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Author(s): Waselius, Tomi; Wikgren, Jan; Penttonen, Markku; Nokia, Miriam

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1 **Breathe out and learn: expiration-contingent stimulus**
2 **presentation facilitates associative learning in trace eyeblink**
3 **conditioning**

4 Tomi Waselius^{1*}, Jan Wikgren^{1,2}, Markku Penttonen¹ & Miriam S. Nokia^{1,3}

5 ¹Department of Psychology, P.O. Box 35, 40014 University of Jyvaskyla, Finland

6 ²Centre for Interdisciplinary Brain Research, University of Jyvaskyla, Finland

7 ³Neuroscience Center, Helsinki Institute of Life Science, University of Helsinki, Finland

8 *corresponding author: tomi.waselius@jyu.fi

9

1 **ABSTRACT**

2 Rhythmic variation in heart rate and respiratory pattern are coupled in a way that optimizes
3 the level of oxygen in the bloodstreams of the lungs and the body as well as saves energy in
4 pulmonary gas exchange. It has been suggested that the cardiac cycle and respiratory pattern
5 are coupled to neural oscillations of the brain. Yet studies on how this rhythmic coupling is
6 related to behavior are scarce. There is some evidence that, for example, the phase of
7 respiration affects memory retrieval and the electrophysiological oscillatory state of the
8 limbic system. It is also known that the phase of the cardiac cycle and hippocampal
9 electrophysiological oscillations alone affect learning. Here we studied whether the timing of
10 training trials to different phases of respiration affects learning trace eyeblink conditioning in
11 healthy adult humans. Trials consisting of a neutral conditioned stimulus (200-ms tone) and a
12 slightly aversive unconditioned stimulus (100-ms airpuff towards the eye), presented with a
13 600-ms trace interval, were timed to either inspiration or expiration. A control group was
14 trained regardless of respiratory phase. We found that, at the end of training, the rate of
15 conditioned responses was higher in the group trained at expiration than it was in the other
16 two groups. That is, brain state seems to fluctuate as a function of respiratory rhythm and this
17 fluctuation is also behaviorally relevant, exerting its effect on, at the least, a simple form of
18 associative learning.

19

20 **Keywords:** memory, respiration, respiratory sinus arrhythmia

21

22

1 1. INTRODUCTION

2 The effect of rhythmic variation of bodily functions on brain oscillations and cognition is an
3 emerging field of research. Since the 1950s, it has been known that respiratory pattern and
4 cardiac cycle are synchronized, a phenomenon termed *respiratory sinus arrhythmia* (RSA)
5 (Begher & Hubay, 1955). In short, the cardiac cycle is longer during expiration (breathing
6 out) and shorter during inspiration (breathing in) (Berntson, Cacioppo, & Quigley, 1993;
7 Hirsch & Bishop, 1981; Katona & Jih, 1975). One function of RSA in vertebrates is to
8 optimize gas exchange in the lungs and periphery (Yasuma & Hayano, 2004). This
9 cardiorespiratory regulation is driven by the pons of the brainstem via baroreceptors located
10 on the walls of major blood vessels (Farmer, Dutschmann, Paton, Pickering, & McAllen,
11 2016). It is known that the phase of the cardiac cycle alone affects neural processing of
12 external stimuli in humans (Gray et al., 2012; Martins, McIntyre, & Ring, 2014; Park,
13 Correia, Ducorps, & Tallon-Baudry, 2014; Waselius, Wikgren, Halkola, Penttonen, & Nokia,
14 2018). For example, negative pictures are judged to be more intense when presented during
15 systole (Gray et al., 2012). In addition, in rabbits, learning trace eyeblink conditioning
16 (TEBC), a hippocampus-dependent variant of classical Pavlovian conditioning (Holland &
17 Bouton, 1999; Solomon, Vander Schaaf, Thompson, & Weisz, 1986), is most effective if the
18 conditioned stimulus is timed to the diastolic phase of the cardiac cycle (Waselius, Wikgren,
19 Halkola, Penttonen, & Nokia, 2018). Thus, rhythmic variation in bodily functions seems to
20 have an impact on the encoding of external information in the brain.

