Universidade de Aveiro Departamento de Educação e Psicologia
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JACQUELINE HELENA TAVARES FERREIRA

EMOÇÕES DE MEDO E NOJO: RESPOSTAS SUBJETIVA E CARDÍACA EM FUNÇÃO DE DIFERENTES ESTÍMULOS SENSORIAIS

EMOTIONS OF FEAR AND DISGUST: SUBJECTIVE AND CARDIAC RESPONSES AS A FUNCTION OF DIFFERENT SENSORY STIMULI

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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Psicologia, realizada sob a orientação científica da Doutora Sandra Soares, Professora Auxiliar do Departamento de Educação e Psicologia da Universidade de Aveiro, e coorientação do Doutor Carlos Fernandes da Silva, Professor Catedrático do Departamento de Educação e Psicologia da Universidade de Aveiro, e do Doutor Mats J. Olsson, Professor Catedrático do Departamento de Neurociências, Divisão de Psicologia, do Instituto Karolinska (Suécia).

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Dedico este trabalho a todos que de alguma forma me apoiaram ao longo deste percurso.

o júri

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palavras-chave

Emoções, resposta cardíaca do nojo e do medo, variabilidade da frequência cardíaca, propensão e sensibilidade ao nojo, entropia do ruído, classificador automático, odores corporais.

resumo

O trabalho apresentado nesta tese teve como objetivo explorar a resposta cardíaca das emoções de nojo e de medo usando estímulos visuo-auditivos e olfativos. Esta tese está organizada em três grandes partes. A primeira parte apresenta uma breve revisão das teorias das emoções, uma descrição sobre o reconhecimento automático da emoção baseado no sinal do ECG e uma revisão acerca dos sinais químico-sensoriais transmitidos pelos odores corporais, assim como os seus efeitos nas respostas fisiológicas, cognitivas e subjetivas. A segunda parte apresenta os quatro estudos que foram realizados. No Estudo 1 usámos filmes para induzir as respostas emocionais de nojo, de medo e neutras e examinámos se a entropia do ruído do sinal de ECG pode funcionar como um potencial biomarcador para discriminar as três condições emocionais. Os resultados mostraram que é possível discriminar as três condições emocionais usando a entropia do ruído do sinal de ECG com 88% ($p < .05$) de precisão e que o valor da mediana da condição de nojo foi superior, quando comparado com as condições de medo e neutras. No Estudo 2 usámos a entropia do ruído do sinal de ECG para desenvolver e testar um algoritmo que classifica as emoções automaticamente. O classificador obteve um bom desempenho na identificação de nojo e medo (com 60% de sensibilidade e 80% de especificidade) e um desempenho perfeito na condição neutra. Para além da resposta a estímulos visuais, também avaliámos a resposta cardíaca usando estímulos olfativos, nomeadamente os odores corporais de nojo, medo e neutros. De forma a controlar as diferenças individuais da propensão e sensibilidade ao nojo na perceção dos odores corporais, no Estudo 3 examinámos as características psicométricas da versão Portuguesa da DPSS-R. Os resultados confirmaram a existência de dois fatores independentes, propensão e sensibilidade ao nojo. Adicionalmente, a escala obteve uma validade convergente e discriminante aceitável e confiabilidade satisfatória. No Estudo 4, investigámos como é que os odores corporais recolhidos em condições emocionais específicas influenciam a resposta de odores corporais emocionais apresentados subsequentemente, ao nível subjetivo e da resposta cardíaca. Os resultados demonstraram uma redução da variabilidade da frequência cardíaca (HF-HRV) quando os participantes cheiraram os odores corporais neutros depois dos odores corporais de nojo e de medo. O efeito da ordem de apresentação dos odores corporais também se verificou nas avaliações subjetivas, sendo os odores corporais neutros avaliados como mais intensos depois da apresentação dos odores de medo e de nojo. Este efeito foi independente da agradabilidade atribuída aos odores corporais. Finalmente, na terceira parte, apresentamos a discussão geral dos principais resultados, as limitações dos estudos, bem como propostas para estudos futuros e potenciais implicações e aplicações dos resultados. Em síntese, os resultados dos estudos descritos neste trabalho sugerem que o ruído do ECG contém informações significativas que podem permitir reconhecer emoções e que a ordem de apresentação do odor corporal pode afetar a resposta cardíaca e subjetiva dos participantes.

keywords

Emotions, disgust propensity and sensitivity, cardiac response of disgust and fear, heart rate variability, entropy of noise, automatic classifier of emotions, body odors.

abstract

The work presented in this thesis aimed to explored the cardiac response of the emotions of disgust and fear using visuo-auditory and olfactory stimuli. This thesis is organized into three major sections. The first section provides a brief revision of the theories of emotions, a brief description of automatic recognition of emotion based on ECG (electrocardiogram) and a review of chemosensory signals transmitted via body odors, as well as their effects in human's physiological, cognitive and behavioral responses. The second section presents the four studies that were conducted. In Study 1we used movies to induce disgust, fear and neutral emotions and examined whether noise entropy of ECG can work as a potential biomarker to discriminate disgust from fear and neutral conditions. The results showed that it is possible to discriminate such emotions based on ECG noise entropy with 88% ($p<.05$) accuracy and that the median value of the disgust condition was higher when compared with the fear and neutral conditions. In Study 2 we developed and tested a classifier to automatically classify emotions using noise entropy of ECG. The performance of the classifier was good for fear and disgust identification (60% of sensitivity and 80% of specificity) and perfect for identification of the neutral condition (100%). In addition to the responses to the visual stimuli, we also evaluated the cardiac response using olfactory stimuli, namely the body odors collected in conditions of disgust, fear and neutral. To control for potential individual differences in disgust propensity and sensitivity on body odor perception, in Study 3 we examined the psychometric properties of the Portuguese version of DPSS-R. The results confirmed the existence of two distinct factors, disgust propensity and sensitivity. Moreover, the scale showed an acceptable convergent and discriminant validity and a satisfactory reliability. In the Study 4 we investigated how a BO prime affects the emotional tone of a subsequent BO message, on cardiac and subjective responses. The results demonstrated a reduction in heart rate variability (HRV-HF) when the participants smelled the neutral body odors after they smelled the disgust and fear body odors. The effect of order of presentation was also evident in the subjective ratings, with the neutral odors being perceived as more intense when the receivers smelled the neutral odors after they smelled the negative body odors. Such effects were independent of the pleasantness of the body odors. Finally, in the third section we presented the general discussion of the main results, the current limitations of the studies as well the future directions and the potential implications and applications of the results. Overall, the findings of the studies described in this thesis suggest that the ECG noise contains meaningful information that can allow emotion recognition and that the order of presentation of body odor can affect the cardiac response and subjective response of the receivers.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ANS	Autonomic Nervous System
SNS	Sympathetic Nervous System
PSN	Parasympathetic Nervous System
ECG	Electrocardiogram
HR	Heart Rate
HRV	Heart rate variability
LF	Low frequency
HF	High Frequency
R-R intervals	Interval between consecutives R waves
ROC curve	Receiver operating characteristic curve
NN	Normal to normal beats
SDNN	Standard deviation of NN intervals
SFS	Sequential forward selection
SBS	Sequential backward selection
EMG	Electromyography
EDA	Electrodermal activity
EEG	Electroencephalogram
IQR	Interquartile Range
AVNN	Average of all NN intervals
BOs	Body Odors
LMMS	Linear Mixed Models
DN	Disgust Neutral
ND	Neutral Disgust
FN	Fear Neutral
NF	Neutral Fear

PART I: THEORETICAL BACKGROUND

CHAPTER I: INTRODUCTION

1. Emotion

Emotion have been shaped by the pressure of evolution. The critical role of evolution in emotion is based, above all, on the significant discoveries about the facial expression of emotions, written by Darwin in his book, *The Expression of Emotion In Man and Animals* (Darwin, 1872). Besides considering that expressions of emotions are hereditary and have communicative functions, Darwin defended that they evolved and serve adaptive purposes. These expressions emerged from a process that protects and prepares the organism for action. Throughout evolution, our ancestors faced diverse challenges such as escaping from predators and finding food (e.g., Nesse, 1990; Nesse & Ellsworth, 2009). Facing these emotional stimuli repeatedly forced the organism to develop an effective system to optimize its adequacy with the environment. Emotions then evoke special systems that coordinate and produce organized responses from multiple components, by preparing the organism to approach or avoid certain situations (e.g., Nesse, 1990; Tooby & Cosmides, 2008).

Although the environmental challenges we face today are very different from those our ancestors faced in the past, emotion is crucial to process information, hence influencing our cognitive processes, such as perception, attention, and memory (e.g., Kensinger, 2009; Öhman, Flykt, & Esteves, 2001; Vuilleumier, 2005; Yiend, 2010), as well as physiological responses, such as heart rate and immune function (e.g., Kreibig, 2010; Salovey, Rothman, Detweiler, & Steward, 2000; Schaller & Park, 2011; Stevenson et al., 2012), and our behavioral responses (e.g., Baumeister, Vohs, Nathan DeWall, & Zhang, 2007; Tooby & Cosmides, 2008). Therefore, emotion influences the body's major systems.

Everyone knows what an emotion is,
until asked to give a definition.
Then, it seems, no one knows.
(Fehr & Russell, 1984, p. 464)

1.1. Theories of emotion

What is an emotion? This question has been an issue for a long period of time and still there is no consensus about its definition (e.g., Barrett, 2006a; Hamann, 2012; Kleinginna & Kleinginna, 1981; Solomon, 2008). However, all theorists agree that emotional responses result from an interaction between three major components: cognitive, physiological and behavioral (e.g., Ekman & Davidson, 1994; Izard, 2010; Lang et al., 1993; Nesse, 1990; Scherer, 2005). Moreover, most contemporary researchers assume that emotion has biological roots with an adaptive function and, essentially, two major theories have been adopted to study emotion: discrete and dimensional theories (e.g., see reviews Hamann, 2012; Mauss & Robinson, 2009).

1.1.1. Discrete and dimensional theories of emotion

Darwin's work had a great influence in the study of emotions, especially on discrete theories, which assume that evolution played a crucial role in designing emotion's characteristics and functions (e.g., Coppin & Sander, 2013). This theory considers that some emotions are discrete or basic (e.g., Ekman, 1992; Tomkins, 1980). These emotions are fear, anger, disgust, sadness, happiness and surprise, and other emotions have also been added to this list, such as contempt (Ekman, 1992; Izard, 1991; Tomkins, 2008) and interest (e.g., Izard, 1991; Panksepp, 2004). The combination of these emotions would result in more complex emotional states, called secondary emotions (e.g., embarrassment, guilt, pride, Ortony & Turner, 1990; Tangney, Miller, Flicker, & Barlow, 1996).

Basic emotions share some properties, such as: a) they are present in humans and non-humans (e.g., the facial expression of fear produced by primates is similar to the human expression of fear; b) they have specific triggers (threatening situations trigger the emotion of fear); and c) they are triggered automatically and are displayed for a short duration (provoke

rapid changes to mobilize the organism) (e.g., Coppin & Sander, 2013; Ekman, 1992). Moreover, they have specific facial expressions and functions. For example, the facial expression of fear is characterized by raised brows, increased eye and nasal aperture, whereas the facial expression of disgust is characterized by slightly narrowed brows, decreased eye and nasal aperture (nose wrinkling) associated with lowered lips (e.g., Darwin, 1872; Izard, 1971; Matsumoto, Keltner, Shiota, O'Sullivan, & Frank, 2008; Susskind et al., 2008). Thus, the facial expression of fear seems to be the opposite of disgust. While the facial expression of fear increases sensory acquisition by enhancing eye movements and breathing speeding, the facial expression of disgust decreases sensory acquisition by reducing eye movements and breathing speeding, which is related to their specific functions (e.g., Ekman & Friesen, 2003; Susskind et al., 2008). Indeed, fear motivates the organism to escape from or avoid dangerous situations (e.g., encounters with predators, Adolphs, 2013; Öhman, Carlsson, Lundqvist, & Ingvar, 2007; Öhman et al., 2001; Soares, Maior, Isbell, Tomaz, & Nishijo, 2017), while disgust motivates the organism to avoid or reject pathogenic agents (e.g., Olatunji, Haidt, McKay, & David, 2008; Tsao & McKay, 2004; Tybur, Lieberman, Kurzban, & DeScioli, 2013). Both emotions have distinctive functions or goals, which might require specific organism responses. Therefore, according to this theoretical approach, each basic emotion should activate a specific psychophysiological response (e.g., Ekman, 1992; Stemmler, 2004).

According to Wundt, the emotional experience is characterized in three dimensions: a) pleasure/displeasure; b) excitement/inhibition; and c) tension/relaxation (Wundt, 1980). Influenced by this view, the dimensional theory of emotions considers that an emotional experience results from two fundamental neurophysiological dimensions - valence and arousal (Russell, 1980). Although there is some discussion about the number of dimensions [for example, Fontaine, Scherer, Roesch, and Ellsworth (2007) proposed four dimensions, pleasantness, potency-control, activation-arousal, and unpredictability] and about the term for the dimensions adopted by researchers [for example, Watson, Wiese, Vaidya, and Tellegen (1999) suggested positive and negative affect)], the most commonly adopted ones are the two dimensions of the circumplex model of affect, proposed by Russell (1980). Smith and Ellsworth (1985), state that valence and arousal are considered as essential elements to characterize an emotion. Valence represents the extent of pleasantness (positive valence) and unpleasantness (negative valence), while arousal represents the extent of calmness (low arousal) and excitation (high arousal) (e.g., Lang, Greenwald, Bradley, & Hamm, 1993; Russell, 1980). Instead of a categorical representation, the emotional experience is represented

on a continuum, which includes an unlimited number of emotions (e.g., Barrett & Russell, 1999). Thus, according to the dimensional theories of emotion, fear and disgust share the same valence and arousal, i.e., they are both represented by negative valence and high arousal (Hamann, 2012; Lang et al., 1993) (see Figure 1).

In summary, both theories have provided significant advances in the comprehension of emotion, but both have been criticized. Discrete theory has been criticized especially because of its lack of explanation in situations where emotions overlap, which represent a great challenge for comorbid psychological disorders (e.g., mood disorders, Coppin & Sander, 2013; Posner, Russell, & Peterson, 2005). In turn, dimensional theory has been criticized because of its lack of ability to differentiate some emotions; for instance, as mentioned above, fear and disgust share the same valence and arousal (e.g., Coppin & Sander, 2013). Importantly, although these theories diverge in the way they describe emotions, some researchers consider that they can complement each other, since combining these two approaches allow to capture both the general and the specific aspects of emotion (e.g., Levenson, 1988; Russell, 2003).

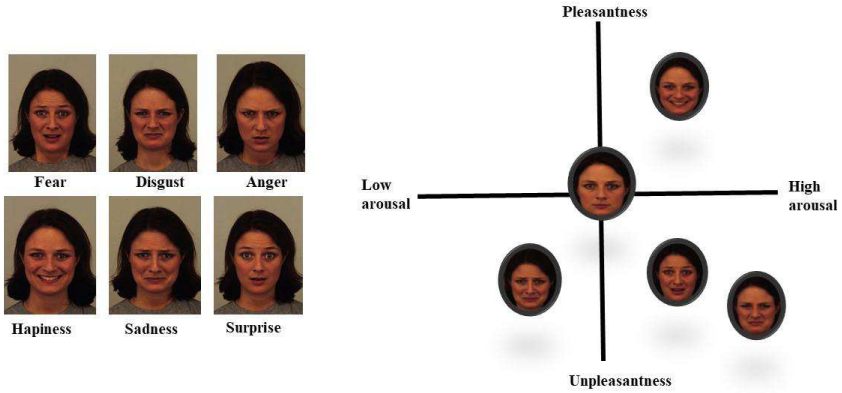

Figure 1. Representation of basic facial expressions within the framework of dimensional approach. Adapted from "The implicit processing of categorical and dimensional strategies: an fMRI study of facial emotion perception." by Y. T. Matsuda, T. Fujimura, K. Katahira, M. Okada, K., Ueno, K. Cheng, and K. Okanoya, 2013, Frontiers in human neuroscience, 7, p. 2. Adapted with permission. The faces used in the figure were from The Karolinska Directed Emotional Faces, Lundqvist, D., Flykt, A., & Öhman, A. (1998). The Karolinska Directed Emotional Faces (DEF). Stockholm: Department of Clinical Neuroscience, Psychology Section, Karolinska Institutet [CD-ROM, ISBN 91-630-7164- 9].

2. Physiological responses of emotions

One of the main goals of psychophysiology is to study the physiological component of emotional response (e.g., Cacioppo, Berntson, Larsen, Poehlmann, & Ito, 2000). The peripheral nervous system has been more investigated because it was studied and described first, as its study is more accessible than the central nervous system (e.g., Berntson, Quigley, & Lozano, 2007; Cacioppo et al., 2000; Larsen, Berntson, Poehlmann, Ito, & Cacioppo, 2008). The peripheral nervous system includes the somatic and autonomic nervous systems (ANS), which are generally associated with skeletal muscle voluntary innervation of body movements (e.g., facial muscle) and involuntary body functions, respectively (e.g., stimulation of the digestion, innervation of the cardiac muscle, respiratory rate) (e.g. Berntson et al., 2007; Larsen et al., 2008). The ANS is divided into a sympathetic nervous system (SNS), which is associated with body activation and a parasympathetic nervous system (PSN), related to body relaxation or restorative functions (e.g., Berntson et al., 2007; Larsen et al., 2008). In general, these two systems work in a dynamic balance and in a complementary way (e.g., Berntson et al., 2007; Larsen et al., 2008). The activities of these two systems can be affected by environmental changes, such as physical or psychological stress. Some emotional events, such as dangerous or threatening situations, can activate the sympathetic system, which triggers the fight-flight response (e.g., increased heart rate and blood flow) while in a context of perceived safety, the parasympathetic system becomes dominant (e.g., stores the energy and regulates basic body functions such as digestion) (e.g., Bradley & Lang, 2007; McCorry, 2007; Porges, 2009). Therefore, the activities of these two systems can be affected by emotional response. However, whether ANS activities are specific or general for basic emotions is a question that remains to be answered.

2.1. Emotion elicitation

Selecting effective techniques to induce emotions is one of the critical steps to identify the autonomic response patterns of the emotional response. Researchers have used different techniques, such as facial action tasks, pictures, movies, music and odors to induce emotions (e.g., Kreibig, 2010; Palomba, Sarlo, Angrilli, Mini, & Stegagno, 2000; Rainville, Bechara, Naqvi, & Damasio, 2006; Rottenberg, Ray, & Gross, 2007). The major goal is to select a technique that can be effective in inducing the target emotion and in triggering physiological responses (e.g., Christie & Friedman, 2004). The presentation of images and films are the most commonly used techniques to induce emotions, regardless of the theoretical approach, that is, discrete or dimensional (e.g., Christie & Friedman, 2004; Kreibig, 2010). They do not involve deception and can be easily standardized (e.g., Codispoti, Ferrari, & Bradley, 2006; Uhrig et

al., 2016). However, few studies have compared the effectiveness of images and film presentation in emotional elicitation. Uhrig et al. (2016) compared the effectiveness of these two techniques and found that the images were more effective in triggering negative emotions. However, they only measured subjective responses and the participants knew most of the films used, which was not the case with the images. Nevertheless, although images are an effective method to induce emotions, many researchers that used films have been successful in eliciting emotion to study both its subjective and physiological responses (e.g., Kreibig, 2010; Schaefer, Nils, Sanchez, & Philippot, 2010). Contrarily to static images, films are dynamic stimuli and multisensorial since they include both visual and auditory stimulation, hence are more effective in capturing attention and it have greater ecological validity (e.g., Gross & Levenson, 1995; Hewig et al., 2005). Although they can induce other emotions, films can be quite reliable to induce target emotions (e.g., Hewig et al., 2005; Rottenberg et al., 2007; Schaefer et al., 2010).

2.2. Measuring the Autonomic Nervous System activity

Emotional responses trigger physiological reactions (body sensations) that are partially unconscious and outside the individuals' voluntary control (e.g., Cacioppo, Tassinary, & Berntson, 2007; Mauss & Robinson, 2009). To measure these reactions, researchers have used several measures, which besides accessing emotional states that individuals are less aware of, are very useful for people who have difficulties in identifying or expressing emotion, such as people who suffer from autism spectrum disorders (e.g., Cacioppo et al., 2007; Codispoti, Surcinelli, & Baldaro, 2008; Mauss & Robinson, 2009; Mendes, 2009). These measures also reduce the cognitive and social bias (e.g., social desirability constraints) that are inherent to self-report measures (e.g., Jang, Park, Park, Kim, & Sohn, 2015; Mauss & Robinson, 2009; Mendes, 2009). Electrodermal and cardiovascular activities are the most commonly used (e.g., Kreibig, 2010; Mauss & Robinson, 2009; Mendes, 2009), but this thesis will only focus on the electrocardiogram (ECG).

2.3. The Electrocardiogram

The ECG is a non-invasive tool that records the electrical activity of the heart using electrodes placed at different locations on the surface of the body (e.g., Cacioppo et al., 2007; Larsen et al., 2008; Limaye & Deshmukh, 2016). The electrical activity of the heart is represented by different phases: P wave - atrium depolarization (atrium contraction), QRS complex - depolarization of right and left ventricles (ventricles contraction), T wave - repolarization of ventricles (ventricles relaxation) and U waves (e.g., Berntson et al., 2007;

Wasilewski & Poloński, 2012). The ECG frequency ranges from 0.67 to 120 Hz. The P and T waves are low frequency components (5-9 Hz), whereas the QRS complex is in the high frequency component (e.g., Clifford, Azuaje, & Mcsharry, 2006; Hoti & Khattak, 2014). The most common cardiovascular indices used by researchers are heart rate and heart rate variability (e.g., Kreibig, 2010). The heart rate (HR-number of heart beats per minute) reflects the activity from both branches of the autonomic system. The dynamic balance between both branches of the ANS controls the heart rate change by influencing the sinoatrial node activity (primary pacemaker), which is responsible for starting the heartbeat (e.g., Berntson et al., 2007; Shaffer, McCraty, & Zerr, 2014). Sympathetic activation increases the HR by increasing the rate of spontaneous depolarizing of pacemaker cells, whereas parasympathetic activation (vagal modulation) decreases the heart rate by reducing the rate of spontaneous depolarizing of pacemaker cells (e.g., Shaffer et al., 2014). A faster heart rate produces a shorter interbeat interval and this can be due to increased SNS or decreased PNS activities, while a slower heart rate results in a longer interbeat interval, which could result from increased PNS or decreased SNS activities (e.g., Appelhans & Luecken, 2006). Both systems influence the heart rate continuously, but the parasympathetic activity predominates at rest (ranging from 60-100 beats per minute-bpm) with an average of 75 bpm (e.g., Quintana & Heathers, 2014; Shaffer et al., 2014). These heartbeat oscillations become apparent when the heart rate is analyzed on a beat-to-beat interval, often named heart rate variability (HRV, e.g., Shaffer et al., 2014). The HRV is composed by high frequency (HF, 0.15-0.40 Hz) and low frequency (LF, 0.01-0.15 Hz) components. The lower frequency is mediated by both systems, whereas the higher frequency is essentially mediated by the parasympathetic system (e.g., Shaffer et al., 2014; Thayer, Åhs, Fredrikson, Sollers, & Wager, 2012). An increased activity in the sympathetic system is related with an increase in heart rate, which reduces the HRV. In contrast, an increased parasympathetic activity is associated with reduced heart rate and higher HRV (e.g., Thayer et al., 2012).

2.4. Cardiac responses of disgust and fear

William James (1894/1994) was one of the first psychologists to propose that each emotional state is activated by specific patterns of ANS. Ever since, searching for a specific pattern of ANS has been one of the central topics of psychological research, as humans often use categories and body sensations to describe emotional experiences (e.g., Barrett, 2006b; Larsen et al., 2008). Furthermore, investigating the physiological response of emotions would help to understand the mechanisms underlying emotional responses, especially in

psychopathology (e.g., Codispoti et al., 2008). Many studies investigating the autonomic pattern of discrete emotions have reported some cardiac specificities for the emotions of fear and disgust. For instance, studies examining the heart rate response of six basic emotions during voluntary production of facial expression (directed facial action task) or during a relived emotion task (relive a past emotional experience), have found greater cardiac acceleration for fear than for disgust (e.g., Boiten, 1996; Ekman, Levenson, & Friesen, 1983; Levenson, Ekman, & Friesen, 1990). Furthermore, results from studies using images showed that fear images (e.g., snakes, angry faces) increased the heart rate, and that disgust images (e.g., mutilation pictures) reduced the heart rate, when compared with neutral images (e.g., Aue, Flykt, & Scherer, 2007; Lang et al., 1993; Stark, Walter, Schienle, & Vaitl, 2005). Moreover, studies using movie presentations showed that a disgust movie, depicting surgery and blood, activated the parasympathetic system (reflected in a decreased heart rate), while a fear movie depicting violent threats increased the sympathetic activity (thus with an increase in heart rate) (e. g., Gross & Levenson, 1993; Palomba et al., 2000). These results suggest that disgust seems to reduce the heart rate while fear seems to accelerate it. Indeed, lower heart rate is related with an increased activity in the parasympathetic system and is, therefore, often considered as an autonomic marker of the disgust response. In contrast, a higher heart rate, associated with increased sympathetic activity, is considered as an autonomic marker of the fear response (e.g., Levenson et al., 1990; Rohrmann & Hopp, 2008; Woody & Teachman, 2000). However, results from some studies do not support this cardiac specificity. Specifically, when disgust is compared with the neutral condition, the results seem less consistent. The results of some studies found no evidence of differences between the disgust and neutral conditions (e.g., Klorman & Ryan, 1980; Levenson, Carstensen, Friesen, & Ekman, 1991), while others have found an increased heart rate for the disgust condition (e.g., Prkachin, Williams-Avery, Zwaal, & Mills, 1999; Vrana, 1994). Moreover, Rohrmann and Hopp (2008) found evidence of a co-activation between both systems in response to disgust stimuli, instead of a dominance of the parasympathetic system. Furthermore, some studies have suggested that the heart rate response seems to be influenced by different types of disgust stimuli (see review, Kreibig, 2010). These stimuli have been grouped in two categories: a) contamination and pollution stimuli; and b) mutilation, blood and injuries stimuli. In fact, studies have found evidence of an increased heart rate towards contamination stimuli and a decreased heart rate to mutilation stimuli (see review, Kreibig, 2010). However, this evidence is insufficient to claim such cardiac specificity.

