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Research article

Respiratory modulation of intensity ratings and psychomotor response times to acoustic startle stimuli

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ABSTRACT

Respiratory interoception may play an important role in the perception of respiratory symptoms in pulmonary diseases. As the respiratory cycle affects startle eye blink responses, startle modulation may be used to assess visceral-afferent signals from the respiratory system. To ascertain the potential impact of brainstem-relayed signals on cortical processes, we investigated whether this pre-attentive respiratory modulation of startle (RMS) effect is also reflected in the modulation of higher cognitive, evaluative processing of the startle stimulus. Twenty-nine healthy volunteers received 80 acoustic startle stimuli (100 or 105 dB(A); 50 ms), which were presented at end and mid inspiration and expiration, while performing a paced breathing task (0.25 Hz). Participants first responded to the startle probes by 'as fast as possible' button pushes and then rated the perceived intensity of the stimuli. Psychomotor response time was divided into 'reaction time' (RT; from stimulus onset to home button release; represents stimulus evaluation) and 'movement time' (MT; from home button release to target button press). Intensity judgments were higher and RTs accelerated during mid expiration. No effect of respiratory cycle phase was found on eye blink responses and MTs. We conclude that respiratory cycle phase affects higher cognitive, attentional processing of startle stimuli.

1. Introduction

Interoception, the perception of bodily processes, plays an important role for mental and physical health [54]. Stimulation of receptors (i.e. interoceptors) in the viscera and afferent signal transmission from the viscera to the central nervous system represent the neurophysiological basis for interoception [22]. Chronic respiratory conditions, such as chronic obstructive pulmonary disease (COPD) [29,40,63] or asthma [9,17,18,34], are associated with alterations in signal transmission from the respiratory system and their perception, which may contribute to the development of anxiety symptoms in these patients [28,37,62]. It remains unclear, however, whether altered respiratory interoception (e.g., symptom perception) in respiratory diseases is due to organic damage (bronchial obstruction, emphysema) or to catastrophizing attribution styles associated with respiratory sensations [29]. Resolving this issue, however, necessitates a methodology that is independent of self-reports of respiratory sensations, which may be subject to cognitive biases.

Startle eye blink responses represent a psychophysiological approach to investigate visceral-afferent signal transmission associated with interoception, which is independent of active cooperation and self-reports of bodily sensations by the participant. The 'cardiac modulation of startle' (CMS) paradigm is an example for such a methodology that reflects visceral-afferent signals from the cardiovascular system. Cardiac cycle time modulates the startle responsiveness to an acoustic stimulus in that responses are attenuated during the early phase (R-wave +230 ms) compared to its late phase (R-wave +530 ms) [39,45,47–51,53]. As the CMS is largely diminished in individuals with diabetic autonomic neuropathy, intact baro-afferent neural feedback may be required for this effect [47]. Due to its quick responsiveness, it has been argued that mainly brainstem-relayed circuits (e.g., primary acoustic startle circuit, arterial baroreflex) are involved in mediating the CMS [47].

We recently showed that the respiratory cycle also modulates startle eye blink responses ('respiratory modulation of startle' – RMS) [52], which is in line with other, preliminary findings [13]. More specifically, higher startle response magnitude can be observed at mid expiration, compared to all other time points within the respiratory cycle. Afferent signals from the respiratory system may be responsible for this effect, although its exact neurophysiological mechanisms remains unclear. As this RMS effect was identical between instructions for paced vs. spontaneous breathing [52], one could conclude that the conscious

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perception of respiratory signals (mainly required for paced breathing) does not play a role for the RMS.

Although brainstem-relayed visceral-afferent signals represent a neural correlate of interoception [22,59], an intact representation of those signals in higher brain areas is required for conscious interoception. This afferent information is transmitted over the visceroceptive and the lamina I spinothalamocortical pathway, represented and integrated in brain areas, such as the thalamus, the anterior cingulate and the insula [15,16]. Irrespective of the conscious processing of respiratory signals during paced breathing, the advantage of the CMS/RMS paradigm is that the startle response as primary outcome measure is independent of self-reports on bodily sensations, which may be affected by cognitive biases. It is essential, therefore, to ascertain if afferent bodily signals only affect startle responses, which reflect mainly brainstem-mediated mechanisms, or if this outcome measure is also indicative of a higher cognitive, attentional processing of startle stimuli affected by visceral-afferent signals. With regard to CMS, this validation was successful, in that subjectively perceived intensities of the acoustic startle stimuli were lower during the early cardiac cycle phase, whereas the evaluative component (stimulus onset until home button release: 'reaction time') and motor component (home button release until target button press: 'movement time') of the response time to those stimuli were differentially affected, with the 'reaction time' being prolonged and the 'movement time' being accelerated [51].