21 Bodily functions, and especially brain activity, show rhythmic variation or, in other words,
22 they oscillate. It has been suggested that the electrophysiological activity of the brain is
23 organized based on harmonic frequencies, and that the different oscillations serve different
24 physiological functions (Penttonen & Buzsáki, 2003). For example, 3–12 Hz theta

1 oscillations emerge in the mammalian hippocampus during exploratory activities (Buzsáki,
2 and in part regulate the firing of pyramidal cells that encode the location of the subject,
3 so-called place cells (J O’Keefe & Dostrovsky, 1971; John O’Keefe, 1976). In addition,
4 gamma frequency (30–80 Hz) in the hippocampus is phase locked to theta during maze
5 exploration and rapid eye movement sleep (Belluscio, Mizuseki, Schmidt, Kempter, &
6 Buzsáki, 2012). Together, the theta and gamma oscillations pace activity of hippocampal
7 cells: for example, hippocampal interneurons fire action potentials phase-locked to both
8 frequencies (Bragin et al., 1995). Sirota et al. (2008) suggest this kind of synchronization
9 ensures that the timing of information processing is optimal for plasticity.

10 In addition to this harmonic coupling within rhythms of the brain, neural oscillations are also
11 coupled to those of the body (Klimesch, 2013, 2018). For example, breathing rhythm is
12 connected to oscillatory brain activity in widespread brain regions (for a review, see Tort,
13 Brankačk, & Draguhn, 2018). More specifically, using depth electrodes in human epilepsy
14 patients, it was found that the power of electrophysiological oscillations in the delta (0.5–4
15 Hz), theta (4–8 Hz) and beta (13–30 Hz) frequency bands in the piriform cortex, the
16 amygdala and the hippocampus increases during inspiration. Interestingly, this phase-
17 amplitude coupling between respiration and limbic system electrophysiological activity was
18 specific to nasal breathing. Similar observations have also been made in mice (Tort, et al.,
19 2018; Yanovsky, Ciatipis, Draguhn, Tort, & Brankačk, 2014). For example, in urethane-
20 anesthetized mice the olfactory bulb and the hippocampus exhibit respiration entrained
21 oscillations distinct from spontaneous hippocampal theta activity (Yanovsky et al. 2014).
22 Furthermore, oscillatory activity (2-14 Hz) in the hippocampus (and other brain areas)
23 follows breathing rhythm and is coherent with the hippocampal theta oscillation during
24 exploratory activity (Tort et al. 2018b). However, these oscillations also occur incoherently

1 with the theta oscillation. Simply put, respiration-driven neural activity in the hippocampus is
2 often distinct from hippocampal theta (Tort et al. 2018b).

3 In addition to theta and gamma, electrophysiological oscillations typical for the limbic system
4 also include the so-called sharp wave-ripples (SPW-R). These are fast bursts of hippocampal
5 neural activity (110–200 Hz) that are present usually during sleep and immobility (Buzsáki,
6 1986; John O’Keefe, 1976). SPW-Rs represent synchronous excitation of CA1 pyramidal
7 neurons (Buzsáki, 1986) and are thought to reflect memory consolidation (for a review, see
8 Buzsáki, 2015). However, SPW-Rs also occur while awake and, in fact, presenting
9 conditioning stimuli contingent on SPW-Rs enhances learning of TEBC in rabbits (Nokia,
10 Penttonen, & Wikgren, 2010). Interestingly, SPW-Rs more likely occur during expiration in
11 mice (Liu, McAfee, & Heck, 2017) and, even more curiously, this SPW-R modulation is
12 dependent on an intact olfactory bulb.

