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Body-Brain interactions in visual perception

VOLUME 1

Thesis submitted to the University of Coimbra in fulfilment of the requirements of the Master's Degree in Physics Engineering under the scientific supervision of Ph. D. Maria José Ribeiro and Ph. D. Marco Simões and presented to the Physics Department of the Faculty of Sciences and Technology of the University of Coimbra

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Supervisors:

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Ph.D. Marco Simões

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To my parents, thank you for the opportunity to pursue a higher education and, in a more general way, thank you for pretty much everything. Obviously, to the rest of my family, as well, - especially my sisters and grandparents - I am thankful for all the love, affection and knowledge you have always shared with me.

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Resumo

A respiração é mais do que simplesmente um sinal vital. Está profundamente ligada à atividade cerebral, processamento sensorial e função cognitiva. Estudos sobre a sincronia entre as oscilações cerebrais e a respiração sugerem que a atividade respiratória é modulada para melhorar a perceção. Nesta dissertação, propus-me a estudar a interação entre a atividade respiratória e a perceção visual numa tentativa de compreender melhor tais interações. Para isso, desenhei uma tarefa de discriminação visual onde os participantes distinguiam entre duas categorias de estímulos e um aviso sonoro soava alguns segundos antes da apresentação do estímulo visual. As imagens dos estímulos visuais foram apresentadas por um período de tempo muito breve e imediatamente seguidas de uma máscara, utilizando um método de *visual backward masking*, para que os estímulos fossem difíceis de identificar, exigindo uma grande concentração. Durante a recolha dos dados, medimos vários sinais fisiológicos, incluindo, obviamente, a respiração. Conjeturei que: a atividade respiratória é modulada pelo aviso sonoro e o estímulo visual; essa modulação ocorre no sentido de melhorar o processamento visual; a fase da respiração no instante de apresentação dos estímulos modula o desempenho na tarefa. Os meus resultados revelaram que a duração do ciclo respiratório foi modulada pela apresentação dos estímulos visuais (a respiração abrandou após a apresentação do estímulo visual), mas não foi modulada pelo aviso sonoro. No entanto, a fase do ciclo respiratório no momento do estímulo não estava relacionada com a modulação da duração do ciclo respiratório nem com o desempenho na tarefa. Além disso, observei que períodos de respiração mais acelerada durante o aviso sonoro estão associados a um melhor desempenho. Estes resultados sugerem que: os participantes tendem a abrandar a respiração com a exibição de estímulos visuais, talvez para melhorar o processamento visual; uma respiração mais rápida no som de alerta poderá melhorar o desempenho. De forma geral, as conclusões a que cheguei confirmam que a respiração é modulada durante tarefas

cognitivas e oferecem uma base para investigações futuras.

Abstract

Respiration is much more than a vital sign of life. It is deeply connected to brain activity, sensory processing and cognitive function. Studies regarding the synchrony of brain oscillations with respiration have suggested that breathing activity is modulated in order to improve perception. In this dissertation, I aimed to study the interaction between breathing activity and visual perception in an attempt to better understand these interactions. I designed a visual discrimination task where participants had to distinguish between two categories of stimuli, with an auditory warning cue sounding a few seconds before visual stimulus display. The target stimuli images were presented for a very brief period of time and immediately followed by a mask, using a visual backward masking technique, so that the stimuli were hard to identify, requiring a great level of concentration. During data acquisition, we measured several physiological signals, including, obviously, respiration. I hypothesized that: breathing activity is modulated by attentive anticipation elicited by the warning cue and by visual stimuli presentation; this modulation happens in a way that enhances visual processing; breathing phase at the onset of the visual stimuli modulates visual discrimination abilities. My results revealed that the duration of breathing cycle was modulated by the presentation of the visual stimuli (breathing slowed down after visual stimulus presentation) but was not modulated by the warning cue. Phase of breathing cycle at stimulus onset did not affect modulation of breathing cycle duration nor did it predict task performance. Besides this, I found that a faster breathing rate at the time of the auditory cue was associated with a better accuracy. I concluded that: participants tend to decelerate respiration with visual target display, perhaps in order to improve visual processing; a faster respiration during the pre-stimulus period might enhance accuracy. Overall, these results confirm that breathing is modulated during cognitive tasks and can be the blueprint for future investigations.

List of Acronyms

EDA electrodermal activity

EEG electroencephalogram

EGG electrogastrography

EKG electrocardiogram

EMG electromyography

EOG electrooculography

PPG photo plethysmogram

'NaN' not a number

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Chapter 1

Introduction

Breathing is the process we use to exchange gas with the external environment, by moving oxygen into our lungs and flushing out carbon dioxide. In the lungs, this gas exchange occurs in the alveoli through diffusion, and the circulatory system then transports these gases to and from the cells [1, 2].

This process repeats itself in cycles of inhalation (air entering the lungs) and exhalation (air leaving the lungs) [3]. Our breathing rate - number of respiratory cycles per minute - is a primary vital sign of life [4].

Under normal conditions, breathing activity is controlled automatically and unconsciously by homeostatic mechanisms, maintaining the partial pressures of carbon dioxide and oxygen in the arterial blood constant [5].

Breathing, however, is much more than an exchange of gases and plays a role in other important functions - it contributes as a mechanism for laughter, speech, yawning, sneezing, coughing, etc [6–9].

In certain conditions, for example at an extreme altitude (low air pressure) or depth (elevated air pressure), the process of breathing is adjusted in order to maintain acceptable levels of oxygen in the bloodstream [10, 11].

Breathing activity can also be related to certain moods - for example, a slower breathing rhythm can encourage relaxation [12, 13]. The same happens during the practise of physical activities - a deeper breathing pattern might be adopted to facilitate greater oxygen absorption and strengthen the body's core [14].

The several ways in which respiration influences our mind and body states are fascinating. In fact, breathing activity might be even more deeply connected to our actions and perception of the outside world than one might think. In my investigation for this dissertation, I attempted to observe and explain some of this connections that run deep in our brains, at a perceptual level.

In this chapter, the context and motivation of the thesis will be explained as well as the outline for the rest of document.

1.1 Contextualisation and Motivation

Usually, in our day-to-day life, we are always moving – we sway our body, we rock our feet, twitch our faces. We are hardly ever still. However, when we are paying attention to any sort of stimulus, there is a tendency to stop all of this activity [15], [16]. In fact, effects such as motor inhibition (behavioural freezing), cardiac deceleration and pupil dilation arise from focusing the mind in anticipation of external events [16–19]. These shifts in body physiology are related to a faster and more sensitive sensorimotor procession. In fact, it is known that cardiac activity, breathing and motor output affect sensory processing, for example:

- Synchronization of visual stimulation with the heartbeat modulates perception [20], [21];
- Accuracy in visuospatial tasks is higher if the stimuli are presented during inhalation in comparison with exhalation [22];
- In mice, spontaneous behaviours modulate activity in the visual cortex [23].

Bodily functions as ongoing **spontaneous behaviours** (blinking, eye movements, twitching, postural adjustments, body sways) and **visceral activity** are inevitable sources of neuronal variability. They modulate sensory perception and need to be considered in order to better understand the interactions between brain activity and behaviour.

Since perception is modulated by these functions (spontaneous behaviours and visceral activity), then cognitive processes that work to optimize sensory processing (like attention) might modulate these bodily functions accordingly. In fact, these modulations are observed during attentive anticipation.

Regarding visceral signals, these can modulate neural activity, perception, memory, decision processes and behaviour [22, 24]. In the same way that cardiac phase modulates sensory perception, emotional processing and action control - which suggests the existence of brain state fluctuations locked to cardiac cycle [25] -, breathing has also been shown to modulate perception, finding higher visuospatial abilities and visual-evoked potentials during inhalation rather than during exhalation [22].

It is also important to understand the relevance of **endogenous attention** (or top-down attention). Endogenous attention refers to the voluntary allocation of attention to a certain object. For example, one can decide either to pay attention to a certain region of space (e.g. the centre of a screen) or to colored items [26]. Both cases are examples of endogenous attention - the first of spatial attention and the latter of feature attention [27, 28]. On the other hand, attention is not only voluntarily directed. Attention can be switched to salient external stimuli even when the subject has no intentions of attending to them [29–31]. For example, regarding visual processing, someone may be focusing on items of a specific color, but their attention may be unconsciously drawn if an object of another color appears suddenly.

Endogenous attention modulates neural activity in a way that increases the processing efficiency of the attended feature. This modulation involves the reduction of neuronal noise and enhancement of sensitivity in the neurons 'targeting' the attended object [32]. Several neural mechanisms have been proposed underlying noise reduction during attentive states, e.g., enhanced inhibitory transmission [32]. The modulation of visceral activity and spontaneous behaviours could work as a complementary mechanism for 'noise' reduction, in order to minimize sources of internal variability. In fact, attention might do just that: for example, somatosensory perception is enhanced during the diastole phase of the cardiac cycle [33] and cardiac deceleration increases the proportion of the cycle that is devoted to the diastole phase. This could be one of the mechanisms through which cardiac deceleration enhances sensory processing.

However, it still remains to be clarified how motor inhibition and modulation of visceral signals affect sensory processing in the brain in a controlled scenario as a laboratory computerized experimental task.

In order to analyze this effect and attempt to push forward this constraint in cognitive neuroscience research, we measured body and eye movements together with brain, cardiac

and stomach electrical activity, breathing, and arousal signals, while participants were engaged in a computerized visual discrimination task.

This thesis is part of a bigger study that intends to investigate several of these processes. The work developed here arises from the interest of understanding how these mechanisms work and will focus on the interaction between **breathing activity** and **visual processing**. For that reason, from here on I will focus more deeply on respiration.

1.1.1 Breathing-related effects in behaviour and sensory-cognitive perception

The effects that breathing-related activity has in cognition and behaviour have been a topic of interest for decades. Therefore, before diving into the work developed in my investigation, it is important to take into consideration some of the work that has already been made in this field.

Since the second half of the 20th century, breathing activity has been studied along with sensory perception. By then, it was found that the breathing phase has an impact on visual signal detection and that signals presented during exhalation were detected more frequently than those presented during inhalation [34]. In fact, Obrist et al. [35] reported that, in order to attenuate task-irrelevant activity during and before task response, respiration slows down, leading to an exhalation period larger than inhalation, which makes sense, since there is less neurological activity during exhalation, as found by Crosby et al. [36]

In fact, breathing activity even plays an important role within the central nervous system. For example, when we sigh, this action works to monitor brain state changes, control arousal and regulate breathing variability, as well as emotions [37]. This is relevant in stressful and challenging situations, which can be the case of a demanding visual task, where attentive anticipation plays a key role. For example, the expectation of a stimulus leads to a deceleration in heart rate that is more pronounced for unconfident decisions [38]. It would be very interesting to see if this effect occurs with respiration.

The purpose of respiration goes beyond the simple exchange of gases that keeps humans alive. There are, indeed, deep and intrinsic relations between respiratory and brain activities: electrical oscillations in the piriform cortex (related to olfactory processes), as well as in the amygdala and hippocampus, fall into synchrony with breathing activity - in phase with the natural rhythm of the breathing cycle - and the intensity of this linkage is higher during

inhalation and less notable when breathing through the mouth instead of nose, underlining the importance of breathing phase and airway in these processes [39]. In fact, regarding visuospatial perception, nasal inhalation has been found to increase brain activity in specific, task-related, regions and improve performance accuracy in a visuospatial task [22].

For a long time, the neuronal oscillations mentioned above were thought to be intrinsic noise that introduces variability in neuronal processing [40]. However, they play a fundamental role in driving neuronal activity [41], forming highly organized patterns that modulate neuronal responses [42–44] and act on sensory perception [45–49].

It has now been observed that cortical activity is phase-locked to respiration and is synchronized by it. Besides, some of this cortical activity (gamma oscillation power and phase transition timing) is involved in cognitive function, which further suggests the association between breathing activity and cognitive processes [50].

Furthermore, the synchrony of these brain oscillations is influenced by the rhythm of the breathing cycle, in a way that adjusts efficiency through neural excitability: the breathing pattern is spontaneously adapted so that inhalation occurs at onsets of cognitive tasks and visual stimuli presented during inhalation evoke stronger neural responses than when presented during exhalation, resulting in a better performance in visuospatial paradigms during inhalation compared to exhalation [22].

In sensory-cognitive paradigms, there is a tendency to align the breathing cycle with the experimental task [51]. Interestingly, Grund et al. [38], noted that participants in a tactile detection task adapted their respiratory cycle to expected stimulus onsets to preferentially occur during late inspiration/early expiration and that the detection rate was highest during the first quadrant after expiration onset. In [51], however, response accuracy did not vary with the breathing cycle as much as reaction times.

By now, it has been observed that respiration modulates perceptual sensitivity, and some suggest that, through respiration, sensory information is aligned with cycles of enhanced excitability in order to facilitate performance [52].

Still, some doubts persist regarding the mechanisms by which this modulation influences cognition, perception and behaviour [53]. Indeed, results found in these investigations have supported the connection between respiration and sensory-cognitive function, hinting that sensation is inherently connected to bodily functions [51].

In this work, I will not be taking on the analysis of brain activity, but I will investigate the

effects between breathing and visual perception and attempt to reach some indications that might explain the presence of such effects. The purpose of this dissertation is to verify and support the discoveries that have been made and attempt to examine further the relation between respiratory phase and performance in a visual detection task.