The startle paradigm has been shown to be useful for the investigation of visceral-afferent signal transmission from the respiratory system. The present study, therefore, aims at validating the RMS effect by investigating whether this pre-attentive, putatively brainstem-mediated modulation of startle eye-blink responses at mid expiration is also reflected by a higher cognitive (attentional) processing of the startle stimuli. This would answer the question whether afferent respiratory signals that modulate startle responses also have the potential to affect behavior (i.e. psychomotor response times) or self-reports (i.e. intensity rating).

We conducted a within-subject experiment with 29 healthy volunteers, combining two earlier study protocols of CMS and RMS [51,52]. While breathing at a paced frequency of 0.25 Hz, participants received 80 acoustic startle probes, presented at four different times within their respiratory cycle: (a) at mid inspiration, (b) at end inspiration, (c) at end expiration and (d) at mid expiration. Furthermore, participants were asked to continuously press a home button until they heard a startle noise, then to release it and press a response button as fast as possible. Afterwards, they rated the intensity of the startle stimulus on a visual analog scale. Startle stimuli had an intensity of 100 or 105 dB(A) to increase variability in psychomotor response times and ratings and to validate response times as an indicator of cognitive processing of the startle stimulus. Considering the recent finding that afferent respiratory signals at mid expiration is associated with higher eye blink magnitude (RMS), we aimed to (i) replicate this finding, and hypothesized that the same afferent feedback mechanism should also result in (ii) higher intensity ratings and (iii) faster 'reaction times', as well as in (iv) slower 'movement times' within this phase of the respiratory cycle.

2. Methods

2.1. Participants

Thirty healthy university-students (twenty-five women) participated in the study. The sample size was identical with a previous study investigating self-report intensity and response time assessment modulated by cardiac cycle phases with 25 participants [51], including an over-recruitment to counter-act the effects of potential drop-outs of approx. 20% [8]. Due to technical malfunction, data of one participant was lost, whereas none of the participant was regarded as non-responder, resulting in a final sample of 29 participants (24 females; mean age: 22.6 [SD = 2.6] years; mean BMI: 21.3 [SD = 2.3] kg/m^2). Participations was reimbursed with a € 10.- gift voucher and course credits. Physical health status was assessed by a customized interview. Exclusion criteria were hearing problems (impairments, tinnitus), any actual health complaints, use of illicit drugs within the last six months, alcohol abuse, medication other than occasional pain killers and oral contraceptives, confirmed physical (especially pulmonary) or mental disorders within the last six months, pregnancy or cutaneous allergies. All participants gave their written informed consent and were made aware of their right to discontinue participation in the study at any time. Ethics approval was sought from a local ethics committee at the University of Luxembourg.

2.2. Stimulus intensity scores

Perceived stimulus intensity was assessed by an Electronic Visual Analog Scale (EVAS). This scale was presented based on pixel positions on an LCD-screen. Intensity ratings were given by clicking on the respective position on the scale with the computer mouse. The anchoring adjectives were 'low' and 'high'. Response resolution of the EVAS device was 960-points. Previous studies confirmed the validity of electronic VAS devices in judging the intensity of startle stimuli [51,57]. We standardized the judgments by the scale resolution and thus provide % EVAS scores.