13 Taken together, inspiration seems to promote rhythmic activity at 0.5–30 Hz in the limbic
14 system (Zelano et al., 2016) while SPW-Rs are more likely to occur in the hippocampus
15 during expiration (Liu et al., 2017). Because previous results also indicate that training
16 contingent on SPW-Rs is beneficial to learning (Nokia et al., 2010), we suggest that the
17 overall neural state could be more favorable for acquisition of new information during
18 expiration than it is during inspiration. To our knowledge, the direct effects of respiratory
19 rhythm on associative learning have not been studied. In this experiment, we trained healthy
20 adult participants in TEBC. In two experimental groups, the training trials consisting of a
21 conditioned stimulus (CS) followed by an unconditioned stimulus (US) were timed either at
22 the inspiration or the expiration phase. A control group was trained at random, irrespective of
23 respiratory rhythm. As explained above, we hypothesized that participants trained at the
24 expiration phase would learn better than those trained at random or at the inspiration phase.

1 **2. METHOD**

2 **2.1 Participants**

3 The participants in this study were recruited via e-mail lists. They gave informed written
4 consent to this study and were free to discontinue participation in the experiment at any point
5 knowing that they would still get a reward (a movie ticket) by participating in the experiment.
6 The study was approved by the University of Jyväskylä Ethical Committee. Thirty-six adults
7 (12 males; aged 20–30 years: mean 24.1) took part in the study. Two of the participants were
8 left-handed. All of the participants were healthy with no history of psychiatric or neurological
9 illnesses. They were not taking medication affecting brain function, and had no disability in
10 hearing or vision.

11 **Experimental procedure**

12 The schematic structure of the experimental procedure is presented in Figure 1.

13 **2.2.1 Recordings**

14 Recording electrodes were attached after participants had signed the written consent.
15 Respiration was recorded and monitored during the experiment with a reusable fabric belt
16 (RESPA00000, Spes Medica, Italy), which was fastened on top of the clothes on the lower
17 chest area. Heart rate was recorded using three electrocardiogram (ECG) electrodes (Kendall,
18 H92SG); one electrode was placed on top of the right clavicle, one on the left lower ribs, and
19 the grounding electrode on the back of the neck. Eyeblinks (see Figure 2B) were recorded
20 using two electrodes (70010-K/12, Ambu, Ballerup, Denmark) that were attached on top of
21 the participant's right eye muscles (orbicularis oculi). All signals were high-pass filtered
22 (0.16 Hz) and low-pass filtered (250 Hz) online and recorded with NeurOne Tesla (with
23 Analog Out Option, Bittium Biosignals Ltd., Finland).

1 2.2.2 Instructions to participants before conditioning
2 The participants sat in a chair in front of a TV screen (Asus VG236 series H, 23"; distance:
3 ~100 cm). They were informed that the aim of the study was to record physiological
4 responses to different types of stimuli while their attention was to be directed at a silent film
5 depicting landscapes and animals. The participants were instructed to follow the film,
6 because there would be questions considering the content of the footage after the session.
7 They were also instructed to sit comfortably in the chair and not pay attention to the
8 disturbing stimuli. In other words, the participants were led to believe that the idea was to
9 study the disturbance caused by beeping sounds and air puffs on their attention towards the
10 film.

11 2.2.3 Trace eyeblink conditioning
12 The conditioned stimulus (CS) was a 200-ms, 440-Hz, 66-dB tone delivered via a
13 loudspeaker situated in the lower right-hand corner of the room. The unconditioned stimulus
14 (US) was an airpuff (0.2 bar source pressure, 100 ms) targeted at the right eye and it was
15 delivered via a plastic tube attached to modified safety goggles. Note that the air pressure was
16 low and none of the participants felt that the air coming to the eye was unbearable. During
17 conditioning trials, a 600-ms trace interval separated the tone offset and the US onset. The
18 presentation of stimuli used for conditioning was controlled by custom software running on
19 an Arduino-based device (ABD).