1.2 Objectives

In accordance with the original study's purpose of researching a variety of body-brain interactions, I acquired, in collaboration with my supervisor, several bodily functions during task performance (even though I only investigated breathing activity):

- brain signal - electroencephalogram (EEG);
- ocular movements and blinking detection - electrooculography (EOG) ;
- eye movements and pupilogram - eye tracker;
- respiration;
- muscle activity - electromyography (EMG);
- cardiac signal - electrocardiogram (EKG) and photo plethysmogram (PPG) ;
- skin conductance - electrodermal activity (EDA);
- stomach activity - electrogastrography (EGG).

To investigate the study hypotheses, I designed a visual discrimination task in which participants engaged while I acquired the aforementioned behavioural and psychophysiological data.

The task applied, described in detail in the following section (2.3), was a warned visual discrimination task where participants were required to determine if the visual stimulus presented was a car or a house.

The two categories selected were cars and houses, because they activate different regions of the visual cortex [54]. This is important, because one objective of the original study is to quantify the effects of visceral signals and body movements on the ongoing activity of the visual areas presenting stronger image classification accuracy. For this, it is important that the stimuli categories activate distinct regions, so it is possible to later differentiate this activity and quantify this effect more clearly.

Each trial started with an auditory warning cue that alerted participants of the upcoming visual stimulus. Immediately after the display of the visual stimulus, a mask was presented,

made out of several parts of images of cars and houses mixed up. This technique is called visual backward masking and is widely used for dissociating awareness and stimulation (figure 1.1).

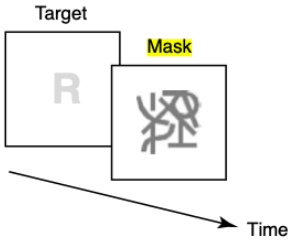


Figure 1.1: Schematic of backward masking. A briefly presented stimulus is erased from awareness by presentation of a 'mask' in close spatial and temporal proximity to the target. (Image from [55].)

With appropriate timing and spatial arrangement of stimulus and mask, the technique works very effectively: an ordinarily visible target can be erased from visual awareness by the mask [56]. The mask 'halts' processing of the target, thereby abbreviating the target's effective duration [55]. After the mask was cleared, a response prompt was shown and the participants selected the stimulus category they saw using their index fingers. Figure 1.2 illustrates this description with an example of a trial.

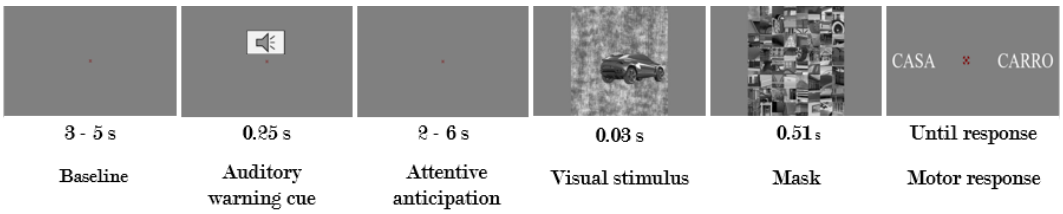


Figure 1.2: Schematic example of one trial type presenting a car stimulus (the response prompt here is not to scale, I increased its size to facilitate visualization).

I proposed that the auditory and visual stimuli might have a crucial effect on the subject's breathing activity and might be linked with neural mechanisms to optimize visual processing.

With this project, I attempted to elucidate if and how visual processing is modulated by breathing activity. In order to do so, I started from the following hypotheses:

- Periods of attentive anticipation of visual stimuli modulate breathing activity;
- The ability to discriminate across categories of visual objects is modulated during the breathing cycle;

- Breathing modulation induced by anticipatory attention improves behavioural performance. This modulation improves image categorization.

My analyses were focused on answering the following questions:

- Is there a modulation in breathing activity with auditory cue and visual stimuli display? Here, I measured the change in breathing cycle duration at the moment of both of these events. At these time points breathing modulation might occur to facilitate perception;

- If there is a modulation, is it related/does it influence task accuracy (i.e. the ability of the brain to discriminate across image categories)? And is this modulation conditioned by the phase of respiration (inhalation/exhalation) at the onset of the events (auditory cue/visual stimuli)? I compared modulation on incorrect trials with modulation in correct trials. The presence of this effect could suggest that breathing activity could, in fact, modulate neural activity in a way that enhances behavioral performance.

1.3 Outline of the Dissertation

The remainder of this document is structured as follows: Chapter 2 gives an overview of the methodology used to acquire and analyze data; in Chapter 3, the results obtained are presented and discussed further in Chapter 4; finally, in Chapter 5, I make a conclusion for the work developed.

Chapter 2

Methods

In this chapter, I will describe the design and conditions chosen for this study, as well as the processes and methods used to gather and analyze the data

2.1 Participants

For this study, I was able to gather and record data from 18 participants, in collaboration with my supervisor. The first participant was the pilot test, and, consequently, I discarded those data since the task design was still far from what I intended and was considerably modified after that (explained ahead - section 2.4). So, after all, we gathered 17 eligible volunteers. All of them agreed to a written consent that was in accordance with the Declaration of Helsinki, in which they were informed about the procedures undertaken in this task. The participants were aged between 20 and 33, where 12 were female and 6 were male.

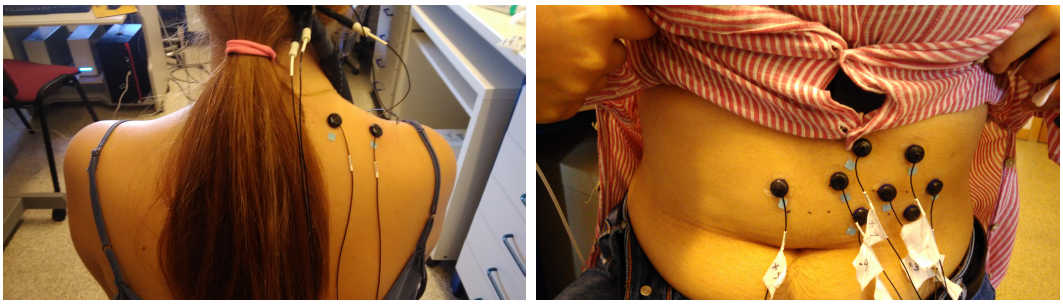
Each participant performed the task at least four times (four runs), each one consisting of 60 trials and a duration of about 10 minutes, which means there was a total of 4080 non-independent trials and almost 700 minutes of physiological activity. Some individual trials and breathing cycles had to be excluded, as I will describe ahead.

2.2 Recording of Physiological Signals

As said before, in this experiment, we recorded signals from several bodily functions (brain signal, ocular movements, respiration, muscle activity, cardiac signal, skin conductance and

stomach activity). The participants wore an EEG cap to record brain activity (64-channel *Quik-Cap* from Neuroscan). This EEG system also includes electrodes that allowed us to measure:

- heart activity - EKG -, which were placed vertically on top of the sternum;
- EOG to detect blinks and eye movements on the horizontal and vertical planes, which were placed near the outer canthus of each eye and above and below the left eye;
- muscle activity - EMG - with electrodes placed on the right side of the trapezius (figure 2.1a);
- stomach activity - EGG - with eight electrodes distributed across the belly, on top of the stomach area (figure 2.1b).



(a) Shoulder EMG electrodes placement. **(b)** EGG electrodes placement.

Figure 2.1: Electrogastrography (EGG) and shoulder electromyography (EMG) electrode distribution.

The layout of the cap is according to the extended 10/20 system.

Besides these electrodes, we also measured EMG on both forearms (2.2a), leg movements with one accelerometer on each ankle (in some cases, we used custom-made accelerometers on the subjects' knees, due to constraints on the systems' availability), PPG and EDA with sensors on the left hand's pinky, middle and ring fingertips (figure 2.2b). All of these were recorded with Biopac's System Bionomadix.

(a) Forearms EMG electrodes placement. **(b)** EDA and PPG electrodes placement.

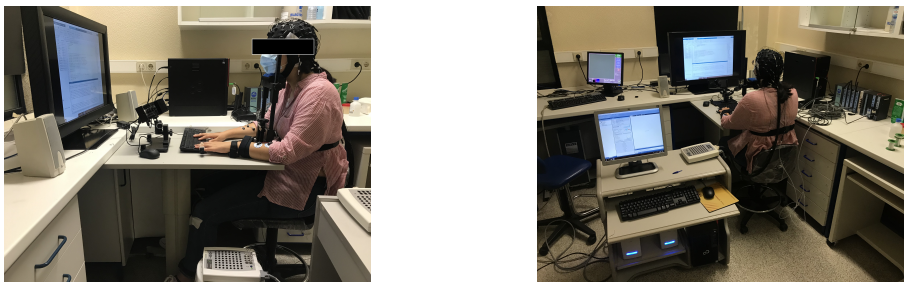
Figure 2.2: Electrodermal activity (EDA), photo plethysmogram (PPG) and forearms electromyography (EMG) electrode distribution.

To keep track of eye movements and pupil dilation, we used the *EyeLink 1000 Plus* (SR Research, Canada), sampling at 1000 Hz.

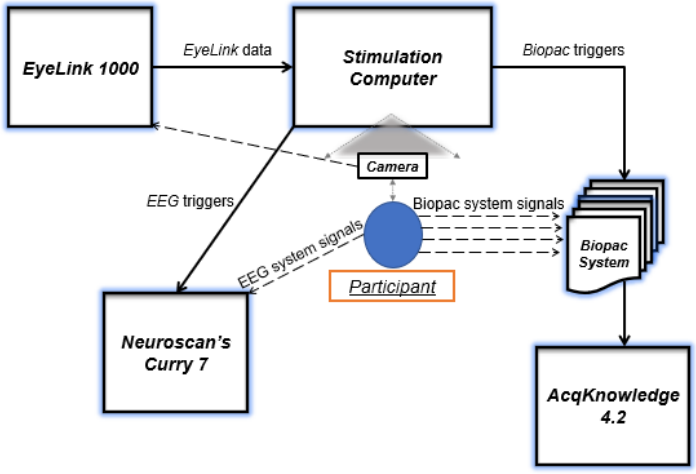
Finally - and more importantly for this thesis -, the participants wore a respiration transducer belt (Biopac's *TSD221-MRI*) to measure respiratory effort associated with circumferential changes in the thoracic region of the torso. The breathing data was transmitted at a rate of 5000 Hz (except for the second subject, for which I used 1000 Hz).

Each data acquisition session took nearly 3 hours and the software we used to acquire the Biopac data was the *AcqKnowledge 4.2* Software and, for the EEG data, we used Neuroscan's Curry 7.

The following images and block diagram show how the acquisition system was set up, as well as the placement of the electrodes described above:



(a) (b)



(c)

Figure 2.3: Representation of the acquisition system setup: (a) side view of the participant position relative to the stimulation computer; (b) photo showing the setup design with all instruments; (c) diagram with each instrument and software identified and how they connect with each other.

2.3 The Visual Task Design

As said before, the task developed was a warned visual discrimination task built with Matlab (version 2021a) using the *Psychtoolbox−3* toolbox extensions [57–59].

The participants were asked to discriminate visual stimuli between two categories: cars and houses (figure 2.4). We used visual stimuli included in the fLoc functional localizer package from [54].

At the beginning of each run, before the task started, there was a 5 second countdown.

(a) **(b)**

Figure 2.4: Example of the two types of stimulus. (a): car stimulus; (b) house stimulus.

Each trial began with a baseline period, which is important to let the participant's bodily signals stabilize and measure spontaneous psychophysiology during rest (during this period the participants are fixating a grey background with no additional information, ranging from 3 to 5 seconds, randomly). This baseline was followed by a warning cue indicating that the visual stimulus would be presented within the next few seconds (the time between cue and visual stimulus varied randomly from 2 to 6 seconds, so that participants would not be able to predict the timing of visual stimulus display, requiring them to be prepared from the sound of warning cue). The warning cue was a pure tone of 1500 Hz and lasted for 0.25 seconds. The visual stimuli were presented for 30ms and immediately followed by a mask (visual backward masking).

The mask was shown for 0.51 seconds and, after that, a response prompt was displayed, consisting of the words "CAR" and "HOUSE" (in Portuguese) next to each other, side by side (the side on which each word was shown was assigned randomly). The participants used their index fingers to answer what they believed was the stimulus they saw, using the keys 'Z' and 'M' to choose the option presented on the left or right, respectively. This design was meant to prevent the participants from answering over the visual stimulus. The original study aimed to use classifiers to discriminate each trial by "house" or "car" using the EEG signal, but only considering the visual data and not if the participant is using the left or right hand. By presenting the response prompt in the described way, the participant does not know which hand to use until the prompt appears, which leaves 500 ms of data where the EEG signal is not affected by the motor response.

After the participant's answer, the task automatically moved to the next trial. At the end of each run, the participant's task performance was presented, with the percentage of

correct trials.

For the 60 trials in each run, I randomized the amount of cars and houses presented. Some runs contained more cars and others more houses, but it averages to about 30 of each category per run (in total, out of the 4080 trials, 2047 displayed houses and 2033 displayed cars).

I took into consideration that if participants had an accuracy of 100 %, that would mean they were discriminating the stimuli perfectly. In that case, there would not be a ground for comparison between what happens in a correct and incorrect trial. On the other hand, if there were a 50 % accuracy, the participants would be simply guessing randomly what the correct answer was without actually focusing and processing the stimuli. For that reason, I aimed at an accuracy of 75 % (which would be in between those scenarios).