2.3. Technical parameters

Physiological data were collected using a Biopac® MP150 system (Biopac Systems, Inc.) with 16-bit resolution and a sampling rate of 1 kHz. Electromyogram (EMG) startle responses were recorded with Kendall Arbo H124SG electrodes, one placed 0.5 cm below the left eyelid in a vertical line with the pupil when looking straight ahead, and another one lateral at a distance of 1.5 cm to assess electrical activity of the Musculus orbicularis oculi [8]. Startle stimuli consisted of acoustic white noise probes (intensities: 100 and 105 dB(A), 50 ms duration, instantaneous rise time, binaural presentation). Hardware band-pass filter settings (Biopac EMG100C) were 10–500 Hz, followed by online software filtering (28 Hz high-pass filter) [60]. The raw signal was rectified and integrated with a time constant of 10 ms [6]. Electrodes for electrocardiogram (ECG) recording (ECG Tyco Healthcare H34SG Ag/AgCl electrodes of 45 mm diameter) were placed on the thorax according to a standard lead II configuration. Respiratory activity was measured with a respiratory belt (Biopac Systems, Inc.) placed between the fifth and eighth ribs on the skin. The signal was band-pass filtered (0.05–10 Hz) before it was digitized. Psychophysiological signals were manually inspected for data quality before recording. Breathing phases were identified online by a DASYLAB-8.0 (National Instruments, Inc.) algorithm, based on pattern detection. For each participant and each of the four respiratory time points, an individual reference template pattern was defined from a sequence of individual breathing cycles [52]. When the difference between the current respiratory signal and the reference template pattern crosses a fixed threshold and a local minimum of this function occurs, the pattern is considered as 'detected' and a startle stimulus is presented. Offline manual evaluation showed that 15% of the breathing patterns were not accurately detected, leading to an omission of startle stimulation in the respective trial. In this case, the stimulus was presented in the next trial, in which the pattern was detected (usually the subsequent trial). No false positive detection of a breathing phase occurred.

2.4. Response times data collection

A home button and a response button were located on an E-Prime Serial Response Box (PST Software, Inc.) with a distance of 1 cm between buttons. While the middle key of the response box was always defined as the home button, the response button was located right of the home button. Participants were instructed to keep the home button

pressed throughout the experiment, except for responding to a startle stimulus by pressing the response button. As in previous studies [20,21,25,35], the time from onset of stimulus presentation to releasing the home button was defined as 'reaction time' (RT), representing the pre-motor component of the response time, i.e. the cognitive processing of the response stimulus. The interval between releasing the home button and pressing the response button was defined as 'movement time' (MT), representing the motor component of the response time. The RT-component was interpreted as a measure of cognitive processing speed [20], and the MT-component as the speed of sensorimotor processes [21]. Although 'reaction times' as defined by Doucet and Stelmack are not identical with pre-motor reaction times as based on the onset of an EMG response of the limb, strong correlations with latencies in psychophysiological signals [20,32] suggest a substantial overlap between both. Due to the skewed distribution of response times median values were calculated for each condition for each individual participant and included in the statistical model. Means of medians across participants are reported in the results section.

2.5. Procedure

Participants were seated in a comfortable chair in front of a LCD computer screen. Glasses were removed, and electrodes for ECG-measurement and EMG electrodes were attached to the chest and below the left eye, respectively. Participants were then allowed to put their glasses back on. A respiratory belt was placed around the thorax, and headphones (Sennheiser Electronic GmbH & Co. KG, Wedemark, Germany) were attached. Participants were informed about the experimental procedure on the computer display. They were asked to relax, to neither speak nor move, to avoid longer periods of eye closure, and to listen carefully to all acoustic stimuli.

After a 5-min resting phase at the beginning of the experimental session, six startle probes of 105 dB(A) intensity without any contingence to the participants' breathing phases served as habituation trials. Participants neither had to respond to nor to judge the intensity of these acoustic stimuli, and EMG-responses on these trials were not further analyzed.