20 First, four US-alone trials with an inter-trial interval (ITI) of 5 s were presented to make sure
21 that the participants felt comfortable enough to proceed with the experiment. After this, 2
22 minutes of resting data were recorded, followed by five CS-alone trials (random ITI 20-40 s).
23 Then, 50 CS+US conditioning trials (ITI 20-40 s) were presented either at inspiration,
24 expiration or at a random phase of breathing.

1 To this end, the respiration and EMG signals were conveyed to a custom script running in
2 LabVIEW (National Instruments). Both signals were sampled at 1 kHz. At each time point,
3 the last second of both EMG and respiration signals were analyzed. EMG was evaluated for
4 spontaneous eyeblinks, that is, the signal had to stay below a set amplitude threshold in order
5 to proceed with presenting the conditioning trials. The respiration signal was analyzed in two
6 consecutive 500-ms windows. In order to trigger a trial, the signal amplitude during the latter
7 500-ms time window had to cross a set absolute threshold value (peak for inspiration, trough
8 for expiration) and the signal had to either rise (inspiration) or fall (expiration) at a certain
9 rate between the two consecutive time windows. Note that the threshold values for the EMG
10 and for the respiration peak and rise (inspiration) and for the trough and fall (expiration) were
11 set individually for each participant during the 2-minute baseline recording prior to
12 conditioning. As an end result, when the participant was not spontaneously blinking and
13 respiration was at a desired phase, LabVIEW sent a TTL pulse to the ABD, which then
14 presented the actual conditioning stimuli (see above).

15 Two minutes of spontaneous breathing without any external stimuli were recorded after the
16 conditioning to visually confirm on-line that the experimental manipulation had not changed
17 the respiration pattern overall and that the respiration belt signal quality had remained similar
18 to that recorded before experimental manipulations. The whole procedure lasted about 40
19 minutes depending on the random ITI.

20 2.2.4 Questions to participants after conditioning

21 After the experiment, participants answered background questions about age, gender, and
22 handedness and five questions concerning the silent film (e.g., “What kind of footwear did
23 the man use in the snow at the beginning of the film?”). One open question about the
24 disruptive stimuli was also presented: “When did the airpuff occur?”

1 2.3 Data analysis

2 2.3.1 Conditioned responses

3 The percentage of conditioned responses (CR) performed by each participant was analyzed

4 off-line using MATLAB (The MathWorks Inc.). The signal was low-pass filtered (40 Hz) and

5 the absolute values for the signal were calculated. The mean amplitude during a 500-ms pre-

6 US period (MEANpre) was calculated. In addition, the mean of the standard deviation of the

7 amplitude during the 500-ms pre-CS period (SDpre) was determined. Learned responses were

8 detected from a 200-ms time window immediately preceding the US. To qualify as a learned

9 response, the eye muscle activity had to exceed the following threshold: MEANpre +

10 2*SDpre. Trials were grouped into five blocks of 10 trials (CC1, CC2, CC3, CC4 and CC5)

11 for analysis and the percentage of learned responses per block was calculated.

12 2.3.2 Respiration, ECG and the respiratory sinus arrhythmia (RSA)

13 RSA was analyzed off-line using MATLAB by searching epochs of respiratory phases

14 (inspiration and expiration) with at least two consecutive R-peaks within a phase and

15 calculating the mean interval (in ms) between these R peaks. Epochs with only one R-peak

16 were rejected from the analysis (Grossman, Beek, & Wientjes, 1990).

17 2.3.3 Statistics

18 A paired samples *t* test was used to compare R-peaks during inspiration vs. expiration.

19 Repeated-measures analysis of variance (ANOVA), with five training blocks of 10 trial

20 averages each as a within-subjects factor and group (3) as a between-subject factor, was used

21 to analyze changes across training and differences between experimental groups in learned

22 responding. In case the assumption of sphericity was violated, *p* values were corrected using

23 Huynh-Feldt estimates. A one-way ANOVA was used for comparisons between groups at

24 each trial block. For post hoc comparisons, Bonferroni-corrected *p* values are reported.