We asked each participant to perform at least 4 runs. Some did more than 4 (in cases where we thought it could be better to repeat the recording), but I only analysed four runs out of each participant for consistency.

After the second and fourth runs, we also included a different visual task, with videos from a separate study being acquired at the same time that will not be considered for this work.

2.4 Task Adjustments

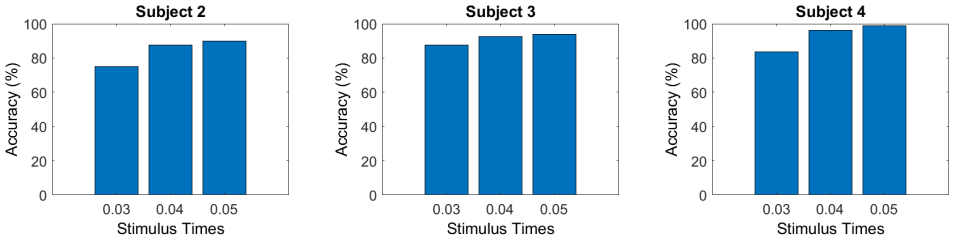
The description in the previous sub-section was the basis of the task for most of the study. However, it is essential to note that the task suffered minor changes throughout the study to improve what we believed should be slightly corrected, as mentioned below:

- For the pilot test, the backward masking method was not used. Instead, the same stimuli were presented with gaussian noise. This did not work properly because the gaussian noise appeared to cover the house stimuli much more than car stimuli, making it only possible to discriminate houses by knowing it was not a car;

- For the first three subjects, there were three levels of stimuli duration: 30 ms, 40 ms and 50 ms. Each of these levels occurred the same number of times (from the 60 trials in each run, there were 20 trials for each level). As figure 2.5 shows, I noticed that participants were performing too well (way above 75 % accuracy), so I decided

to only use the first, most difficult, level from there on. For these subjects, on visual stimulus-related analysis, I only considered trials with a duration of 30 ms;

- For subjects 3 to 7, the triggers for the Biopac and Neuroscan systems were sent through the same port using a splitter. Here, I found that some triggers were missing because the time between triggers was too small for the system to process.



(a) **(b)** **(c)**

Figure 2.5: Percentage of correct answers on each 'level' of stimulus display time for subjects 2, 3 and 4. It is visible that in longer stimuli the accuracy is much higher than desired.

Before moving on to the next section, I will use table 2.1 as an overview of the main parameters that were used for each participant and went through adjustments.

Subject	Runs	Stimuli Duration (Levels)	Biopac Sampling Rate (Hz)	Leg Accelerometers
2	4	3 (0.3ms, 0.4ms, 0.5ms)	1000	3, both knees + ankle (bionomadix)
3	5	3 (0.3ms, 0.4ms, 0.5ms)	5000	3, both knees + ankle (bionomadix)
4	5	3 (0.3ms, 0.4ms, 0.5ms)	5000	3, both knees + ankle (bionomadix)
5	4	1 (0.3ms)	5000	3, both knees + ankle (bionomadix)
6	4	1 (0.3ms)	5000	3, both knees + ankle (bionomadix)
7	4	1 (0.3ms)	5000	3, both knees + ankle (bionomadix)
8	4	1 (0.3ms)	5000	3, both knees + ankle (bionomadix)
9	4	1 (0.3ms)	5000	3, both knees + ankle (bionomadix)
10	4	1 (0.3ms)	5000	3, both knees + ankle (bionomadix)
11	4	1 (0.3ms)	5000	2, ankles (bionomadix)
12	4	1 (0.3ms)	5000	2, ankles (bionomadix)
13	4	1 (0.3ms)	5000	2, ankles (bionomadix)
14	4	1 (0.3ms)	5000	2, ankles (bionomadix)
15	4	1 (0.3ms)	5000	2, ankles (bionomadix)
16	4	1 (0.3ms)	5000	2, ankles (bionomadix)
17	4	1 (0.3ms)	5000	2, ankles (bionomadix)
18	5	1 (0.3ms)	5000	2, ankles (bionomadix)

Table 2.1: Main task parameters that suffered changes. All the other parameters mentioned remained unchanged.

2.5 Analysis of Breathing Data

2.5.1 Preprocessing

I used Matlab (version 2020b) to analyse all of the data considered for this thesis. The breathing signals were initially downsampled from 5000 Hz (1000 Hz for participant 2) to 100 Hz. This rate is adequate for breathing signals, given that the average number of respirations for a non-respiratory compromised healthy adult is between 12 and 20 breaths per minute [60]. The data were then filtered using a high-pass filter at 0.01 Hz to remove low frequency drifts from the breathing signal and a low-pass filter at 2 Hz to remove high-frequency noise. Both the downsampling and filtering of the data were made with the EEGLAB toolbox version 2021.0 [61].

The next preprocessing step was a smoothing of all data using the Matlab function *smoothdata* with the *'loess'* method. I did this because the raw data contained too much high frequency noise to analyse. I tried several methods and then calculated the difference between the original and smoothed data to find out which method presented a difference closest to zero. I chose the *'loess'* method because it was the one that distorted less the data and kept it closest to the original. Figure 2.6 shows a comparison of the data before and after preprocessing (the high-pass filter corrects the signal average to zero).

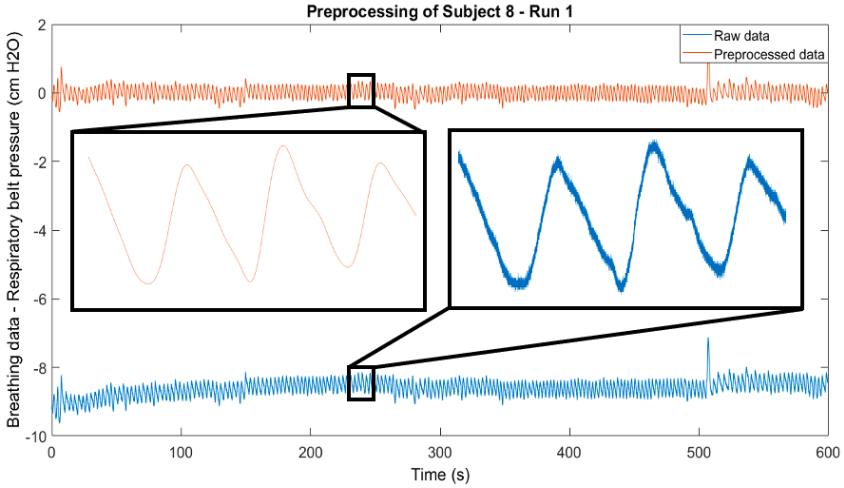


Figure 2.6: Comparison between raw and preprocessed breathing data. The preprocessed data contains much less noise, as it is shown in the zoomed-in parts side by side, taken from the same time frame.

The next step was to include the triggers I had defined when designing the task. The triggers were numbered from 1 to 16 in the following way:

Trigger Number	Event
1	Start of experiment
2	Auditory cue
3	"House" stimulus with duration 30ms
4	"House" stimulus with duration 40ms (only for subjects 2, 3 and 4)
5	"House" stimulus with duration 50ms (only for subjects 2, 3 and 4)
6	"Car" stimulus with duration 30ms
7	"Car" stimulus with duration 40ms (only for subjects 2, 3 and 4)
8	"Car" stimulus with duration 50ms (only for subjects 2, 3 and 4)
9	Response prompt with "house" option on the left
10	Response prompt with "car" option on the left
11	Response: participant chooses the option on the left correctly
12	Response: participant chooses the option on the left incorrectly
16	Response: participant chooses the option on the right correctly
13	Response: participant chooses the option on the right incorrectly
14	End of experiment

Table 2.2: Triggers and the corresponding events.

Note: the numbers of the triggers are not in order because of the adjustments that were made regarding the absence of some triggers in one of the designs I made initially.

I also removed the breathing data acquired before trigger 1 and after trigger 14 - "residual" data -, since they were not relevant for the analyses. After that, I made sure every subject's sampling rate was the same to facilitate the following approaches. The triggers, when acquired in the Biopac system, are converted to binary code, so, in my analysis, I used a Matlab script to convert them to decimal system and obtain the following data:

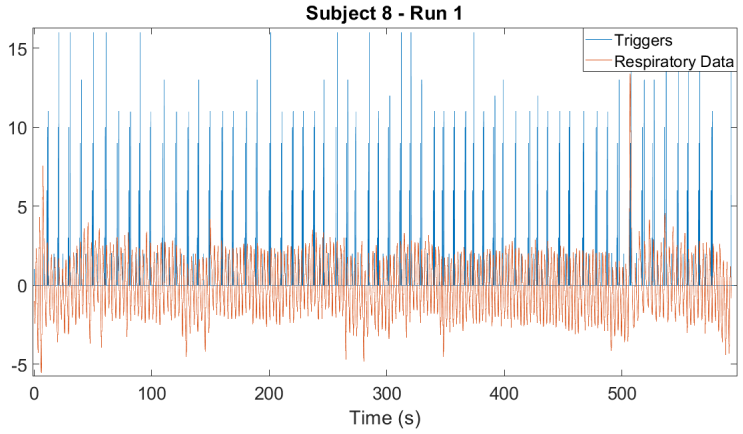


Figure 2.7: Example of one subject's run, representing which trigger happened at what time during the run and the breathing activity in that moment (the breathing data representation was vertically stretched to help visualization).

Breathing data is composed of peaks and valleys, where peaks correspond to the end of the inhalations and start of exhalations and vice versa - valleys correspond to the end of the exhalations and start of inhalations. From here, I defined the duration of an individual respiratory cycle as the length between two valleys - from the start of inhalation to the end of exhalation.

By visualizing the data, I noticed the presence of **artefacts**, occurring as odd or unexpected breathing behaviour: small deviations to the surrounding breathing pattern (figure 2.8). So, my next and last preprocessing step was to remove all breathing cycles with artefacts.

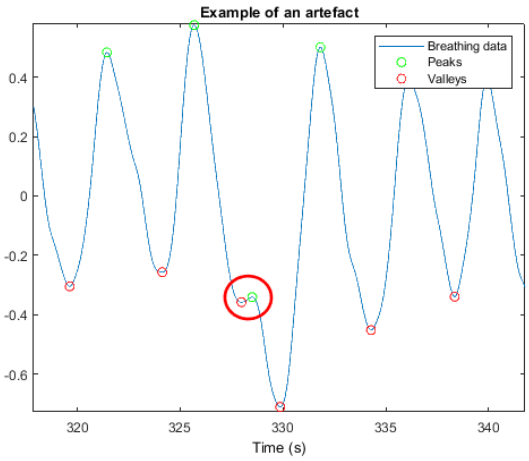


Figure 2.8: Example of an artefact. The expected and trending respiratory behavior would be a full exhalation. However, this small deviation is enough to lead to the detection of a peak and a valley, incorrectly.

I began with a peaks-and-valleys approach, by using Matlab's function *'findpeaks'* to determine the beginning and end of inhalations and exhalations. This function return local maxima by identifying data sample that is larger than its two neighboring samples. To obtain valleys, I inverted the signal, so that what used to be valleys were then peaks, and use *'findpeaks'* again. Figure 2.9 shows an example of one run from one subject.

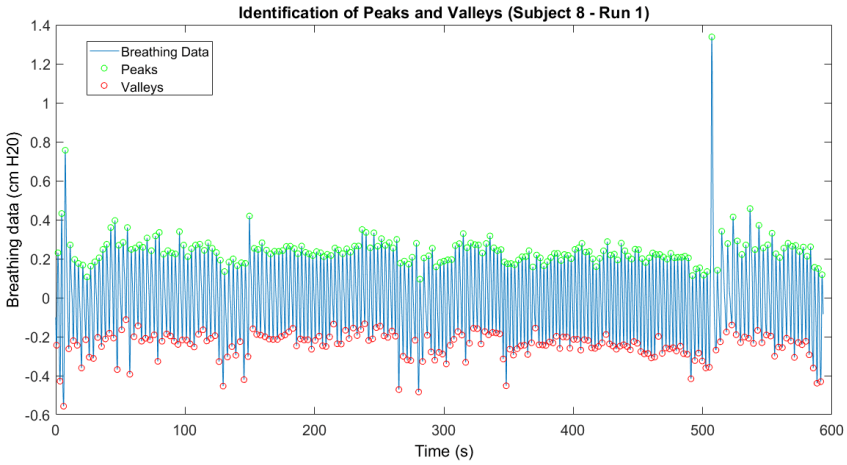


Figure 2.9: Output obtained from using *'findpeaks'* (example from one run from a single subject).

This procedure was quite accurate and allowed me to easily obtain the duration of the breathing cycle from the 'distance' between two valleys, i.e., the difference between the position of adjacent valleys, obtained with the *'findpeaks'* function. This simplified the process of removing artefacts, because, as I will explain ahead, it is a reliable way of discriminating artifacts from normal breathing patterns, by telling me the abnormal duration of those 'cycles'.

Then, I defined artefacts as cycles with a duration below a certain threshold. My initial intention was to perform a linear interpolation on every cycle identified as an artefact, as in figure 2.10. Nevertheless, this method could not tackle every occurrence and missed a large number of artefacts.

