal Respiration-Driven Rhythm Distinct from Theta Oscillations in Awake Mice. *J*  
513 *Neurosci* 36: 162–177, 2016.
- 514 40. **Ito J, Roy S, Liu Y, Cao Y, Fletcher M, Lu L, Boughter JD, Grün S, Heck DH.** Whisker barrel  
515 cortex delta oscillations and gamma power in the awake mouse are linked to respiration. *Nat*  
516 *Commun* 5: 3572, 2014.
- 517 41. **Karalis N, Sirota A.** Breathing coordinates limbic network dynamics underlying memory  
518 consolidation. 2018. doi: <http://dx.doi.org/10.1101/392530>, 2018.
- 519 42. **Buzsáki G.** Neural syntax: cell assemblies, synapsembles, and readers. *Neuron* 68: 362–385, 2010.
- 520 43. **Fell J, Axmacher N.** The role of phase synchronization in memory processes. *Nat Rev Neurosci*  
521 12: 105–118, 2011.
- 522 44. **Fries P.** A mechanism for cognitive dynamics: neuronal communication through neuronal  
523 coherence. *Trends Cogn Sci* 9: 474–480, 2005.
- 524 45. **Tsanov M, Chah E, Reilly R, O'Mara SM.** Respiratory cycle entrainment of septal neurons  
525 mediates the fast coupling of sniffing rate and hippocampal theta rhythm. *Eur J Neurosci* 39: 957–  
526 974, 2014.
- 527 46. **Dahmani L, Patel RM, Yang Y, Chakravarty MM, Fellows LK, Bohbot VD.** An intrinsic  
528 association between olfactory identification and spatial memory in humans. *Nat Commun* 9: 4162,  
529 2018.
- 530 47. **Nokia MS, Mikkonen JE, Penttonen M, Wikgren J.** Disrupting neural activity related to awake-  
531 state sharp wave-ripple complexes prevents hippocampal learning. *Frontiers in Behavioral*  
532 *Neuroscience* 6: 2012. <https://doi.org/10.3389/fnbeh.2012.00084>.
- 533 48. **Nokia MS, Penttonen M, Wikgren J.** Hippocampal ripple-contingent training accelerates trace  
534 eyeblink conditioning and retards extinction in rabbits. *J Neurosci* 30: 11486–11492, 2010.
- 535 49. **Jacobson SW, Stanton ME, Dodge NC, Pienaar M, Fuller DS, Molteno CD, Meintjes EM,**  
536 **Hoyme HE, Robinson LK, Khaole N, Jacobson JL.** Impaired delay and trace eyeblink  
537 conditioning in school-age children with fetal alcohol syndrome. *Alcohol Clin Exp Res* 35: 250–  
538 264, 2011.



539 50. **Woodruff-Pak DS.** Eyeblink classical conditioning differentiates normal aging from Alzheimer's  
540 disease. *Integr Physiol Behav Sci* 36: 87–108, 2001.

541 **Figure captions**

542 **Figure 1. Experimental design and examples of breathing and ECG signals used for**  
543 **timing the conditioning trials.** (A) After presenting five US-alone trials the participants  
544 started to watch a documentary film. Then there was a five-minute resting period followed by  
545 five CS-alone trials. Next, 50 pairs of CS+US trials were timed to a certain cardiorespiratory  
546 phase (Ins-Sys, Ins-Dia, Exp-Sys or Exp-Dia). At last, five CS-alone trials were presented as  
547 an extinction training (EXT) followed by a two-minute rest period before the experiment  
548 ended. (B) Breathing signal and ECG were recorded and followed online to time conditioning  
549 trials to a certain cardiorespiratory phase. During the systolic phase (Sys) of the cardiac cycle,  
550 the ECG shows the QRS complex, reflecting ventricular depolarization, and the T wave,  
551 reflecting ventricular repolarization. Between the end of the T-wave and next R-peak, is the  
552 diastolic phase (Dia). The diastolic phase during expiration is marked with grey bars for  
553 demonstrating the hypothesized optimal phases for stimulus presentation.