1 **3. RESULTS**

2 3.1 R-peak intervals were longer during expiration
3 To first verify the existence of RSA in our current data we analyzed the interval between R-
4 peaks at inspiration and expiration. A paired samples *t* test showed that there was a
5 statistically significant difference in R-peak interval (in milliseconds) between inspiration and
6 expiration throughout the experiment (in all participants): The interval between R-peaks was
7 longer during expiration (mean \pm standard deviation: 764 ± 83 ms) compared to inspiration
8 (737 ± 78 ms); $t(35) = 4.96, p < 0.0001, d = 0.836$. In other words, the heart rate was slower
9 during expiration and the overall heart rate was around 80 beats per minute.

10 3.2 Timing of trials to expiration enhanced learning
11 Next, we studied the effects of TEBC (5 training blocks) and respiration phase (Inspiration,
12 Expiration, Random) on learned responding (see Figure 3) using a repeated measures
13 ANOVA. There was a significant interaction between group and training block: $F(8, 132) =$
14 $2.478, p = 0.019, p\eta^2 = 0.14$ [main effect of training block: $F(4, 132) = 8.413, p < 0.0001$,
15 but the main effect of group: $F(2, 33) = 0.522, p = 0.600$] was not significant. These results
16 suggest learning curves in the three groups were different. Further analysis of the significant
17 group and training block interactions showed that the effect of training on learned responding
18 was significant in the Expiration group [$F(4, 44) = 5.182, p = 0.002$] and in the Random
19 group [$F(4, 44) = 5.681, p = 0.001$] but not in the Inspiration group [$F(4, 44) = 2.248, p =$
20 0.079].
21 To further analyze at which stage of the learning process the groups differed from each other,
22 separate one-way ANOVAs were conducted at each trial block. This analysis did not reveal a
23 significant difference in the CR percentage until the last (5th) training block [$F(2, 35) =$

1 4.275, $p = 0.022$, $\eta^2 = 0.26$; blocks 1 to 4: $F(2, 35) = 0.92 - 1.282$, $p = 0.291 - 0.912$]. The
2 CR percentage (%) during the 5th training block was highest in the Expiration group ($73.33 \pm$
3 30.55) and lower in the Inspiration (45.0 ± 28.44) and Random (47.5 ± 18.15) groups. Post
4 hoc comparisons (Bonferroni) indicated that the difference was significant between the
5 Expiration and Inspiration groups: $p = 0.038$. The difference was also very close to
6 significance between the Expiration and Control groups: $p = 0.065$. There was no difference
7 in CR percentage between the Control and Inspiration groups: $p = 1.00$. To conclude, the
8 three groups acquired the CR at different rates, and this difference is due to the Expiration
9 group performing more CRs than the other two groups at the end of conditioning.

10 3.3 Participants paid attention to their primary task but also became aware of the stimulus
11 contingency

12 All of the participants were instructed to pay attention to a silent film while ignoring other
13 stimuli. Of the 36 participants, 35 were able to answer close to 100% correctly on the
14 questionnaire considering the landscape footage and animals (only one wrong answer from
15 180 answers). However, all of the participants also correctly answered the open question
16 about the TEBC stimulus presentation (“When did the airpuff occur?”), stating, for example,
17 “At the beginning, there were a few tones alone and after a while a tone followed by an
18 airpuff started to occur” or “The tone was always followed by an airpuff.” In other words, the
19 participants became aware of the CS-US contingency even though they had been
20 concentrating on the film.

1 **4. DISCUSSION**

2 We studied whether human participants concentrating on a silent film would learn TEBC
3 better if the conditioning trial was presented at the expiration phase of respiration than if it
4 was presented at the inspiration phase or at a random phase. All of the participants showed
5 consistent RSA throughout the experiment, which indicates stability of the autonomic
6 nervous system function. As a result of conditioning, all participants became aware of the
7 CS-US contingency. At the end of the training, the participants explicitly trained during
8 expiration performed more learned responses than those trained during inspiration. This
9 suggests that the respiration phase during training has an effect on the outcome of TEBC.