2.5. Autonomic pattern of emotional response: multivariate approach

Researchers have suggested that using multiple autonomic measures at the same time on one subject can help to establish a more accurate interpretation of the autonomic pattern of an emotional response (e.g., Berntson & Cacioppo, 1999; Cacioppo, Klein, Berntson, & Hatfield, 1993; Rainville et al., 2006; Thayer & Friedman, 2000). However, in general, regardless of the number of measures used, most studies have adopted a univariate approach to data analysis (e.g., Cacioppo et al., 1993; Kreibig, 2010). The univariate approach analyzes the relationship between a single dependent measure and one or more independent variables (e.g., Kragel & LaBar, 2013; Meyers, Gamst, & Guarino, 2006). However, the emotional response integrates a complex interaction among physiological components that might have a specific influence on different emotional states. Therefore, a univariate approach might allow only a partial characterization of an emotional state (e.g., Kragel & LaBar, 2013; Meyers et al., 2006) and, for that reason, some researchers have adopted a multivariate approach to analyze the data.

Often, the data set collected during an experimental setting includes multiple dependent and independent variables. The multivariate approach examines simultaneously the relationship among three or more variables, contrarily to the univariate approach, which analyzes variables separately (e.g., Kragel & LaBar, 2013; Meyers et al., 2006). The general purpose of this approach is to examine, predict and explain the relationships among variables. There are many multivariate techniques, which can be broadly classified as dependency and interdependency techniques (e.g., Meyers et al., 2006). The dependency technique, such as multivariate analysis of variance (MANOVA), discriminant analysis and multiple regressions, are applied when the criterion/dependent variables and the predictor/ independent variables are known (e.g., Meyers et al., 2006). The interdependency techniques, such as factor analysis, cluster analysis and multidimensional scaling, can be used when variables are interrelated without dependent or independent characterization (e.g., Meyers et al., 2006).

Using multivariate approach, Christie and Friedman (2004) analyzed the data from multiple autonomic measures (electrocardiogram, skin conductance and blood pressure), while participants viewed emotional films (amusement, anger, contentment, disgust, fear, and sadness) and a neutral film. The results from pattern classification analyses showed a distinct autonomic pattern for all emotions, except for disgust. Specifically, the percentage of correct prediction of the fear emotion was 52.94 % while for disgust was 20.59%. Adopting a similar approach, Stephens, Christie and Friedman (2010) showed that, overall, 44.6% of the observations were correctly classified into the predicted emotional conditions (amusement,

anger, contentment, fear, sadness, surprise and a neutral state), which was above chance level ($z=16.05$, $p<.001$). Kragel and Labar (2013) also found it was possible to differentiate emotions (fear, anger, sadness, surprise, contentment and amusement) with an accuracy of 58.0% for autonomic measures (ECG, electrodermal, gastric and respiratory activity), which was significantly above the chance level (14%). Although the results from Christie and Friedman (2004) did not find an autonomic pattern for disgust, these studies have shown promising results in identifying a specific pattern of autonomic response of distinct emotions, which indicates that using multiple measures and improving the analyses' approaches or adopting new ones to analyze the data from autonomic measures can also help to identify the autonomic pattern of emotions more accurately.

CHAPTER II: AUTOMATIC RECOGNITION OF EMOTIONS

3. Pattern recognition

Recognizing patterns is critical for human survival and has an essential role in human brain development (e.g., Mattson, 2014). The primary function of the brain is to process the environmental information received from the sensory systems, and then produce adaptive behavioral responses, such as finding food sources and identifying potential predators (e.g., Mattson, 2014). Humans are excellent at recognizing patterns; we have the ability to recognize patterns at a very early stage in our lives (e.g., Mattson, 2014; Simion & Giorgio, 2015). For instance, newborns within the first few days of live showed preference towards happy faces compared to fearful ones (e.g., Farroni, Menon, Rigato, & Johnson, 2007). Moreover, newborns with 3 months old can differentiate human faces from non-human faces (monkey and gorilla faces, Heron-Delaney, Wirth, & Pascalis, 2011). Thus, our brain has developed an effective cognitive and neural network for pattern recognition (e.g., Leppänen & Nelson, 2009; Parr, 2011).

According to Watanabe (2014), patterns can be represented by some entity, such as the image of a fingerprint or a human face. Pattern recognition can be described as a process of collecting information from the environment and processing it, and deciding based on those patterns or categories (Watanabe, 2014). Teaching a machine how to recognize a pattern is a challenge for researchers. Pattern recognition applied to machines means building a machine with the ability to collect information from the environment, which would then learn to select patterns of interest from the environment and make a reasonable decision about the categories of the patterns (e.g., Duda, Hart, & Stork, 2001; Jain, Duin, & Mao, 2000).

Automatic pattern recognition has been applied in several areas for different purposes, for example, for biometric recognition based on features such as faces, fingerprints and voices (e.g., Chen & Te Chu, 2005; Jain, Hong, & Pankanti, 2000; Jain et al., 2000; Jain, Ross, & Prabhakar, 2004); for bioinformatics to perform sequence of analysis of DNA (e.g., Kumar, Nei, Dudley, & Tamura, 2008; Kumar, Tamura, & Nei, 2004; Stormo, 2000), and for others applications (e.g., see review by Jain et al., 2000). Recently, researchers have been trying to develop computers with the ability to recognize emotional states (e.g., Jerritta, Murugappan, Nagarajan, & Wan, 2011; Picard, Vyzas, & Healey, 2001; Picard, 2002).

3.1. Affective computing

As previously mentioned, emotion represents an essential component of human interaction with the environment. Emotions add meaning to the human experience, since they can trigger an appetitive or aversive response towards objects, people or even ideas (e.g., Tooby & Cosmides, 2008). Moreover, emotion plays a critical role in rational thinking and decision-making (e.g., Bechara, 2004; Bechara, Damasio, & Damasio, 2000; George & Dane, 2016). Traditionally, machine systems, such as computers, were built based primarily on a cognitive approach, i.e., ignoring the emotional component. However, nowadays researchers from computer and affective science are interested in integrating emotions to build more intelligent systems, with the ability to sense and respond to the user's emotional state in an appropriate way, that is, a machine with an interface capable of knowing the user's emotional state (e.g., André, Rehm, Minker, & Bühler, 2004; Picard & Klein, 2002; Picard et al., 2001; Picard, 2002). In an attempt to build a computer that can recognize the user's emotional state, researchers have trained and tested computer algorithms to classify emotions based on physiological responses (e.g., see review by Jerritta et al., 2011; Picard et al., 2001).

3.2. Automatic recognition of emotions

The process of emotion recognition using computational algorithms includes the following steps: a) selecting the theoretical approach; b) emotion elicitation and data acquisition; c) pre-processing the signal; d) processing the signal (feature extraction and feature reduction), and e) classification (Figure 2). As in psychophysiological research, researchers in this area have adopted discrete or dimensional approaches and have used similar procedures to induce (usually images and movies) and to measure the emotion response (ECG being the most commonly used) (e.g., Bong, Murugappan, & Yaacob, 2012; Jerritta et al., 2011; Selvaraj, Murugappan, Wan, & Yaacob, 2013). Below we will focus on pre-processing and processing of the signal (feature extraction and feature reduction), as well as in classification, since the remaining steps were already described in the first chapter of this thesis.

3.3. ECG pre-processing phase

Electrocardiogram (ECG) has been widely used for screening and diagnosing heart diseases, and therefore, a clear or clean ECG signal is required for these purposes (e.g., Clifford et al., 2006; Limaye & Deshmukh, 2016; Rangayyan, 2015; Sörnmo & Laguna, 2006). However, the ECG record is often subject to several sources of noise and artifacts, which are unwanted information that complicate the manual and automatic analysis and the interpretation

of the ECG signal (e.g., Clifford et al., 2006; Limaye & Deshmukh, 2016; Sörnmo & Laguna, 2006).

3.3.1. ECG noise characterization

Noise and artifacts can be produced by internal factors of the body, i.e., other physiological activities, or by external factors from the environment (e.g., noise from other device systems) and are broadly classified into two categories: low frequency noise, such as baseline wander, and high frequency noise, such as power line interference (e.g., Haritha, Ganesan, & Sumesh, 2016; Rangayyan, 2015; Sörnmo & Laguna, 2006). The most common sources of noise that affect the ECG are: electromyography, baseline wander and power line interference (e.g., Haritha et al., 2016; Limaye & Deshmukh, 2016; Sörnmo & Laguna, 2006). Electromyography noise are produced by electrical activities from other body muscles besides the heart. Their frequency components overlap substantially with those of the QRS complex, which makes their detection difficult (e.g., Limaye & Deshmukh, 2016; Luo & Johnston, 2010). The baseline wander, in turn, is produced by electrode-skin impedance (poor or loose contact of the electrode and the skin), participants' movements and respiration. It lies within the low-frequency component of the ECG, usually restricted to frequencies below 0.5 Hz (e.g., Łęski & Henzel, 2005; Limaye & Deshmukh, 2016; Sörnmo & Laguna, 2006). Power line interference originates from the electromagnetic fields of devices coupled to an electric power system, with an interference within 50 Hz or 60 Hz frequency components of the ECG (e.g., Limaye & Deshmukh, 2016; Maggio, Bonomini, Leber, & Arini, 2012). Therefore, it is often necessary to apply filtering techniques to remove or mitigate these types of interferences on the signal, hence allowing a more correct analysis of the signal (e.g., Limaye & Deshmukh, 2016; Maggio et al., 2012).

3.3.2. Noise reduction techniques

The main purpose of the ECG pre-processing is filtering, which allows removal or reduction of the unwanted information that may contaminate the signal of interest and, therefore, enables the improving of the ECG quality, especially for clinical analysis (e.g., Gregg, Zhou, Lindauer, Helfenbein, & Giuliano, 2008; Maggio et al., 2012; Sörnmo & Laguna, 2006). As mentioned above, noise can be present in different frequency bands, which can overlap or be relatively close to the information of interest. Therefore, often this phase consists of applying multiple filters.

Several techniques have been used in the literature to eliminate noise, including traditional filters, such as a low pass (pass low frequencies and suppress or attenuate higher frequencies) and high pass bands (pass high frequencies and suppress or attenuate low frequencies) or more sophisticated filters (e.g., Gacek, 2012; Gregg et al., 2008; Sörnmo & Laguna, 2006). Power line interference has often been removed using the notch filter, which allows selecting only the frequency of interference, i.e., the frequencies we want to remove (e.g., Gregg et al., 2008; Limaye & Deshmukh, 2016; Rangayyan, 2015; Sörnmo & Laguna, 2006). The baseline wander, in turn, is commonly removed using high-pass filtering based on moving average to remove or attenuate the low frequencies' interference (e.g., Gregg et al., 2008; Łęski & Henzel, 2005). The moving average filter allows examining a set of data points that are created from a series of averages of different subsets of the full data set (e.g., Rangayyan, 2015). The Butterworth filter allows as flat a response as possible in the passband (e.g., Rangayyan, 2015).

Although these approaches have been used with some degree of success to remove noise from the ECG, it is well known that filtering techniques can provoke distortion in the signal, especially when the unwanted information overlaps with wanted information, as in the case of electromyography (e.g., Gregg et al., 2008; Łęski & Henzel, 2005). Thus, researchers have been actively seeking to improve filtering techniques (e.g., Luo et al., 2013; Maggio et al., 2012; Sörnmo & Laguna, 2006). However, the main concern of such investigations is to obtain an ECG signal that allows to signal the singularities that may indicate the presence or absence of pathologies in a medical setting. For instance, unwanted information, such as electromyography, may be catastrophic for screening and diagnosing cardiovascular disease (e.g., early diagnosis of myocardial ischemia can prevent myocardial infarction) but for emotion identification it could be useful since the emotional response influences the heart and other muscles in the body (e.g., Ackerl, Atzmueller, & Grammer, 2002).

According to Kaur (2015), a filtering technique must be chosen based on the signal characteristics, such as extraction of the type of waves, the time required for pre-processing, the complexity involved and reconstruction of the signal, as well as the practical application of the ECG analysis. Considering that the filtering techniques applied by researchers in psychophysiological and affective computing to analyze the ECG of emotional response has been similar to that used in the clinical setting, which might leave out a part of information that may be important for emotion recognition, we believe it is necessary to perform more than inspection or quantification of noise information.

3.4. ECG processing (feature extraction and selection)

Feature extraction is performed after pre-processing the ECG signal. This process consists of extracting statistical information or ECG features that can be used to classify the emotional content (e.g. Bong et al., 2012; Jerritta et al., 2011; Selvaraj et al., 2013). These features can include statistical information in the time domain (e.g., R-R intervals, standard deviation of NN intervals -SDNN), frequency domain (e.g., low frequency, 0.04-0.15 Hz; high frequency 0.16-0.4 Hz), time-frequency domain, among others (e.g., Bong et al., 2012; Jerritta et al., 2011). Feature selection consists of selecting features that are relevant or correlated with emotions because those features that do not contain relevant information for emotion classification can reduce the classifier's performance (e.g., Jerritta et al., 2011). Many feature reduction algorithms, such as sequential forward selection (SFS), sequential backward selection (SBS) and Ficher projection, have been used to select such relevant features (e.g., see review by Jerritta et al., 2011).

3.5. Classification

In this step, the selected relevant features for classifying emotional states are subdivided in two data sets that will be used to train and test the classifier (Jerritta et al., 2011). The training classifier is created based on the labeled data set that contains the selected features and the true corresponding emotional label that was presented to the individual. Once an emotion classifier is trained, it can automatically recognize the emotional state of the individual with a certain accuracy, without knowing the label of the corresponding emotion. The test classifier is created based on another data set of selected features, but without a corresponding label of the individual's emotional state. Then, the accuracy of the emotion recognition can be calculated by dividing the number of correct decisions by the total number of decisions (e.g., Bong et al., 2012; Kappeler-Setz, 2012).

The results from studies that have used the electrocardiogram signal have showed that the performance of many algorithms developed to automatically recognize emotions seems to vary between 30% and 95% (e.g., Bong et al., 2012; Li & Chen, 2006; Selvaraj et al., 2013). The performance of the algorithms can be influenced by variables such as difficulty in eliciting the target emotion, reduced number of participants (using the data from same participant to training and test data set) and by type of signal and data processing (e.g., Bong et al., 2012; Wagner, Kim, & Andr, 2005). Considering that affective computing science is still in its infancy, more studies are needed to improve the performance of these algorithms to automatically recognize emotions.

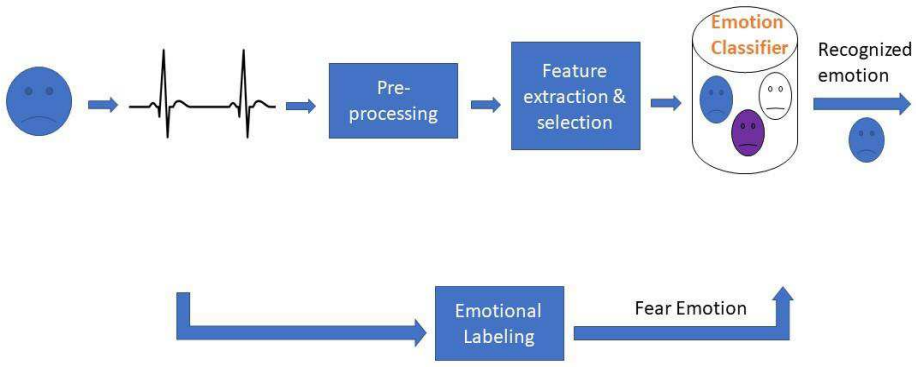

Figure 2. Process of supervised emotion recognition. For training the emotion classifier, a data set collected during emotional induction is labeled with the correspondent condition. Adapted from "Multimodal Emotion and Stress Recognition", by C. Kappeler-Setz, 2012, ETH Zurich, Switzerland.

CHAPTER III: NOSE FOR EMOTIONAL BODY ODORS

> We humans are overwhelmed with semiochemicals at all times in our lives. Some like to say that we live in a "chemical soup"—not referring to factory wastes, but to the everyday chemical information that plants, storms, babies, books, foods, and so forth produce… Some of this "soup" apparently cues us about the moods in the air, but not in such a way that we are usually aware of it. (Haviland-Jones and Wilson, 2005, p. 244.)

4. Olfactory stimuli

Visual and auditory cues play an essential role in affective communication. Indeed, humans often rely on visual and auditory cues, such as facial expression, body posture and vocal tone to assess the emotional state of others (e.g., de Gelder, 2006; de Gelder, Pourtois, & Weiskrantz, 2002). Similarly to visual and auditory cues, olfactory signals, especially body odors, have also been shown to influence and mediate social interactions (e.g., Pause, 2012; Pause, Adolph, Prehn-Kristensen, & Ferstl, 2009; Pause, Ohrt, Prehn, & Ferstl, 2004). However, chemical communication between humans is usually unconsciously perceived, and therefore, the role of olfactory cues in emotion communication has been overlooked until recently.

4.1. Human Body odors

Body odors can result from different body fluids, such as urine, sperm, sweat secretions, etc. (e.g., Lenochova, Roberts, & Havlicek, 2009; Lübke, Gottschlich, Gerber, Pause, & Hummel, 2012; Wongchoosuk, Lutz, & Kerdcharoen, 2009). So far, the body odor that has been most used for investigating chemosensory communication among humans is sweat from the armpits, which is segregated essentially by the apocrine glands (e.g., Lenochova et al., 2009; Liuzza, Olofsson, Sabiniewicz, & Sorokowska, 2017; Pause, 2012, 2017). The characteristic malodor of sweat samples is due to the action of bacterial enzymes present on the skin surface (Shelley & Hurley, 1953). The secretion of apocrine glands is mediated by the sympathetic nerves, which is associated with autonomic activation and can, therefore, also be influenced by the emotional response (e.g., Parma et al., 2017; Pause, 2012, 2017).

4.2. Body odors as stimuli with high social and ecological relevance

Animals rely greatly on olfactory cues to send and receive messages from their related or unrelated conspecifics. They can extract information from odors, such as conspecific recognition, dominance, aggression and mating signals (e.g., Lepri, 2003; Wyatt, 2003). Olfactory cues can be carried by wind and water so, consequently, cover a long distance. Unlike other senses, such as vision, they may also be crucial in ambiguous/dark situations (e.g., Lübke, 2010; Parma et al., 2017; Pause, 2012). These characteristics make the olfactory system an effective form of communication (e.g., Lübke et al., 2012; Parma et al., 2017; Pause, 2012).

Although humans are more conscious about visual and auditory cues, recently many studies have demonstrated that olfactory cues influence humans' social interactions (e.g., Gaby & Zayas, 2017). Body odors, in particular (especially axillary sweat), have been considered a stimulus with high social and ecological relevance for two major reasons: first, they are processed by neuronal networks different from those that involved in the processing of common odors, which involves the anterior and posterior cingulate cortex and angular gyrus and occipital cortex (Lundström, Boyle, Zatorre, & Jones-Gotman, 2008). Moreover, friend/kin and stranger body odor also activate different brain regions. While a friend's body odor seems to activate the same regions previously seen during kin recognition (e.g., dorsomedial prefrontal cortex), a stranger's body odor seems to activate the same regions that are involved in the processing of visual fearful stimuli (e.g., fearful faces), namely the amygdala and the insula (e.g., Lundström et al., 2008; Lundström, Boyle, Zatorre, & Jones-Gotman, 2009). Furthermore, emotional body odors (i.e., body odors collected under emotional conditions), namely anxiety chemical cues, recruit neuronal correlates that are involved in processing social emotional stimuli (fusiform gyrus) and in the regulation of empathic feelings (insula, precuneus, cingulate cortex; Prehn-Kristensen et al., 2009). Moreover, stress related chemical cues, have been shown to activate the amygdala (e.g., Mujica-Parodi et al., 2009). Second, body odors convey a great diversity of information that can be detected by humans, such as age (e.g., Mitro, Gordon, Olsson, & Lundström, 2012), gender (e.g., Penn et al., 2007), mating signals (e.g., Gildersleeve, Haselton, Larson, & Pillsworth, 2012; Grammer, Fink, & Neave, 2017), healthy status (e.g., Olsson et al., 2014) and emotional status (e.g., Chen & Haviland-Jones, 2000; de Groot et al., 2015; de Groot, Smeets, Kaldewaij, Duijndam, & Semin, 2012). Studies have showed that humans can identify their own body odors as well as differentiate family body odors from strangers' body odors (e.g., Mallet & Schaal, 1998; Olsson, Barnard, & Turri, 2006). Thus, like visual and auditory stimuli of high ecological relevance, body odors

are processed by specific neuronal networks and provide relevant information about the individual's identity.

4.3. Can humans smell emotions from body odors?

Communication via olfactory cues entail chemical signals, which are produced by a sender and can be detected by a receiver from the same or a different species (e.g., Parma et al., 2017; Pause, 2017). Many studies have demonstrated that humans can smell emotions from chemical cues. For instance, humans can smell and differentiate fear from happiness and neutral body odors (Ackerl et al., 2002). Humans can also smell chemical cues from body odors of anger (Mutic, Parma, Brünner, & Freiherr, 2016), anxiety (Pause et al., 2004), and disgust (de Groot et al., 2012). Furthermore, chemical cues can generate emotional contagion between humans. Emotional contagion can be described as a tendency for a receiver to automatically reproduce the emotional state of the sender by mimicking and synchronizing facial expressions, vocalizations, posture and movements (e.g., Hatfield, Cacioppo, & Rapson, 1993). Indeed, de Groot et al. (2012) showed that when the receivers smelled the fear signal, they produced a fearful face, which was related to sensory acquisition (by enhancing the sniff magnitude and eye scanning), whereas when they smelled disgust they produced a disgust face, which was associated with sensory rejection (by reducing the sniff magnitude and eye scanning). Such emotional contagion can optimize the chances of survival since fear signals inform about potential environmental danger, while disgust inform about potential contaminants in the environment (e.g., Susskind et al., 2008).

4.4. The effect of emotional body odor on the receiver's response

So far, most of the studies from the chemosensory field have often used body odors as context (or primes) for visual stimuli to investigate the effects of body odors on receiver's information processing and behavior (e.g., Pause et al., 2004; Zernecke et al., 2011; Zhou & Chen, 2009). Odors are considered as powerful context cues since they can influence behavior outside of consciousness, they can trigger strong emotional responses and specific memories and can be easily and rapidly associated with other environmental stimuli (e.g., Smeets & Dijksterhuis, 2014). These characteristics make odors a special type of prime (e.g., Bargh, Williams, Huang, Song, & Ackerman, 2010; Smeets & Dijksterhuis, 2014). According to Lundström et al. (2008) and Mujica-Parodi et al. (2009), the effects of body odors on receivers seem to occur mostly outside consciousness. Contrarily to other sensory modalities, olfactory information can be directly projected to the amygdala and the hypothalamus, without thalamic

processing (related to conscious olfactory chemical cues processing), and thus trigger behavioral and endocrine responses without conscious perception (e.g., Plailly, Howard, Gitelman, & Gottfried, 2008).

The context of chemical fear-related cues affects a wide range of responses in the receiver. Such effects have been shown mainly using behavioral tasks. For instance, under the influence of anxiety/fear chemical, the perception of happiness induced by a happy face was reduced (Pause et al., 2004; Zernecke et al., 2011), while ambiguous faces (morphed faces between happy and fearful facial expressions) were rated as more fearful (Zhou & Chen, 2009). Fear related chemical cues can also improve receivers' cognitive performance, by increasing the accuracy in a word-association task, with their reaction to ambiguous word pairs being slower (Chen, Katdare, & Lucas, 2006). Moreover, viewed ambiguous faces (morphed face neutral to angry) in such context, recruits additional attention resources, since the neural activity that is involved in attention to salient environmental stimuli was increased in receivers (increased late positive potential component of event related potential; Rubin, Botanov, Hajcak, & Mujica-Parodi, 2012). Furthermore, anxiety chemical cues seem to activate physiological responses by priming defensive behavior in humans. For instance, Pause et al. (2009) and Prehn, Ohrt, Sojka, Ferstl, and Pause (2006) showed an increase startle reflex amplitude (an aversive reflex) by measuring the receivers eyeblink reflex in such context. The enhanced startle reflex has been related with negative emotional contexts (as a response to fear stimuli), while a diminished startle reflex is associated with positive emotional contexts (as a response to pleasant stimuli) (e.g., Lang, Bradley, & Cuthbert, 1990; Miltner, Matjak, Braun, Diekmann, & Brody, 1994). Thus, these results suggested that humans seem to be very sensitive to this context, since the exposure to fear/anxiety chemical cues seems to prepare the organism to deal with dangerous situations by increasing the vigilance for visual stimuli that is related with the threat, while the perception of safety seems to decrease. Considering that, in everyday life, the perception of odors is often accompanied by a wide variety of contextual information that include not only visual and auditory, but also olfactory information, it is possible that the information from fear related chemical cues contexts can be sustained for a longer period of time, which could work as a prime for a subsequent context, especially if it is a neutral context.