554 **Figure 2. Participants trained during expiration-diastole (EXP-DIA) made more**  
555 **conditioned responses than those trained during inspiration-systole (INS-SYS).** (A) The  
556 percentage of conditioned responses per 5-trial block was used as a measure of learned  
557 behavior. There was no difference in responding to the tone-CS prior to conditioning (CS  
558 alone, one way ANOVA). Participants in all groups learned the trace eyeblink conditioning  
559 task (TEBC, main effect of block) and participants trained in diastole made more conditioned  
560 responses than those trained in systole (rm ANOVA, main effect of cardiac cycle). Further, in  
561 accordance with our hypothesis, participants trained at EXP-DIA made more conditioned  
562 responses during TEBC than those trained at INS-SYS (independent samples t-test). (B)  
563 Conditioned responding at best was higher in participants trained at diastole than at systole  
564 (univariate ANOVA) and higher in participants trained at EXP-DIA than those trained at

565 INS-SYS (independent samples t-test). Asterisks refer to statistical significance: \*  $p < 0.050$ ,  
566 \*\*  $p < 0.010$ , \*\*\*  $p \leq 0.001$ . Vertical lines in panel A indicate standard error of mean.  
567 Horizontal lines in panel B refer to the mean.

568 **Figure 3. The conditioned stimulus evoked a larger N1 response in participants trained**  
569 **during expiration than in those trained during inspiration.** (A) Joint plot of the grand-  
570 average ERP waveform and topographic maps (depicted at the N1 and P2 peak) of all  
571 participants with valid data ( $n = 40$ ). The butterfly plot of the ERP waveform is spatially  
572 colored by the channel locations. (B) Left: ERP waveform at region of interest (channels 6, 7  
573 and 106) for the four groups separately ( $n = 10$  in each group). Right: Topographic maps of  
574 two major components for the four groups separately: the auditory N1 peaks at around 112  
575 ms and auditory P2 component peaks around 189 ms. N1 amplitude was larger in participants  
576 trained at EXP than at INS (univariate ANOVA) and larger in participants trained at EXP-  
577 DIA than at INS-SYS (independent samples t-test). Asterisks refer to statistically significant  
578 differences between groups in N1 amplitude: \*  $p < 0.050$ , \*\*\*  $p \leq 0.001$ .

579

580

581 **Acknowledgements**

582 We thank Jan Wikgren, Lauri Viljanto, Viki-Veikko Elomaa and Petri Kinnunen for technical  
583 help in building the recording systems. Special thanks to Jan Wikgren for helping with the  
584 manuscript.