10 Our results can be interpreted in the light of Prokasy's (1987) theory, which states that
11 learning proceeds in two stages. In the first phase an association is formed between the CS
12 and the US and the appropriate CR is selected. In the second phase, the likelihood of
13 performing a CR increases and the CR is adjusted to maximize the adaptivity of the response.
14 It seems that in our current study the experimental manipulation affected mostly the latter
15 phase of learning. That is, once acquired, the performance of the adaptive learned response
16 was more likely during expiration than during inspiration. However, there was no significant
17 difference in learning between subjects trained at random and either of the experimental
18 groups. The fact that TEBC conducted irrespective of respiration phase resulted in learning to
19 a degree that falls between the two experimental groups can be interpreted to further
20 underline the different effects of inspiration and expiration phases of respiration on learned
21 responding.

22 Our current results support the idea that bodily rhythms such as breathing and heart rate
23 regulate cognition, possibly via their connection to rhythms of the brain. Recent studies
24 indicate respiration-driven slow electrophysiological oscillations in the hippocampus

1 (Nguyen Chi et al., 2016; Tort, et al., 2018; Zelano et al., 2016). In addition, a study in mice
2 reported that SPW-Rs (Buzsáki, 2015), the fast bursts of activity thought to mediate memory
3 consolidation into the neocortex, occur in the hippocampus more likely during expiration, but
4 only when the olfactory bulb is intact (Liu et al., 2017). The mechanisms behind these
5 respiration-related hippocampal rhythms are still unclear. However, it is known that the
6 rhythm of respiration is driven by the preBötzinger complex (preBötC) in the ventrolateral
7 medulla of the brainstem (Pilowsky & Feldman, 2001; Smith, Ellenberger, Ballanyi, Richter,
8 & Feldman, 1991). The cardiovascular function is also controlled by cells in the ventrolateral
9 medulla (Brown & Guyenet, 1985). Physiologically and anatomically, the neurons pacing the
10 respiratory and cardiovascular function are intertwined (Dergacheva, Griffioen, Neff, &
11 Mendelowitz, 2010) and could have similar effects on the overall neural state. Interestingly,
12 activation of Cdh9/Dbx1 neurons in the preBötC seems to evoke behavioral arousal whereas
13 genetic ablation of Cdh9/Dbx1 neurons has opposite effects (Yackle et al., 2017). It has been
14 suggested that activation of large neural populations in the preBötC also affects the overall
15 state of the hippocampus and other brain regions (for a review, see Del Negro, Funk, &
16 Feldman, 2018). Thus, it could be that both bodily and brain rhythms are paced by common
17 orchestrators in the brainstem.

18 It is likely that there are bidirectional connections between the limbic system and the
19 cardiorespiratory system. At any given time, the cardiorespiratory state is the outcome of the
20 net balance between the parasympathetic and sympathetic nervous system. Yet through which
21 mechanisms exactly these processes connect with the changing state of the limbic system
22 remains unclear. Although the activation of the sympathetic–parasympathetic nervous system
23 can modulate the RSA, it has been stated that respiration rate and tidal volume are only
24 affected during, for example, repetitive mental tasks and aerobic exercise, but not in a steady
25 state when an autonomic tone is constant (Grossman & Taylor, 2007; Grossman & Wientjes,

1 2001). This means that, in principle, in our current experiment, watching the silent film or the
2 TEBC itself could have affected the state of the autonomic nervous system and thus
3 respiration. However, according to our results, participants had a high level of RSA during
4 the task, which indicates a constant state of the autonomic nervous system.