To create variability in intensity ratings, acoustic startle-stimuli were presented at two intensities of either 100 or 105 dB(A), which reliably produce startle responses in a yet unpublished study [55] and were previously used in studies addressing subjective intensity of startle [58]. Startle stimuli were presented at a defined time point during each of the four different phases within the respiratory cycle: (a) at end of expiration (minimal volume based on breathing belt), (b) at midpoint of on-going inspiration, (c) at end of inspiration (maximal volume) and (d) at midpoint of on-going expiration [52]. During each of these 8 conditions (2 intensities × 4 respiratory phases), 10 startle stimuli were presented, resulting in 80 stimuli in total. One trial of each of the eight conditions was presented in a randomized order before replacement. Participants received computer-controlled verbal instructions throughout the experiment to inhale ('in') and exhale ('out') over headphones, guiding them to breathe at a frequency of 0.25 Hz with an inspiration-expiration ratio of 2:3. This frequency was selected because it ensures comparable systolic blood pressure between mid inspiration and mid expiration phases [52]. During the experimental condition, breathing frequency of the participants was M = 0.25 Hz (SD = 0.01 Hz). During startle presentation, this auditory stimulation had to be interrupted without affecting the participants' breathing rhythm, since the auditory instructions could otherwise act as pre-pulses to the processing of the startle stimulus [5]. The experimental condition was, therefore, subdivided into trials consisting of five respiratory cycles each. During the first three cycles, participants were verbally instructed to breathe, while they saw a green light on the LCD screen. During cycles four and five, they were asked to continue breathing in the paced rhythm without verbal instructions. This part of the trial was marked by a blue light on the screen. Participants were instructed to keep pressing the home button of the response box with the index finger of their dominant hand. All startle stimuli appeared during the fifth respiratory cycle if the respective respiratory event was detected (for details: see [52]), implying that the minimal time period from last verbal instruction to the startle stimulus onset was 4 s. When hearing a startle stimulus, participants were asked to respond as fast as possible by releasing the home button and pressing the response button with the same finger. After each stimulus, they were asked to indicate the perceived noise intensity by the question 'Please indicate the perceived intensity of the sound by clicking on the corresponding position on the scale below' with the two anchors 'low' and 'high'. The experimental session, including instructions, lasted for approx. 60 min.

2.6. Analysis of physiological data

A customized C++ based semi-automated PC program was used to analyze EMG-responses. The algorithm identified response peaks in the rectified and integrated signal during a time interval of 20 to 150 ms after the startle probe onset. The baseline period was defined by a 50 ms interval prior to acoustic stimulation. All response data were inspected manually after algorithmic detection. Signals with electrical and physiological artifacts, such as coinciding blinks or other facial muscular activities, which introduced noise to the baseline period, were rejected from analysis and defined as missing. The percentage of invalid trials ranged from 3.8 to 7.6% per condition (M = 5.3%). If a participant's responses were not visible (zero amplitude) at their typical response latency, response amplitude was set to zero. Zero response data were included in the averaging procedure, with startle response magnitude as the final output measure [8]. The minimal number of visible responses per cell was two. Averaging was done within-participant, calculated separately for each startle stimulus intensity and each respiratory phase. For each participant, startle response magnitudes were T-scored over all four respiratory phases and both intensity conditions. Respiratory cycles were automatically detected with WinCPRS software (Absolute Aliens Oy, Turku, Finland) and manually confirmed, from which breathing frequency data was derived.

2.7. Statistical analysis

A 2 × 4 ANOVA with repeated measurement was employed for each dependent variable with the within-subject factors 'startle stimulus intensity' (100 and 105 dB(A)) and 'respiratory cycle phase' (mid inspiration, end inspiration, mid expiration, end expiration). Dependent variables were (i) T-scored startle response magnitude, (ii) RT to the startle stimulus, (iii) MT and (iv) stimulus intensity ratings. Critical α-level was set to 0.05. If the assumption of sphericity was violated, Greenhouse–Geisser corrected p and df values are reported. We calculated simple contrasts as post-hoc comparisons, with 'mid expiration' as reference category, as it has previously been shown that this particular respiratory phase significantly modulates startle eye blink responses compared with the other phases [52]. All statistics were conducted with SPSS 23.0 (SPSS, Inc.).

3. Results

3.1. EMG startle response magnitude

ANOVA of startle response magnitude revealed a significant main effect for 'startle stimulus intensity' (see Fig. 1;F [1,28] = 13.86; p = 0.0009; η^2 = 0.33). Startle response magnitude was higher in the condition of '105 dB(A)' compared to the condition of '100 dB(A)' intensity. There was neither a significant main effect for 'respiratory cycle phase' (F[3,84] = 0.56; $p > 0.10$) nor a significant 'startle stimulus intensity' × 'respiratory cycle phase' interaction (F [3,84] = 0.85; $p > 0.10$).