4.5. Interindividual differences in odor perception

The way odors are perceived varies among individual's due to factors, such as age, smoking habits or gender (e.g., Doty et al., 1984; Doty & Cameron, 2009; Doty & Laing, 2015).

Moreover, whether females and males differ in their ability to perceive odors has been vastly investigated. Females, in general, perform better in odor identification than men (e.g., Doty & Cameron, 2009; Doty & Laing, 2015; Platek, Burch, & Gallup, 2001; Wallace, 1977). In addition, gender differences are also observed in subjective ratings with females tending, on average, to rate body odors as more intense and less pleasant than men (e.g., Doty & Cameron, 2009; Doty & Laing, 2015; Platek et al., 2001). Disgust sensitivity is another factor that might influence individuals' odor perception. Disgust propensity is the tendency to respond with disgust, whereas disgust sensitivity is the tendency to feel disgust as aversive or unpleasant (e.g., Goetz, Lee, Cougle, & Turkel, 2013; van Overveld, de Jong, Peters, Cavanagh, & Davey, 2006). Studies have demonstrated that individuals with high disgust sensitivity tend to adopt more avoidance behaviors towards potential disease cues (e.g., Oaten, Stevenson, & Case, 2011; Stevenson, Case, & Oaten, 2009). Body odor is a powerful disgust trigger because it can carry potential signals of disease (e.g., Curtis, Aunger, & Rabie, 2004; Shirasu & Touhara, 2011). Indeed, Olsson et al. (2014) showed that individuals can differentiate odors of sickness from odors of health, since participants rated sickness odors as more unpleasant, more intense and more unhealthy. Furthermore, previous findings have shown that we experience more disgust towards body odors from strangers than from our own or from family and friends' body odors (Case, Repacholi, & Stevenson, 2006; Stevenson & Repacholi, 2005). Thus, it is possible that individual differences in disgust response might influence how individuals perceive body odors, particularly those from strangers.

AIMS OF THE THESIS

The general aim of this thesis was to explore the cardiac response of the emotions of disgust and fear, using visual/auditory and olfactory stimuli. In study I, we explored noise information to test whether the ECG filtering techniques applied to emotion recognition are the most accurate, since to the best of our knowledge no study has yet investigated filtering techniques for emotion recognition. Thus, in the first study, the aim was to differentiate disgust and fear using noise information. In study II, we used noise entropy to train a classifier for automatic emotion recognition.

Similarly to visual stimuli, as mentioned above, previous studies have demonstrated that body odor is processed by neuronal networks, different from that recruited to process common odors (Lundström et al., 2008), and affect human cognitive, physiological and behavioral responses (e.g., Mujica-Parodi, et al., 2009; Zhou & Chen, 2009; Chen et al., 2006; de Groot et al., 2012). Considering that the way individuals perceive body odors can be modulated by the disgust response, in study III, we validated the Disgust Propensity and Sensitivity Scale (DPSS-R) for the Portuguese population, to be used in study IV to evaluate the individual differences in disgust response related with the perception of body odors (BOs). Finally, in study IV we investigated how a BO prime affects the emotional tone of a subsequent BO message, both at the subjective and psychophysiological levels.

GENERAL METHODOLOGY

The general methodology of the 4 studies was presented in Table 1. In the studies I and II, we used movies to induce fear, disgust and neutral response. The participants watched 25 minutes of videos containing disgusting scenes ("Pink Flamingos", Rottenberg et al., 2007), horror scenes ("The Shining", Rottenberg et al., 2007) and a nature documentary for the neutral condition ("Easter Island-Solar Eclipse" National Geographic). Since the duration of each movie used in this study was 25 minutes, all three movies were edited (see in the appendix section Table 21 the scenes that were used). While the participants watched the movies, the ECG was recorded to collect physiological responses of emotional and neutral conditions. The participants rated their subjective responses using the Likert Scale (1-7) before and after movies visualization and filled the STAI-State questionnaire. After the movie visualization, they also filled the Positive and Negative Affect Scale, the STAI-Trait Questionnaire and the Disgust Scale (the responses of STAI Trait and Disgust Scale were used only in study IV).

To examine the psychometric properties of the Portuguese version of Disgust propensity and Sensitivity Scale-Revised (DPSS-R) in study III, a web-based survey was performed. We examined the reliability (internal consistency and stability) and validity (criterion validity, content validity and construct validity). Moreover, we explored the associations between DPSS-R and measures of psychopathologies namely, Maudsley Obsessive Compulsive Inventory (MOCI), Spider Phobia Questionnaire-Revised (SPQ-R15) and State-Trait Anxiety Inventory (STAI Form Y-2 Trait).

Concerning study IV, we explored how the emotional body odors could affect the cardiac activity and the subjective response. Thus, we realized two separate studies: a donation study and a transmission study. The donation study included the same sample that participated in the studies I and II, but it is important to stress out some main differences. Although the emotional body odors were collected while all the participants were watching movies, in this study we only needed 20 participants/donors and all donors had a normal level of disgust sensitivity and anxiety trait (for more detailed information see the donation study in the study IV). Moreover, although the analysis approach that we used to analyze the results from studies I and II (Non-parametric Wilcoxon signed-rank tests and one-way ANOVA) differed from the study IV, (donation study- Linear Mixed Models analysis), it is also important to mention that the results found were similar since both approaches showed that the target emotions were successfully induced in participants/donors.

The transmission study included different participants from those that participated in the donation study. To control the level of anxiety trait and disgust propensity and sensitivity, the receivers filled the Stai-Trait and the DPSS-R questionnaires. The ECG response was recorded while the receivers were exposed either disgust, fear and neutral body odors. They also had to rate the intensity and the pleasantness of each body odor (see transmission study for more detailed information).

Table 1. General methodology.

I. Experimental Studies

ELIGIBILITY CRITERIA DEFINED FOR STUDIES I, II AND IV:

- University students
- No cardiovascular, respiratory, allergic or metabolic diseases
- Not medicated
- No psychiatric or psychological disorders antecedents
- Dietary and hygienic restrictions

WITHIN SUBJECT's DESIGN

Study I (n= 25)		**Study II (n= 25)**	
Aim: To explored if ECG noise entropy can be used as a new biomarker in emotion identification.		Aim: To explore if ECG noise allows the classification of emotions, while using its entropy as an input in a decision tree classifier.	
Stimuli	**Data collection**	**Stimuli**	**Data collection**
Videos to elicit emotional response of: – Disgust – Fear – Neutral 1-week interval between each video	**Psychophysiological measures:** – ECG using MP100 system	**Videos to** elicit emotional response of: – Disgust – Fear – Neutral 1-week interval between each video	**Physiological measures:** – ECG using MP100 system **Self-report measures:** – State-Anxiety Inventory| STAI Form Y-1 – Positive and Negative Affect Scale | PANAS – Likert Scale (1-7)

Study IV

Aim: To explored how a BO prime affects the emotional tone of subsequent BO message, both at the subjective and cardiac levels

Experiment 1 – Body Odors Sampling (N=20)-Within subject design		**Experiment 2 – Body Odor Receivers (N=69)- Between subject design**	
Stimuli	**Data collection:**	**Stimuli**	**Data collection**
Videos to elicit emotional response of: – Disgust – Fear – Neutral 1-week interval between each video	– ECG using MP100 system – Emotional body odors **Self-report measures:** – Disgust Scale-Revised| DS-R – Trait Anxiety Inventory | STAI Form Y-2 – Positive and Negative Affect Scale | PANAS – Likert Scale (1-7)	**Odors:** – Disgust body odors – Fear body odors – Neutral body odors	**Physiological measures:** ECG using MP100 system **Self-report measures:** – STAI Form Y-2 – Disgust Propensity and Sensitivity Scale-revised | DPSS-R – Visual Analogic Scale (0-100)

II. Validation Study

STUDY III (N=229)

SAMPLE: Participants from general population collected through a web-based survey.

Aim: To examine the psychometric properties of the Portuguese version of DPSS-R

Measure: Disgust Propensity and Sensitivity Scale-revised | DPSS-R	**Additional self-report measures:**	– DS-R – Maudsley Obsessive Compulsive Inventory | MOCI – Spider Phobia Questionnaire-Revised | SPQ-R15 – STAI Form Y-2

PART II: THE EXPERIMENTAL STUDIES

LIST OF STUDIES

STUDY I

Brás, S., **Ferreira, J.**, Soares, S. C., & Silva. C.F., (2015). Psychophysiology of Disgust: ECG Noise Entropy as a Biomarker. *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS,* 2351 – 2354. doi: 10.1109/EMBC.2015.7318865.

STUDY II

Ferreira, J., Brás, S., Silva. C.F., & Soares, S. C. (2016). An automatic classifier of emotions built from entropy of noise. *Psychophysiology*, *54*(4), 620-627. DOI: 10.1111/psyp.12808. SCImago/Scopus© SJR 2016: 1.540/Q1; ISI JCR® Impact factor (2016): 2.668

STUDY III

Ferreira, J., Bem-Haja, P., Alho, L., Rocha, M., Silva. C.F., & Soares, S. C. (submitted). Subjective Experience of Disgust: Psychometric Properties of the Portuguese Version of Disgust Propensity and Sensitivity Scale -Revised.

STUDI IV

Ferreira, J., Parma, V., Soares, S. C., Alho, L., Silva. C.F., Olsson, M., & Soares, S.C. (submitted). Emotional body odor contexts: Priming effects on cardiac and subjective response.

STUDY I. PSYCHOPHYSIOLOGY OF DISGUST: ECG NOISE ENTROPY AS A BIOMARKER.

Psychophysiology of Disgust: ECG Noise Entropy as a Biomarker

Abstract

The identification or classification of emotions allows the description of the person's state and, therefore, the inference of their preferences. The basic emotion of disgust, in particular, allows the organism to protect itself against diseases. Usually, the decrease in heart rate is associated with this emotion. As an avoidance behavior, when facing with disgust stimuli, the body reacts with movements, such as muscle contraction, etc. These reactions are evidenced in the electrocardiogram (ECG) as noise responses. In this paper, we propose the amount of ECG noise measured by the noise entropy as a new biomarker in emotion identification, which has been neglected in the literature. Our results showed that the noise entropy was able to discriminate between disgust, fear and neutral conditions in 88% ($p < .05$). It was also evidenced in this dataset that the median noise entropy in disgust was higher than in neutral and in fear conditions.

Introduction

Disgust is a basic emotion that is associated with response patterns that are universally recognized (Ekman & Friesen, 2003). The main function of this emotion is to protect the organism against disease (e.g., Curtis et al., 2004). Many pathogens agents can be easily found in stimuli like feces, vomit, blood and rotting meat. These stimuli work like a cue that signal disease and when the organism detects such stimuli, the disgust response is elicited in order to distance the individual from potential sources of contamination (Oaten, Stevenson, & Case, 2009). Disgust response includes a characteristic facial expression (wrinkling of the nose, slightly narrowed brows and retraction of the upper lip) that can allow spell out or avoid the ingestion of poison food or noxious odors (Oaten et al., 2009). It also includes behavioral avoidance from the disgust stimuli, which can be an object or a person, subjective feelings like fear of contamination, as well as physiological changes. In general, the decrease in heart rate or the elevated parasympathetic activity are indicators of physiological disgust responses (Rohrmann & Hopp, 2008). Although many studies only focus on heart rate, the electrocardiogram (ECG) signals contain other types of information that can be considered for analysis. In this study, we propose the quantity of ECG noise as a new biomarker of disgust response. To accomplish the paper goal, the noise entropy is calculated with specifications that emphasize transient singularities in the signal.

Method

Participants

Twenty-five individuals (10 males and 15 females, age range 18-37 years) with a mean age of 22.25 years (*SD* = 4.21), participated in this study. The participants had no cardiovascular, respiratory, allergic or metabolic diseases, were not taking medication, and had no previous history of psychiatric or psychological disorders. Participants were recruited at the University of Aveiro and received course credits for their participation. They gave their written consent and were informed about the possibility of withdrawing from the experiment at any time. The study was approved by the Ethics Committee of the University of Aveiro, Portugal, the guidelines of the Declaration of Helsinki and standards of the American Psychological Association were followed.

Stimuli and Procedure

In a within-subject experimental design, participants were shown three types of video - disgust, fear and neutral (one each week). The disgust film contained disgust scenes from movie, Pink Flamingos, the fear film contained horror scenes from movie, The Shining, and the neutral film displayed a documentary about (Easter Island- Solar Eclipse). The disgust and fear films had been successfully used to induce disgust and fear, respectively, in previous studies (e.g., de Groot et al., 2012;Vianna & Tranel, 2006). The duration of each film was 25 minutes and the order of presentation of movies was counterbalanced across participants.

In each experimental session, the experimenter sat in an adjacent room from which the assessment room could be viewed through a glassed window. Before the film presentation, the participants watched a film of 4-min resting baseline period (video displaying a beach sunset with acoustic guitar soundtrack) and were instructed to sit quietly and to relax as much as possible. Also, they were instructed to avoid looking away or shut their eyes if they found the films too distressing.

The acquired ECG was sampled at 1000Hz, using the MP100 system and AcqKnowledge software (Biopac Systems, Inc.). During the preparation phase, the adhesive disposable Ag/AgCl-electrodes were fixed in the right hand, as well as in the right and left foot. The physiological channels were continuously sampled during the film presentations. Figure 3 exemplifies the experimental setup.

Figure 3. Data collection setup. The participant is watching the video on a computer monitor, and hearing the video sounds by the headphones. The experimenter is sat in the adjacent room, and a replica of the video is displayed on a mirror, only for control.

Data analysis

The ECG revealed to be contaminated by noise, therefore a sequence of filters where implemented in order to remove unwanted noise. The power line interference was removed by the use of a notch filter at 50Hz, with a quality factor Q = 10. The baseline wander was taken off using a moving average filter with window width of 1000 samples. Also, a Butterworth low pass filter of order 10 was implemented with a cut off frequency of 40Hz.

The Noise

The noise signal was calculated by the difference between the raw ECG and the filtered signal (eq. 1).

$$noise = ECG - filtered \quad (1)$$

Quantity of noise

The entropy measures the order/ disorder of a time series (Thakor & Tong, 2004). In this paper, the entropy will represent/ quantify the amount of noise in each window. We used time dependent entropy that is calculated directly from the ECG noise time-series (Thakor & Tong, 2004), evaluating the signal transitions.

The ECG noise amplitude range W is divided in M disjoint and consecutive intervals Ii (eq. 2) (Brás et al., 2014). The probability distribution is calculated as the number of samples (Ni) in each interval Ii divided by the total number of samples N in each ECG noise window (eq. 3). The entropy is calculated based on equation (4), where log represents the natural logarithm.

$$W = \bigcup_{i=1}^{M} I_i \quad (2)$$

$$p_i = \frac{N_i}{N} \quad (3)$$

$$TE = -\sum_{i=1}^{M} p_i \log(p_i) \quad (4)$$

Since we wanted to find transient singularities on the noise signal, we chose adaptive amplitude partitions; i.e. the W is adjusted to the amplitude variation in each window (Ii). In this study, the interest is in the ECG noise trend; therefore, the ECG noise window length should not be too small (1-2 samples, Thakor & Tong, 2004). So, the entropy was evaluated in 1000 samples windows.

The first 15 participants data were used to build our hypothesis and the last 10 participants data were used to test this hypothesis. The results will be presented considering both each group and also the global sample. The analysis was performed evaluating each participant individual reaction, i.e., we compared data from the same participant in different scenarios. A non-parametric statistical approach was used in order to evaluate the differences between the segments. The Wilcoxon rank-sum test evaluates if two independent samples came from the same distribution with a different median. In this study, we assumed independence

between the three segments because there was a week interval between the different videos. This analysis allowed us to infer the ability of the biomarker in the emotion differentiation.

Results

The ECG data segment is composed by baseline and active stimuli, so each data segment is divided in the two parts. Considering the baseline of each segment, the hypothesis of equal medians cannot be rejected ($p > .05$) among the three conditions, which indicate that participants start the stimuli exposure in a similar condition. The raw ECG, the filtered ECG and noise are represented in Figure 4. The noise entropy is represented in Figure 5.

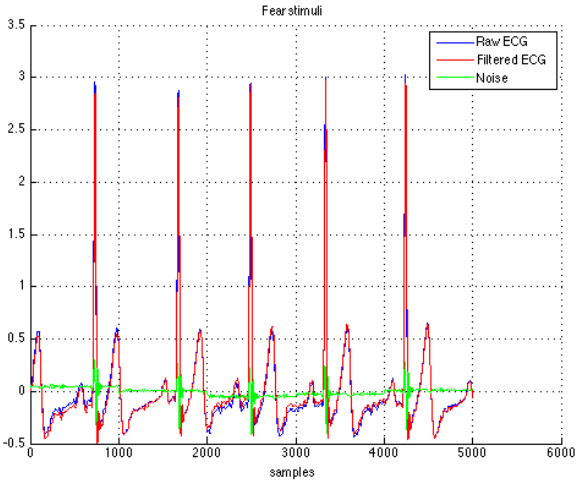

Figure 4. An excerpt of raw ECG (blue), filtered ECG (red) and noise (green) of participant 6 in the fear condition.

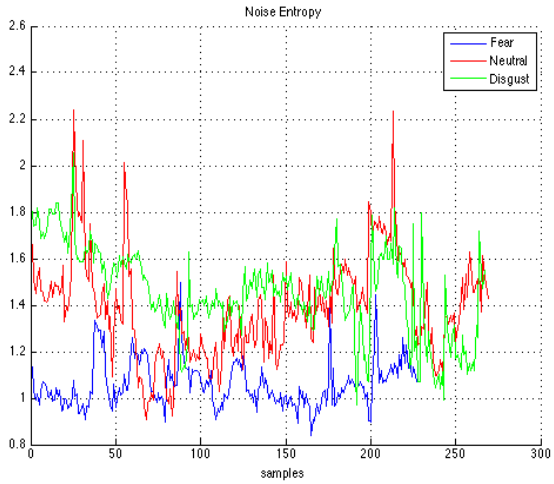

Figure 5. Participant 6 noise Entropy in fear (blue), neutral (red), and disgust (green) condition.

Regarding the initial dataset (first 15 participants) concerning the comparison between the emotions, 86.7% (39 in 45), comparisons revealed a significant difference, from which 6 in 15 participants revealed higher median in neutral condition than fear, 9 in 15 revealed higher median to disgust in comparison to fear, and finally 9 in 15 revealed higher median noise entropy for disgust comparing to neural condition.

In the test dataset, 90% (27 in 30) comparisons presented a significant difference ($p < .05$) among conditions. From which 7 in 10 participants revealed higher median for neutral in comparison to fear, 4 in 10 revealed higher median for disgust than in fear, and 5 in 10 revealed higher median in disgust comparing to neutral.

Considering the global dataset, we found that 88% (66 in 75) comparisons revealed significant differences ($p < .05$), from which 13 in 25 participants had a higher median entropy value in neutral than in fear, 13 in 25 revealed higher median in disgust comparing to fear, and 14 in 25 revealed higher median in disgust in comparison to neutral.

In summary, in median, the participants in the disgust condition showed higher entropy values ($p < .05$), in contrast with the fear condition, which presented the lowest entropy values ($p < .05$). Table 2 summarizes the previous results. Figure 6 is an illustrative example of the presented analysis.

Table 2. Results from initial, test and global dataset.

	Initial Dataset	Test Dataset	Global Dataset
# Neutral>Fear	6	7	13
#Disgust>Fear	9	4	13
#Disgust>Neutral	9	5	14
Participants	15	10	25
Differences	39	27	66
# Tests	45	30	75

Note. Columns represent the three analyses datasets, the initial dataset was used to build the hypothesis, the Test dataset was used to test the hypothesis and the global dataset is the combination of both datasets. The first three lines (# Neutral>Fear, #Disgust>Fear, #Disgust>Neutral) represent the number of participants that revealed higher median values (e.g., neutral than fear condition), considering that there are statistical differences between the two groups. Participants represent the number of participants in each dataset; Differences is the number of comparisons that revealed significant differences; and #Tests corresponds to the number of comparisons that were done in the study (15x3).

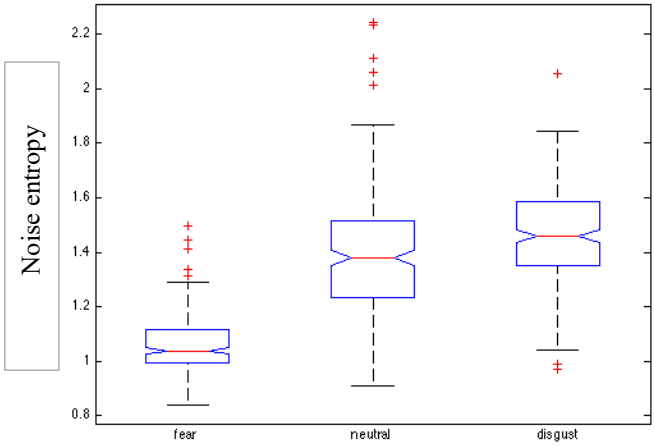

Figure 6. Boxplot comparing the fear, neutral, and disgust noise entropy for participant 6.

This approach is a proof of concept, and it is planned to implement data mining and machine learning methods to allow an automatic classification of the participant emotional status.

Discussion

This investigation attempted to demonstrate that the ECG noise can be a new biomarker of physiological response of disgust identification and differentiation from other emotions. Results showed that noise entropy is able to discriminate the three conditions used in this study (disgust, fear, and neutral). In this dataset, the median noise entropy value tended to be higher in disgust than in the other conditions, and lower in the fear condition. Since the participants were asked to sat in a rest position, the ECG noise should be minimal. However, an increase in noise was observed, which is probably justified as an avoidance behavior, as they possibly tried to find a way of scape, for example by muscular contraction or movement, which was revealed in ECG as an increase in noise. The increase in muscular tension in the disgust condition can be explained by fear of contamination. Many contaminants like germs and viruses are present in the environment and they can cause harm to the organism. Generally, humans respond to these stimuli with rejection or with physical distancing from the offensive object or situation (e.g., Rozin & Fallon, 1987). The emotion of disgust is also at the core of some psychopathologies such as obsessive-compulsive disorder, spider phobia and blood-injection-injury (e.g., Cisler, Olatunji, & Lohr, 2009). This research opens a new avenue of research while proposing that muscle tension may be an important physiological biomarker in the study of disgust.

STUDY II. AN AUTOMATIC CLASSIFIER OF EMOTIONS BUILT FROM ENTROPY OF NOISE

An automatic classifier of emotions built from entropy of noise

Abstract

The electrocardiogram (ECG) signal has been widely used to study the physiological substrates of emotion. However, searching for better filtering techniques in order to obtain a signal with better quality and with the maximum relevant information remains an important issue for researchers in this field. Signal processing is largely performed for ECG analysis and interpretation, but this process can be susceptible to error in the delineation phase. In addition, it can lead to the loss of important information that is usually considered as noise and, consequently, discarded from the analysis. The goal of this study was to evaluate if the ECG noise allows for the classification of emotions, while using its entropy as an input in a decision tree classifier. We collected the ECG signal from 25 healthy participants while they were presented with videos eliciting negative (fear and disgust) and neutral emotions. The results indicated that the neutral condition showed a perfect identification (100%), whereas the classification of negative emotions indicated good identification performances (60% of sensitivity and 80% of specificity). These results suggest that the entropy of noise contains relevant information that can be useful to improve the analysis of the physiological correlates of emotion.

Introduction

Emotions play an important role in our lives as they signal important events in our environment and trigger action programs to enable adaptive responses (e.g., Oatley & Jenkins, 1996). These events range from immediate survival pressures (e.g., Soares, Lindstrom, Esteves, & Ohman, 2014) to social communication (e.g., Öhman, Dimberg, & Öst, 1985). Emotions are then crucial elements of our lives while facilitating the interplay between the organism and the environment. Thus, it comes as no surprise that several scientific disciplines, such as psychophysiology (e.g., Cacioppo et al., 2000), cognitive psychology (e.g., Oatley & Johnson-Laird, 1987), neuroscience (e.g., Davidson, Jackson, & Kalin, 2000), and social sciences (e.g., Keltner & Haidt, 1999), have devoted a good deal of attention to the study of emotions. More recently, and although emotions have been largely neglected for so long in computer science, there has been an exponential interest and a large bulk of studies to date showing that emotions are crucial to building more intelligent and interactive machines (André et al., 2004). Consequently, human-computer interactions are increasingly more efficient, which may be of

great value in several contexts, namely, in clinical settings for patients with emotional difficulties (e.g., autism; see Jerritta et al., 2011; Picard et al., 2001).

Emotions involve synchronized alterations in physiological (e.g., heart rate), subjective (e.g., likes or dislikes), and behavioral responses (e.g., approach or avoidance; see Lang et al 1993). Here, we will focus our attention on the physiological dimension of emotions. This dimension enables the collection of responses without conscious effort (i.e., not subject to voluntary conscious control), thus providing more reliable information than that collected from facial expressions or from speech, which can both be more easily masked (e.g., Jang et al., 2015). The interest in identifying and recognizing emotions through physiological signals is not new. Although there is still no consensus in the literature about the specific psychophysiological patterns of some specific emotions (e.g., Kreibig, 2010), it is well known that physiological responses of emotion can cause alterations in the autonomic nervous system (e.g., in heart rate; see Kreibig, 2010). However, the majority of these studies base their interpretation of the results in statistical analysis such as the *t* test or analysis of variance comparisons (e.g., Kreibig, 2010). Other methods like data mining enable the identification of patterns in the data that are not accessible when researchers only rely on descriptive statistical analysis or hypothesis testing. In addition, machine learning algorithms complement these identifications while allowing the classification and prediction of the behavior of data associated with different emotions and, consequently, their automatic recognition from physiological signals. Importantly, the combination of these methodologies can improve the diagnosis and intervention in mental health settings (e.g., Nasoz, Alvarez, Lisetti, & Finkelstein, 2004), particularly in disorders in which the emotional systems are hampered (e.g., Picard et al., 2001).