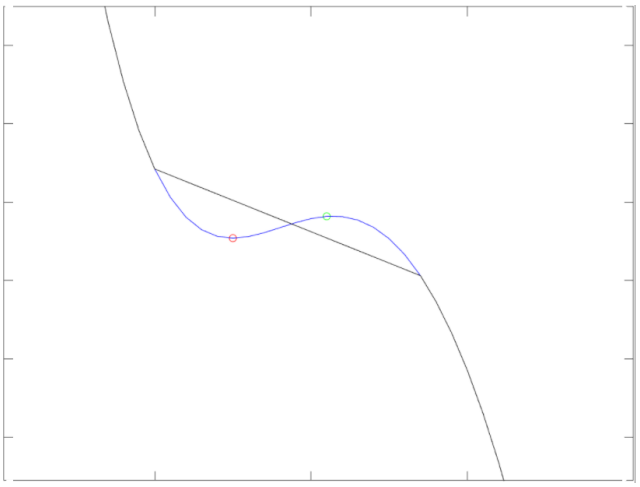


Figure 2.10: Correcting artefacts by linear interpolation.

In a second attempt, I opted for a statistical procedure: if a cycle is too small compared to the mean duration of all cycles in that run, then it should be removed. Quantitatively, this is done using the z-score, measuring how many standard deviations below or above the mean a cycle duration is. Having done this calculation with Matlab's function *'zscore'*, I rejected all cycles with a z-score below -2.5 (z-score $<$ -2.5).

In this process, I considered removing cycles with z-scores higher than 2.5 (in that case, it would be $|z\text{-score}| > 2.5$), although longer breathing cycles most likely are not artefacts, but just deep breaths instead, in which case it is interesting to keep them for analysis.

After doing this, I noticed that this was not the most accurate strategy, because a lot of artefacts happened in the middle of an inhalation or exhalation. For example, a slight increase in breathing data in the middle of an exhalation would cause a peak-valley pair to be identified where it should not. This could happen at a point where the distance between the previous and following valleys are valid (inside the threshold defined), without being considered an artefact, even though it clearly is one. Figure 2.11 shows that: what was expected to be a complete exhalation is interrupted by a very small inhalation, instead, and an artefact occurs without being considered as one, since the distance between valleys is accepted.

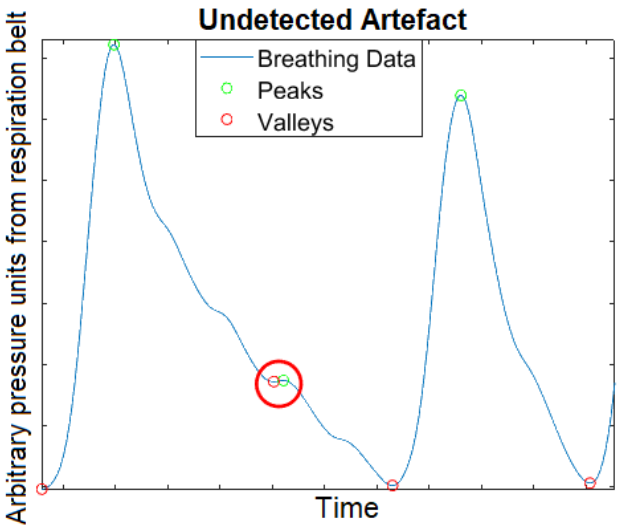
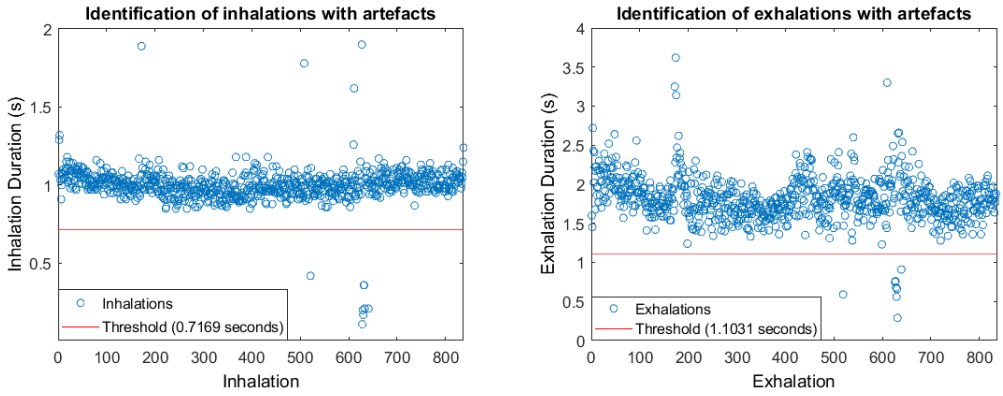


Figure 2.11: Example of an artefact not detected by the z-score method.

To avoid this, I realized that I would need to take the peaks into consideration, examining also inhalation and exhalation lengths. This procedure would take care of situations like the one described above, where the small increase would now be identified as an artefact given the abnormal inhalation length. This procedure showed to be the most effective. Figure 2.12 shows how this was done.



(a) **(b)**

Figure 2.12: Visualization of how the z-score method identifies artefacts (data from subject 8). The red line in all of the graphs corresponds to the defined threshold (z-score < -2.5). The graph on the left represents inhalation cycles and the one on the right exhalation cycles. It is possible to see here that inhalations are considerably shorter than exhalations.

Every identified artefact was then substituted by blank values - not a number ('NaN') values -, and I was able to remove every cycle with 'NaN' in them, which left me with the data looking like what is represented in figure 2.13. From this point on, everything was ready to proceed to the intended analyses.

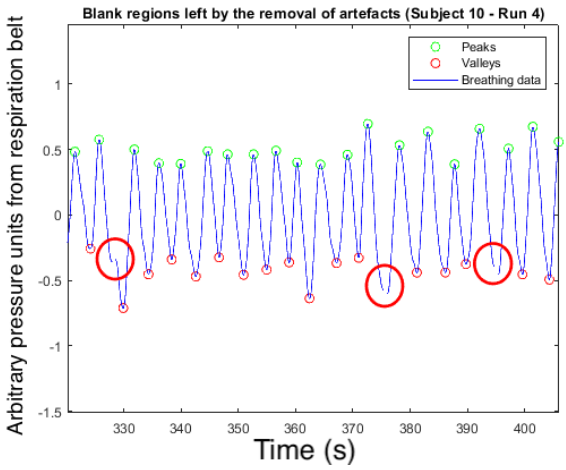


Figure 2.13: Representation of 'NaN' values in data after removing artefacts.

It is important to note that, when performing the z-score method, I noticed some abnormalities. The graphs in 2.14(a) and 2.14(b) show the same as the previous graphs but for subject 17. I observed that the threshold I defined was not excluding any artefacts, even though there clearly are several inhalation/exhalation lengths that are very short. However, there are so many of these cases and they happen so regularly, that the mean is skewed and these points end up being accepted instead of rejected. This happens for both inhalation and exhalation. Looking at the data, I understood that this participant did long pauses in between each breathing cycle, and a lot of tiny cycles arise from the peaks-and-valleys approach (figure 2.14(c)). This happened throughout all the data. Unfortunately, after spending quite some time trying to deal with this, I decided it was best to leave these participant's data out of the analyses, even though there was nothing wrong with the collecting of the data whatsoever. The same problem happened for subject 7. Therefore, in analyses that involve breathing data, I only considered 15 participants.

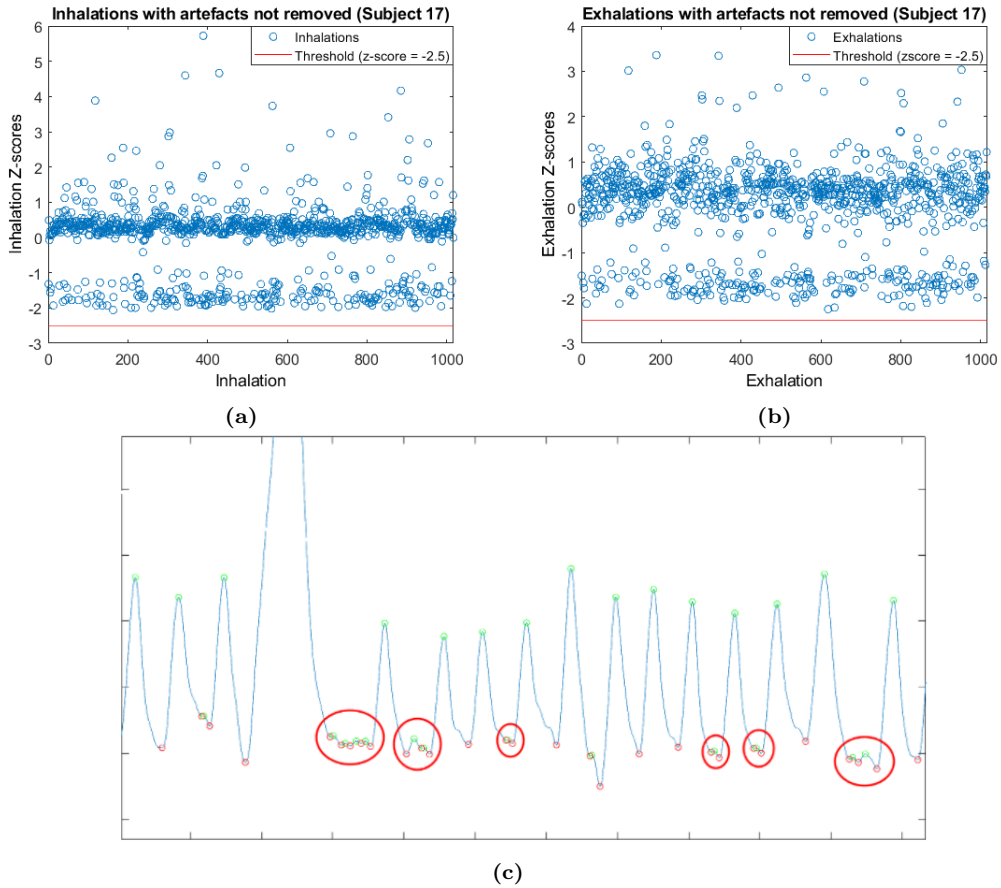


Figure 2.14: Breathing data from subject 17 showing artefacts. (a) and (b): No artefacts identified by the z-score method - all durations are above the threshold (red line); (c) is the visualization of artefacts at the end of several breathing cycles due to the breathing activity of this participant throughout the whole task, occurring repeatedly.

2.5.2 Analyses Performed

In my analyses, I considered two moments of special interest: the moment at which the auditory cue happens and the moment at which the visual stimulus (car or house images) is displayed. These might have a crucial effect on the subject's breathing activity and might be linked with neural mechanisms to optimize visual processing. From here on, I will refer to these moments as 'events' and I will consider both of them into every investigation. There were 2 analyses I set out to conduct:

- The first was to determine whether those events led to a modulation of the breathing activity, comparing the duration of breathing cycles' at the moment of these events

and just before they occur. I also did this for both inhalation and exhalation, to find out if, in case of existing modulation, that difference came specifically from one of those phases;

- Secondly, I took into account the task performance of each subject and evaluated if there was any relation between the accuracy (correct/incorrect) of each trial and the phase of breathing cycle at which both events occurred as well as the breathing modulation for each scenario.

Modulation of breathing activity with auditory cue and visual stimuli

As explained, to explore this modulation, I determined, for each trial, the duration of the breathing cycle before the events. I called this variable $D1$. I then did the same thing for the cycle occurring at the moment of the events and called that variable $D2$. Having determined both lengths, I then proceeded to calculate the difference between the two, in order to obtain the modulation. To that variable, I called D, defined by $D = D2 - D1$ (figure 2.15). I calculated these values and averaged them for each run of each participant. I also took into account one more variable, which I called $D3$, corresponding to the mean duration of all breathing cycles of that run.

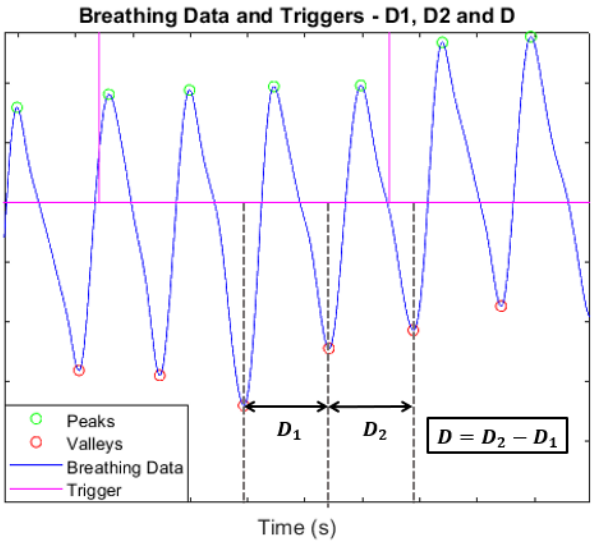


Figure 2.15: Definition of variables $D1$, $D2$ and D.

Since, for this analysis, I considered pairs of breathing cycles (the one occurring at the moment of the events and the one before), it is important to note that if any of the two cycles contained 'NaN' values (due to the presence of artefacts), I would not consider the other, as well. Figure 2.16 shows an example of this (in this case, these are image triggers).