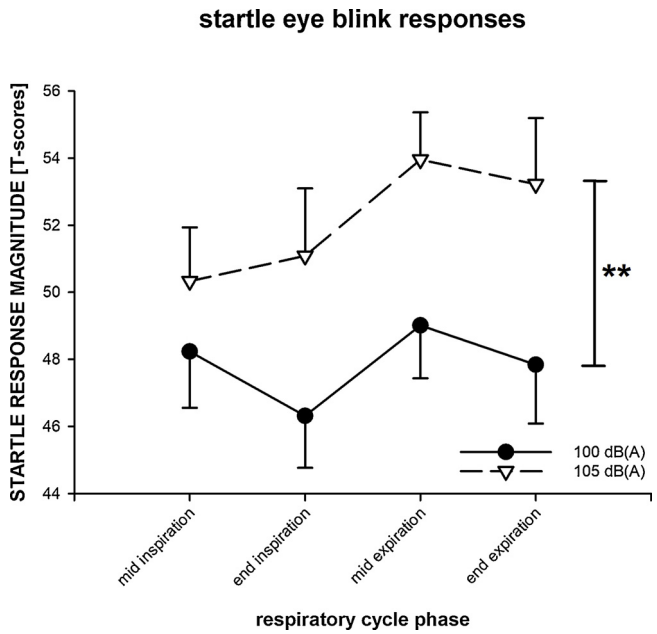

Fig. 1. The startle eye blink responses assessed by unilateral EMG for all four time points within the respiratory cycle. Only the main effect of stimulus intensity is significant ($p < 0.01$). T-scores are reported. Error bars represent SEM.

3.2. Perceived intensity

There was a main effect for 'startle stimulus intensity' on intensity ratings (F [1,28] = 35.05; p = 0.000002; η^2 = 0.56). Furthermore, we observed a significant main effect for 'respiratory cycle phase' (F [3,84] = 3.44; p = 0.03; η^2 = 0.11). A significant within-subject contrast (F [1,28] = 7.46; p = 0.01; η^2 = 0.21) between end inspiration and mid expiration indicated a higher rating of startle stimulus intensity during the latter (see Fig. 2). No other significant differences were observed. There was no significant interaction of 'startle stimulus intensity' and 'respiratory cycle phase' (F[3,84] = 2.16; p = 0.10).

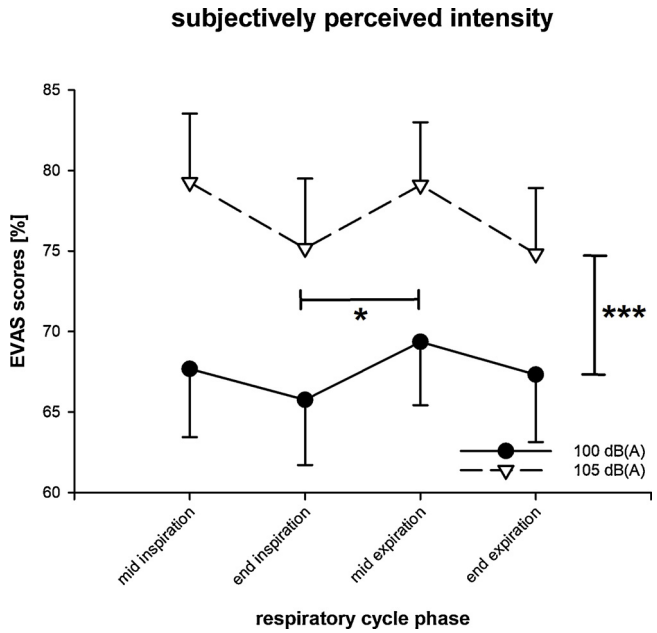

Fig. 2. Subjectively reported stimulus intensity scores. Both the main effects of stimulus intensity ($p < 0.001$), as well as respiratory cycle phase ($p < 0.05$) are significant, indicating a higher rated intensity at mid expiration compared to end inspiration. Error bars represent SEM.