5 Our current results add to findings from our previous study in which TEBC was enhanced
6 when the conditioned stimulus was timed to the diastolic phase of the cardiac cycle (Waselius
7 et al., 2018). Our previous results (Waselius et al., 2018) suggest that the brain processes
8 information differently during the diastolic and systolic phases. In short, responses to external
9 stimuli are attenuated during the diastolic phase and amplified during the systolic phase. It
10 could be that the large populations of neurons that drive the cardiovascular pace in the
11 brainstem are less active during the diastolic phase of the cardiac cycle. It is very plausible
12 that these neurons should drive the systolic phase, when the heart contracts. This could lead
13 to a state of the nervous system that is more favorable for plasticity, such as enhanced SPW-
14 R activity in the hippocampus (Buzsáki, 1989; Nokia et al., 2010). In further studies it would
15 be interesting to study how respiration phase (or other bodily rhythms) affects for example
16 neural activity in the amygdala, which also seems to play a significant role in TEBC (Büchel,
17 Dolan, Armony, & Friston, 1999; Chau & Galvez, 2012).

18 When taken as a whole, our results indicate that the state of the brain could be even more
19 optimal for learning at the diastolic phase of the cardiac cycle during expiration (Figure 4).
20 Monitoring both respiration and the cardiac cycle could be useful when trying to facilitate
21 learning.

22 4.1 Limitations

23 First, the sample size in this study is rather small, even though it is comparable with norms in
24 the field. Second, recent studies have shown that nasal breathing vs. oral breathing have

1 different effects on the limbic system (Zelano et al., 2016). While we did not explicitly
2 instruct the participants to breathe only through the nose, we excluded participants with
3 respiratory health problems such as the common cold. Furthermore, the experimenter
4 monitored the recording via video camera throughout the conditioning session. In cases of
5 excessive mouth breathing such as yawning, the experiment would have been discontinued.
6 However, we observed no such activity during the experiment.

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5

1 **FIGURE LEGENDS**

2 Figure 1 Experimental design

3 The participants were instructed to concentrate on watching a silent film. First, four air puffs
4 later used as the unconditioned stimulus (US) were presented. After this, two minutes of
5 resting data was recorded followed by five presentations of the tone-conditioned stimulus
6 (CS) alone. Then, 50 CS+US conditioning trials were presented either at inspiration,
7 expiration or a random phase of breathing. The trace period was 600 ms and the inter-trial
8 interval varied between 20 and 40 s. Two minutes of data were recorded also after the
9 conditioning procedure.

10 Figure 2. Conditioning procedure and an example of a learned response

11 A) Conditioned stimulus (CS) and unconditioned stimulus (US) were both timed either at
12 inspiration, expiration (presented in the same figure) or at a random phase of respiration
13 (Resp). B) Participants were trained in trace eyeblink conditioning (TEBC) with a 200-ms,
14 440-Hz, 66-dB tone as a CS and a 100-ms air puff as an US. The trace period between CS
15 and US was 600 ms. A typical well-timed learned eyeblink response observed from one
16 person during one trial is illustrated.

17 Figure 3. Participants trained at the expiration phase of respiration made more conditioned
18 responses at the end of the conditioning procedure compared to those trained at the
19 inspiration phase.

20 Blocks from CC1 to CC5 represent groups of 10 trials. There was insignificant spontaneous
21 blinking during tone-alone trials. Learning in the Expiration group was linear whereas in the
22 Inspiration and Random groups it was not. The Expiration group also performed better at the
23 end of the training compared to the Inspiration group. Error bars equal to standard error of

1 mean. Asterisk refers to one-way ANOVA, indicating statistically significant difference,

2 Bonferroni-corrected post-hoc comparisons, $p < 0.05$.

3 Figure 4. The large population activation in the preBötzinger complex might project to the
4 limbic system and therefore affect learning

5 We suggest that the brain is in a more favorable neural state for learning during the diastolic
6 phase of the cardiac cycle especially during the expiration phase of respiration. The lighter
7 the area is, the more favorable the timing is for plasticity, according to our suggestion.

8 Continuous line = raw ECG, dotted line = respiration.