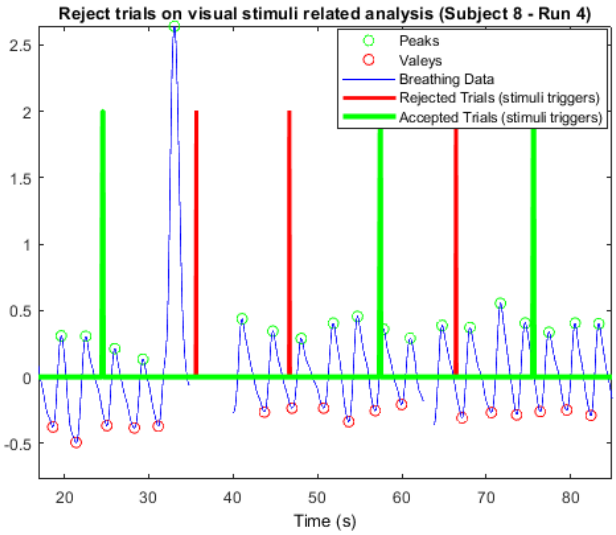


Figure 2.16: Representation of the rejection of three trials. The red lines correspond to stimuli triggers on trials that were rejected either because they occur on a breathing cycle containing 'NaN' values or because the previous breathing cycle contained 'NaN' values.

After doing this for both events, I ended up with 3337 trials for auditory cue-related analysis - with an average trial acceptance rate of 92.7 % ($SD = 4.37\%$) per participant, ranging from 82.9 % to 97.9 % - and 2913 trials for visual stimulus-related analysis - averaging 93.2 % ($SD = 4.78$) rate of accepted trials per participant, ranging from 82.9 % to 97.9 % -, while keeping the amount of trials with cars and houses balanced - for auditory cue analysis, 50.3 % were house trials and 49.7 % were car trials, whereas, for visual stimuli, 49.2 % corresponded to house trials and 50.8 % to car trials.

The findings I arrived at, which I will explain in detail later, led me to examine the breathing cycles surrounding the visual stimulus - more specifically, the breathing cycle at the onset of the visual stimulus and the two breathing cycles before and after the visual stimulus (to which I will refer to as $C1$, $C2$, C, $C3$ and $C4$ - see figure 2.17).

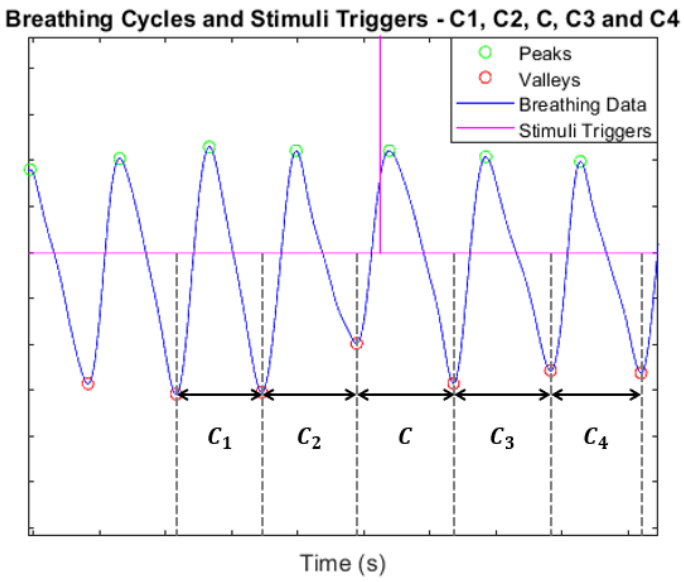


Figure 2.17: Definition of variables $C1$, $C2$, C, $C3$ and $C4$.

I also hypothesized that if there was any modulation, it would depend on the phase of the cycle at which the event occurs, for example: if the visual stimuli are presented at the end of exhalation, that is too late to provoke any modulation on that cycle.

Therefore, I took another approach and used circular statistics, dividing the length of each breathing cycle into radians, from 0 to 2π (where 0 represents the start of inhalation and 2π the end of exhalation) and determined at which angle each event occurs. This also allowed me to visualize if there is a significantly predominant angle (a predominant phase of the cycle at which the events happened). Consequently, the final output consisted of two arrays: one with the phase of each trial and one with the measured modulation (variable D) on each trial.

Relation between breathing modulation and task performance and phase of breathing cycle

When accounting for task performance, I first evaluated if there was a relation with the number of runs, i.e., if the participants improved along the task. For this evaluation, I considered all 17 participants, since this did not involve breathing data. In case of finding something relevant here, I would then investigate if this effect was induced by any breathing

pattern - namely, using $D3$ - with the expectation of observing a similar variation in overall breathing rhythm and performance along runs.

Then, I wondered if the accuracy could be related to the breathing modulation caused by each event (auditory cue and visual stimuli). In other words, does a stronger modulation lead to a higher accuracy (or maybe the other way around)? I also thought that the accuracy of participants could depend on how soon or late in the breathing cycle the event happens. In that sense, I used the same circular statistics procedure as before but now applied only in correct trials and incorrect trials and compared the outcomes.

Here, it is important to note that I had to reject some data: some subjects had very few incorrect trials and, given that I had already removed trials in the preprocessing stage, several of those overlapped, leaving me without any available trials for some subjects; on top of this, as I mentioned earlier, I only considered 30ms trials for subjects 2, 3 and 4 (corresponding to one third of the data of these participants), which, once again, reduced the amount of data available. Therefore, for this analysis, I considered fewer subjects: 12 when evaluating auditory cue-related effects and 11 for visual-stimuli effects.

2.6 Statistical analysis

To verify if the results I obtained were statistically significant, I used the SPSS software to run the Repeated Measures ANOVA tests.

To analyse the relation between phase of breathing cycle at the moment of events and modulation (D) statistically, I used Matlab custom scripts to run a circular-logistic regression to test whether phase predicts modulation at the single-trial level. Phases are sine- and cosine transformed and used as circular predictors of the modulation in a regression model with coefficients β_1 and β_2 [62]:

$$D_i = \beta_0 + \beta_1 cos\Phi_i + \beta_2 sin\Phi_i + \epsilon \tag{2.1}$$

where D_i is the modulation on trial i, Φ_i is the phase at which the event occurred in trial i, β_0 the intercept term and ϵ the error term. To quantify the performance of the fit, a p-value for each participant can be obtained. To do this, I used Matlab's function *'regress'*, which returns the coefficients β_0, β_1 ans β_2, as well as the p-value. I ran this for each participant and determined the percentage of participants for which this regression

was significant ($p < 0.05$). Additionally, I also ran a one-sample t-test on the cosine and sine coefficients, β_1 and β_2, to find out whether or not these coefficients are, on average, significantly different from zero. The size of the coefficient for each independent variable represents the size of the effect that variable has on the dependent variable [63]. I ran this test with Matlab's function *'ttest'*, which returns the p-values for each coefficient and tells me if phase of breathing cycle has a significant effect on predicting breathing cycle modulation.

For the last analysis, where I investigated the relation between task performance and phase of breathing cycle on event presentation, I performed a Watson test, which was most appropriate for this case [64]. The Watson test is the nonparametric version of the Watson-Williams two-sample test. It computes a test statistic U^2, which is based on the ordering of the phases and computing the cumulative relative frequency distributions. Once again, I ran this test for each participant and calculated the percentage of participants for which this regression was significant. To determine the p-values, I made use of the script '*watsons_U2_approx_p.m*' from the 'Simulations phase statistics' function developed by Wolpert, N, & Tallon-Baudry, C. in [65].

Chapter 3

Results

In this chapter, I will present the analyses I performed and the results they led to, by using the methods described in the previous section.

3.1 Results obtained

3.1.1 Task performance variation along runs

Regarding task performance, an initial approach showed that the mean accuracy percentage across all participants was 80.1 % ($SD = 8.73$ %), ranging from 62.9 % to 95.4 %. The mean accuracy on houses was 75.5 % ($SD = 11.9$ %), from 47.1 % to 96.2 %, and on cars 84,2 % ($SD = 11,7$ %), from 54.9 % to 100 %.

I will refer to "Effect of Run" as the change in modulation from run to run. In my following analyses I will include "Effect of Time", which refers to the comparison between the breathing cycle duration at the moment of event, $D2$, and the duration of breathing cycle just before that, $D1$, and "Effect of Accuracy", when comparing scenarios between correct trials and incorrect trials. The interaction effect represents the combined effects of the two factors on my measure, i.e., if the impact of one factor depends on the level of the other factor.

While running the experiment in the laboratory, I noticed that, for almost all participants, the accuracy increased with each run. This was interesting to see, and, in fact, some participants would comment that in the beginning they found it hard to discriminate

between cars and houses, but after some trials they would start to get better at this task. Therefore, to confirm this, I ran a repeated-measures ANOVA test only with run (4 levels) as within-subject factor. Indeed, **this relation was significant**:

Effect of Run	$\boldsymbol{F_{(3,48)} = 10.4}$	$\boldsymbol{p < 0.001 (0,000023)}$

Table 3.1: Results from repeated-measures ANOVA with run as within-subject factor suggest an increase of task accuracy along runs.

Figure 3.1 shows the same result:

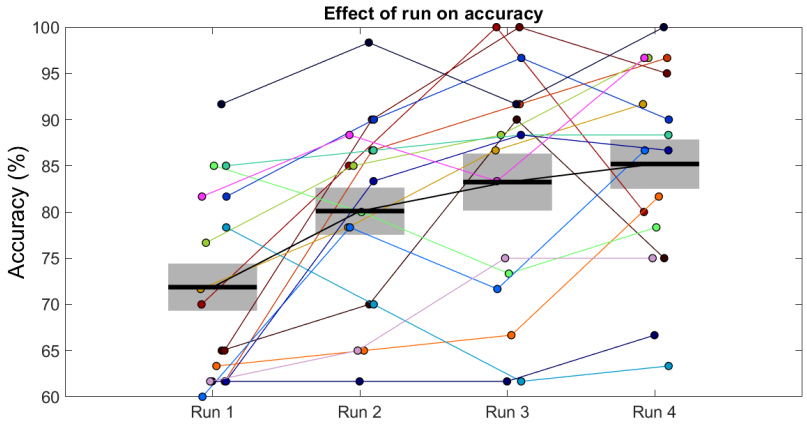


Figure 3.1: Effect of run on accuracy (the black horizontal line depicts mean across participants and the grey box represents ± standard error of the mean; different participants are represented with different colours).

This result was interesting to observe and I then wondered whether this improvement in accuracy could also be related to breathing activity - there could be a significant adjustment in breathing cycle durations from run to run. To analyse this, I made use of the variable $D3$ mentioned earlier (corresponding to the mean duration of all breathing cycles of each run) and, as before, performed a repeated-measures ANOVA test for $D3$ with run (4 levels) as within-subject factor (table 3.2 and figure 3.2).

Effect of Run	$F_{(3,42)} = 0.562$	$p = 0.643$

Table 3.2: Results from repeated-measures ANOVA with run as within-subject factor revealed no consistent pattern of $D3$ along runs.

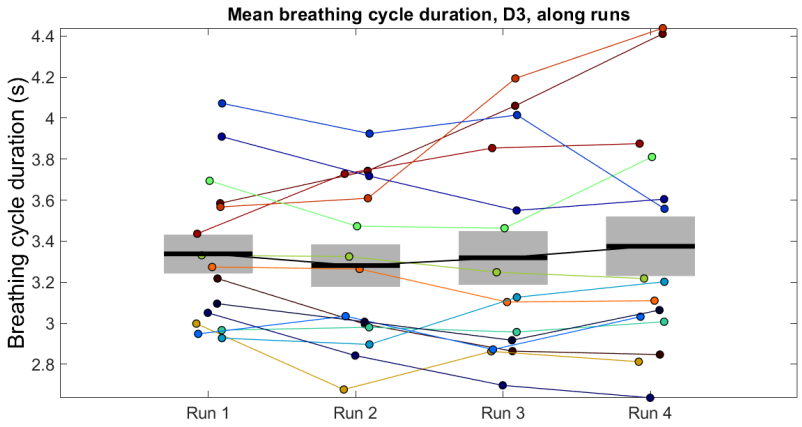


Figure 3.2: Effect of run on breathing cycle duration (the black horizontal line depicts mean across participants and the grey box represents ± standard error of the mean; different participants are represented with different colours).

I found that the effect I was expecting turned out to not be statistically significant - **the mean duration of the breathing cycle does not change across runs** and, therefore, the improvement in accuracy is not associated with mean breathing cycle duration. Besides this, I also analysed a correlation between $D3$ and the percentage of correct trials, which did not reveal any statistical significance, as well (figure 3.3).

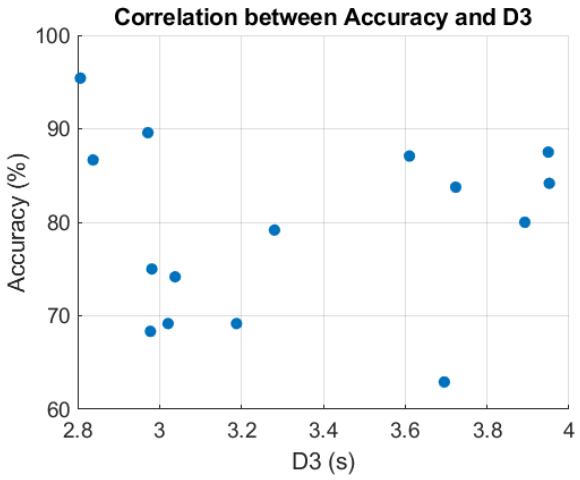


Figure 3.3: Task performance plotted against the mean duration of all breathing cycles. Each dot represents one participant.