Several studies on affective computing or human-computer science have developed algorithms for automatic emotion recognition (e.g., Nasoz et al., 2004) using a wide range of physiological signals, such as electroencephalogram (EEG; e.g., Liu, Sourina, & Nguyen, 2011), electrocardiography (ECG; e.g., Xu & Liu, 2009), electromyography (EMG; e.g., Nakasone, Prendinger, & Ishizuka, 2005), electrodermal activity (EDA; e.g., Henrique, Paiva, & Antunes, 2013), or multimodal approaches (e.g., Li & Chen, 2006). The ECG signal, in particular, is widely studied and seems to be very sensitive to the influence of emotions, with several studies successfully classifying emotions using ECG features such as heart rate, heart rate variability, P-QRS-T waves, among other features (e.g., Agostinelli, Giuliani, & Burattini, 2014; Xu & Liu, 2009; Xu, Liu, Hao, Wen, & Huang, 2010). In addition, various

methodologies have been applied to extract and select the emotional features from raw ECG in order to achieve a better classification of emotions (e.g., Valenza, Citi, Lanatá, Scilingo, & Barbieri, 2014). Most of these studies have showed recognition accuracies of over 60% (e.g., Li & Chen, 2006; Picard et al., 2001; Wagner et al., 2005) of different basic emotions, which are the most widely studied ones.

Although studies have demonstrated promising results regarding emotion recognition, extracting and selecting the features that contain emotional information can be a difficult process, since emotion-specific patterns are not well defined yet (e.g., Jerritta, Murugappan, Wan, & Yaacob, 2012; Li & Chen, 2006). Furthermore, the presence of noise in the ECG signal represents one of the biggest challenges during both the data acquisition and the feature extraction. This difficulty is valid both in the laboratory and in real-time collection, especially with negative emotions (e.g., fear and disgust), since the ECG signal is very sensitive and the signal voltage level is low, between 0.5 and 5 mV (e.g., Nayak, Soni, & Bansal, 2012). Based on the hypothesis that the ECG entropy of noise calculation is not based on the ECG wave delineation, therefore involving a decrease in the preprocessing error, we recently investigated this measure as a possible biomarker for emotional differentiation of some of the basic emotions -fear and disgust (and a neutral emotional condition; Brás, Ferreira, Soares, & Silva, 2015). The results confirmed our prediction while showing that the entropy of noise was indeed able to discriminate between the different emotions in 88% of cases. In the present work, our goal was to implement an automatic classifier in order to evaluate the automatic ability of the measure in the differentiation of the same emotional conditions as in Brás, Ferreira et al.'s (2015) work.

Method

Participants

Twenty-five students from the University of Aveiro (10 males and 15 females, age range 18–37 years), with a mean age of 22.25 years (*SD* = 4.21) volunteered for the study and received course credits for their participation. Participants signed a written informed consent and were given the opportunity to withdraw from the study at any time. None of the participants had a previous history of cardiovascular, respiratory, allergic, metabolic, psychiatric, or psychological disease or were taking medication. Authorization from the Ethics and Deontology Committee of the University of Aveiro, Portugal, was given, and the guidelines of

the Declaration of Helsinki and standards of the American Psychological Association respected.

Stimuli

Participants were presented with three types of videos—eliciting disgust, fear, and an emotionally neutral video—in a within-participant experimental design. The presentation of each video was done with a 1-week interval. The film eliciting disgust included disgust scenes (*Pink Flamingos*), while the film eliciting fear contained horror scenes (*The Shining*), and the neutral film presented a documentary about Easter Island (*Solar Eclipse*). These films had been successfully used in previous studies (e.g., de Groot et al., 2012; Vianna & Tranel, 2006). The order of the presentation of films was counterbalanced between and within participants. The duration of each video was 25 min.

Electrocardiogram

The acquired ECG was sampled at 1000 Hz, using the MP100 system and AcqKnowledge software (Biopac Systems, Inc.).

Procedure

Participants were informed about the goal of the study and asked to read the instructions and sign the written informed consent. Following this, three ECG Disposable Biopac EL503 Ag-AgCl snap electrodes were placed following a standard Lead II configuration (right arm, left leg, and right leg ground; Berntson et al., 2007) and connected to the Biopac MP100, ECG Module, (Biopac Systems, Inc.). The experimenter sat in a room contiguous to that in which the experimental session took place, and from where the participant could be viewed. Prior to the presentation of each video, participants were asked to rate their subjective anxiety level and to watch a 4-min video displaying a beach sunset with acoustic guitar soundtrack, which served as the resting baseline period. During this time, participants were instructed to sit quietly and to relax as much as possible. Afterward, they were instructed to avoid looking away from the monitor or shutting their eyes whenever they found the disgust and fear scenes too distressing. The physiological channels were constantly sampled during the presentation of the videos. Finally, participants rated the subjective emotions experienced during the presentation of the videos, and filled in the Portuguese version (Silva & Spielberger, 2007) of the State-Trait Anxiety Inventory (STAI Form Y-2, Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983), as well as the Portuguese version (Galinha & Pais-Ribeira, 2005) of the Positive and Negative Affect Scale (PANAS; Watson, Clark, & Tellegen, 1988).

Biomarker: Signal analysis

The entropy measure

Entropy enables the evaluation and quantification of the order or disorder of a time series (Thakor & Tong, 2004). It is based on the notion of probabilistic distribution of a signal characteristic (e.g., time, frequency). Since our goal was to evaluate the signal transitions, the implemented entropy was time-based, calculated directly from the signal. The signal amplitude range (*W*) is divided in disjoint (*M*) and consecutive intervals (*Ii*) in Equation 5 (Brás et al., 2014). In each *Ii* interval, the probability distribution (*Pi*) is calculated by the ratio between the number of samples (*Ni*) in the interval, and the total number of samples (*N*) in the window, Equation 6. Equation 7 allows for the calculation of the entropy, where log represents the natural logarithm.

$$\boldsymbol{W = \bigcup_{i=1}^{M} I_i} \tag{5}$$

$$\boldsymbol{P_i = \frac{N_i}{N}} \tag{6}$$

$$\boldsymbol{TE = -\sum_{i=1}^{M} P_i log(P_i)} \tag{7}$$

In each signal window, the amplitude range is adapted to the signal window characteristics (i.e., the *W* will be updated to the amplitude variation in each signal window). Considering the method theory description and the goal of the present study, our interest was in the trend. Therefore, the window's length should not be too small (1–2 samples; Thakor & Tong, 2004), which motivated the selection of a 1,000-sample window.

The noise signal

The ECG noise signal was calculated by the subtraction of the filtered ECG from the raw ECG in Equation 8. We used visual inspection before and after the filtering process to verify the quality of the signal and of the peaks. The filtered ECG was calculated by the removal of power line interferences by the use of a notch filter at 50 Hz and by a quality factor $Q = 10$. The baseline wander was filtered by means of a moving average filter with a window width of 1,000 samples. The high frequency noise was filtered using the application of a Butterworth low-pass filter of order 10 and a cutoff frequency of 40 Hz.

$$\boldsymbol{Noise = ECG - Filtered} \quad (8)$$

The classifier

The implementation of classifiers is common when the aim is the discrimination between groups. These methods are, by definition, able to extract information from the data that is not accessible by itself. They combine information in order to find dependencies that are not obvious (e.g., Brás, Silva, Ribeiro, & Oliveira, 2015). The processing of automatic emotion recognition involves four phases: (1) acquisition phase, (2) feature extraction, (3) feature selection, and (d) automatic classification (e.g., Jerritta et al., 2011; Xu & Liu, 2009). In this study, we implemented a decision tree to categorize each emotion. This method was chosen due to its characteristics. As inputs of the decision tree, we tested different combinations of ECG entropy of noise values characterizing the participants' emotional status. The output provided by the decision tree is the evaluated emotion (fear, neutral, disgust). The decision trees are described by linguistic rules (e.g., if the value of entropy of noise was higher than *x*—a predefined value—then it is disgust; otherwise, the analysis continues) that allow a straightforward interpretation by both technical and nontechnical staff. Basically, in each node, the decision tree evaluates the given information and decides the node's outcome: either the final classification or another test node. The data go through the tree until a leaf is reached, corresponding to the class that classifies the input data. For the tree split, Gini's diversity index is used, which evaluates the level of diversity in a sample (e.g., Kantardzic, 2011). To correctly deal with the overfitting, after the general decision tree training, it is inspected and pruned (Wieben, Afonso, & Tompkins, 1999). The pruning level is found as the level producing the smallest tree, which is within the minimum cost subtree. For building the decision tree, the data were divided into two groups, the test and the training data set. The test data set is defined as 50% of each emotion associated with the ECG entropy of noise, randomly chosen. The training data set corresponds to the other 50%. To evaluate possible overfitting of the tree, the classifier was trained and tested 100 times, and the input data randomly chosen in each tree (Brás, Silva et al., 2015).

Performance measures of the classifier

In the evaluation of the classifier's performance, three measures were used: the error, the sensitivity, and the specificity in Equation 9 and 10. The error translates the degree of correctly classified records by the classification system (in equation 11). Sensitivity evaluates the

probability of the method classifying an emotion in a particular class, when it effectively belongs to it. On the other hand, specificity evaluates the probability of the method not classifying an emotion in a specific class, when it actually belongs to it. *TP* corresponds to the true positive evaluations, *TN* is the true negative, *FN* belongs to the false negative, and *FP* represents the false positive matches.

$$\boldsymbol{Sensitivity} = \frac{\boldsymbol{TP}}{(\boldsymbol{TP} + \boldsymbol{FN})} \tag{9}$$

$$\boldsymbol{Especificity} = \frac{\boldsymbol{TN}}{(\boldsymbol{FP} + \boldsymbol{TN})} \tag{10}$$

$$\boldsymbol{Error} = \mathbf{1} - \frac{\boldsymbol{TP}}{\boldsymbol{N}} \tag{11}$$

Results

In order to verify if the emotions were successfully elicited, we analyzed the participant's subjective ratings, the level of anxiety state, and the positive and negative affect. In these analyses, the data from one participant were eliminated due to an error that occurred in the program, making up a total of 24 participants. The significance level was set at alpha < .05.

Subjective Ratings

Since the normality assumptions were violated for the subjective ratings, the nonparametric Wilcoxon signed-rank tests (df = 24) were used (Table 3). The Bonferroni correction was applied, resulting in a significance level set at $p < .017$. The results seem to confirm our manipulation (e.g., the fear film induced more fear than other emotions), while showing that, in the disgust condition, participants reported significantly more disgust and surprise than in the fear ($z = 4.33$, $p < .001$, $r = .88$; $z = 3.40$ $p = .001$, $r = .69$) and in the neutral condition ($z = 4.38$, $p < .001$, $r = .89$; $z = 4.02$, $p < .001$, $r = .82$). Moreover, in the disgust condition, participants rated the disgust video as significantly less pleasant than the fear, ($z = 2.84$, $p = .005$, $r = .58$) and the neutral film, ($z = 4.15$, $p < .001$, $r = .85$). The fear film was also rated as significantly less pleasant than the neutral film ($z = 4.08$, $p < .001$, $r = .83$). In the fear condition, participants also reported significantly more fear than in the disgust ($z = 3.69$, $p < .001$, $r = .74$) and neutral conditions ($z = 4.32$, $p < .001$, $r = .88$). Finally, in the neutral condition, participants reported significantly more happiness than in the disgust ($z = 3.52$, $p < .001$, $r = .72$) and fear conditions ($z = 4.11$, $p < .001$, $r = .82$).

Table 3. Participants' subjective ratings of the emotions induced by the movies.

	Disgust Condition	Fear Condition	Neutral Condition
Self-reported disgust	7(2-7)	1(1-5)	1(1-1)
Self-reported fear	1(1-7)	3(2-7)	1(1-2)
Self-reported happiness	1(1-4)	1(1-4)	3.5(1-6)
Self-reported anger	1(1-6)	2(1-5)	1(1-5)
Self-reported sadness	1(1-4)	2(1-5)	1(1-2)
Self-reported surprise	5(2-7)	3(1-6)	2.5(1-5)
Self-report pleasantness	1(1-3)	2(1-5)	5(3-7)

Note. *Subjective ratings were measured in 7-point Likert scales (1 = not at all; 7 = very) and are presented as medians (range).*

STAI State Inventory

A paired *t* test was run to compare the effect of anxiety levels before and after emotion elicitation for each condition. The level of anxiety state after disgust elicitation was not significantly different from baseline, $t(23) = -1.63$, $p = .12$, $d = .68$; from $M = 32.25$, $SD = 5.60$, to $M = 34.50$, $SD = 6.83$. However, in the fear condition, the anxiety state levels significantly increased after fear elicitation, $t(23) = -3.69$, $p = .001$, $d = 1.54$; from $M = 31.71$, $SD = 5.06$ to $M = 35.33$, $SD = 6.09$. Finally, in the neutral condition, the level of anxiety state was not significantly different from the baseline, $t(23) = -.17$, $p = .87$, $d = .07$; from $M = 32.71$, $SD = 7.45$ to $M = 32.86$, $SD = 5.81$.

Positive and Negative Affect Scale

We conducted two separate one-way analyses of variance (ANOVAs) to compare the effect of negative and positive affect on fear, disgust, and neutral conditions. The analysis of variance showed the main effect of negative affect, $F(2,46) = 42.12$, $MSE = .178$, $p < .001$, $\eta p2 = .647$. Bonferroni post hoc tests indicated that the negative affect experienced by the participants in the disgust condition did not significantly differ from the negative affect experienced by participants in the fear condition ($p > .05$). On the other hand, Bonferroni tests also showed that, in the disgust and fear condition, participants experienced significantly more negative affect than in the neutral condition ($p < .001$). For the analysis of the positive affect, the Mauchly test indicated that the assumption of sphericity had been violated, $\chi2(2) = 5.85$, $p < .05$. Therefore, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon = .81$). The results showed a main effect of positive affect, $F(1.62,37.30) = 26.16$, $MSE = .482$, $p < .001$, $\eta p2 = .53$. Bonferroni tests showed that the positive affect experienced by participants in the disgust condition did not significantly differ from the positive affect experienced by participants in the fear condition ($p = .47$). Moreover, participants in the disgust and fear conditions experienced significantly less positive affect than in the neutral condition ($p <. 001$).

Biomarker: Quantity of Noise Analysis

To prevent overfitting of the tree (as described in Method), the tree was run 100 times for each input combination, and considered a randomly selected sample for both the train and the test. The classifier error was calculated and presented as mean ± *SD*. Two approaches were considered for the classifier evaluation: (1) selection of 75% of the participants to the train data set, and test in the remaining 25%; (2) selection of 75% of the samples in each participant for

train, and the remaining 25% of the samples for test. The second approach was used in order to verify if the interindividual variability may have interfered with the results. This approach is not feasible in real conditions and, therefore, was only used for evaluation and comparison. We observed that the lowest value in the train data set was verified in the combination of normalized and differentiated median and interquartile range (IQR) ECG entropy of noise values. The lowest error in the test data set was observed for the inputs' combination of normalized and differentiated ECG entropy of noise median values, after splitting the data from all the participants between train and test data sets, which was already expected. However, the difference did not influence the classification performance, and the intravariability of the participants' data did not negatively influence the classifier's performance. Therefore, the chosen classifier (Figure 7) is the one that uses as inputs the combination of normalized and differentiated median and IQR ECG entropy of noise values, with performance values of 73.40% ± 21.48 for sensitivity and 86.70% ± 9.51 for specificity. Table 4 resumes the classifier's performance in terms of error, using the considered inputs:

1. Absolute ECG entropy of noise values.
2. Normalized ECG entropy of noise values (ratio between the median ECG entropy of noise in each condition and the median value in the neutral condition).
3. Differentiated ECG entropy of noise values (difference between the median ECG entropy of noise in each condition and the median value in the neutral condition).
4. Normalized and differentiated ECG entropy of noise values.
5. Normalized and differentiated ECG entropy of noise values, considering both the median and interquartile difference (IQR) values as inputs.
6. Normalized and differentiated ECG entropy of noise values**,** considering only the median values as input, splitting the participant's data between train and test data set. Considering that the train and test data set contain information from all participants, we randomly selected an interval sample of ECG entropy of noise values and assigned it to the train data set, whereas the remaining data were used in the test data set.
7. Normalized and differentiated ECG entropy of noise values, considering both the median and interquartile difference values as inputs, splitting the participant's data between train and test data set. Considering that the train and test data set contain information from all participants, we randomly selected an interval sample of ECG

entropy of noise values and assigned it to the train data set, and the remaining data were used in the test data set.

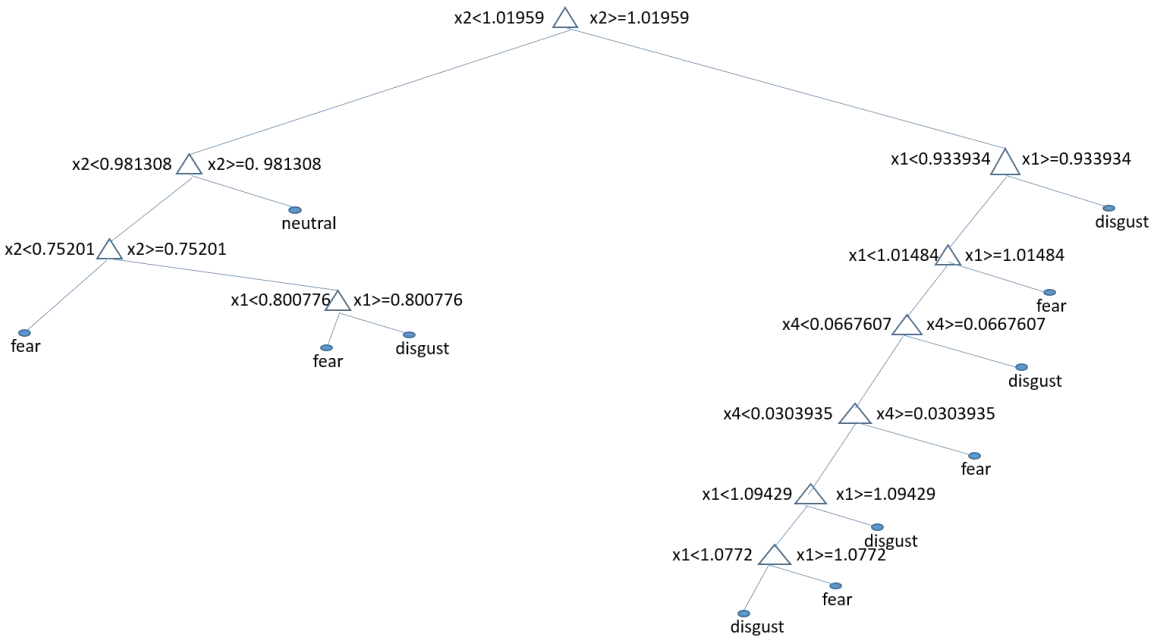

Figure 7. Decision tree classifier selected for problem solving.

Note. x1 - median of the normalized ECG entropy of noise values (ratio between the median ECG entropy of noise in each condition and the median value of the neutral condition); x2 - iqr of the normalized ECG entropy of noise values (ratio between the iqr ECG entropy of noise in each condition and the iqr value of the neutral condition); x3 - median of the differentiated ECG entropy of noise values (difference between the median ECG entropy of noise in each condition and the median value of the neutral condition); x4 - iqr of the differentiated ECG entropy of noise values (difference between the iqr ECG entropy of noise in each condition and the iqr value of the neutral condition).

Table 4. Classifier error evaluation, which is assessed as the ratio between the true positive (correctly classified cases) and the total number of cases.

	Train	Test
Absolute	52.75 ± 19.18%	66.72 ± 5.78%
Normalization (median)	36.98 ± 15.92%	61.06 ± 8.67%
Differentiation (median)	39.98 ± 18.17%	61.33 ± 8.24%
Normalization + Differentiation (median)	21.82 ± 9.45%	32.58 ± 5.73%
Normalization + Differentiation (median + iqr)	**19.44 ± 8.84%**	**34.36 ± 5.04%**
Normalization + Differentiation (median) split	25.42 ± 4.36%	28.13 ± 2.30%
Normalization + Differentiation (median + iqr) split	26.00 ± 7.45%	31.19 ± 3.68%

Note. The tree error was calculated by the evaluation of 100 runs, which evaluates randomly selected test and train datasets in order to prevent overfitting of the tree (tree dependent on the used sample). The bold evidences the selected input that corresponds to the lowest train error.

The classifier's performance (over the 100 random runs of the classifier) was also evaluated considering each one of the classes (see Table 5 and Table 6). The neutral condition was always correctly identified. For the fear and disgust conditions (i.e., both negative emotions), the performance was similar: 60% of sensitivity. This result indicates that the classifier correctly distinguished among the three conditions, which was confirmed by a ROC (receiver operating characteristic) curve (Figure 8). The curve shows that we obtained 1.0 for the neutral condition, indicating a perfect prediction, and 0.85 for the fear and 0.79 for the disgust conditions, which indicate good prediction levels.

Table 5. Classifier performance by class.

	Sensitivity	Specificity
Fear	58.04± 13.67%	82.38 ± 6.46%
Neutral	100.00 ± 0.00%	97.00 ± 6.03%
Disgust	62.16 ± 11.19%	80.72 ± 5.73%

Note. Sensitivity evaluates the probability of the method classifying an emotion in a class when it effectively belongs to it; specificity evaluates the probability of the method not classifying an emotion in a class when it actually does not belong to it.

Table 6. Confusion matrix presented as the mean±standard deviation of the classifier performance over the 100 random runs.

	Confusion matrix train dataset		
	Fear	Neutral	Disgust
Fear	68.62% ± 19.31%	1.85% ± 5.38%	29.54% ± 19.46%
Neutral	0.00% ± 0.00%	100.00% ± 0.00%	0.00% ± 0.00%
Disgust	27.62% ± 14.77%	0.69% ± 3.62%	71.69% ± 15.62%
	Confusion matrix test dataset		
	Fear	Neutral	Disgust
Fear	48.00% ± 13.75%	4.33% ± 11.00%	47.67% ± 14.92%
Neutral	0.00% ± 0.00%	100.00% ± 0.00%	0.00% ± 0.00%
Disgust	48.25% ± 15.00%	4.17% ± 8.92%	47.58% ± 16.00%

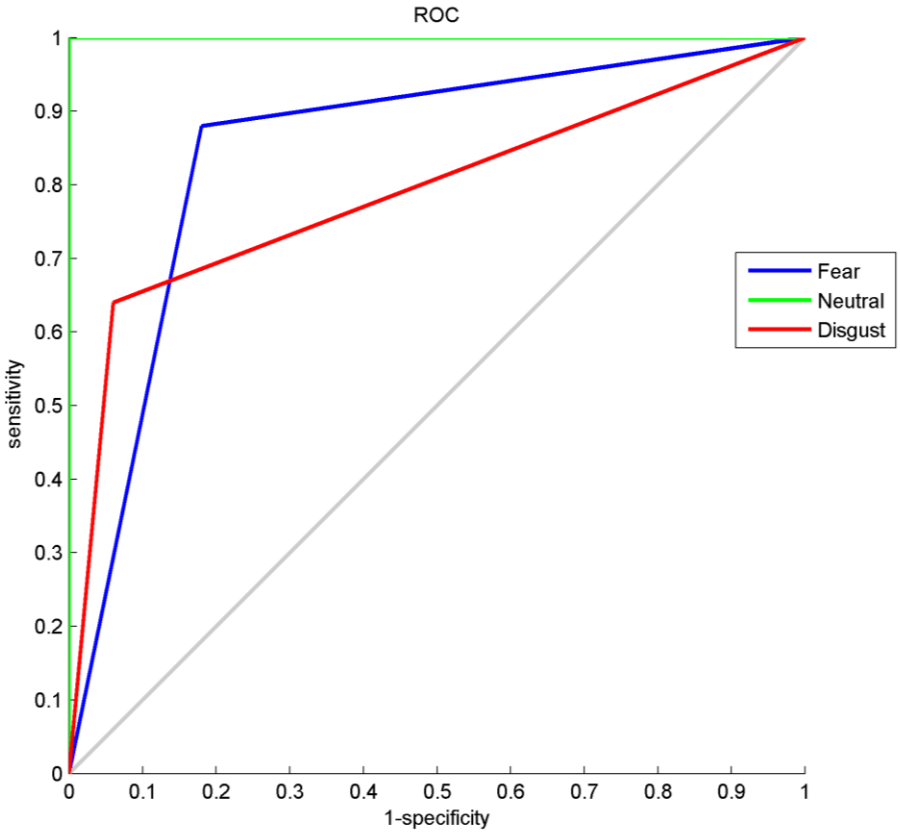

Figure 8. ROC curve evaluating the relation between sensitivity and specificity for chosen classifier.

Note. The perfect match corresponds to the point in the top left corner.