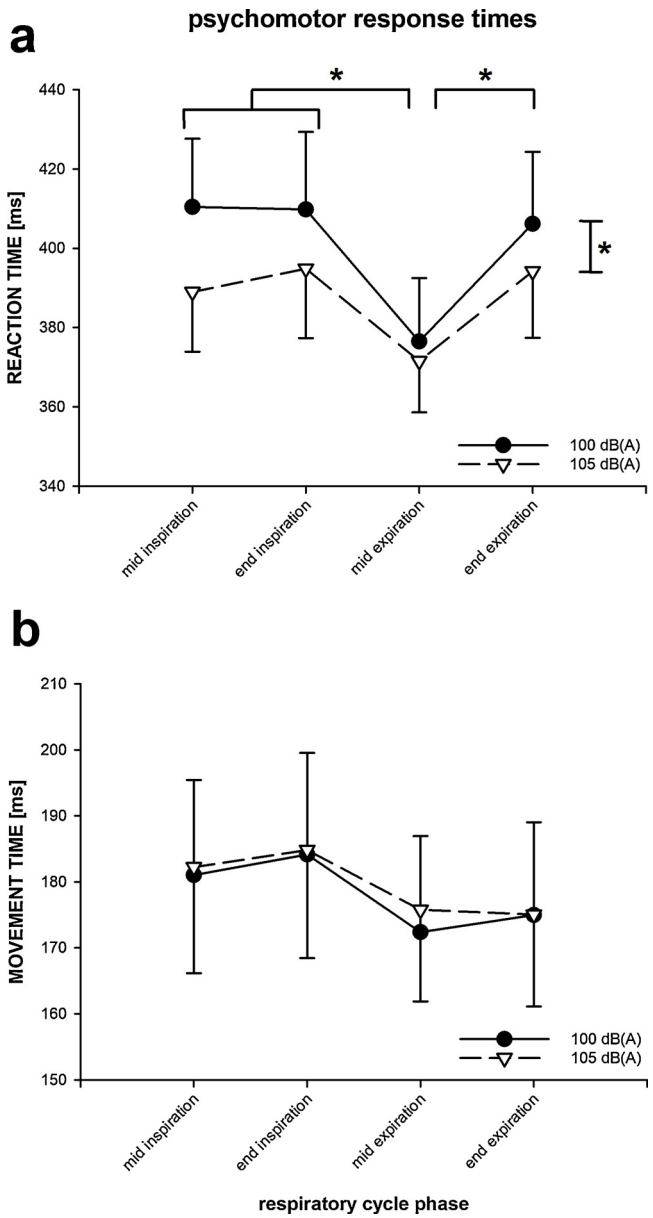

Fig. 3. The total response time is divided into evaluative component (a); 'reaction time': RT) and motor component (b); 'movement time': MT). Means of medians are reported. Error bars represent SEM.

3.3. Reaction time

The ANOVA of the central, evaluative, component RT revealed a significant main effect for 'stimulus intensity' (F [1,28] = 6.69; p = 0.02; η^2 = 0.19). RT was shorter at a startle stimulus intensity of '105 dB(A)', compared to the '100 dB(A)' stimulus intensity. Furthermore, there was a significant main effect for 'respiratory cycle phase' (F [3,84] = 3.25; p = 0.03; η^2 = 0.10). RT was significantly shorter during mid expiration compared to mid inspiration (F [1,28] = 5.20; p = 0.03; η^2 = 0.16), end inspiration (F [1,28] = 8.88; p = 0.006; η^2 = 0.24) and end expiration (F [1,28] = 8.87; p = 0.006; η^2 = 0.24; see Fig. 3). No other significant differences were observed. There was no significant interaction of 'stimulus intensity' and 'respiratory cycle phase' (F[2.16,58.71] = 0.45; $p > 0.10$).

3.4. Movement time

For the analysis of MT, the data of three further participants had to be excluded, due to a technical problem with the response button (whereas the home button and, therefore, the 'reaction times' were unaffected). Neither were there main effects for 'startle stimulus intensity' (F [1,25] = 0.18; $p > 0.10$) or for 'respiratory cycle phase' (F [1.64, 40.92] = 0.67; $p > 0.10$; see Fig. 3), nor was there a significant interaction between 'stimulus intensity' and 'respiratory cycle phase' (F [3,75] = 0.11; $p > 0.10$).

4. Discussion

This is the first study to demonstrate that self-report intensity of and reaction times to startle stimuli are affected by the respiratory cycle phase. In partial accordance with hypothesis (ii), we found higher intensity ratings when startle probes were presented at mid expiration compared to end inspiration. In addition, the analysis of response times to the startle stimuli showed a shorter pre-motor component of the response time (RT) at mid expiration compared to all other phases of the respiratory cycle (iii). This may imply that the cognitive processing of the startle stimulus is affected by the respiratory cycle.