585 **Grants**

586 The work was supported by the Academy of Finland grant number 321522 to MSN.

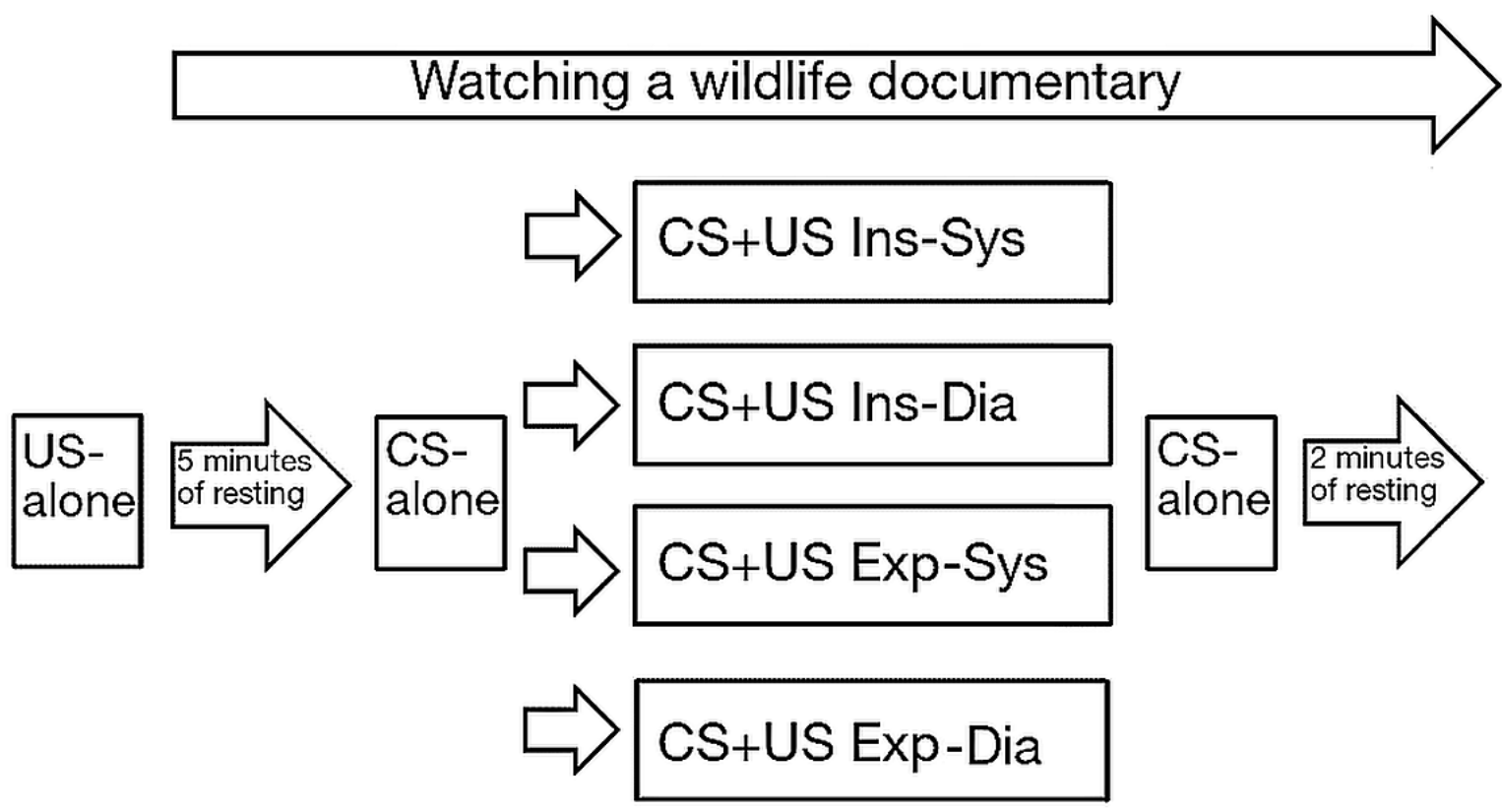
587 **Disclosures**