HRV Measures

The HRV measures used in the literature are not equivalent and should be carefully selected according to the goals of each study. Indeed, there is not a standardization of the methodology, which undermines the comparison between studies and the generalization of the results. Considering the different approaches and measures used for the HRV analysis, we selected the methods that are appropriate for short-term segments. The short-term measures evaluate segments of 15-min duration, as reported in the Physionet HRV Toolkit (Goldberger et al., 2000). However, in our study we have collected 25 min of data. Therefore, since the 24-h methods are not reliable, only the 15-min measures may be applied in our study. Notwithstanding this, because the duration of the segments may compromise the analysis, temporal and frequency domain measures were also tested. The evaluation of the performance of the measures in the three separate conditions showed that the time domain measures presented the best results, especially the average of all NN intervals (AVNN).

In order to compare the performance of the ECG entropy of noise classifier with the performance of the HRV measures classifier, a decision tree was implemented for a

combination of inputs based on statistics over the HRV measure. The selected statistics were the same as those reported for the ECG entropy of noise classifier. As expected, the classifier with better performance was not the same input combination as in the entropy of noise. However, because a direct comparison was not possible between the results of the classifiers, we selected the classifier with lower error. A comparison between error performance allowed inference of the input measure that is better suited to differentiate the emotional conditions. Consequently, we obtained an error rate of 21.49% ± 7.61 in the train data set, considering the normalized and differentiated AVNN median values as an input of the decision tree. In the chosen method, we decided to implement an ECG classification independently of the participant, so the data split between train and test data set was not included in the comparison.

ECG Signal Entropy

Along with the evaluation of the ECG entropy of noise as an emotion biomarker, it is also relevant to validate the filtered ECG signal entropy and the raw ECG signal entropy as possible biomarkers. Thus, a classifier (decision tree) was designed considering as input values the ECG (filtered/ raw) signal entropy. Following the previous approach, the best results were associated with the input combination of the median and interquartile difference from the normalized and differentiated ECG signal entropy values, with an error rate of 25.56% ± 8.06 and 36.53% ± 6.92 in train and test data set, respectively (Table 7). In the raw ECG signal entropy, the error was 23.72% ± 9.34 and 36.64% ± 7.75 in train and test data sets, respectively (Table 8).

Table 7. Classifier error evaluation using the filtered ECG, which is assessed as the ratio between the true positive (correctly classified cases) and the total number of cases.

	Train	Test
Absolute	59.04 ± 16.35%	66.39 ± 3.20%
Normalization (median)	36.89 ± 18.76%	62.06 ± 8.90%
Differentiation (median)	37.32 ± 18.13%	62.22 ± 10.08%
Normalization + Differentiation (median)	25.36 ± 8.76%	39.56 ± 4.35%
Normalization + Differentiation (median + iqr)	**25.56 ± 8.06%**	**36.53 ± 6.92%**
Normalization + Differentiation (median) split	27.13 ± 8.85%	30.89 ± 3.05%
Normalization + Differentiation (median + iqr) split	24.36 ± 6.17%	29.76 ± 3.84%

Note. The tree error was calculated by the evaluation of 100 runs, which evaluates randomly selected test and train. The bold evidences the selected input that corresponds to the lowest train error.

Table 8. Classifier error evaluation using the raw ECG, which is assessed as the ratio between the true positive (correctly classified cases) and the total number of cases.

	Train	Test
Absolute	49.82 ± 0.00%	66.28 ± 0.00%
Normalization (median)	34.82 ± 15.14%	57.78 ± 10.11%
Differentiation (median)	37.47 ± 17.31%	60.22 ± 8.38%
Normalization + Differentiation (median)	31.41 ± 16.69%	60.44 ± 6.90%
Normalization + Differentiation (median + iqr)	**23.72 ± 9.34%**	**36.64 ± 7.75%**

Note. The tree error was calculated by the evaluation of 100 runs, which evaluates randomly selected test and train datasets. The bold evidences the selected input that corresponds to the lowest train error.

The results using the raw and the filtered ECG showed that the better performance was still associated with the entropy of noise biomarker, thus indicating that there is useful information in higher frequencies of the ECG that may aid in the discrimination of emotions.

Discussion

In the present study, we present an automatic classifier of emotions based on the ECG entropy of noise, since the results from a previous study (Brás, Ferreira et al., 2015) indicated that, although the filtering process is planned to eliminate/attenuate some interferences in the ECG signal, it seems to involve the loss of relevant information for emotion recognition. Our results are in line with those of Brás, Ferreira and colleagues (2015), showing a perfect or good prediction in the three emotional conditions—fear, disgust, and neutral, which confirms the ability of ECG entropy of noise for emotion recognition. The evaluation method was performed independently of the participant. More specifically, by evaluating a new ECG segment, the method was able to predict the specific emotion without information from the participants. In order to correctly validate the method, the split of the participants' data was evaluated by dividing the data from each participant in both test and train data sets. The chosen classifier considered the median and interquartile difference values from the normalized and differentiated ECG entropy of noise with train and test data sets exclusive from each other. There are a few studies that have implemented the user-independent system, and the results showed a lower classification rate (Jerritta et al., 2011). In our study, however, we obtained a sensitivity of 73.40% ± 21.48 and a specificity of 86.70% ± 9.51, revealing that when the classifier predicts a class (fear, neutral, or disgust) it is likely to be true.

The use of techniques aiming at reducing the noise and improving the quality of the signal in the ECG is highly advisable (e.g., Agostinelli et al., 2014). In fact, in clinical practice, it is unusual that the characteristics of the interference are even described or recognized, which may obviously affect the design of the filter to be applied to the signal. Several filtering techniques are used to detach the noise from the signal (e.g., Agostinelli et al., 2014). However, it is not possible to accomplish this without also removing relevant information from the signal (e.g., Brás et al., 2013, 2014). The removed noise contains both high frequency external noise and physiological noise, such as respiration, gastric movements, and muscle contraction. Given that previous studies have been able to recognize emotions using biosignals like respiration and EMG (e.g., Li & Chen, 2006; Liu et al., 2011), it is then likely that this information, which is traditionally discarded in the analyses, may be important in emotional identification from ECG features. Although using multiple measures could lead to better emotion recognition accuracies, the ECG may be useful in conditions in which the use of more than one measure is not possible for emotion recognition. In this work, we proposed an approach that uses the ECG

signal information in higher frequencies for the classification of emotions, which was successful in classifying specific emotions.

The entropy measure used in the present study adapted itself to the signal characteristics and, therefore, the proposed method did not present restrictions on abrupt changes of the signal, which are usually present in noise and noisy signals. We showed that the ECG entropy of noise, compared with the HRV measure, raw ECG, and the filtered ECG signal entropy as inputs for the classifier, was the variable that better discriminated between the three emotional conditions. As predicted, this indicates that the information that is usually discarded from the analysis also contains relevant indications for emotional classification, as previously shown in Brás, Ferreira et al.'s (2015) study. Although the loss of some information during the filtering techniques may not be relevant for clinical pathological reasons (e.g., Agostinelli et al., 2014), our results show that they may be useful for emotion identification. With this work, we intended to demonstrate that all the information should be inspected, even the usual "noise," which, in many cases when we are eliciting emotions may be justified by the participant's reaction to the emotional elicitation. For instance, the emotion of disgust involves synchronized behavioral responses, such as wrinkling of the nose, slightly narrowed brows, retraction of the upper lip (e.g., Darwin, 1872; Ekman & Friesen, 1978; Izard, 1971; Susskind et al., 2008), and related with increased bradygastria, with the goal of avoiding disease contamination (e.g., the ingestion of poisonous food; e.g., Meissner, Muth, & Herbert, 2011). Therefore, it is somehow expected that the noise on the ECG signal increases in the presence of such negative emotional stimuli. However, future studies should test whether this is also the case for other emotions, namely, positive ones.

Automatic classification of emotions through physiological responses can be an important addition for the development of measures that can allow real-time assessment and the manipulation of human emotions (e.g., Wu et al., 2010). Moreover, automatic classification of emotions may enable the development of machines (e.g., computers and robots) that are able to recognize human emotions and interact with humans in different settings, namely, in learning processes (e.g., Craig, Graesser, Sullins, & Gholson, 2004), in affective gaming (e.g., Conati, 2002), in driving (e.g., Katsis, Katertsidis, Ganiatsas, & Fotiadis, 2008), and in health care settings (e.g., Picard, 2002)

STUDY III. SUBJECTIVE EXPERIENCE OF DISGUST: PSYCHOMETRIC PROPERTIES OF THE PORTUGUESE VERSION OF DISGUST PROPENSITY AND SENSITIVITY SCALE-REVISED

Subjective Experience of Disgust: Psychometric Properties of the Portuguese Version of Disgust Propensity and Sensitivity Scale-Revised

Abstract

Individual differences in the experience of disgust are known to influence the development and maintenance of several psychopathologies. This study examined the psychometric properties of Portuguese version of the Disgust Propensity and Sensitivity Scale-Revised (DPSS-R) for use in Portuguese-European populations. Two-hundred and twenty-nine participants filled the DPSS-R. The confirmatory factor analysis of DPSS-R with 11 items provided better goodness-of-fit indices than the original DPSS-R with 12 items. Moreover, the DPSS-R also revealed acceptable convergent and discriminant validity, as well as significant correlations with the Disgust Scale, Maudsley Obsessive Compulsive Inventory, Spider Phobia Questionnaire-Revised, and State-Trait Anxiety Inventory. The internal consistency, test-retest reliability, and composite reliability of this instrument were appropriate. Finally, women reported higher levels of disgust propensity and sensitivity than men. The Portuguese version of DPSS-R can be a valid and reliable measure of disgust propensity and sensitivity and, therefore, a useful instrument in both research and clinical practice.

Introduction

The emotion of disgust has an enormous impact in our health and welfare since it involves the protection of the organism against disease (e.g., Curtis & Biran, 2001; Davey, 2011; Schaller & Park, 2011). Studies have shown that stimuli that signal the presence of disease (e.g., dirty places, body fluids, wounds, and images indicative of individuals with infectious diseases) have the ability to trigger disgust responses, which include behavioral avoidance from these stimuli to prevent contamination or infection (e.g., Curtis, et al., 2004). Recently, it has been shown that this adaptive function of disgust also impacts the immune system, while preparing the organism for infection on various immune parameters upon exposure to disease cues [e.g., elevating the core body temperature (Stevenson et al., 2012) and increasing proinflammatory cytokine interleukin-6 (Schaller, Miller, Gervais, Yager, & Chen, 2010)]. Additional studies have also showed that when the immune system is suppressed (such as following periods of illness or in the first trimester of pregnancy), an enhanced vigilance to and subsequent avoidant behavior away from disease stimuli are observed (for an overview, see Schaller & Park, 2011), together with higher disgust and nausea, both with the ultimate goal of counteracting the vulnerability of the immune function.

Although disgust involves the adaptive function of protecting us from being in contact with potential contaminants or diseases, individual differences in disgust have been pointed as having an important contribution to the development and maintenance of several psychopathologies, such as contamination-based obsessive–compulsive disorder, blood-injection-injury phobia, spider phobia (e.g., Cisler et al., 2009), eating disorders (e.g., Davey & Chapman, 2009; Harvey, Troop, Treasure, & Murphy, 2002), sexual dysfunction in woman (e.g., de Jong, van Overveld, Schultz, Peters, & Buwalda, 2009), and hypochondriasis (e.g., Davey & Bond, 2006). However, the exact role of disgust in psychopathology is not yet conclusive, mainly because other aversive emotions, such as fear, are also involved. Thus, disentangling the role of disgust in psychopathology is deemed as highly relevant for the development of more comprehensive theoretical models and, as a consequence, to the development of more effective psychological treatments.

In the last decades an increasing number of studies have included additional measures to further understand disgust, as well as its association with psychopathology (e.g., behavioral avoidance tasks, as well as psychophysiological, neural and immunological correlates) (e.g., for a review, see Davey, 2011). Yet, self-report measures have been the most common and widely used instruments to help understand individual differences in disgust (Davey, 2011). The first self-measures of disgust, like the Disgust and Contamination Sensitivity Questionnaire (DQ; Rozin, Fallon, & Mandell, 1984), the Disgust Scale (DS; Haidt, McCauley, & Rozin, 1994), and the Disgust Emotion Scale (DES; Walls & Kleinknecht, 1996), were developed to assess the disgust propensity (i.e., the frequency of the disgust experience, e.g., Olatunji, Cisler, Deacon, Connolly, & Lohr, 2007; Olatunji, Williams, et al., 2007; van Overveld, de Jong, & Peters, 2010; van Overveld, et al., 2006). However, none of these measures assess the disgust sensitivity (i.e., the level of unpleasantness of the disgust experience), which represents a component of disgust that is highly correlated with the development and maintenance of psychopathology (e.g., Olatunji, Cisler, et al., 2007; van Overveld et al., 2006). In order to counteract this limitation, Cavanagh and Davey (2000) developed the Disgust Propensity and Sensitivity Scale (DPSS-32 items), which was further revised by van Overveld et al. (2006), resulting in a 16-item revised version of the DPSS (DPSS–R). Finally, in a subsequent revision, Fergus and Valentiner (2009) proposed a shorter version of the revised scale, composed by 12 items, and with improved psychometric qualities.

The disgust propensity and sensitivity are two components of the disgust response (e.g., Fergus & Valentiner, 2009). The first is considered as a trait and the second as a state

component (e.g., Davey & Bond, 2006), each influencing psychopathology. For instance, the disgust propensity seems to predict spider phobia (e.g., van Overveld et al., 2006) and is associated with obsessive–compulsive disorder (OCD, e.g., Goetz et al., 2013; Olatunji, Tart, Ciesielski, McGrath, & Smits, 2011), while disgust sensitivity predicts emetophobia (fear of vomiting) (e.g., van Overveld, de Jong, Peters, van Hout, & Bouman, 2008) and both disgust propensity and disgust sensitivity predict blood fear disorder (e.g., van Overveld et al., 2006). Thus, studying both components is indispensable for better determining the mediating role of individual differences in disgust and psychopathology.

Based on the increasing emergence of studies aimed at understanding the disease avoidance nature elicited by disgust and the mediating role of individual differences, the goal of the present study is to adapt and validate the Portuguese versions of the Disgust Propensity and Sensitivity Scales-Revised. More specifically, we will analyze the reliability (internal consistency and stability) and validity (criterion validity, content validity and construct validity). Moreover, we will examine the associations between DPSS-R and measures of psychopathologies such as, Maudsley Obsessive Compulsive Inventory (MOCI), Spider Phobia Questionnaire-Revised (SPQ-R15) and State-Trait Anxiety Inventory (STAI Form Y-2 Trait).

Method

The guidelines of the Declaration of Helsinki and the standards of the American Psychological Association were followed. The project was also approved by the Scientific Council of the University, which evaluated the scientific, legal, and ethical issues.

Participants

Two-hundred and twenty-nine healthy Portuguese participants (176 women), with ages between 18 and 65 years (M = 26, SD = 8.71), filled the DPSS-R online. All participants read the instructions in the informed consent form and agreed to voluntarily participate in the study. After reading the instructions, participants were then informed that they had the right to withdraw from the study at any point in time and that the data would be strictly confidential. Nine participants were excluded because they were not Portuguese speaking.

Instruments

Disgust Propensity and Sensitivity Scale-Revised (DPSS-R; Fergus & Valentiner, 2009)

The reduced-item DPSS-R includes 12-items and is composed by two subscales: disgust propensity and disgust sensitivity. Both the total scale and the two subscales present good internal consistency (Disgust Propensity α = .83 and Disgust Sensitivity α = .80) (Fergus & Valentiner, 2009).

Disgust Scale-Revised (DS-R; Haidt, McCauley & Rozin, 1994, modified by Olatunji, Cisler et al. 2007)

The DS-R consists of 27 items, but only 25 items are considered in the analysis. This scale measures the disgust response for different disgust elicitors. It is composed by three subscales: core disgust, animal-reminder disgust, and contamination-based disgust. The subscales have an acceptable internal consistency (all $\alpha > 0.70$) (Olatunji, Cisler et al., 2007). Participants from the current study filled the Portuguese version that was translated and adapted by Ferreira-Santos, Martins Sousa, and Mauro (2011).

The Maudsley Obsessive Compulsive Inventory (MOCI; Hodgson and Rachman, 1977)

The MOCI includes 30 items that assess the obsessive-compulsive symptoms. The inventory is composed by four domains: checking, cleaning, doubting, and slowness. Participants filled the Portuguese version (Nogueira et al., 2012), which includes three domains: doubting and rumination, which has an adequate internal consistency (α = .72), checking (α = .66) and the cleaning domain (α = .63) that has a questionable internal consistency according to Nunnally and Bernstein (1994).

The Spider Phobia Questionnaire-Revised (SPQ-R15; Klorman, Hastings, Weerts, Melamed, & Lang, 1974; modified by Olatunji, Woods et al., 2009*)*

The SPQ-R15 consists of 15 items that measure fear and avoidance of spiders. This scale has good internal consistency (α =.89) (Olatunji, Woods et al., 2009). Participants filled the Portuguese version that was translated and adapted by Silva, Soares, and Esteves (in preparation).

The State-Trait Anxiety Inventory (STAI Form Y-2 Trait, Spielberger et al., 1983)

The STAI Form Y-2 Trait includes 20 items that asses the level of trait anxiety. Participants filled the Portuguese version (Silva & Spielberger, 2007), which according Nunnally and Bernstein (1994), has a good internal consistency (α = 0.89 for men and α = 0.90 for women).

Procedure

The DPSS-R was translated into European Portuguese by two bilingual individuals and reviewed by one researcher highly proficient in English. Next, the DPSS-R was submitted to a think-aloud procedure in order to adjust the vocabulary and improve the comprehension of the DPSS-R. The scale was then back-translated by a bilingual researcher with no prior knowledge of the DPSS-R. The back-translation was sent to one of the authors of the revised scale for the official approval of the Portuguese version. For the test-retest reliability of the Portuguese version of the DPSS-R a second phase was conducted 1 week later, in which 23 participants from the original sample were asked to complete the DPSS-R again.

Data analysis

Data Analysis was run with SPSS (v.22). The factorial validity of the instrument was assessed through a confirmatory factor analysis with Asymptotically distribution-free (ADF) Method, using Amos suite from SPSS (v.22). The overall goodness-of-fit of the factor model was evaluated in accordance with the indices: χ^2/df; CFI; PCFI; GFI; PGFI; RMSEA; P [rmsea ≤ 0.05] and MECVI. The adjustment of the original model was made by removing the item that damaged local adjustment and individual reliabilities. The local adjustment was evaluated by factor weights and the individual reliability of the items. The Average Variance Extracted (AVE; Convergent and Discriminant Validity) and the Composite Reliability (factorial reliability) for each factor were evaluated, as described by Fornell and Larcker (1981). The internal consistency was evaluated by Cronbach's alpha (Inter-item and item-total correlations are presented) and the test-retest reliability (temporal constancy) by the Intraclass Correlation Coefficient (ICC). A t-test for independent samples was run to compare whether the subscales scores of DPSS-R differed between women and men participants.

Results

The mean overall score obtained for the Portuguese version of DPSS-R (11 items, see CFA analysis below) was 30.498 (SD = 6.311). The overall mean (and standard deviation) of each subscale, and also by sex, is presented in Table 9. Descriptively, women reported higher levels of propensity and sensitivity than men, and this difference was statistically significant for the two factors, $t_{propensity}$ (227) = 2.118, $p < .05$; d = -.332 and, $t_{sensitivity}$ (227) = 3.003, $p < .001$; d = -.471.

Table 9. Overall mean (SD) of the DPSS-R and means (SD) according to the sex of the participants, based on our adjusted model of the DPSS-R 11 items.

	Overall Score	Men	Women
Propensity	18.74 (3.43)	17.86 (3.54)	19.00 (3.37)
Sensitivity	11.76 (3.94)	10.36 (3.33)	12.18 (4.02)

Construct Validity

The construct validity of the Portuguese version of DPSS-R was evaluated by calculating its three sub-components: the factorial validity, the convergent validity, and the discriminant validity. Regarding the first sub-component, a confirmatory factor analysis with the ADF method (towards multivariate normality violation) was produced to test the two-factor solution - 'Propensity and 'Sensitivity' - found in validation studies from other countries (e.g., Fergus & Valentiner, 2009). The two-factor model of the DPSS-R with a sample of 229 participants, revealed a poor goodness-of-fit ($\chi^2(53) = 182.019$. $p < .001$; $\chi^2/df = 3.434$; CFI = .846; PCFI = .679; GFI = .881; PGFI = .599; RMSEA = .105; P [rmsea ≤ 0.05] < .001; MECVI = 1.069). Additionally, one item (Item 12) revealed low factor weight ($\lambda \leq 0.5$) and inadequate individual reliability ($R^2 \leq 0.25$), which was obstructing the factor validity of the instrument.

Consequently, based in global and local adjustment, the model was adjusted by eliminating the item that suggested a saturation in different factors from those suggested in the literature (Brown, 2006; Marôco, 2014). This change resulted in the adjusted model presented in Figure 9.

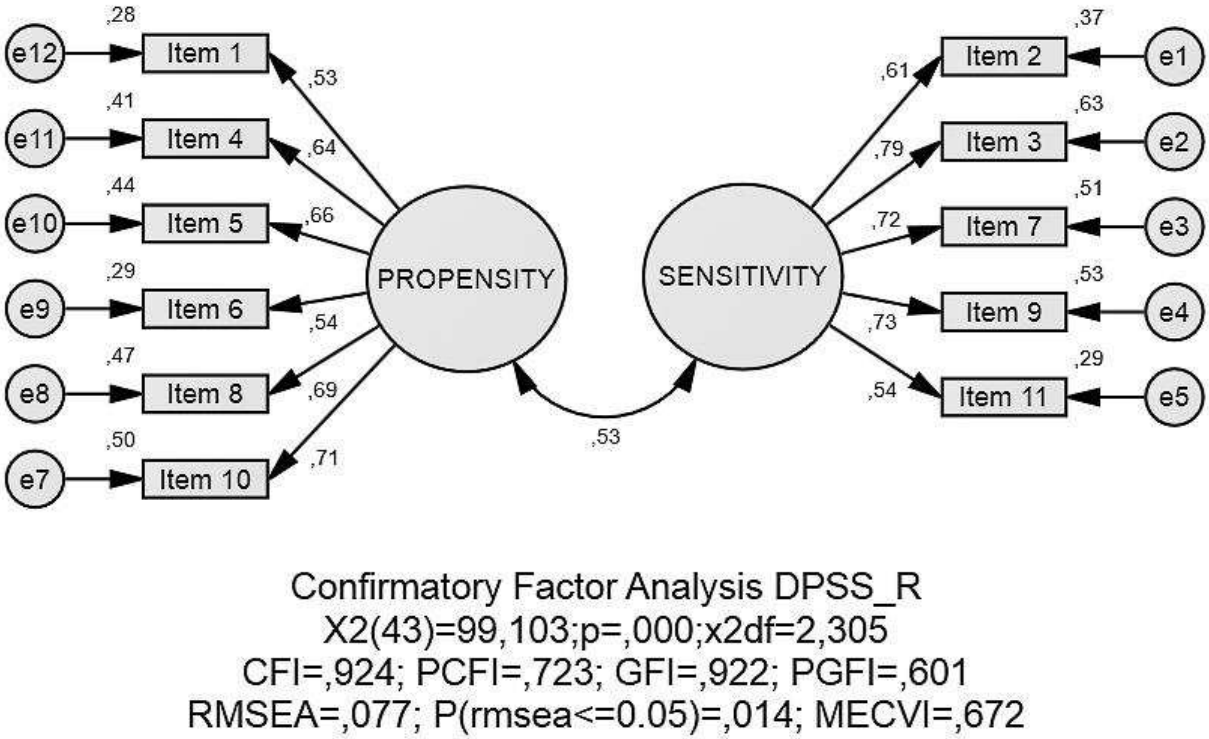

Figure 9. Standardized path coefficients for two-factor model.

Overall, the goodness-of-fit indices of the adjusted model can be considered good (CFI; PCFI; GFI; PGFI; RMSEA), with only the χ^2/df revealing a marginal value between the tolerable and good (see values in Figure 9). A comparison between the indices of the original model and those of the adjusted model, as indicated by MECVI (comparison index - less is better), revealed that the adjusted model produces better adjustment qualities, confirming that the latter is a better model than the former. Thus, the adjusted model constitutes a better fit to the observed correlation structure among the items in our sample. Additionally, all the items of the two factors in the adjusted model obtained high factor weights ($\lambda \geq 0.5$) and appropriate individual reliabilities ($R^2 \geq 0.25$), indicating to be a reflection of the latent factor being measured. In sum, these data confirm the factorial validity of the Portuguese version of DPSS-R with the adjusted model.

The convergent validity of the instrument was assessed by the average variance extracted (AVE), calculated using the following formula:

$$\widehat{AVE_J} = \frac{\sum_{i=1}^{k} \lambda_{ij}^2}{\sum_{i=1}^{k} \lambda_{ij}^2 + \sum_{i=1}^{k} \varepsilon_{ij}}$$

(λ_{ij} are the standardized factor weights and $\varepsilon_{ij} = 1 - R_{ij}^2 \cong 1 - \lambda_{ij}^2$ are the residues of each item)

The factors "Propensity" and "Sensitivity" obtained AVE values of 0.40 and 0.47, respectively. These values are close to those that are usually regarded as adequate (Hair, Anderson, Tatham, & Black, 1998) and are considered relatively good for validation studies in psychology (Marôco, 2014). The discriminant validity was calculated by comparing the AVE of each factor with the square of the correlation between the two factors (Anderson & Gerbing, 1988). Both AVE reached values above the square of the correlation between the two factors (.281) revealed that the factors only have 28.1 % of common information, thus confirming their discriminant validity.