One possible explanation (a) may be that the previously established RMS effect is associated with visceral-afferent signals from the respiratory system [52]. The specificity of the effect of respiratory phases on startle [52], intensity ratings and RTs (this study) to the mid expiration phase supports the assumption that receptors sensitive for transient changes (phasic receptors) may be involved in mediating this effect, such as slowly adapting pulmonary stretch receptors. As lower baro-afferent signal transmission is associated with higher startle responses [47], unloading of phasic respiratory receptors may also facilitate the central representation and processing of incoming startle stimuli. We previously found a similar concordance between lower startle responses and lower perceived intensity during the early compared to the late cardiac cycle phase for the CMS effect [51]. We would argue, therefore, that bottom-up transmission of afferent cardiac and respiratory signals affects the perceived intensity of auditory signals. This may be due to a lower competition-of-cues during this respiratory phase [41,42], i.e. lower respiratory phasic receptor input would release resources to process the startle stimulus and execute the motor response. If afferent respiratory signals are responsible for this effect, it remains for future studies to investigate if respiratory modulation of startle is associated with conscious respiratory interoception.

The results show shortened RTs to the startle stimuli at mid expiration. Hence, the central evaluation of the startle stimulus can be executed more quickly and potentially affected less by visceral-afferent signals, which supports previous research that found shorter response times during the late phases of the cardiac cycle, particularly regarding RTs [4,23].

A second explanation (b) concerns the potential involvement of afferent information from respiratory muscles. At mid inspiration, increased activity of inspiratory neurons induce a contraction of inspiratory muscles, such as the diaphragm, intercostal and abdominal muscles, whereas expiratory neurons silencing the inspiratory neurons make the inspiratory muscles relax again during expiration [3]. Reduced feedback from respiratory muscles, therefore, may also modulate the processing of the startle stimulus at mid expiration.

A third possible explanation (c) may lie in the consciously controlled efferent motor code generation. Spontaneous breathing rhythms are mostly automatically generated by brainstem centers in the pons, rostral and ventral medulla (e.g., pre-Bötzinger and Bötzinger complex) [38,44], whereas in paced breathing higher cortical structures (e.g., supplementary motor area, cortico-subcortical network) exert control over these centers [14,30]. At rest, the inspiratory phase is considered the 'active' phase of respiration, whereas the expiration is seen as a more 'passive' phase [43]. In our study, the reduced control of cortical structures over medullary centers in this 'passive' mid expiration phase may provide more resources to process the startle stimulus, resulting in increased subjective intensity and faster RTs. Furthermore, a lower potential for distraction and more focused attention on respiratory signals during the 'active' and the 'passive' breathing phase could also have contributed to this effect.

Startle eye blink was substantially higher in response to the stronger stimulus intensity, as previously reported [7,65]. In addition, higher stimulus intensity (105 dB(A)) resulted in higher intensity ratings of startle stimuli and accelerated RT [12,51].

In contrast to previous findings [13,52], however, there was no significant modulation of startle response magnitude as a function of respiratory cycle phase (i). As we aimed at investigating the impact of RMS on higher cognitive processing, we aligned our sample size to the study of Schulz et al. [51]. First, the differences in sample size (42 in the previous RMS study vs. 29 in the present study) may have contributed to the current null findings concerning effects in startle response magnitudes, although descriptively a similar pattern emerged as in the previous RMS study. Second, the fact that we presented 80 stimuli of 105 dB(A) in the previous study, but only 40 stimuli of 105 dB(A) in the current study may have accounted for discrepant findings. Third, the study design of Schulz et al. [52] did not include additional response time and intensity ratings, both requiring attentional resources allocated towards the auditory modality (and potentially away from visceral sensations). Increased attention may prompt startle response modulation [10]. For example, it may well be that the RMS effect requires attention allocated towards respiratory sensations, which was overridden by the instruction to focus on auditory stimuli. Another candidate mechanism could be higher cortical excitability induced by the preparation for elicitation of a motor response [56]. Higher respiratory-related evoked potentials, another neurophysiological indicator for afferent signals from the respiratory system, are increased when attention is focused towards respiratory sensations [64], suggesting the potential for a top-down modulation of processing of afferent respiratory signals. Fourth, although we presented approx. 15% catch trials, in which no startle stimulation occurred, we cannot rule out that an anticipation effect may have reduced the total startle responses [24] and overridden the RMS effect, given that all startle stimuli occurred in the fifth respiratory cycle. This scenario, however, seems to be unlikely, as in our previous study we demonstrated that there was no difference in RMS between the current paced breathing and a spontaneous breathing condition [52].