3.1.2 Modulation of breathing activity with auditory cues and visual stimuli

For this analysis, I will present side-by-side the results obtained for the auditory warning cue with the ones for the visual stimuli. All the analyses were the same in both cases.

I started by looking for a breathing cycle modulation with the auditory cue, so I compared $D1$ and $D2$ with respect to this trigger (effect of time). My line of thought was to also do this for the inhalation and exhalation of breathing cycles in these trials, in case I found a significant modulation, to investigate whether this modulation comes particularly from inhalation or exhalation. In these analyses I included the effect of run because as accuracy increased with run, we wanted to test if the modulation also changed with run. The first result showed that **there was no significant effect of auditory cue on breathing cycle duration** (according to the values obtained in table 3.3 and represented in figure 3.4).

Effect of Run	$F_{(3,42)} = 0.407$	$p = 0.749$
Effect of Time	$F_{(1,14)} = 0.023$	$p = 0.882$
Interaction *Run vs. Time*	$F_{(3,42)} = 1.15$	$p = 0.340$

Table 3.3: Results from repeated-measures ANOVA with run and time as within-subject factors for breathing modulation with auditory cues.

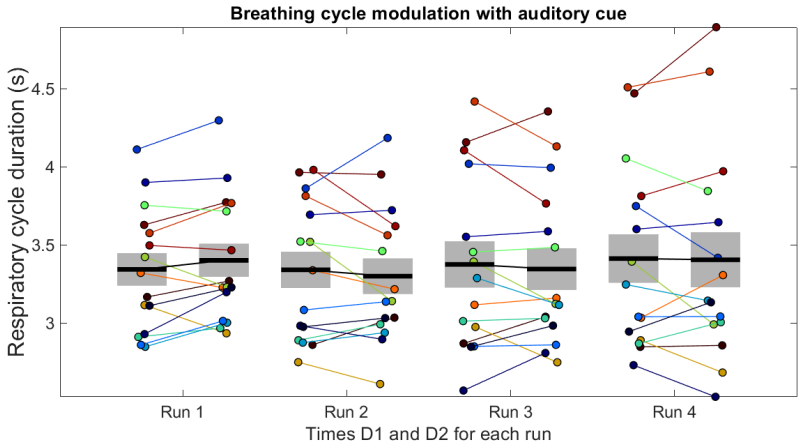


Figure 3.4: Breathing cycle modulation with auditory cue (the black horizontal line depicts mean across participants and the grey box represents ± standard error of the mean; different participants are represented with different colours).

Given that no relation was found, it did not make sense to explore the modulation on inhalation and exhalation, as explained earlier.

As for the visual stimuli, the result was different. I found that, on average, the value of $D2$ was higher than $D1$ for the majority of participants across all runs (only one participant showed a negative mean value of $D2 - D1$). Table 3.4 shows the results of performing, once again, a repeated-measures ANOVA with run (4 levels) and time (2 levels) as within-subject factors, where it is clear that the effect of time is significant, with $p = 0.001$ (highlighted in bold). This allowed me to conclude that **there was a significant effect of the visual stimuli on breathing cycle**. Figure 3.5 represents this modulation and appears to suggest that participants with a longer breathing cycle present a more accentuated breathing modulation.

Effect of Run	$F_{(3,42)} = 0.472$	$p = 0.704$
Effect of Time	$\boldsymbol{F_{(1,14)} = 17.2}$	$\boldsymbol{p = 0.001}$
Interaction *Run vs. Time*	$F_{(3,42)} = 1.24$	$p = 0.307$

Table 3.4: Results from repeated-measures ANOVA with run and time as within-subject factors for breathing modulation with visual stimuli.

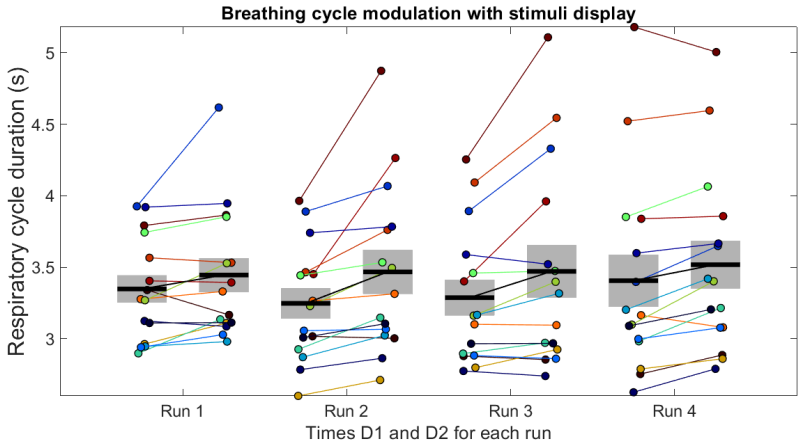
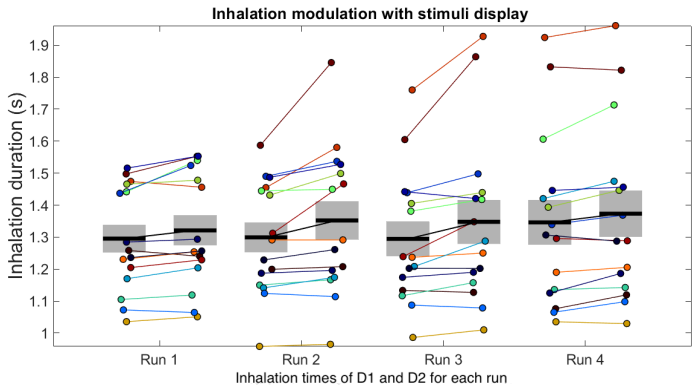


Figure 3.5: Breathing cycle modulation with visual stimuli (the black horizontal line depicts mean across participants and the grey box represents ± standard error of the mean; different participants are represented with different colours).

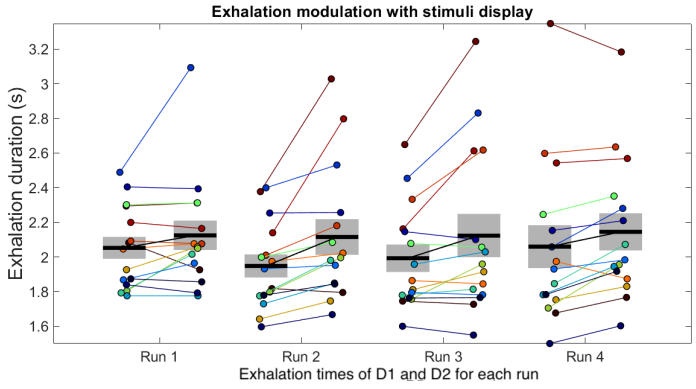
The next step was to look into the modulation on each phase - inhalation and exhalation. Here, the results are presented as before (in table 3.5 and figures 3.6a and 3.6b).

Inhalation:		
Effect of Run	$F_{(3,42)} = 0.841$	$p = 0.479$
Effect of Time	$\boldsymbol{F_{(1,14)} = 16.9}$	$\boldsymbol{p = 0.001}$
Interaction *Run vs. Time*	$F_{(3,42)} = 1.39$	$p = 0.260$
Exhalation:		
Effect of Run	$F_{(3,42)} = 0.483$	$p = 0.696$
Effect of Time	$\boldsymbol{F_{(1,14)} = 14.4}$	$\boldsymbol{p = 0.002}$
Interaction *Run vs. Time*	$F_{(3,42)} = 1.10$	$p = 0.358$

Table 3.5: Results from repeated-measures ANOVA with run and time as within-subject factors for inhalation and exhalation modulation with visual stimuli.



(a)



(b)

Figure 3.6: Inhalation and exhalation modulations with visual stimuli (the black horizontal line depicts mean across participants and the grey box represents ± standard error of the mean; different participants are represented with different colours).

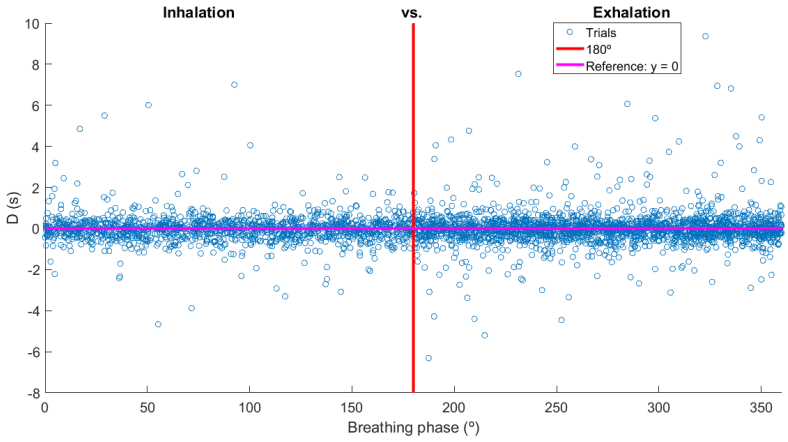
There was a significant modulation on inhalation and exhalation provoked by the visual stimuli.

I then analysed if the occurrence of modulation depends on the timing of the events. In other words, when the events take place, which breathing phase leads to a higher change in D ($D = D2 - D1$)?

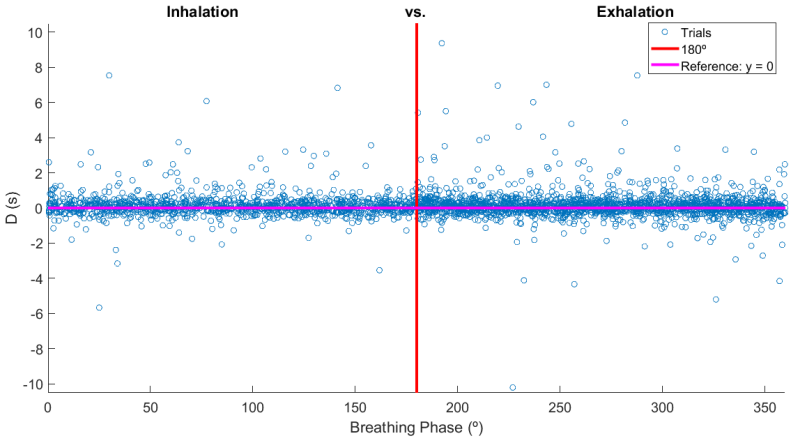
To answer this, I transposed the breathing cycle into an interval from 0^{o} to 360^{o}. The exhalation is usually longer than inhalation, which means that 180^{o} does not correspond to the middle point of one breath, where one starts to exhale. That middle point occurs around 130^{o} to 150^{o}.

For this analysis, I measured, for each trial, the angle at which the auditory cue and

visual stimuli were presented and the corresponding value of D . Having done this for all subjects, ended up with a table with the angles in one column and D values in another. To visualize this effect, I plotted these phase-modulation pairs in a scatter plot (for visualisation purposes, the graphs represent all trials from all participants, but the statistical analysis was done for each participant separately, since within-participant trials are not independent). The result was the following:



(a) Auditory cue.



(b) Visual stimuli.

Figure 3.7: Relation between breathing modulation, D, and angle at which the events occur, relative to the breathing cycle. For a matter of visualization, I assigned the first half (0^{o} - 180^{o}) to inhalation and the second half (180^{o} - 360^{o}) to exhalation. These graphs contain all trials relative to all participants.

It is interesting to note that on the bottom graph there are much less large negative values, which can imply that it is rare to find very long cycles before the presentation of visual stimuli (possibly associated with a deep audible breath, as in weariness or relief).

For the auditory cue analysis there were a total of 3337 points and for the visual stimuli there were 2913 points (corresponding to the total of valid trials in each case).

For the auditory cue, and using the circular-logistic regression aforementioned, there was only statistical significance for 1 participant (6.67 %) out of the 15 eligible and, for the visual stimuli, 4 out of 15 participants showed p-values lower than 0.05 (26.7 %).

This last result could have some relevance - 27 % is still a considerable amount. To examine this significance, I ran a one-sample t-test on the cosine and sine coefficients, β_1 and β_2, from the circular-logistic regression (see equation 2.1). I did this for all 15 participants and found that these coefficients are, on average, not significantly different from zero, meaning that phase does not have a significant effect on predicting breathing cycle modulation. The next table shows the p-values obtained with this test for the coefficients β_1 and β_2 of both events (auditory cue and visual stimuli), which are all larger than 0.05:

Event	**Coefficient**	**p-value**
Auditory Cue	β_1	0.837
	β_2	0.635
Visual Stimuli	β_1	0.560
	β_2	0.932

Table 3.6: p-values obtained from one-sample t-test on circular-logistic regression coefficients.

Therefore, from these results, I found that there is **no significant relation between the modulation and the timing of the events relative to the breathing cycle**.

Upon these findings, I speculated about the breathing variations along each trial (instead of event-related modulation only), since the breathing rhythm slows down with visual stimuli but suffers no change with auditory cue and the existing modulation is not linked with the moment in breathing cycle at which the events occur. Perhaps there might be an acceleration of breathing rhythm somewhere along the trial that compensates for the slowing down of breathing activity. As mentioned in the Methods section (2.5.2), to analyse this I will consider the breathing cycles surrounding the visual stimulus trigger and refer to each of them as $C1$, $C2$, C, $C3$ and $C4$ (see figure 2.17).

The results I found appeared to be very interesting: a noticeable change in breathing cycle duration occured around the visual stimuli display. I had already observed that breathing cycle slows down (duration increases) with presentation of visual stimuli, but here it is also possible to see that the modulation I noticed before is now countered in a symmetrical way and the breathing cycle after the visual stimuli exhibition is faster (duration decreases), restoring the breathing rhythm (figure 3.8 illustrates this).