Criterion Validity

In this analysis, 86 participants were excluded because we included only the completed answers from all the questionnaires. The criterion validity of DPSS-R was obtained through the Spearman correlation coefficient (rs), as shown in Table 10. The DPSS-R showed evidence of a good criterion validity since the correlation between DPSS-R and its two subscales with others measures were statistically significant (low to moderate), with the exception between Disgust Propensity and Contamination Domain of DS that was not statistically significant. The internal concistency values for DS and its domains, MOCI, FSQ-R15 and STAI Y-2 were apropriate (all $\alpha \geq 0.71$). However, we obtained poor internal concisttency for the contamination domain of DS, Checking and Cleaning subscale of MOCI and STAI Y-2 (ranged from .500 to .662) (see Tbale 14 in the appendix section).

Reliability

To test the DPSS-R reliability, we assessed the internal consistency (Cronbach's α), the test-retest reliability, and the composite reliability for each of the factors that resulted from the adjusted model. The results regarding the first two measures on each of the factors are presented in Table 10, according to the sex of the participant.

Table 10. Reliability of the DPSS-R

	Total	Men	Women
Factor 1: Propensity			
Cronbach's α	.778	.795	.769
Mean corrected item-total correlation	.532	.565	.519
Test-retest reliability (ICC)	.889	.814	.939
Factor 2: Sensitivity			
Cronbach's α	.808	.571	.812

Mean corrected item-total correlation	.599	.604	.529
Test-retest reliability (ICC)	.885	.622	.947

Both factors obtained a good internal consistency considering that their alpha values are well above the cut-off value of 0.7 (Nunnally & Bernstein, 1994). The ICC values are above the cut-off points defined by Fleiss, Levin, and Paik (2003) for an acceptable test-retest reliability. The mean of corrected item-total correlation also revealed appropriate values (see Table 10). Regarding the composite reliability, which reflects the internal consistency of the items of the factor, calculated using $\widehat{CR_j} = \frac{\left(\sum_{i=1}^{k} \lambda_{ij}\right)^2}{\left(\sum_{i=1}^{k} \lambda_{ij}\right)^2 + \sum_{i=1}^{k} \varepsilon_{ij}}$, the values obtained for the two factors were above 0.7, indicating an appropriate construct reliability ($CR_{Propensity}$ = 0.798; $CR_{Sensitivity}$= 0.812).

Discussion

In the current study we analyzed the psychometric properties of DPSS-R from a nonclinical sample of the Portuguese population. The results showed that the Portuguese version of DPSS-R can be a valid and reliable measure to assess the disgust propensity and sensitivity in European-Portuguese population. The analysis of the validity of DPSS-R consisted of content, construct and criterion validity. The content validity of the DPSS-R was warranted through the procedures of spoken reflection and independent back-translation, thus maintaining the semantic and conceptual structure of the original scale. Moreover, and supporting construct validity, the confirmatory factor analysis showed that the disgust propensity and sensitivity are two independent factors, which corroborate the results from others studies (e.g., Fergus & Valentiner, 2009; van Overveld et al., 2006). However, and contrarily to the results presented by Fergus and Valentiner (2009), one item of the sensitivity subscale was removed (item 12). This deletion was based on the low factor weight and inadequate individual reliability of the item, which compromised the factorial validity of disgust sensibility since this item did not reflect the latent factor that was supposed to be measured by this factor. As a result, the global and local adjusted model with 11 items fitted the data significantly better than the model with 12 items, which revealed a poor goodness-of-fit. The differential factor loadings across these studies can be due to the cross-cultural differences in the interpretation of this item. The results also confirmed the convergent validity of the DPSS-R, showing the positive correlation between the items of each factors and

discriminant validity, showing that the items of the disgust propensity factor did not correlate with the items of the disgust sensitivity factor.

Regarding the criterion validity, the results showed low to moderate correlations between DPSS-R (total scale and its subscales) and DS (total scale and its domains). Although these two measures are related, the DPSS-R seems to assess others aspects of the disgust response that are not limited to the context or to the disgust elicitors as presented in the DS. Importantly, these results corroborate the results from others studies that use the DPSS-R (e.g., van Overveld et al., 2006; van Overveld et al., 2010). However, compared to the disgust sensitivity, disgust propensity presented the lowest correlation with DS, which was not expected since both are likely to measure the disgust propensity (e.g., van Overveld et al., 2006).

Similar to other studies that used different versions of disgust scales, the DPSS-R and its subscales also showed significant correlations with the Maudsley Obsessive Compulsive Inventory (e.g., Schienle, Stark, Walter, & Vaitl, 2003), the Spider Phobia Questionnaire-Revised (e.g., Olatunji, Cisler, et al., 2007), and the State-Trait Anxiety Inventory (e.g., Davey & Bond, 2006). Overall, our results showed that the disgust propensity scale was more associated with those measures than disgust sensitivity, since disgust propensity revealed the highest values of coefficients in the correlations. These results support previous findings, which implicate disgust propensity in development of some specific psychopathologies, namely spider phobia (Olatunji, Cisler, et al., 2007; van Overveld et al., 2006) and OCD (e.g., Goetz et al., 2013; Olatunji et al., 2011). Although in our study we did not measure negative affect, that can be an artifact that could influence these associations. Olatunji, Cisler, et al. (2007) demonstrated that the relationship between DPSS-R and its subscales with anxiety disorder symptoms was maintained even when the negative affect was controlled for. The emotion of disgust has evolved in order to protect us from being contaminated or being harmed from disease. Since fear of contamination is a relevant symptom in some psychopathologies (e.g., OCD, spider phobia and BII, Davey, 2011), this adaptive function of disgust may play an important role in the vulnerability to some psychopathologies, particularly when individuals are actively engaged in health-related behaviors (Olatunji, 2015). In relation to the reliability of the DPSS-R, the results from this study showed adequate internal consistency, which is similar to the study results found by Fergus and Valentiner (2009). Moreover, the test-retest and the composite reliabilities were appropriate.

Finally, the results showed that women reported higher levels of disgust, especially of disgust propensity, when compared to men. Although this result can be influenced by the

discrepancy between the number of women and men in our sample, studies that have previously used the DPSS-R with 16 items (e.g., Olatunji, Cisler, et al., 2007), the Disgust Scale (e.g., Haidt et al., 1994) or images (e.g., Curtis et al., 2004) have shown a similar pattern of results. The higher levels of disgust in women have been accounted for as the product of an evolutionary pressure to the protection of the self and of the offspring (e.g., Fessler, Eng,& Navarrete, 2005; Fleischman, 2014). Although in general women tend to report higher levels of disgust than men, future research with more balanced samples is needed in order to confirm this tendency. Studies using clinical sample are also necessary in order to further explore the relationship between disgust propensity and sensitivity and psychopathologies and, therefore, provide more specific information regarding this association. Additional studies should also try to assess the behavioral and psychophysiological signature of disgust propensity and sensitivity, as this would provide a more reliable an integrative approach to the study of individual differences in disgust. In sum, the psychometric assessment of the Portuguese version of the DPSS-R was positive since we provide the first valid instrument to assess disgust in the research practice and clinical settings in European-Portuguese populations (see the DPSS-R scale in appendix section).

STUDY IV. EMOTIONAL BODY ODOR CONTEXTS: PRIMING EFFECTS ON CARDIAC AND SUBJECTIVE RESPONSE

Emotional Body Odor Contexts: Priming Effects on Cardiac and Subjective Responses

Abstract

Many studies have indicated that the chemical cues from negative body odors (BOs) can influence the psychophysiological and behavioral responses of the receivers. However, these olfactory signals have been used mainly as contextual information for processing visual stimuli. Here, for the first time, we focus on BO-BO effects in order to evaluate how a BO prime affects the emotional tone of a subsequent BO message. The axillary sweat samples were taken from 20 donors in three separate sessions while they watched fear, disgust and neutral videos. In a double-blind experiment, we analyzed the cardiac and subjective responses from 69 participants who were either exposed to fear and neutral BOs or to disgust and neutral BOs. Our results showed a reduced cardiac parasympathetic activity (HF%) when participants smelled the negative BOs before the neutral BOs. The intensity of the neutral odor also increased following the exposure to both negative BOs. These findings provide evidences that the order of odor presentation of odor-odor priming can greatly affect the subsequent decoding of the message both at the physiological and at the subjective levels. Furthermore, BO-BO priming effects seem to be driven by valence (negative vs. neutral) rather than emotion-specific (fear vs. disgust vs. neutral) effects.

Introduction

Despite the erroneous notion that humans have an impoverished sense of smell humans, like other mammals, have a sophisticated olfactory system, as well as an excellent olfactory ability (McGann, 2017). Specifically, humans have proven to be very skilled in detecting social information from olfactory cues of body odors (BOs, Parma et al., 2017). For instance, humans are able to identify kinship (Porter, Balogh, Cernoch, & Franchi, 1986), discriminate age (Mitro et al., 2012), sex (Penn et al., 2007), and detect emotional states from olfactory cues alone (e.g., de Groot et al., 2015). The study of the emotional communication mediated via BOs has mainly focused on negative emotions, such as fear and disgust (e.g., Parma et al., 2017; de Groot & Smeets, 2017), to investigate one of the main functions of the sense of smell, namely protecting us from danger (Stevenson, 2010). For instance, studies have shown that chemosignals of hazardous stimuli receive a preferential cognitive processing (Li, Howard, Parrish, & Gottfried, 2008; Parma, Ferraro, Miller, Åhs, & Lundström, 2015) and elicit behavioral and psychophysiological responses that operate to promote avoidance and improve the chances of survival (Parma et al., 2017). Threat detection and discrimination are highly efficient and

seldom dependent on conscious processing (Lundström & Olsson, 2010). Indeed, their effects have been revealed via the analysis of implicit measures, including among others facial electromyography and eye scanning (de Groot et al., 2012). As an example, smelling the BO of a person experiencing fear elicits fearful facial expressions and a correspondent increased muscle activity of the *medial frontalis*, whereas smelling the BO of a person experiencing disgust produces a disgust facial expression that magnifies the activity of the *levator labii* muscle. This suggests emotional contagion as one of the basic mechanisms regulating human chemosensory communication (see also the emotional complementarity approach; Mutic et al. 2016). However, the physiological responses elicited by BOs are still being uncovered, especially when considering a renown marker of stress responses, the heart rate variability (HRV). A reliable way of measuring stress responses is to look at the HRV, namely the variation in time intervals between heartbeats (Task Force of the European Society of Cardiology, 1996). Greater stress responses correspond to the reduction in the percentage of high frequency (HF%) of the total HRV, in the absence of concurrent reduction of total spectrum power. In other words, these parameters can be interpreted as a selective reduction of vagal activity, which corresponds to a heightened stress state (Hjortskov et al., 2004). Within the olfactory field, only a handful of published studies investigated how common odors impact the cardiac response. For instance, unpleasant odors, such as isovaleric acid and rancid butter, are associated with heart rate increase (e.g., Alaoui-Ismaïli, Robin, Rada, Dittmar, & Vernet-Maury, 1997; Bensafi et al., 2002; Pichon et al., 2015). To date, and to the best of our knowledge, the only study using BOs to assess its effects on cardiac response is that of Albrecht and colleagues (2011), in which they showed that both neutral and anxiety odors decreased the recipients' heart rate over the time of exposure to the olfactory stimuli. Thus, it remains unclear how cardiac activity reflects the processing of olfactory negative stimuli.

Considering that the odors are greater elicitors of emotional responses (see Parma et al., 2017) and that the processing of chemical cues in general, and of BOs in particular, is highly plastic and dynamically regulated by the context in which such stimuli are interpreted (Wilson, Best, & Sullivan, 2004), many studies have used either discrete or dimensional approaches to explore how BOs serve as contextual information for other sensory modalities (preferentially visual). These studies have investigated the cognitive, behavioral and psychophysiological modulations that odors induce in the processing of visual information (Pause et al, 2004; Zernecke et al, 2011; Zhou and Chen 2009). In line with the concept of affective priming, odors can modulate the processing of stimuli and events in a manner congruent with the valence of

the odor (Smeets & Dijksterhuis, 2014). For instance, smelling a fear BO while looking at faces biases the perception of the facial expression in a negative manner: happy faces are perceived as less happy than when exposed to a neutral context (Pause et al., 2004). Moreover, neutral or ambiguous faces are perceived as more fearful in the fear context than in happy and in the neutral context (Zhou & Chen, 2009). However, the role of BOs in serving as contextual stimuli for other sensory information is still in its infancy. The very same affective effects highlighted in the visuo-olfactory domain may emerge also when considering odor-olfactory stimulations. This would fit the idea that if, as it has been claimed, affective priming phenomena have an adaptive function, they facilitate the quick response to opportunities and threats in the environment (Klauer, 1997). Considering that the temporal dynamics of olfactory stimulation make these stimuli more long-lasting than visual stimuli (Yeshurun & Sobel, 2010), the role of BO as context in modulating other olfactory messages has yet to be determined.

Here, for the first time, we focus on BO-BO effects in order to evaluate how a BO prime affects the emotional tone of a subsequent BO message, both at the subjective and psychophysiological levels. Specifically, participants were asked to smell, in different orders, the BOs of a person who either experienced fear or disgust as well as an emotionally neutral state. During this task, the cardiovascular activity was measured to reveal stress responses. After smelling each odor, subjective perceptual ratings of odors were collected. This design allows us to test the effect of odor priming on another BOs. If the BO communication is indeed dynamic and highly dependent on contextual factors, we shall observe a differential processing of emotional and neutral BOs depending on the temporal dynamics of the stimuli presentation. Specifically, we foresee that if fear and disgust BOs are presented first, their effects shall be maintained and alter both the subjective and psychophysiological responses of the subsequently presented neutral stimuli (increased perception of intensity and unpleasantness and a selective reduction of the cardiac parasympathetic activity, respectively), whereas the reversed pattern is expected for when neutral BOs are presented first. Also, this design allows us to disentangle whether such odor priming is based on the valence of the BO (negative vs. neutral) or if is emotion-specific (fear vs. disgust vs. neutral). This would emerge specially in the psychophysiological level. We hypothesize that if chemosensory communication of danger-related stimuli is emotion-specific, a differential pattern in psychophysiological responses should be found between fear and disgust BOs, in accordance with the categorical accounts (de Groot et al., 2012). If chemosensory communication of danger is instead valence-based, we expect unspecific psychophysiological effects between fear and disgust, that is, an overall reduction in the HF% of the total HRV for both fear and disgust. Given that emotional

chemosensory communication occurs mostly unconsciously (but see Parma et al., 2017 for exceptions), no dissociations in the participants' conscious perceptions of intensity and pleasantness between fear and disgust are expected.

Method

All the experimental procedures of this study were approved by the scientific council of the University of Aveiro, and were performed in accordance with the Declaration of Helsinki and the standards of the American Psychological Association. Written informed consent was obtained from each donor and participant. Below, we will separately report the materials and methods for the Donation study and the Transmission study.

Study 1- Donation Study

Donors. Twenty donors came to the laboratory to donate their BO samples during three separate video sessions (disgust, fear and neutral condition), one week apart from each other. The donors (10 males: $M = 21.30$ years; $SD = 2.36$ years, range = 18-25; 10 females: $M = 21.70$ years; $SD = 2.54$ years, range = 19-28) were heterosexual and had no cardiovascular, respiratory, metabolic, psychiatric, or psychological disease nor were they taking any medication, during the three weeks of BO collection. All female donors were taking oral contraceptives. Donors underwent dietary and hygienic restrictions before and during BO collection to reduce sweat contamination with exogenous and endogenous odorants. Starting from the evening before sweat collection to the end of the collection, donors were asked to refrain from eating odorous food (e.g., garlic, onion, cabbage, spices), to drink coffee and alcoholic beverages, to smoke, as well as to engage in physical exercise (de Groot, Semin, & Smeets, 2014). Donors filled the Trait Anxiety Inventory (STAI-T, Silva & Spielberger, 2007), to assess their level anxiety. This questionnaire includes 20 items and the range scores is 20-80, the higher score indicating greater anxiety symptoms. Donors also filled the Disgust Sensitivity (DS) scale (Ferreira-Santos, Martins, Sousa, & Mauro, 2011) to assess their level of disgust propensity. This scale contains 27 items and the averages run from 0 (lowest possible disgust sensitivity) to 4 (maximum possible disgust sensitivity). Since both measures revealed in all participants no deviations from the norm and therefore granted inclusion in the donor's sample.

Procedure

To allow the collection of emotional BOs, the donors watched 25 minutes of disgust videos, containing sickening scenes ("Pink Flamingos", Rottenberg et al., 2007), fear videos containing horror scenes ("The Shining", Rottenberg et al., 2007), and a nature documentary for the neutral condition ("Easter Island-Solar Eclipse" National Geographic). Such videos were presented once per week, in three separate sessions, in counterbalanced order. The disgust and fear videos have been used successfully in prior studies (de Groot et al., 2012; Vianna & Tranel, 2006). The neutral video was selected based on subjective emotional ratings made by 35 young adults to confirm whether the video induced subjective ratings of disgust and fear (see Table 15 in appendix section).

On the day before the BO collection, donors received a kit that included a cotton towel (Jumbo, Portugal) and a hypoallergenic bath gel (Lactacyd Derma, Omega Pharma Portuguesa), to be used for a shower at home before coming to the laboratory. The towels were washed with odorless soap (Blancotex, Jumbo, Portugal) and were packed separately in zip-lock bags prior to each BO collection (Alho et al., 2015). On the day of the BO collection, the donors took a shower with the odor-free soap and were not allowed to use any other body hygiene and cosmetic products. In the laboratory, after they washed and dried their armpits one additional time, they wore a cotton t-shirt (SportZone, Portugal), with nursing pads positioned under both armpits (Mercurochrome Baby, Laboratoires JUVA, Portugal). The nursing pads were secured to the armpit area with a portion of medical tape (6 cm, Omnifix, Paul Hartmann LDA) placed on the external side of nursing pads (i.e., the side that was not in contact with the axillary area and never came in contact with the side of the pad in touch with the skin). Donors were then asked to rate their perceived disgust and fear by using two separate 7-point Likert scales. Subsequently, donors were presented with one video per session and instructed to avoid looking away from the monitor. The compliance to this rule was assessed visually by an experimenter. No donor had to be excluded due to this reason. Immediately after each video presentation, donors rated their perceived emotional experience, by using two separate 7-point Likert scales, and completed the Portuguese version of the Positive and Negative Affect Scale (Galinha and Pais-Ribeiro 2005). To control whether participants did not leave the laboratory stressed after the emotional induction, after a 10-min pause, the donors rated once again their perceived fear and disgust and, at the completion of the ratings, the cotton pads were removed from the t-shirt. Each of the two pads from each of the three sessions were cut into four equally-sized quadrants (24 quadrants per donor), stored in sealed zip-locked bags, frozen at -20°C and,

defrosted 1h before the beginning of the experimental session. This freezing procedure has been adopted in other studies and does not seem to change the hedonics characteristics of the BO samples (Alho et al., 2015; Lenochova et al., 2009).

Data analysis

All data were analyzed via R using the lme4 (Bates, Mächler, Bolker, & Walker, 2015) and BayesFactor packages (Morey & Rouder, 2013). To determine whether the videos were effective in inducing disgust and fear, respectively, we performed separate linear mixed models (LMMs) to analyze the subjective emotional ratings (fear and disgust), as well the positive and negative affect reported by the donors. The LMMs used for these analyses had the subjective emotional ratings, the positive or the negative affect as dependent variables, the Subject ID as a random factor and the following fixed factors: video condition (3 levels- fear, disgust and control), session code (2 levels- before or after video presentation), order of presentation (2 levels- emotional to control and control to emotional) and sex (2 levels- males and females). Sex, group and order differences are only discussed when significant, since this was not the main goal of the study.

Results

The videos successfully induced disgust and fear experiences in the donors

Donors reported significantly lower levels of positive affect after watching the disgust and the fear video, as compared to when watching the neutral video (M = 1.75, SD =1.07). No significant differences between the positive affect elicited by the disgust (M = 0.40, SD = 0.70) and fear videos were retrieved (M = 0.80, SD = 0.42). In contrast, negative affect significantly decreased after viewing the disgust video (M = 0.80, SD = 0.63) than the fear video (M = 1.50, SD = 0.71), and both elicited increased negative affect, as compared to the negative affect reported following the vision of the neutral video (M = 0.10, SD = 0.31). To verify that the negative experience reported by the donors specifically reflected the emotional tone of each video, we evaluated the subjective ratings of disgust and fear before and after the vision of each video. Before watching the disgust, fear or neutral video the donors reported to experience similar levels of disgust and fear (Table 11). Following the vision of the disgust video, the donors reported significantly greater disgust (M = 3.18, SD = 0.11) than fear M = 1.2, SD = 0.11), whereas the opposite pattern was revealed for the fear video, which elicited greater reports of fear M = 2.6, SD = 0.37) than disgust feelings [M = 1.2, SD = (1.9); see result of the mixed model in Table 11]. In other words, we verified that the sweat samples were collected within-subject by donors experiencing disgust (during the viewing of the disgust video) and fear (during the viewing of the fear video).

Table 11. Disgust and fear ratings by donors before and after each video condition. The values reported in the first row of each cell represent beta values, whereas the values in brackets the significance of such beta values. The constant values refer to the intercepts of the models considered. Empty cells represent the variables used as reference for the other calculations.

	Dependent variable:			
	Disgust Rating on 7-point Likert Scale			
	Pre	Pre	Post	Post
Disgust		-0.100		4.100***
		(0.070)		(0.444)
Fear	0.100		-4.100***	
	(0.070)		(0.444)	
Neutral	-0.000	-0.100	-4.400***	-0.300
	(0.060)	(0.060)	(0.385)	(0.385)
Constant	1.000***	1.100***	5.400***	1.300***
	(0.049)	(0.049)	(0.314)	(0.314)
Observations	40	40	40	40
Log Likelihood	12.450	12.450	-56.049	-56.049
Akaike Inf. Crit.	-14.900	-14.900	122.099	122.099
Bayesian Inf. Crit.	-6.845	-6.845	130.154	130.154
	Fear Rating on 7-point Likert Scale			
	Pre	Pre	Post	Post
Disgust		-0.500		-2.392***
		(0.359)		(0.433)
Fear	0.500		2.392***	
	(0.359)		(0.433)	
Neutral	0.250	-0.250	-0.154	-2.546***
	(0.311)	(0.311)	(0.366)	(0.366)
Constant	1.100***	1.600***	1.204***	3.596***
	(0.254)	(0.254)	(0.307)	(0.307)
Observations	40	40	40	40
Log Likelihood	-48.177	-48.177	-55.189	-55.189
Akaike Inf. Crit.	106.354	106.354	120.377	120.377
Bayesian Inf. Crit.	114.409	114.409	128.432	128.432
Note:				*p<0.1; **p<0.05; ***p<0.01

Transmission Study

BO Receivers. Ninety-two participants took part in this study as receivers. None of them was included in the donation part of the study. Twenty-three participants were excluded, 20 due to technical issues with ECG and 3 for not having completed all questionnaires or for being older than 35 years. The remaining 69 participants (37 males: *M* = 22.76 years; *SD* = 4.16, range = 18-35; 32 females: *M* = 21 years; *SD* = 3.66 years, range = 18-31) followed the same selection criteria that were used for the donors. They were instructed to avoid using scented body products and to abstain from caffeine and physical exercise at least 12h before the experimental session. Using a double-blind, between-subject design (neutral-disgust; disgust-neutral; fear-neutral and neutral-fear), recipients from each group smelled 20 odors, including 10 odors from the neutral condition (5 male and 5 female) and 10 odors from the emotional condition, either fear or disgust, which were presented twice. Smell abilities were ensured using the odor identification subtest of Sniffin' Sticks test (Burghart Instruments, Wedel, Germany; Hummel, Kobal, Gudziol, & Mackay-Sim, 2007). All participants were included in the final sample, since their scores were 11/16 or above this cut point. To control for potential individual differences across groups, we analyzed the STAI-T inventory and Disgust Propensity and Sensitivity Scale (Ferreira et al., 2016). The DPSS-R contains 11 items and the range of scores for propensity subscale is 6-30 and the range of scores for Sensitivity subscale is 5-25. The results did not show any clinical or extremes signs of anxiety or disgust sensitivity and propensity, as a result, no participant was excluded. Results are detailed in Table 12.

Table 12. Description of the recipients' sample, mean and standard deviation presented.

	Groups							
	DN		FN		ND		NF	
N	16		17		18		18	
Gender	8F		8F		8F		8F	
Age	21.50	3.43	24.12	5.28	20.63	3.61	22.39	3.79
STAI – Trait Anxiety	36.75	7.76	31.82	10.61	31.61	6.02	34.56	4.64
Disgust Propensity	18.13	3.09	17.94	3.85	17.50	3.09	17.78	2.92
Disgust Sensitivity	14.87	5.08	12.76	3.85	12.11	3.29	13.11	3.91

Note: DN (Disgust-Neutral Group); FN (Fear-Neutral Group); ND (Neutral-Disgust Group); NF (Neutral-Fear Group);

Procedure

The experimental session started with the completion of the informed consent and the questionnaires (STAI-T inventory and DPSS-R). Subsequently, three ECG Disposable Biopac EL503 Ag-AgCl snap electrodes were placed on the recipient's body following a standard lead II configuration (right arm, left leg and right leg ground; Berntson et al., 2007) and connected to a Biopac MP100, ECG Module (Biopac Systems, Inc.). The recipients were then instructed to sit quietly to avoid sudden movements and to sniff when the experimenter presented each odor in an open jar, positioned 2 cm away from the nostrils. Subsequently, the participant smelled one odor for 3 seconds and was asked to rate that odor's perceived intensity and pleasantness, using VAS scales (anchored to the extremes of 0 and 100). After 10 odor trials, the recipients rested for 5 minutes, before they were exposed to a new set of 10 odors. The order of the odor presentation was counterbalanced across participants and across groups. Finally, the participants underwent the evaluation of olfactory functionality via the Sniffin' Sticks identification subtest (Hummel et al., 2007).