588 The authors declare no conflict of interest.

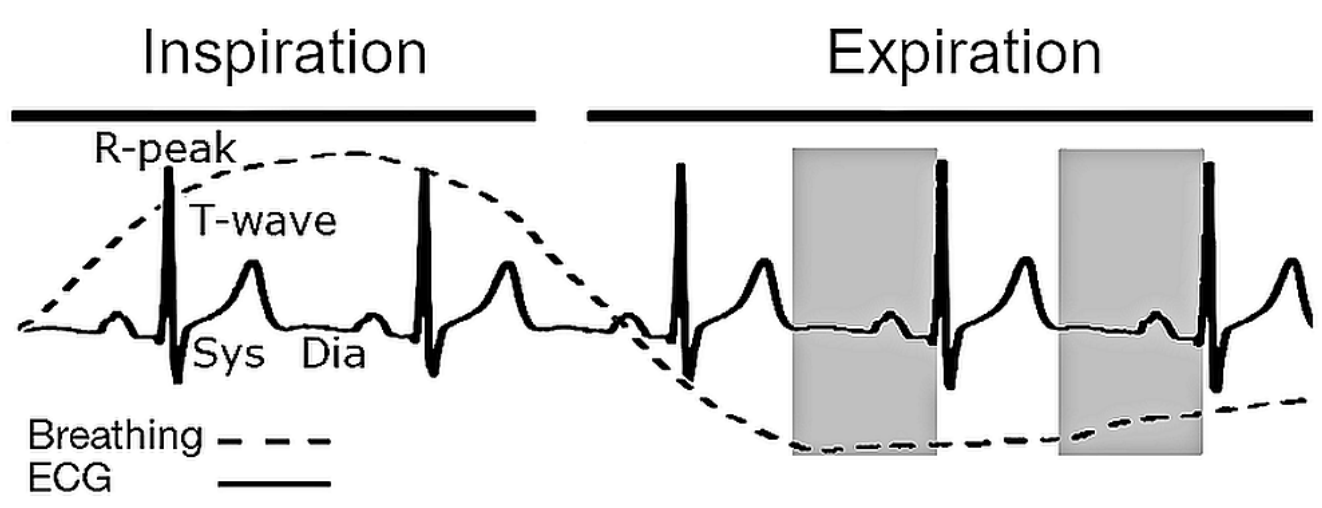
589

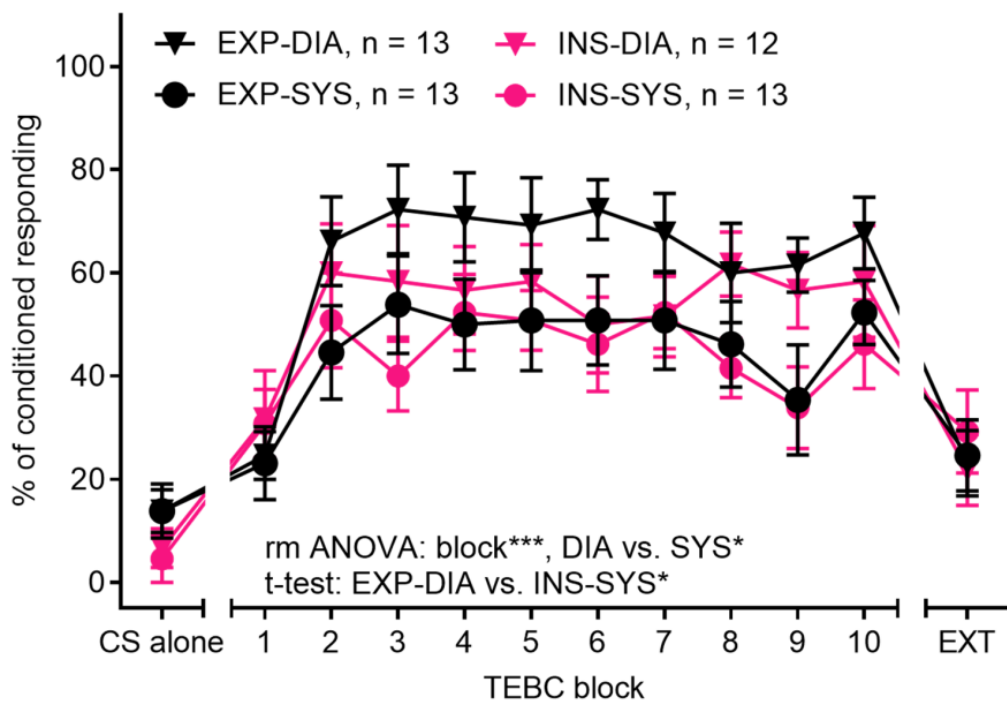
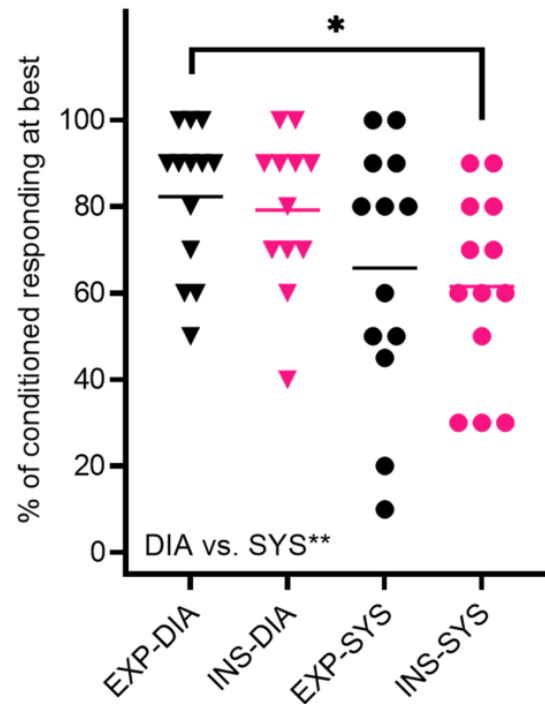
590