Finally, we did not observe any effect of the respiratory cycle phase on MT (hypothesis iv). As a simple motor reaction in response to an eliciting stimulus requires less cognitive load and its execution may not be affected in the conflicting presence of visceral-afferent input [51], this may have led to comparable MTs in all the time points of the respiratory cycle.

Previous studies on psychomotor response times yielded mixed findings including faster reaction times during inspiration [2,25,26,31] and faster reaction times during expiration [11,27]. There are, however, important methodological differences between these studies and the current investigation. First, the methodology of pattern detection in this study allows for a more precise definition of respiratory events than realized in previous studies, in which manual elicitations of stimulus presentation or post-hoc assignments into different respiratory phases were used. This algorithm is also suitable to detect respiratory phases in airflow assessment. Second, none of the previous studies investigated responses to startle stimuli, which may account for stronger effects in psychomotor response times. Third, the separation into evaluative and motor response time components (RT and MT) represents a novel aspect, which suggests that the evaluative component accounts for effects of the respiratory cycle on total psychomotor response times.

In contrast to previous findings [51], MTs were not affected by the startle stimulus intensity. In Schulz et al. [51] faster MTs were only found when comparing the intensity condition of 105 dB(A) to that of

85 dB(A). In the current study, we selected startle stimuli intensities, which would not be easily distinguishable (100 dB(A) and 105 dB(A)), to prevent participants from noticing that only two different intensities were presented. Nevertheless, the minor differences between intensities might explain why there was no effect of stimulus intensity on 'movement time'.

Our previous results suggest that startle responses, a mainly pre-attentive mechanism [36], are modulated by the respiratory cycle [52], potentially by afferent respiratory signals. The current findings indicate that the respiratory cycle also affects higher cortical processing of startle stimuli. We would argue, therefore, that the RMS is not only suitable to reflect the impact of afferent respiratory signals at brainstem level (startle eye blink responses), but also at higher, cortical levels (as indicated by response times and intensity ratings). It may serve, therefore, as an indirect indicator of afferent respiratory signals processed at a cortical level independent of self-reports of bodily sensations. The same conclusion is possible for the CMS, as cardiac cycle phases also affect eye blink responses, perceived intensity, and psychomotor response times [51].

5. Limitations

A volume-based assessment may have a higher timely precision in assessing respiratory flow and is less susceptible to artifacts. Notwithstanding, we decided against this approach, as these techniques require breathing through mouthpieces and may, therefore, be associated with uncommon or unpleasant sensations for the participants, which may interfere with startle responses [1]. The sample size was in line with previous research on cardiac modulation effects on perceived intensity and psychomotor response times. Yet, it may have been too small to replicate the RMS effect. Further, the uneven sex distribution across the sample (predominantly female participants) may compromise the generalizability of these findings. Unfortunately, the sample was not large enough to test for sex differences in a systematic manner. Nevertheless, in previous research, startle modulation by visceral-afferent signals was reliably observed in samples with a similar age- and sex-distribution [45,47,48,50–52]. These issues should be considered in possible replications or extensions of the present study. Moreover, we only investigated healthy participants. As neuropsychological alterations, which concern cognitive capacities (e.g. attention, psychomotor pace, accuracy) are associated with pulmonary diseases such as COPD [19,33,46], it is mandatory to test individuals with pulmonary disorders of different degrees of severity. Investigating whether their specific deficits (e.g., in cognitive capacities, respiratory physiology and mechanics) [61] influences the effect of respiratory cycle phase on startle eye blink responses may reveal the neurophysiology behind the RMS. Currently, conclusions on potentially involved neurophysiological mechanisms have to be drawn with caution.

6. Conclusion

Respiratory cycle phase has been shown previously to modulate eye blink responses to startle stimuli (RMS). In the current study, this modulating effect of respiratory cycle phase was reflected in higher perceived stimuli intensities at mid expiration and in accelerated evaluative 'reaction times' (RT) to the startle probes in this particular cycle phase. Hence, respiratory cycle not only affects the pre-attentive modulation of startle responses, but also higher cognitive, attentive processing of acoustic startle stimuli.

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