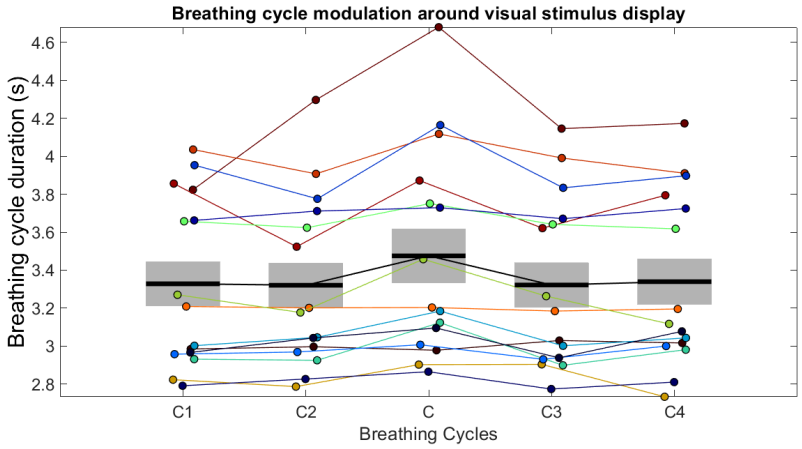


Figure 3.8: Mean durations of breathing cycles ahead ($C3$ and $C4$), behind ($C1$ and $C2$) and on visual stimulus onset (C). The black horizontal line depicts mean across participants and the grey box represents ± standard error of the mean; different participants are represented with different colours.

To verify statistical significance, I used, once again, a repeated-measures ANOVA test with time (5 levels) as the only within-subject factor (since I am comparing durations of 5 breathing cycles).

Effect of Time	$\boldsymbol{F_{(4,56)} = 7.14}$	$\boldsymbol{p < 0.001 (0.000103)}$
Effect of Run	$F_{(3,42)} = 0.321$	$p = 0.810$
Interaction *Run vs. Time*	$F_{(12,168)} = 0.879$	$p = 0.570$

Table 3.7: Results from repeated-measures ANOVA with time as within-subject factor reveal a significance effect in change of cycle duration.

The effect is significant: there is an effect of time among the 5 cycles surrounding the visual stimulus trigger, using the averages of each run. If the effect of run is also included, it does not have a significant effect, neither is there an interaction between the time and run factors (however, the effect of time remains), which might indicate that there is no relation

between this breathing pattern and accuracy (since accuracy increased significantly with run).

By running paired t-tests on all pairs of cycles, it is confirmed that the central cycle, C, is significantly different from all the others, while cycles $C1$, $C2$, $C3$ and $C4$ do not differ from each other (analysis not shown).

3.1.3 Relation between breathing modulation and accuracy

Once I started exploring task performance, one question that obviously arose was if the accuracy could be related to the breathing modulation caused by each event (auditory cue and visual stimuli). In other words, does a stronger modulation lead to a higher accuracy (or maybe the other way around)? In order to do this, I compared the modulation measured in **incorrect trials** against the modulation measured in **correct trials**.

I started by analysing this for the auditory cue. I already knew that no modulation occurred with auditory cues, but, to my surprise, a significant effect of accuracy was present (table 3.8, figure 3.9).

Effect of Accuracy	$\boldsymbol{F_{(1,11)} = 4.89}$	$\boldsymbol{p = 0.049}$
Effect of Time	$F_{(1,11)} = 0.214$	$p = 0.653$
Interaction *Accuracy vs. Time*	$F_{(1,11)} = 0.335$	$p = 0.575$

Table 3.8: Results from repeated-measures ANOVA with accuracy and time as within-subject factors for the correlation between modulation with auditory cues and task performance.

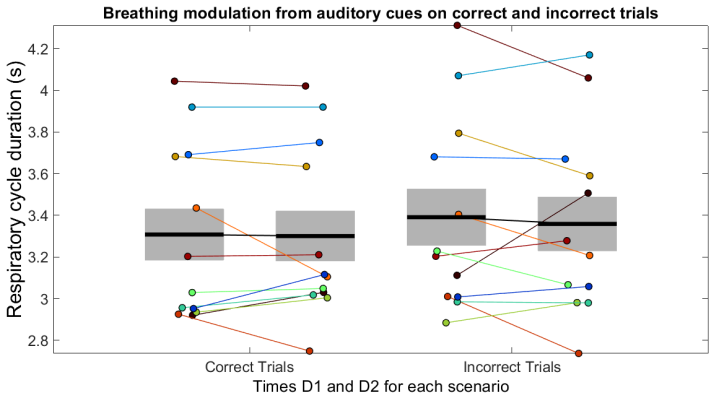


Figure 3.9: Relation between task performance and breathing modulation in relation to the auditory cue (the black horizontal line depicts mean across participants and the grey box represents ± standard error of the mean; different participants are represented with different colours).

This significant effect of accuracy reflected the fact that, **in incorrect trials, the breathing cycle duration is longer**. This significant effect of accuracy indicates that the means of $D1$ and $D2$ on correct trials are different from incorrect trials. It suggests that periods where breathing duration is longer are associated with a higher probability of incorrect responses. However, there was no significant effect of time, nor interaction effect time x accuracy, suggesting that there was no modulation of breathing duration with the auditory cue either in correct or incorrect trials.

After this, I moved on to the visual stimuli. The modulation observed before, with the visual stimuli, left me hopeful of finding an interesting relation here.

Effect of Accuracy	$F_{(1,10)} = 3.02$	$p = 0.113$
Effect of Time	$\boldsymbol{F_{(1,10)} = 10.8}$	$\boldsymbol{p = 0.008}$
Interaction *Accuracy vs. Time*	$F_{(1,10)} = 0.193$	$p = 0.67$

Table 3.9: Results from repeated-measures ANOVA with accuracy and time as within-subject factors studying the modulation of the breathing cycle with visual stimuli and task performance.

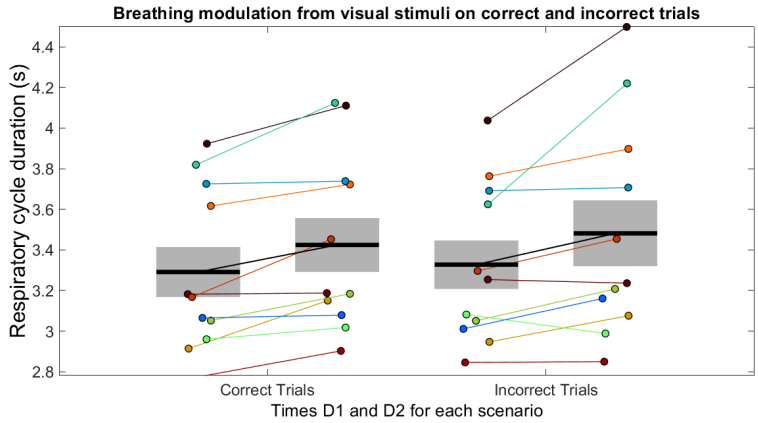
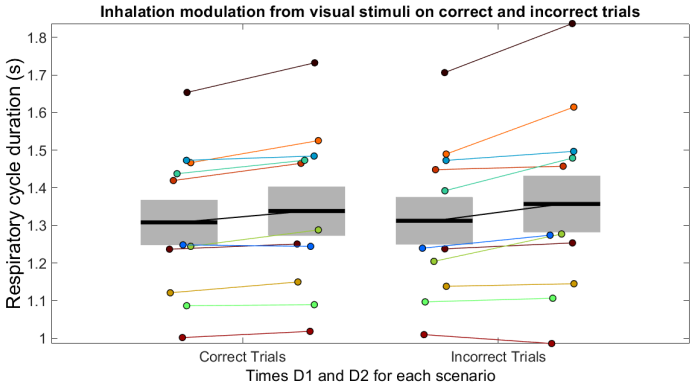


Figure 3.10: Relation between task performance and breathing modulation in relation to the visual stimuli (the black horizontal line depicts mean across participants and the grey box represents ± standard error of the mean; different participants are represented with different colours).

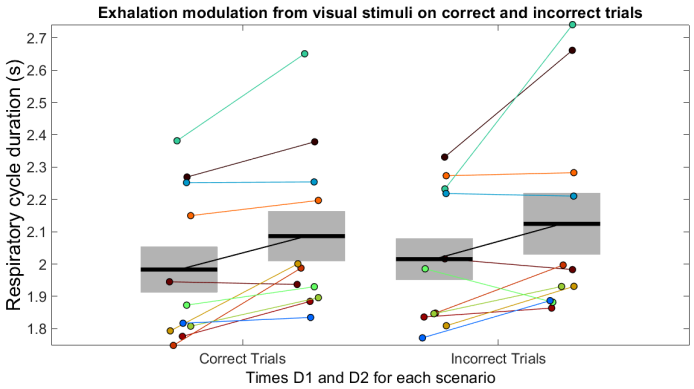
Consequently, I analysed the same for inhalation and exhalation:

Inhalation:		
Effect of Accuracy	$F_{(1,10)} = 1.52$	$p = 0.246$
Effect of Time	$\boldsymbol{F_{(1,10)} = 11.9}$	$\boldsymbol{p = 0.006}$
Interaction *Accuracy vs. Time*	$F_{(1,10)} = 1.73$	$p = 0.217$
Exhalation:		
Effect of Accuracy	$F_{(1,10)} = 3.45$	$p = 0.093$
Effect of Time	$\boldsymbol{F_{(1,10)} = 7.86}$	$\boldsymbol{p = 0.019}$
Interaction *Accuracy vs. Time*	$F_{(1,10)} = 0.023$	$p = 0.883$

Table 3.10: Results from repeated-measures ANOVA with accuracy and time as within-subject factors studying the modulation of inhalation and exhalation with visual stimuli and task performance.



(a)



(b)

Figure 3.11: Relation between task performance and inhalation/exhalation modulation in relation to the visual stimuli (the black horizontal line depicts mean across participants and the grey box represents ± standard error of the mean; different participants are represented with different colours).

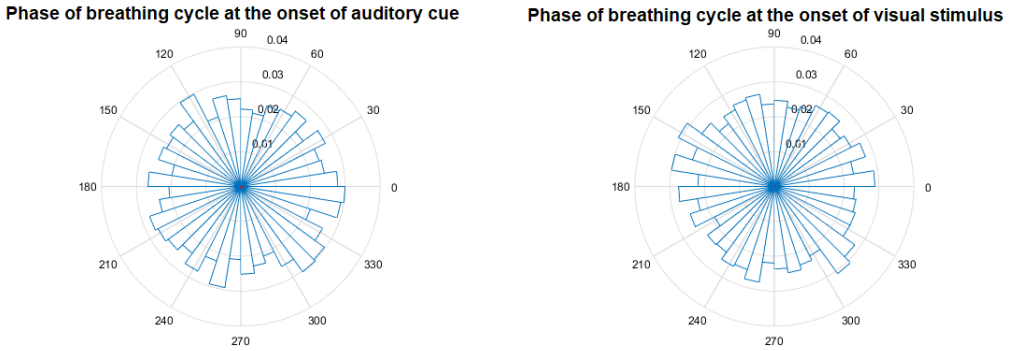
In the results presented above, I found that there was a significant effect of time, i.e., **there is a significant modulation in breathing cycle on both correct and incorrect trials** (this was already expected from the modulation observed across all trials). The same happened with inhalation and exhalation. The absence of an effect of accuracy means that there is no difference in breathing pattern associated with outcome (correct/incorrect) - breathing cycle duration is not significantly different when comparing correct and incorrect trials. Furthermore, the absence of an interaction between accuracy and time means that there is not any relation between accuracy and breathing modulation.

3.1.4 Effect of breathing phase on accuracy

Still regarding task performance, I followed the same line of thought as before and analysed if the breathing phase at which the auditory cue or the visual stimulus occur is related to the accuracy. In other words, does the phase of breathing cycle where the auditory cue or the visual stimulus are presented have an impact on the number of correct answers?

To represent these results, I plotted a circular histogram, with angles from 0^{o} to 360^{o} (as in the unit circle). The circle is divided in 40 bins and each bin is as long as the number of events in that interval - in this case, I am referring to the amount of trials with event triggers in that phase interval.

Firstly, I started simply by analysing all trials, just to have an idea of where the events occur, relatively to the breathing cycle (figure 3.12).



(a) **(b)**

Figure 3.12: Phase of breathing cycle at the onset of auditory cue (a) and visual stimulus (b). Once again, only for illustrative purposes, these plots include all trials from all participants.