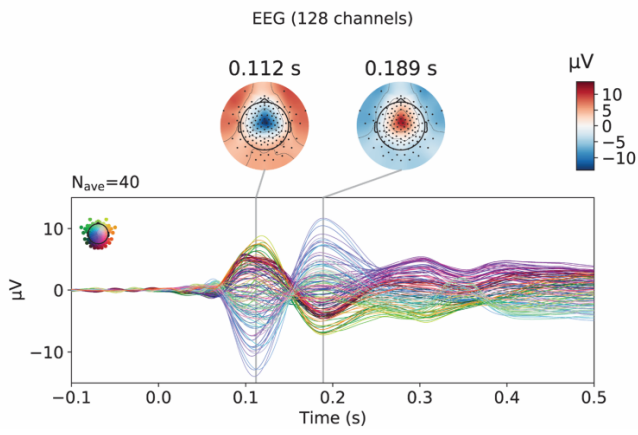
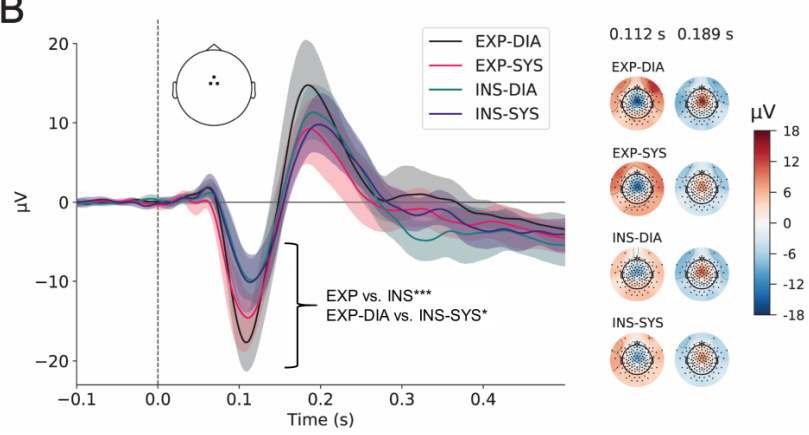
A



B



**A****B**

**A****B**

## APPENDIX 1: A FULL QUESTIONNAIRE FOR PARTICIPANTS

The researcher fills in: ID\_\_\_\_\_ Date\_\_\_\_\_ Time\_\_\_\_\_

**Please fill in the missing information and answer the following questions.**

Age:

Sex:

Handedness:

1) What equipment did the man in the film use for travelling in the snow?

2) Were there any light phenomena in the film?

3) Name two to three animals you saw in the film.

4) Did the fishermen catch any fish?

5) What large bird appeared at the beginning of the second film?