Dependent variables and data analysis

The analyses of odor intensity and pleasantness differences were conducted by separate LMMs. To fit the models, each rating was introduced as the dependent variable, the Subject ID was a random factor and the fixed factors were: Group [4 levels: Disgust-Neutral (DN), Neutral-Disgust (ND), Fear-Neutral (FN), Neutral-Fear (NF)], Order (2 levels- odor 1 and odor 2), Odor Condition (3 levels- Disgust, Fear Neutral) and Sex (2 levels, male and female)]. Additionally, to examine whether negative odors induced any stress response on the cardiac activity, we performed the frequency-and-time domain HRV analysis, using Kubios software (Tarvainen, Niskanen, Lipponen, Ranta-aho, & Karjalainen, 2014; University of Eastern Finland, Kuopio, Finland). The frequency-domain was calculated using the power spectrum analysis on the inter-beat-intervals (for more details, see Trinder et al., 2001). We considered the following measures: *total power* (reflecting total HRV, ms^2), High Frequency an index of pure vagal tone, expressed as absolute power in arbitrary units, *percentage of HF* (0.15-0.40Hz) over total power. For a time-domain approach, we calculated the time interval between consecutive R-waves (RR), which reflects the myocardial contraction frequency (ms). To correct for non-normality, HRV variables were log-transformed (Task Force of the European Society of Cardiology, 1996). To further determine the reliability of our analyses, we applied Bayesian statistics which, beyond determining potential differences between groups (as LMM), also provide evidence towards determining conclusions about a "no group difference", as well

as informing on whether inconclusive evidence exists (i.e., data are not informative enough to provide support for either a difference or no difference between groups; Dienes, 2016). Importantly, Bayesian analyses (anovaBF with Subject as a random factor) allow to predict the likelihood of our hypotheses (a difference between the two groups exposed to the different odor conditions, as well as the direction of such difference). As a commonly accepted rule, a Bayes Factor (BF) value = 1 indicates no evidence of a difference, whereas BF between 3 and 10 indicated moderate evidence of difference between groups. BF comprised between 1 and 3 provide anecdotal evidence.

Results

The cardiac parasympathetic activity is selectively reduced when participants smell the negative odors before the neutral odor

The results of HRV measures, time- and frequency-domain, revealed no significant differences between groups at the baseline, when no odor was presented (please, refer to appendix section Table 16). Considering that the duration of the exposure to the odor is different across groups (see in the appendix section Table 17 and Figure13 with the results of the model), we included the duration of the session as a covariate in the model assessing whether HRV variables are different based on the group and the odor condition. The only measure reaching the level of significance was the percentage of cardiac parasympathetic activity (HF%). As depicted in Figure 10, a significant reduction of the high frequency proportion (HF%) appeared when participants smelled the negative BOs as compared to the neutral BO. Although no differences were evident between DN and FN groups, they both showed reduced HF%, as compared to ND and NF. Please refer to appendix section Table 18 for details on the other measures.

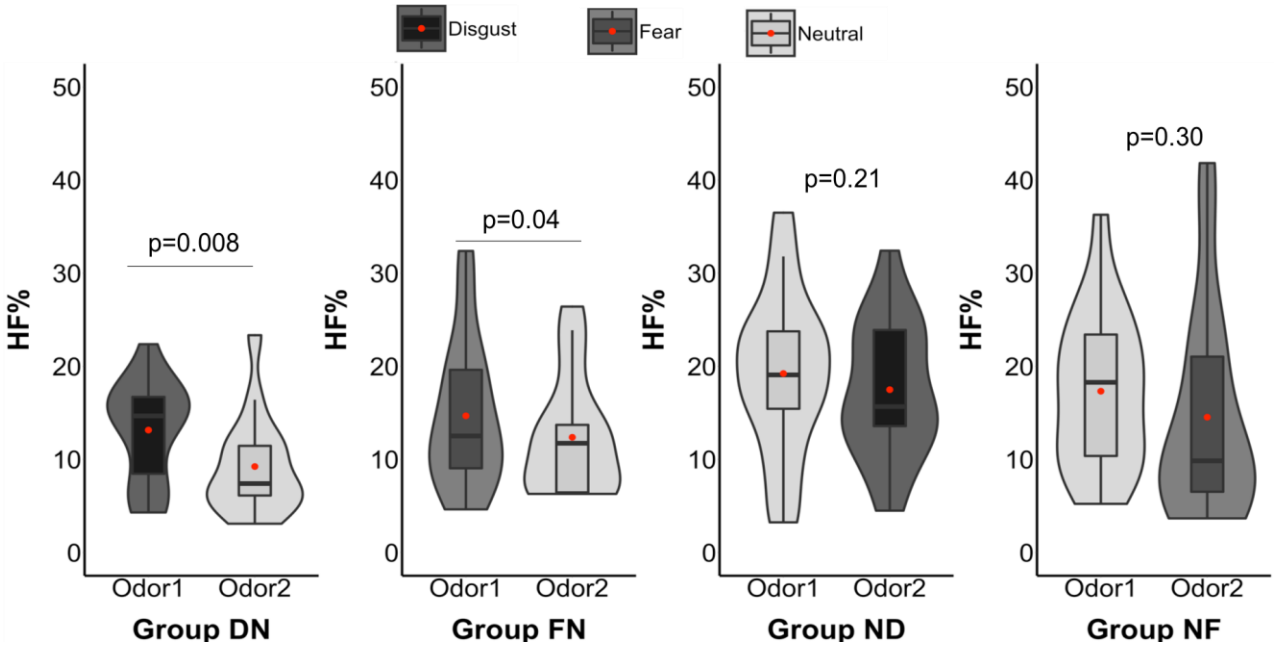

Figure 10. Graphical representation of the proportion of HF in each Group per emotional and neutral BO and color coded by order of odor exposure.

Smelling a negative BO increases the perceived intensity of a subsequently presented neutral BO.

The LMM on intensity ratings revealed no significant main effect of Group [X^2 (3, N = 2760) = 4.89, p = 0.18] or interaction involving this factor (p > .05). Furthermore, a significant main effect of Odor Condition [X^2 (2, N = 2760) = 18.00, p = .0001] was found. Post-hoc contrasts indicated that the neutral odor was perceived as more intense than the disgust [Z=3.06, p = .007, CI (2.71-5.33)], but not the fear odor [Z = 2.01, p = .13, CI (2.26-6.71)]. Importantly, a significant main effect of Odor Presentation Order [X^2 (1, N = 2760) = 6.24, p = 0.01] was retrieved. The second odor was perceived as more intense than the first odor presented. As evident from Figure 11, this pattern is significant for the DN (neutral = 47.63; disgust = 40.33, p < .004) and the FN groups (neutral = 45.63; fear = 39.23, p < .02), but not for the ND and NF groups. This entails that the neutral BO was perceived as more intense following the presentation of a negative BO, irrespective of the emotional characterization of the odor itself (disgust or fear). The Bayesian analysis confirmed that there was no evidence of a difference across the intensity ratings of the groups (BF = 0.21±0.88%). For the full results, please refer to appendix section Table 19.

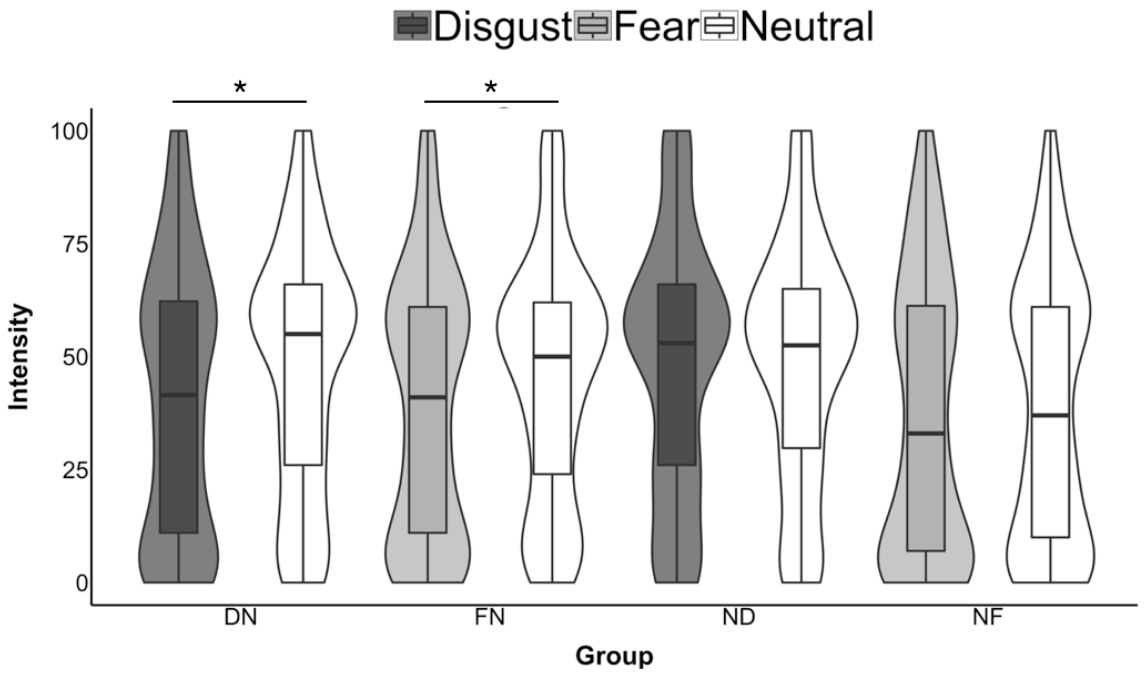

Figure 11. Intensity ratings across groups and conditions. * = p<0.05.

Within group, the neutral and emotional odor conditions are perceived as iso-pleasant

The LMM on pleasantness ratings did not reveal significant main effect or interactions ($p > .05$, Table 13 for full model details), besides the effect of Group [X^2 (3, N = 2760) = 12.28, $p = .006$]. However, post-hoc contrasts revealed that the NF group rated the fear samples as more pleasant than the FN group [Z = 3.66, p = .007, CI (6.84-11.97)], as showed in the Figure 12. The Bayesian analysis support only up to anecdotal evidence of Group differences in the pleasantness ratings (BF= 1.04±0.66%). For the full results, please refer to appendix section Table 20.

Table 13. ***R***esults of the mixed model pleasantness ~ Group*Odor+Session. The ND group represents the reference group.

	Pleasantness Odor		
	B	CI	p
Fixed			
(Intercept)	38.76	33.51 – 44.01	**<.001**
Group: DN	0.31	-5.16 – 5.78	.911
Group: FN	-3.67	-8.69 – 1.34	.152
Group: NF	4.35	-0.60 – 9.31	.086
Odor: Disgust	-1.16	-4.52 – 2.19	.497
Odor: Fear	0.58	-1.38 – 2.53	.563
Session	1.38	-0.58 – 3.33	.168
Group: DN*Odor: Disgust	3.44	-2.14 – 9.01	.228
Random			
σ^2		347.523	
$\tau_{00,\ ID}$		40.114	
N_{ID}		69	
ICC_{ID}		0.103	
Observations		2760	
R^2 / Ω_0^2		.139 / .136	

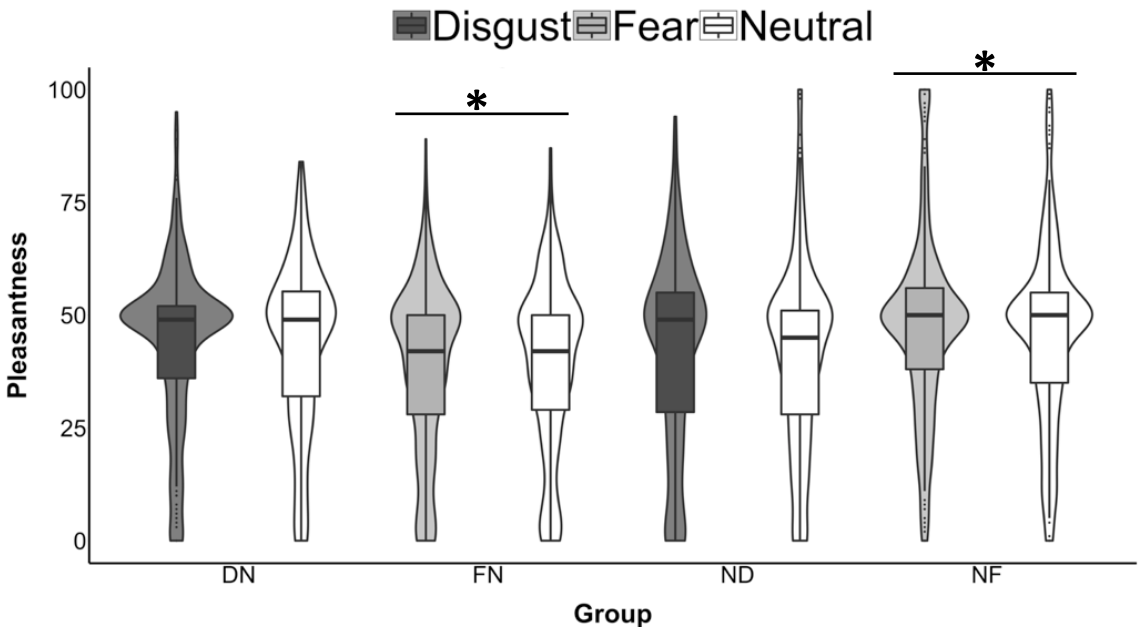

Figure 12. Pleasantness ratings across groups and conditions.

Discussion

The goal of the current research work was to investigate the ability for an emotional or neutral BO to act as context for the decoding of a subsequently presented BO, as reflected in subjective and psychophysiological responses. First and foremost, the analysis of the cardiac activity revealed that smelling the negative emotional odors before smelling the neutral odor reduced the cardiac parasympathetic activity measured during the presentation of the second block of odors (the neutral BOs, in this instance). Instead, when participants smelled the negative emotional odors preceded by the neutral odor, such reduction in the cardiac parasympathetic activity did not emerge when the second odor block (in this case, the negative emotional odors) was presented. This is expected since the emotional tone of the odor is negative, whether it is fear or disgust (e.g., Parma et al., 2017; de Groot & Smeets, 2017). No specific modulation was retrieved based on the specific emotion transmitted, supporting the idea that BOs presented first and serving as context for BOs presented subsequently may be based on the communication of the valence of the stimulus but not its specific emotional tone (e.g., Chen, Katdare, & Lucas, 2006; Prehn et al., 2006). This result suggests that being merely exposed to negative chemical cues, influenced the HRV response of subsequent neutral stimuli. In the animal kingdom, many studies have demonstrated that fear chemical cues act as warning signals by impacting the physiological responses of the recipients and increasing their level of vigilance for environmental cues (Wyatt, 2003). For example, Horii, Nagai, and Nakashima (2013) conducted a study where they investigated the effect of the order of odor exposure on

the ANS in rats. They found that an unpleasant odor (i.e., the odor of a predator) induced a stress response in the recipients. They questioned whether the subsequent exposure to a pleasant odor (linalool) would facilitate the stress recovery process. Instead, they showed that the subsequent exposure to a pleasant odor amplified the ANS-mediated stress response. According to the authors, exposing the rats to aversive stimuli, in this case smelling the presence of a predator, generates an hyper-alert state, which facilitates behavioral responses that promote escape or avoidance.

Previous studies that investigated odor priming effects using fear-related chemical cues within the visual domain in humans showed similar results. In the context of fear-related chemical cues, the receivers seem to act with more caution (Pause et al., 2004; Zhou & Chen, 2009). For instance, female recipients when exposed to fear chemical cues (compared to a neutral sweat and a control condition) performed better in a word association task, showing higher accuracy and shorter response times on the meaningful word conditions, compared to conditions where words displayed an ambiguous content (Chen et al., 2006). At the neural level, fear-related chemical cues are encoded in the same way as other biologically-relevant and threatening stimuli. As Mujica-Parodi and colleagues (2009) demonstrate, being exposed to stress chemical cues increases the responses in the amygdala, an area which preferentially responds to relevant and threatening information (Sander, Grafman, & Zalla, 2003). Accordingly, research has also shown that BOs associated with disgust prompt the mobilization of the organism to avoid potential contaminants or diseases. Disgust BOs are known to induce disgusted facial expressions (de Groot et al., 2012), which involve slightly narrowed brows, decreased eye and nasal aperture (nose wrinkling), a facial structure which favors the limitation of the incoming sensory input, reflecting the motivation to avoid or reject pathogenic agents (e.g., Susskind et al., 2008). Indeed, humans are able to detect from BO exposure whether an individual is sick or healthy (Olsson et al., 2014). Hence, as evident for animals, also humans interpret negative emotional chemical cues, such as fear and disgust, as indicators of threat in the environment and implement adaptive responses evident at the physiological level that prepare the organism to deal with dangerous situations. Our study adds to the existing literature, in which BOs are presented in crossmodal priming paradigms, that these adaptive responses are extended to odor-olfactory priming contexts.

Besides a cardiac response compatible with a stress-induced response, the subjective ratings of odor intensity additionally confirmed that the negative BOs communicated the presence of threatening information. This is evident when we evaluate the intensity ratings

performed on the neutral odor. Indeed, the intensity of the neutral odor was greater after having previously being exposed to one of the two negative BOs (i.e., fear and disgust). On the contrary, when the neutral odor was rated during the first session, then no difference in the intensity of the BOs were retrieved. These results suggest that the negative BOs may have induced a hyper attentive state that facilitates the detection of threatening stimuli, which is reflected at the perceptual level with greater ratings of intensity. Indeed, this hypothesis would be in line with evidence obtained via conditioning paradigms, which reveal that the sensitivity to an odor paired with a threatening stimulus is increased after the association is made (see Parma et al., 2015). Contrary to other accounts which suggest that the association of an odor with a threatening stimulus also produces a change in the quality of the stimulus (Li et al., 2008), we were not able to retrieve any changes in the pleasantness of the BOs, irrespective of whether they acted as a prime or a subsequent stimulus. The lack of significant differences in the pleasantness ratings across groups and odor conditions as well as sessions suggests that the odor conditions, irrespective of the emotional tone expressed, were all neutral to mildly unpleasant, as expected from BO samples that are not masked with any fragrance. The comparison with the threatening effect revealed in the intensity ratings suggests that the exposure to negative BOs vs. neutral BOs sensitizes individuals to the presence of the BO, but does not change the quality of the BO.

One may argue that the lack of emotional specificity in the psychophysiology and subjective ratings in the receivers may depend in an emotional improper induction in the donors. However, this is highly unlikely given that our donors subjectively rated their experience as selectively congruent with the emotional tone of the videos they were exposed to. This method has been supported already by many accounts (de Groot et al., 2012). However, we cannot exclude that with other psychophysiological tools an emotional-specific effect would be retrieved. Indeed, as previously demonstrated by de Groot and colleagues (2012), electromyography is able to differentiate stress responses. Here, we demonstrate that we are able to gather the relevant information (danger detection) redundantly with many systems. Future studies should combine these measures and include additional ones to map which are the most effective measures reflecting the decoding of odor priming messages.

A potential limitation of our study is the lack of positive stimuli for a full account of the valence dimension. Therefore, to extend the comprehension of chemosensory modulation on physiological response, we encourage futures studies to examine the effects of positive emotions (e.g., happiness) using a within-subject design. Furthermore, since the results from

Mutic et al. (2016) study showed that chemical cues of anger can communicate a specific type of information, the intention to harm, we also encourage further studies to include this negative emotion, in order to investigate whether its effects on cardiac and subjective response is specific or valence based.

To sum up, we need to be mindful about the order of presentation because odor-odor priming can greatly affect the subsequent decoding of the message. Therefore, trial by trial analyses are warranted when several emotional BO are compared.

PART III: GENERAL DISCUSSION

CHAPTER IV: GENERAL DISCUSSION

5. General discussion

Overall, the goal of the present work was to investigate the cardiac response of the emotions of disgust and fear, using visual/auditory and olfactory stimuli. The ECG is commonly used to identify the autonomic specificity of emotional response. Yet, the ECG is often contaminated with noise, and thus, pre-processing the signal is an important step towards an accurate emotion identification. However, using the same filtering techniques for noise elimination such as those used in a clinical setting might compromise the characterization of the ECG signal for the emotional response, particularly negative emotions, such as disgust and fear, which are associated with more muscle tension in the body (e.g., Ackerl et al., 2002; Woody & Teachman, 2000). Since the ECG can capture electrical activities from other muscles, it is reasonable to speculate that the removed signal may contain more than residual information. Thus, the goal of the Study I was to explore the ECG noise and use noise entropy for emotion identification.

5.1. Cardiac correlates of emotional response using noise entropy

In Study I, we explored ECG noise and used noise entropy for emotion identification. The ECG data was acquired when the participants were watching disgust, fear and neutral movies. As expected, the results from Study I demonstrated that it is possible to differentiate emotions using ECG noise entropy. The ECG from the disgust condition presented more noise entropy than fear and neutral ones. The presence of more noise entropy in the disgust condition might be explained by the presence of electrical activity from gastric electrical activity, since experiencing disgust is associated with increased bradygastria (related with dysrhythmic components of gastric activity (Meissner et al., 2011; Vianna, Weinstock, Elliott, Summers, & Tranel, 2006). Considering that fear is also a negative emotion, the fear condition was expected to have more noise than the neutral condition, but the results failed to demonstrate this, which can lead to the following question: is increased noise entropy specific to the disgust condition? Future studies should inspect the ECG noise for other basic emotions to answer this question.

5.2. Automatic classifier of emotions built from ECG noise entropy

Considering the rapid advancements in technology and the presence of electronic devices in our daily lives, as well as in clinical settings, building devices that can automatically recognize emotions seems more real than ever. Therefore, it is important to consider the adaption of the existing filtering techniques to the emotional response reflected in the ECG. Given that noise entropy seems to differentiate disgust from fear and from neutral emotions, we conducted a second study using noise entropy to build an automatic classifier of emotions, using the same data as in Study I. The results of the subjective ratings regarding emotional induction demonstrated that participants did indeed experience the corresponding target emotions. More specifically, they reported to have experienced more disgust in the disgust condition and fear in the fear condition. The participants also experienced more negative emotions in these conditions than in the neutral condition. Therefore, the results of the subjective ratings showed that these movies were effective in inducing the targeted emotions. These results are in line with results from other studies using these movies to elicit emotions (e.g., de Groot et al., 2012; Vianna & Tranel, 2006).

Amongst the seven inputs that we used to develop a classifier (see results section of Study II), we chose the one that showed the best performance. Classifier performance was assessed by measuring the error value, sensitivity and specificity. Since the error value represents the correctly classified records, the chosen classifier was the one that used, as input, the combination of normalized and differentiated median and iqr ECG entropy of noise values because it had the minimum error value in the training data set (19.44 ± 8.84%). The sensitivity value of this classifier was 73.40% ± 21.48% and the specificity value was 86.70% ± 9.5. Moreover, we assessed the performance of the classifier by calculating the sensitivity and specificity of each emotional class. The neutral condition was always correctly classified, since we obtained a 100% sensitivity value, whereas the fear and disgust emotions were correctly identified 60% of the time. Regarding specificity, the neutral condition displayed, once again, the highest value, that is, 97% of the time the classifier was correct when classifying neutral as not belonging to the condition it did, in fact, not belong to. Concerning the emotions of fear and disgust, the classifier was correct 80% of the time, which means that 20% of the time it classified emotions in the incorrect conditions. These results showed that our chosen classifier had a higher specificity than sensitivity. In general, the sensitivity and specificity values of a test are chosen considering the implications of the test in the real world. For example, in the clinical setting, the desirable value of sensibility and specificity would be 100%, which means correct identification in all cases or no missing identifications (e.g., Lalkhen & McCluskey,

2008). However, it is extremely difficult to accomplish such a goal, and in some cases, the values of both these test parameters will vary. For instance, when the potential outcome represents a higher risk for the individual, it is preferable to have a test with higher sensitivity (e.g., not to miss detecting an individual who has a critical disease) and low specificity (e.g., subjecting an individual to an invasive test analysis) (e.g., Lalkhen & McCluskey, 2008). The results of our study showed lower sensitivity and higher specificity, which might be good considering that our classifier was built using the noise entropy. Overall, the performance of the classifier was good, since for the neutral condition we obtained the perfect prediction (ROC=1) and a good prediction for fear (0.85) and disgust (0.79).

Moreover, we compared the performance of the classifier using noise entropy with a classifier using HRV and both the raw and the filtered ECG signal. Surprisingly, the classifier built from noise entropy performed better since it had the lowest error value (19.44 ±8.84%), compared to the error value of HRV (21.49 ±7.61%), of ECG filtered (25.56±8.06%), and of the raw EGC (23.72± 9.34%). Considering that the noise we used to build a classifier contained all types of noise, i.e., internal and external noise, we expected a better performance, especially when we compared with the filtered ECG, since the external noise that is part of the signal would compromise the signal reducing its performance.