Secondly, I focused on correct and incorrect trials and the result was the following:

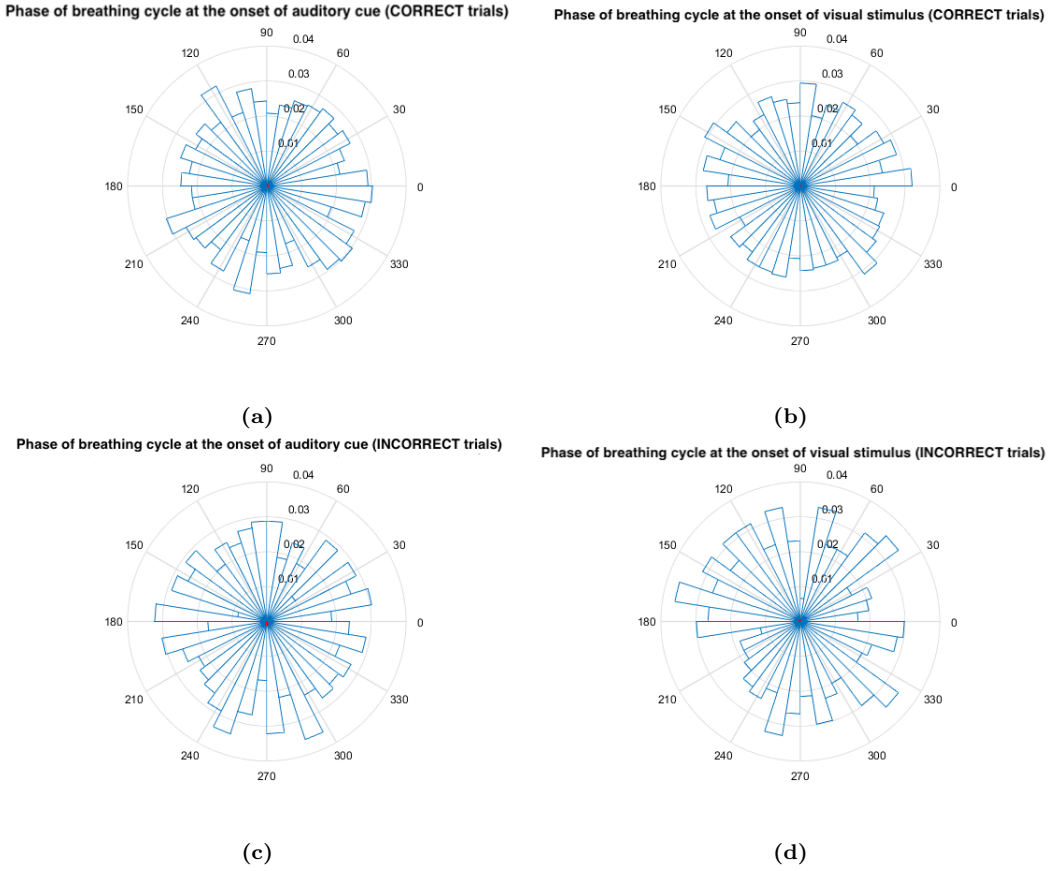


Figure 3.13: Relationship between breathing phase on stimuli display and accuracy.

In each of these graphs, there is a red line in a radial direction, at the centre of the image. This line indicates the direction - the circular mean - and magnitude - mean resultant length - of the mean resultant vector. This magnitude is a statistic between 0 and 1 that gives information about the spread of a circular variable, where 0 means that the spread is large and 1 means that all data are concentrated at a single value [66]. In my representations, however, this red line is barely visible, which might indicate that there is no significant result.

As mentioned before, I used the Watson's test to determine if the phase of the breathing cycle at which the auditory or visual stimuli were presented was related to the accuracy in the respective trial. After performing the Watson's test, I determined the number of participants presenting a significant effect (that is, with $p < 0.05$). The test showed that,

regarding the auditory cue-analysis, the effect was not significant for any participant (0 %). As for visual stimuli-analysis, it was significant for 1 participant out of 15 (6.67 %). I can conclude that **the phase of breathing cycle where the events happen does not have a significant impact on accuracy**.

Chapter 4

Discussion

In my investigation, I asked whether participants engaged in a visual discrimination task modulated their breathing activity, how this modulation was influenced by the breathing phase in which the task events occurred and how it impacted task performance. Across my analysis, I found that there is, indeed, the presence of breathing modulation that manifests significantly at the onset of visual stimuli, although it does not vary with the phase of breathing cycle. Task performance gets significantly better with each run, but this effect is not directly related to respiration, since there was no effect of run on modulation of breathing cycle duration. The covariation of human behaviour with breathing cycle duration - shorter breathing cycles were associated with better task performance - was observed around auditory cue presentation but not at the time of the visual stimulus presentation.

Task performance increases with run, but not because of a breathing modulation

Participants showed an increase in task accuracy along each run. This is something we initially noticed in the laboratory while watching the participants perform the task. Many stated that houses were harder to discriminate and would only answer with that option if the stimulus did not resemble a car, instead of answering because they were sure that it was a house. However, they would comment that this changed as they advanced in the task. It is possible that the visual processing improved, as participants would get accustomed to the task's timings, but this improvement does not appear to be related to the breathing activity.

Breathing activity is modulated by visual stimuli display, but not by the presentation of the auditory warning cue

Initially, I observed that the auditory warning cue does not trigger any kind of modulation in breathing cycle duration, which went against my expectations, since I predicted that the anticipation evoked by the auditory cue would induce a state of alertness that prepared participants for the stimulus presentation. This state of alertness does not seem to be associated with changes in breathing and I noticed, instead, that the breathing modulation arises from the presentation of the visual stimulus (the target stimulus) - there is an increase in breathing cycle duration as the stimulus is displayed.

Besides, from observing the data, it appears that participants with longer breathing cycle reveal a stronger modulation (however, we have not explored this observation in a quantitative manner). I also concluded that the amplitude of the modulation is not related with the phase of breathing cycle at the moment of the events - onset of events on inhalation or exhalation does not allow to predict the impact of modulation on respiration.

From these observations, one possibility is that the positive modulation (increase of breathing cycle duration) could happen because participants tended to relax after seeing the stimulus, as if they had previously accelerated respiration during attentive anticipation. Well, if this was the case, then when does the breathing rhythm accelerate?

Breathing activity slows down when processing visual stimuli

The previous question led me to analyse several cycles surrounding the visual stimulus trigger, in search of an acceleration of breathing cycle duration. At first, I thought that there would be an acceleration at the beginning of the trial, before the auditory cue sounded, which could explain why I could not detect any modulation with the auditory cue. With this analysis, I would be able to find if that was true.

To my surprise, the acceleration in breathing rhythm actually occurs immediately after the visual stimulus is shown. The breathing cycle at the moment of the stimulus had an increased duration and the breathing cycle immediately after that restored the breathing activity back to "normal" by having a short duration, decreasing nearly as much it had increased before.

This observation is very interesting, since it suggests that the presentation of visual stimulus induces a deceleration in breathing activity, maybe as an attempt to facilitate

visual processing.

Nevertheless, there does not seem to exist any relation between this modulation and task performance.

Faster breathing during auditory cue might yield better performance

When analysing the relation between task performance and breathing modulation I came across a curious result. Even though it was borderline significant, there was an effect of accuracy that indicated that on correct trials the breathing cycle duration at the onset of auditory cue was slightly shorter than on incorrect trials. This could be interesting, by suggesting that, in periods where we breathe slower, we are more relaxed and perhaps not so attentive to the surrounding features - there could be attention lapses. One explanation for this could be the linkage between respiration and short-term modulation in sympathetic nerve activity [67]. The sympathetic nervous system activity is associated with high alertness [68] and faster respiratory rate is associated with higher activity in sympathetic nervous system [69]. Therefore, a faster breathing rate could also be associated with increase in performance, but not be the cause.

Relating this to the previous observation, it might be legitimate to say that slower respiration at the onset of auditory cue originates from the breathing rhythm not being completely restored after slowing down significantly with the visual stimulus. This could mean that visual stimuli that induce a stronger modulation might lead to incorrect trials, although we did not test this hypothesis directly and it is important to keep in mind that this effect of accuracy was only borderline significant.

Performing the same analysis with visual stimuli display, I found a significant modulation of breathing cycle duration (effect of time), but not an effect of accuracy nor an interaction between time and accuracy, which means that neither breathing rate nor breathing modulation during visual stimuli presentation have an impact on task performance.

Task performance is not affected by breathing phase at relevant task events

Lastly, I attempted to find a relation between breathing phase at the moments of auditory cue and visual target display and task performance. The result showed that there is no link between these two factors: trial outcome cannot be predicted from the timing of the events relative to breathing cycle.

Even though there is a noticeable modulation arising from visual stimuli display, the moment at which the stimuli is presented (inhalation/exhalation) does not play a role in the amplitude of that modulation, nor in task performance. Perhaps this reveals that participants do not have the tendency to align their breathing cycle with the experimental paradigm.

It is curious to see how some of my results match previous findings, but that does not apply for all: I did not verify the effects of the phase of breathing cycle observed by Perl et al. [22] and Flexman [34] - as a matter of fact, both of those studies revealed opposing observations: Perl et al. [22] found that task accuracy in visuospatial perception is higher when stimuli are presented during inhalation, while Flexman [34] reported that exhalation at task onset would be more beneficial to increase performance.

These mismatches might arise from differences in experimental design. For example, Flexman [34] considers a visual signal detection task, in which the stimulus was a circular spot of light, appearing randomly in one of the four quadrants of the screen. Participants had to press one of four response buttons, positioned in each quadrant of a small response panel and breathing activity was recorded in a method similar to mine, with a bellows pneumograph around the chest, below the ziphoid process. Perl et al. [22], however, measured nasal airflow with a nasal cannula linked to a spirometer, while participants performed three consecutive tasks: a lexical task, a visuospatial task and a mathematics task. In the visuospatial task, more relevant for comparison, two alternatives of three-dimensional shapes were presented simultaneously and participants had to select the one that could exist in the real world (all its facets were correctly joined).

These variations in experimental design could be a factor that leads to different results - for example, it could be possible that a signal detection task induces a stronger effect in respiration due the simpler attentive approach required from participants; the presentation of two similar three-dimensional shapes contains much more information than the simple appearance of a spot of light and might require more attention and visual processing ability in order to identify each detail of stimulus design. Participants might prepare differently for each type of stimulus. The same applies to my experimental design, in which the discriminatory nature of the task, where stimuli are only presented for a very short period, may originate different breathing patterns in order to get ready for the stimuli.

Obviously, the method of recording respiration can also have an impact on the results obtained - for example, the collector in nasal airflow measurement can affect respiratory activity by increasing deadspace, while the use of a pneumograph around the chest might include some motion artefacts.

Chapter 5

Conclusion

5.1 General Conclusion

In this study, I proposed, with the collaboration of my supervisor, to investigate the interactions between human respiration and visual processing. In order to do this, we designed a visual discrimination task, which I programmed from scratch in Matlab. We prepared every acquiring instrument and planned a setup that would be optimal to record the intended signals and acquired data from 18 participants. This great effort allowed me to obtain a large and very rich dataset, containing information from various physiological signals, which provided the unique and valuable groundwork for the development of this research and future ones. Then, I proceeded to elaborate Matlab scripts in order to explore and analyse the breathing data obtained.

We aimed for participants to have an accuracy of 75 % (in reality, this value was slightly higher) and we noticed that there was a significant improvement throughout the task, where participants would learn to better distinguish each stimulus. I attempted to discover what could be the origin of this effect and if it was related with any breathing changes. What my analyses showed, and probably the main result of this study, was that accuracy may be, in part, dictated by the breathing rate during the auditory cue - moments where the participants were breathing faster during the presentation of the auditory cue were associated with better visual performance. This result was only borderline significant but, perhaps, when considering the remaining data from other bodily signals, it is possible to understand

what leads to this phenomenon - one hypothesis is that moments of faster breathing are associated with enhanced arousal, which could be confirmed by looking at the EDA and pupil size data, both measurements of sympathetic activation; also, a look into EEG data could reveal a certain pattern that varies significantly with each run, as does accuracy.

Another interesting result to take home from this research is that respiration slows down when stimuli are presented. Maybe I could speculate that the prolonged breathing rate could help with visual processing in moments of focused attention, but I did not find any evidence of this implication. Nonetheless, although the moment when respiration slows down is locked with visual stimuli, it also coincides with the moment when participants decide and respond, so this deceleration can also be associated, for example, with decision making or the motor response. I speculate that this breathing modulation may even be indirectly related to accuracy variation, since it could affect the breathing rate during the auditory warning cue of each following trial. In fact, the improvement in task performance may result from various factors and the breathing modulation at visual stimuli onset could be just one of those factors, that plays a small role on its own but could show a deeper explanation when putting all of those elements together.

As I have mentioned, it was to my surprise that I did not observe any influence of breathing phase (inhalation/exhalation) on task performance or breathing modulation.

All in all, the results I obtained support that breathing activity is connected to sensory-cognitive function, possibly in a causal way, reinforcing the importance of considering respiration when investigating human cognition.

5.2 Future Work

As I mentioned before, the analysis of the remaining data that I collected could help provide deeper explanations for what I have found, and even reach new conclusions beyond that. This dataset is abundant enough to carry out diverse studies around the same topic on body-brain interactions in visual perception and the work I have developed might pave the way for the next investigators who are interested in this subject, allowing them to incorporate these data in their own work.

As it was planned on the original study, the following steps include the examination of the other physiological signals - EEG, EOG, EMG, EKG, PPG, EDA, EGG, eye movements

and pupillography - and integrate them with the discoveries made in this project. It will be interesting to see what information might come out of that research - perhaps the borderline significant results I observed will reveal to have a stronger impact when combined with other measurements of arousal, for example; it could be found that there is indeed relevance in breathing phase; the EEG data will contribute to a deeper understanding of the impact breathing modulation has on brain function and hopefully explain it in a more extensive and complete way that includes brain-respiration synchronies.

Furthermore, there is still much to be explored regarding respiration and sensory-cognitive interactions and strengthen the framework to explain how the body and brain interact in sensory processing.

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