6) When did the air puff occur?

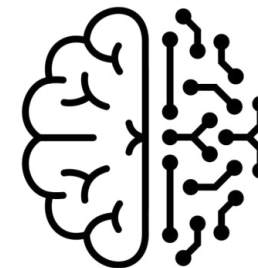
**PLEASE TURN!**



<b>Proposition</b>	True	False
1. The air puff occurred right <i>before</i> the beep.		
2. The air puff occurred right <i>after</i> the beep.		
3. The beep occurred right <i>before</i> the air puff.		
4. The beep occurred right <i>after</i> the air puff.		
5. The beep and the air puff always occurred very close to each other.		
6. The beep and the air puff occurred close to each other only occasionally.		
7. The beep predicted the air puff.		



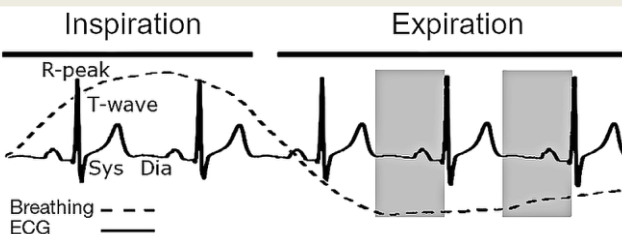
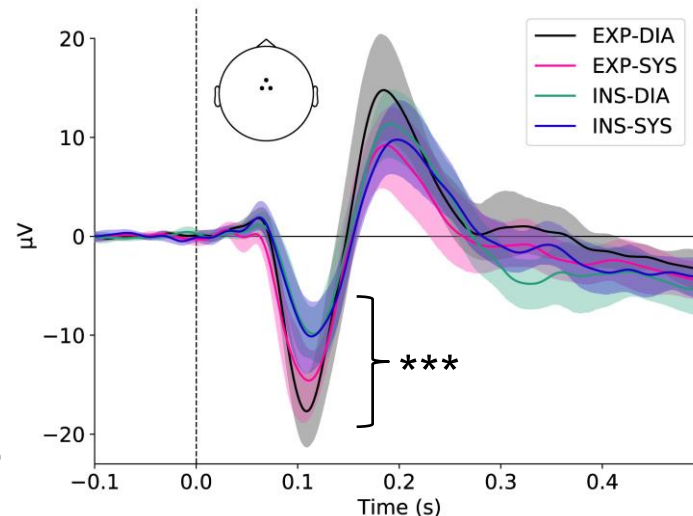
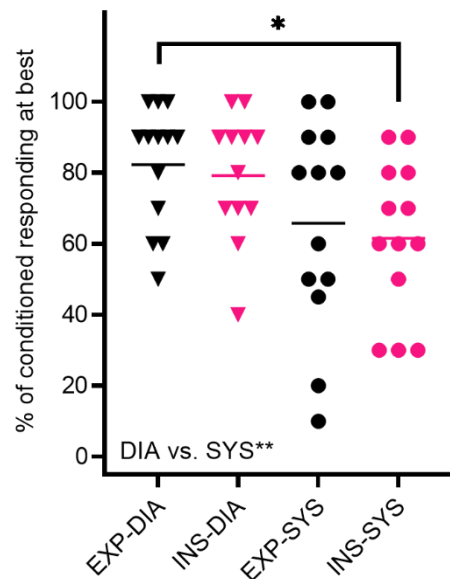
# Bodily rhythms affect conditioning



## METHODS

Breathing (EXPIration vs. INSpiration) and cardiac cycle phase (DIAstole vs. SYStole) were monitored to trigger eyeblink conditioning trials in four different bodily states while participants were watching a wildlife documentary. Conditioned eyeblinks and electroencephalogram were recorded.

## OUTCOME



## CONCLUSION

Breathing and heartbeat have a phase combination (diastole during expiration) that is indicative of a neural state beneficial for conditioning.