5.3. Subjective Experience of Disgust: Psychometric Properties of the Portuguese Version of the Disgust Propensity and Sensitivity Scale-Revised

One of the main goals of this thesis was to investigate how disgust and fear body odors (compared to neutral body odors) can affect the cardiac response. Considering that body odor is a strong disgust elicitor because it carries important information about health status (e.g., Curtis & Biran, 2001 Liuzza, Lindholm, et al., 2017, Liuzza, Olofsson, et al., 2017; Oaten et al., 2009) and since the way individuals perceive body odors can be modulated by disgust, we measured the disgust propensity and sensitivity in Study IV to control such interindividual differences. However, due to the lack of a validated instrument to assess the disgust response in the Portuguese population, in Study III we examined the psychometric properties of DPSS-R and validated it for Portuguese European speaking population. Consistent with Fergus and Valentiner (2009) results, the DPSS presented a structure of two independent factors, although the DPSS-R with 11 items provided better goodness-of-fit indices than with that with 12 items, as proposed by the authors. Item 12 was then removed because it revealed poor goodness-of-

fit. The differences in the factorial solution may be due to cultural influences on how disgust is processed, recognized, conceptualized and verbalized (e.g., Soto, Lee, & Roberts, 2016).

The DPSS-R showed a low to moderate association with the DS total score and its subscales. More specifically, the correlation between disgust propensity and disgust scale was weak, despite the DS tendency to represent a mix of both these factors (Goetz et al., 2013). These results were consistent with those found by van Overveld et al. (2006) and van Overveldet al. (2010). Disgust propensity and sensitivity has been considered by some researchers as a sensitive measure of trait and state of disgust response, respectively (e.g., van Overveld, de Jong, Peters, & Schouten, 2011; van Overveld et al., 2006). Disgust propensity measures the stable tendency to experience disgust and the disgust sensitivity measures the actual or current emotional experience. Thus, this scale is aimed at assessing the disgust response regardless of the context or specific elicitors, as in the case of the DS (Fergus & Valentiner, 2009; van Overveldet al., 2010; van Overveld et al., 2006).

Researchers have been interested in investigating the potential role of disgust propensity and sensitivity in psychopathology (e.g., Cisler, Olatunji, Lohr, & Williams, 2009; van Overveld et al., 2006). In our study, although both subscales were associated with the Maudsley Obsessive Compulsive Inventory, the Spider Phobia Questionnaire Inventory and the STAI-Trait, the results of the disgust propensity scale revealed high values in the correlation coefficients, which corroborates the idea that the role of these two subscales may differ across psychopathologies. For instance, spider phobics seem to score higher in the DP subscale than in the DS subscale (e.g., van Overveld et al., 2006). Spider phobics seem to experience disgust and fear, but some studies have considered that fear is dominant in this case (e.g., Sawchuk, Lohr, Tolin, Lee, & Kleinknecht, 2000; Sawchuk, Meunier, Lohr, & Westendorf, 2002), which might suggest a reduced level of disgust sensitivity. However, other studies have suggested that disgust is a predominant emotion in spider phobia (e.g., Olatunji & Deacon, 2008; Woody, McLean, & Klassen, 2005), and so more studies are needed to clarify the role of both factors in this specific phobia.

Concerning reliability, both subscales of DPSS-R presented adequate internal consistency, indicating that all items of each subscale measured the respective construct associated with each factor, which supports the results of Fergus and Valentiner (2009). Moreover, the results of each subscale showed consistency, since we obtained adequate values on the test-retest. Overall, the criterion validity obtained was also appropriate.

Our results of Study III also showed a higher level of disgust in women than in men, although this result can be affected by the different number of women and men in our sample. Nevertheless, similar results have been found repeatedly in studies using the disgust scale (e.g., Haidt et al., 1994; Olatunji, Cisler et al., 2007) or behavioral tasks (e.g., Curtis et al., 2004). According to evolutionary theories, gender differences can be due to women needing to take care of themselves and their offspring. For example, Fessler et al. (2005) demonstrated a higher level of disgust sensitivity in pregnant woman, more specifically in the first three months of pregnancy, a period that involves a greater suppression of the maternal immune response, which consequently increases the risk of contamination. The lower level of disgust in men, on the contrary, can be related with their ability to take risks with contaminants, which can be associated with a robust immunological system and, therefore, a competitive advantage in attracting women (Fessler, Pillsworth, & Flamson, 2004).

5.4. Emotional body odor contexts: Priming effects on cardiac and subjective response

As mentioned above, in Study IV we aimed to understand the effect of disgust and fear body odors on cardiac activity and subjective responses. As expected, the result from this study showed a reduced cardiac parasympathetic activity when the receivers smelled the neutral odors after being exposed to either fear or disgust odors. A similar pattern of response was found in the subjective responses, with the results showing that the intensity of the neutral odor increased only following the exposure to negative or potentially threatening BOs (i.e., fear and disgust). These results demonstrate that the influence of chemical cues that communicate danger seems to be sustained for a longer period, which influenced the receivers' response at the cardiac and the subjective levels. First, at the cardiac level, a reduced HF of HRV (high level of vagal withdrawal) can be related with adaptive response, since a strong vagal regulation of the heart is related with the ability of an organism to quickly adjust the influence of parasympathetic system in accordance with the environmental demands (e.g., Appelhans & Luecken, 2006; Porges, 2009). Such response may be adaptive, when the individual is dealing with a situation that includes physical or mental stressors (e.g., Porges, 2007; Rottenberg, Salomon, Gross, & Gotlib, 2005). Secondly, at the subjective level, the results are consistent with previous aversive conditioning studies, in which participants who were exposed to an odorant paired with an aversive stimulus (electrical shock) increased their detection sensitivity (see Parma et al., 2015). In fact, such sensitivity change can improve the olfactory capacity to detect threats in the environment. Thus, the intensity of the neutral odor only being increased after the receivers smelled disgust or fear odors may indicate that the odors of fear and aversion

communicate the presence of aversive information, which seems to occur regardless of the quality of the odor, as no significant changes were found in the pleasantness of the body odors.

To the best of our knowledge this study was the first one to show that the order of presentation of emotional body odors can affect the cardiac response of a subsequent neutral body odor in humans. However, there is some evidence from a study with animals that found a similar effect regarding the order of presentation (Horii et al., 2013). The results from this study showed that exposing rats to an unpleasant odor (an odor of a predator) produced a stress response and that such response was augmented when they were exposed to a pleasant odor (linalool) after they smelled the unpleasant odor, which reveals that smelling an aversive odor increases a state of alertness in the rats in order to facilitate behavior responses that promote escape or avoidance. Similarly, the fear-related chemical cues seem to enhance the vigilance in humans. Results from previous studies that have used fear-related chemical cues as a context for visual stimuli in humans have showed that these types of signals carry the threatening message that influences receivers' physiological, cognitive and behavioral responses, which then trigger adaptive responses that prepare the organism to deal with dangerous situations (e.g., Chen et al., 2006; Mujica-Parodi et al., 2009; Pause et al., 2004; Zhou & Chen, 2009). Regarding cardiac discrimination between disgust and fear responses, the results showed no differences, most likely because they are both aversive stimuli, which suggest that the cardiac activity in response to these stimuli seems to reflect the emotional tone which is associated with valence dimension, instead of being associated with specific pattern of emotional response. However, the results from a previous study using similar chemosensory stimuli (fear, disgust and neutral body odors) while recording the facial electromyography activity, showed that disgust generated disgust responses in receivers and that fear body odors produced fear response in receivers, as previous mentioned in the introduction section (de Groot et al., 2012). Thus, future studies should use multiple measures, including electromyography and ECG to investigate which are the most effective measures reflecting the decoding of odor priming messages.

Although, in general, women seem to have a greater sense of smell compared to men (e.g., Brand & Millot, 2001; Garcia-Falgueras et al., 2006; Pause et al., 2009; Zhou & Chen, 2009), the results showed that the effect of the chemical cues on the cardiac and subjective levels seems to be independent of the sex of the receivers, which might suggest that the chemical cues from body odors seems to be perceived equally between male and female receivers. This result is in line with previous studies that did not find any gender effect when

they used fear/ anxiety body odors as a context (e.g., Pause et al., 2009). Regarding the hypothesis that individual differences of disgust sensitivity and propensity might influence the receivers' perception of body odors, we did not perform any further analysis including this variable because the scores of the three conditions did not differ significantly. However, recently, some authors (e.g., Liuzza, Lindholm, et al., 2017) developed a specific scale named Body Odor Trait Disgust Sensitivity that allows to assess the individual differences in disgust response to body odor in general. They showed that even after controlling the general disgust sensitivity, the Body Odor Trait Disgust Sensitivity was a greater predictor of disgust ratings of body odor samples (e.g., Liuzza, Olofsson, et al., 2017). Thus, we encourage further studies to integrate this new scale to assess and to control the individual differences disgust response to body odors.

6. Current limitations, future directions and applications

First and foremost, the common limitation across these studies (I, II and IV) is that we used only the ECG to collect data from physiological component of the emotional response. Considering that emotions can change our physiological responses, such as respiration, muscle and sweat activity, among others, future studies should try to integrate multiple autonomic measures (e.g., EMG, skin conductance) for a better understanding of autonomic pattern emotional responses. Furthermore, although we used stimuli from the three most common sensory modalities that have been studied in the literature, we used them separately. Specifically, in studies I and II we used movies to induce emotions, while in study IV we used olfactory stimuli. However, in everyday life we constantly rely on our vision, auditory, olfactory taste and touch to process the information from the environment, which means that we are often receiving simultaneous information from multiple senses. Specifically, in the affective computing field, to the best of our knowledge no studies have yet included the olfactory stimuli in their research agenda. We believe that it is important that future studies seek to develop an algorithm using a multisensory approach in order to improve performance of classifiers that aim at automatically recognizing emotions. Although the main goal of study IV was to explore how the aversive chemical cues can affect the cardiac response, we argue that for a better characterization of cardiac and autonomic response of emotional responses, it is equally important that future studies should use data from multisensory modalities.

Regarding studies I and II, the analyses from noise entropy provided interesting results about filtering techniques applied in investigating emotional responses using cardiac activity. However, it is important to take into consideration that the noise information we used contained

all types of unwanted information, i.e., external and internal noise. Thus, future studies should solely rely on the noise that is often removed from baseline wander, as well as from electromyography, which might be important for improving ECG characterization and, consequently, might be an important aid in emotion recognition. Moreover, it is important to design filters according to the problem at stake, which is why future studies should explore or test the specific filter cut-offs to apply in emotional research. Furthermore, future studies should also use noise information from positive emotions (e.g., happiness) and from other negative emotions (e.g., anger) to develop an even more reliable classifier based on noise entropy.

Although the results from study IV suggest that the cardiac response of fear and disgust seems to be based on a valence dimension, we must interpret these results with some caution for two major reasons: a) among all HRV variables from time and frequency domain that were analyzed, the only parameter that was statistically significant was HF. Even though the HF of HRV has been considered a reliable indicator to measure the stress response on cardiac activity (e.g., Thayer et al., 2012), future studies are needed to investigate whether the cardiac responses to body odors support valence instead a discrete approach (or even both approaches).

Moreover, we did not use positive emotions for a full account in support of the valence dimension approach. Therefore, further studies should use positive emotions and a within subject design to examine how the order of the body odor presentation might influence the cardiac response. Furthermore, considering that chemical cues of anger communicate the intention to harm (Mutic et al., 2016), we also encourage further studies to include this negative emotion combined with other positive and negative emotions, in order to investigate their effects on cardiac response. Also, future studies should investigate if the cardiac response will be specific for each emotion or if it will be differentiated among positive and negative emotions.

Finally, given the rapid development of computers, as well as their increasing day-to-day use in many contexts, being able to collect psychophysiological data very efficiently and analyze them in real time to recognize emotions, may be extremely useful in the research context, in clinical settings and in everyday life. For instance, developing electronic devices with the ability to recognize emotions would help researchers to improve their understanding of the underlying mechanisms of emotions and, consequently, aid in the development of intervention programs that can be more effective in clinical settings. Automatic recognition of emotions could provide psychologists with more objective information about the individual's

emotional states and with more data, namely data about daily variations of the emotional state, which would increase the effectiveness of psychological interventions, such as in autism spectrum disorder or learning disabilities (Bal et al., 2010; Kaliouby, Picard, & Baron-Cohen, 2006; Martínez et al., 2010). Moreover, using such devices in everyday life can help the individual to cope better with stress situations by keeping a daily track of emotional events, especially stressful ones. For example, such device can be able to identify when the individual is experiencing stress, warning him/her about their emotional state and suggesting relaxation techniques, such as relaxing songs, meditation exercises, among others (e.g., Lisetti & Nasoz, 2004; Nasoz et al., 2004).

7. Conclusion

In two studies (I and II), we examined whether it is possible to identify emotions and build a computer algorithm to automatically classify emotions using ECG noise entropy. We demonstrated that the ECG noise contains information that can be meaningful to recognize emotions (fear, disgust and neutral) and able to build an automatic classifier for emotion recognition. Furthermore, we demonstrated that the HF of HRV decreased when the receivers smelled the neutral body odors after the emotional body odors, either disgust or fear. Our findings provide evidence for the need to adapt the ECG filtering techniques for emotional research and be aware of the effect of the order of body odor presentation on cardiac and on subjective response of the receivers. Moreover, we provided a valid instrument to be used in the European-Portuguese population to rate disgust propensity and sensitivity.

CHAPTER V. REFERENCES

8. References

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CHAPTER VI. APPENDIX

Table 14. Means, standard deviations, internal consistence, and bivariate correlations among DPSS-R and DS, Hypochondria, MOCI, FSQ-R15 and STAI Trait.

Measures	Mean (SD)	1	2	3	4	5	6	7	8	9	10	11	12
DPSS-R													
1. DPSS-R-Disgust Propensity	18.74 (3.70)	(.778)											
2. DPSS-R-Disgust Sensitivity	13.51 (4.53)	.438**	(.808)										
DS													
3. Core Disgust	2,38 (.69)	.320**	.377**	(.771)									
4. Animal Reminder Disgust	2.09 (.84)	.208*	.414**	.676**	(.777)								
5. Contamination	1.56 (.703)	.116	.219**	.481**	.349**	(.500)							
6. Total	2.10 (.59)	.285**	.422*	.922**	.856**	.612**	(.862)						
MOCI													
7. Doubting and Rumination	2.89 (2.20)	.326**	.270**	.207*	.111	.146	.209*	(.754)					
8. Checking	1.99 (1.82)	.304**	.229**	.152	.064	.141	.132	.530**	(.662)				
9. Cleaning	2.44 (1.99)	.263**	.278**	.081	.123	.165	.131	.223**	.340**	(.661)			
10. Total	7.32 (4.66)	.385**	.345**	.201*	.154	.183*	.226**	.818**	.768**	.679**	(.813)		
FSQ-R15													
11. Total	5.85 (4.38)	.342**	.239**	.278**	.161	.121	.239**	.417**	.222**	.124	.198**	(.890)	
STAI Y-2													
12. Trait	49.01 (5.63)	.311**	.259**	.256**	.162	.160	.244*	.153	.233**	.214*	.396**	.153	(.598)

Note. On the diagonal axis, Cronbach alphas are presented. ** Significant at $p < .01$. * Significant at $p < .05$.

Escala de propensão e sensibilidade ao nojo revisto (DPSS-R; Fergus & Valentiner, 2009)

Versão Portuguesa: Ferreira, Soares, Bem-Haja, Alho, Rocha, Madeira & Silva (2016)

Instruções

Este questionário consiste em 11 afirmações sobre o nojo. Por favor, leia cuidadosamente cada afirmação e pense quão frequente é verdadeira para si, assinalando a opção que melhor representa a sua opinião.

		Nunca	Raramente	As vezes	Frequentemente	Sempre
	Evito coisas nojentas.					
	Quando me sinto enojado(a), preocupa-me que possa desmaiar.					
	Assusto-me quando sinto náuseas.					
	Sinto repulsa.					
	Coisas nojentas dão-me voltas ao estômago.					
	Faço cara de nojo quando algo me enoja.					
	Quando reparo que estou nauseado, preocupa-me se vou vomitar.					
	Sinto nojo.					
	Assusto-me quando me sinto a enfraquecer ou a desmaiar.					
	Acho algo nojento.					
	Sinto-me envergonhado(a) quando me sinto enojado(a).					

Table 15. Pre-evaluation of neutral movie.

Neutral Movie	
Fear	1.86 (.28)
Disgust	1 (0)
Anger	1.86 (.37)
Sadness	1.43 (.43)
Happiness	2.63 (1.63)
Surprise	3.14 (1.62)

Note. Subjective ratings were measured in 7-point Likert Scale (1=not all; 7=very) and are *presented as means (standard deviations).*

Table 16. Linear mixed-effects model fit by REML: HRV ~ Group at Baseline. The first three models on p. 35 [(1), (2), (3)] refer to mean RR interval values. Models (4), (5), (6) in such page are performed on Standard Deviation of Normal-to-Normal Intervals (SDNN). Models (1), (2), (3) on p. 36 are performed on Root-Mean Square Differences of Successive R-R intervals (RMSSD). Models (4), (5), (6) in such page are performed on the high frequency of HRV (HF). Models (1), (2), (3) on p. 37 are performed on the percentage of high frequency of HRV (HF%). Models (4), (5), (6) in such page are performed on the total power of the spectrum (Total power). From the top to the bottom line for each variable in the model, it is reported: beta value, confidence interval, t value, p value. P values are rounded automatically by the stargazer package and hereby reported for consistency in that way.

	Dependent variable:					
	mean RR			SDNN		
	(1)	(2)	(3)	(4)	(5)	(6)
Constant	805.931***	770.650***	748.289***	53.340***	51.710***	54.879***
	(749.181, 862.682)	(710.457, 830.843)	(689.893, 806.684)	(43.695, 62.984)	(42.354, 61.067)	(45.786, 63.973)
	t = 27.834	t = 25.093	t = 25.115	t = 10.840	t = 10.832	t = 11.829
	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.000
Group DN	−35.281		22.362		1.630	−1.540
	(−118.008, 47.446)		(−61.503, 106.226)		(−11.808, 15.067)	(−14.795, 11.716)
	t = −0.836		t = 0.523		t = 0.238	t = −0.228
	p = 0.407		p = 0.604		p = 0.813	p = 0.821
Group ND		35.281	57.643	1.540	3.169	
		(−47.446, 118.008)	(−23.786, 139.071)	(−11.716, 14.795)	(−9.878, 16.216)	
		t = 0.836	t = 1.387	t = 0.228	t = 0.476	
		p = 0.407	p = 0.171	p = 0.821	p = 0.636	
Group FN	−57.643	−22.362		−1.630		−3.169
	(−139.071, 23.786)	(−106.226, 61.503)		(−15.067, 11.808)		(−16.216, 9.878)
	t = −1.387	t = −0.523		t = −0.238		t = −0.476
	p = 0.171	p = 0.604		p = 0.813		p = 0.636
Group NF	−9.492	25.790	48.151	5.446	7.075	3.906
	(−89.749, 70.765)	(−56.937, 108.517)	(−33.278, 129.580)	(−7.810, 18.701)	(−5.972, 20.123)	(−8.953, 16.766)
	t = −0.232	t = 0.611	t = 1.159	t = 0.805	t = 1.063	t = 0.595
	p = 0.818	p = 0.544	p = 0.251	p = 0.424	p = 0.292	p = 0.554
Observations	69	69	69	69	69	69
Log Likelihood	−410.634	−410.634	−410.634	−291.610	−291.610	−291.610
Akaike Inf. Crit.	833.268	833.268	833.268	595.219	595.219	595.219
Bayesian Inf. Crit.	846.315	846.315	846.315	608.266	608.266	608.266
Note:						*p<0.05; **p<0.01; ***p<0.001

Table 16. Continued

	Dependent variable:					
	RMSSD			HF		
	(1)	(2)	(3)	(4)	(5)	(6)
Constant	37.431***	27.386***	31.099***	806.135***	438.789*	479.455*
	(28.789, 46.072)	(18.494, 36.278)	(21.933, 40.265)	(412.322, 1,199.947)	(33.559, 844.018)	(61.753, 897.156)
	t = 8.490	t = 6.036	t = 6.650	t = 4.012	t = 2.122	t = 2.250
	p = 0.000	p = 0.00000	p = 0.000	p = 0.0002	p = 0.038	p = 0.028
Group DN	−10.045		−3.713	−367.346		−40.666
	(−22.444, 2.354)		(−16.484, 9.057)	(−932.412, 197.720)		(−622.633, 541.301)
	t = −1.588		t = −0.570	t = −1.274		t = −0.137
	p = 0.118		p = 0.571	p = 0.208		p = 0.892
Group ND		10.045	6.332		367.346	326.680
		(−2.354, 22.444)	(−6.266, 18.929)		(−197.720, 932.412)	(−247.395, 900.755)
		t = 1.588	t = 0.985		t = 1.274	t = 1.115
		p = 0.118	p = 0.329		p = 0.208	p = 0.269
Group FN	−6.332	3.713		−326.680	40.666	
	(−18.929, 6.266)	(−9.057, 16.484)		(−900.755, 247.395)	(−541.301, 622.633)	
	t = −0.985	t = 0.570		t = −1.115	t = 0.137	
	p = 0.329	p = 0.571		p = 0.269	p = 0.892	
Group NF	2.613	12.658*	8.944	66.658	434.004	393.338
	(−9.608, 14.834)	(0.258, 25.057)	(−3.653, 21.542)	(−490.277, 623.593)	(−131.062, 999.070)	(−180.737, 967.414)
	t = 0.419	t = 2.001	t = 1.392	t = 0.235	t = 1.505	t = 1.343
	p = 0.677	p = 0.050	p = 0.169	p = 0.816	p = 0.138	p = 0.184
Observations	69	69	69	69	69	69
Log Likelihood	−288.299	−288.299	−288.299	−536.553	−536.553	−536.553
Akaike Inf. Crit.	588.598	588.598	588.598	1,085.106	1,085.106	1,085.106
Bayesian Inf. Crit.	601.644	601.644	601.644	1,098.152	1,098.152	1,098.152

Note: *p<0.05; **p<0.01; ***p<0.001

Table 16. Continued

	Dependent variable:					
	HF(%)			Total power		
	(1)	(2)	(3)	(4)	(5)	(6)
Constant	14.154***	13.574***	27.671***	3,063.269***	3,247.590***	3,214.700***
	(7.487, 20.822)	(7.106, 20.042)	(21.386, 33.957)	(1,937.986, 4,188.553)	(2,089.683, 4,405.498)	(2,021.157, 4,408.244)
	t = 4.161	t = 4.113	t = 8.628	t = 5.335	t = 5.497	t = 5.279
	p = 0.0001	p = 0.0002	p = 0.000	p = 0.00001	p = 0.00000	p = 0.00001
Group DN	−0.581		−14.098**	184.321		32.890
	(−9.870, 8.709)		(−23.117, −5.078)	(−1,430.303, 1,798.945)		(−1,630.028, 1,695.808)
	t = −0.123		t = −3.064	t = 0.224		t = 0.039
	p = 0.903		p = 0.004	p = 0.824		p = 0.970
Group ND		0.581	−13.517**	151.431	−32.890	
		(−8.709, 9.870)	(−22.680, −4.354)	(−1,488.937, 1,791.800)	(−1,695.808, 1,630.028)	
		t = 0.123	t = −2.891	t = 0.181	t = −0.039	
		p = 0.903	p = 0.006	p = 0.857	p = 0.970	
Group FN	13.517**	14.098**			−184.321	−151.431
	(4.354, 22.680)	(5.078, 23.117)			(−1,798.945, 1,430.303)	(−1,791.800, 1,488.937)
	t = 2.891	t = 3.064			t = −0.224	t = −0.181
	p = 0.006	p = 0.004			p = 0.824	p = 0.857
Group NF	9.003	9.584*	−4.514	421.705	237.384	270.274
	(−0.160, 18.166)	(0.564, 18.603)	(−13.404, 4.375)	(−1,169.686, 2,013.096)	(−1,377.241, 1,852.008)	(−1,370.095, 1,910.642)
	t = 1.926	t = 2.083	t = −0.995	t = 0.519	t = 0.288	t = 0.323
	p = 0.059	p = 0.042	p = 0.324	p = 0.606	p = 0.775	p = 0.748
Observations	69	69	69	69	69	69
Log Likelihood	−267.611	−267.611	−267.611	−604.798	−604.798	−604.798
Akaike Inf. Crit.	547.223	547.223	547.223	1,221.595	1,221.595	1,221.595
Bayesian Inf. Crit.	560.269	560.269	560.269	1,234.641	1,234.641	1,234.641

Note: *p<0.05; **p<0.01; ***p<0.001

Table 17. Linear mixed-effects model fit by REML: Duration~ Group*Odor. The ND group represents the reference group.

	Dependent variable:
	Duration
Constant	516.250***
	(475.650, 556.850)
	t = 24.922
	p = 0.000
Group ND	53.611
	(−2.188, 109.410)
	t = 1.883
	p = 0.060
Group FN	−19.338
	(−75.904, 37.228)
	t = −0.670
	p = 0.503
Group NF	62.917*
	(7.118, 118.715)
	t = 2.210
	p = 0.028
Odor: Disgust	56.719***
	(33.852, 79.586)
	t = 4.861
	p = 0.00001
Odor: Fear	−75.556***
	(−97.115, −53.996)
	t = −6.869
	p = 0.000
Group: ND*Odor: Disgust	−139.080***
	(−170.508, −107.652)
	t = −8.674
	p = 0.000
Group: FN*Odor: Fear	137.908***
	(106.974, 168.843)
	t = 8.738
	p = 0.000
Observations	138
Log Likelihood	−730.075
Akaike Inf. Crit.	1,480.151
Bayesian Inf. Crit.	1,509.423
Note:	*p<0.05; **p<0.01; ***p<0.001

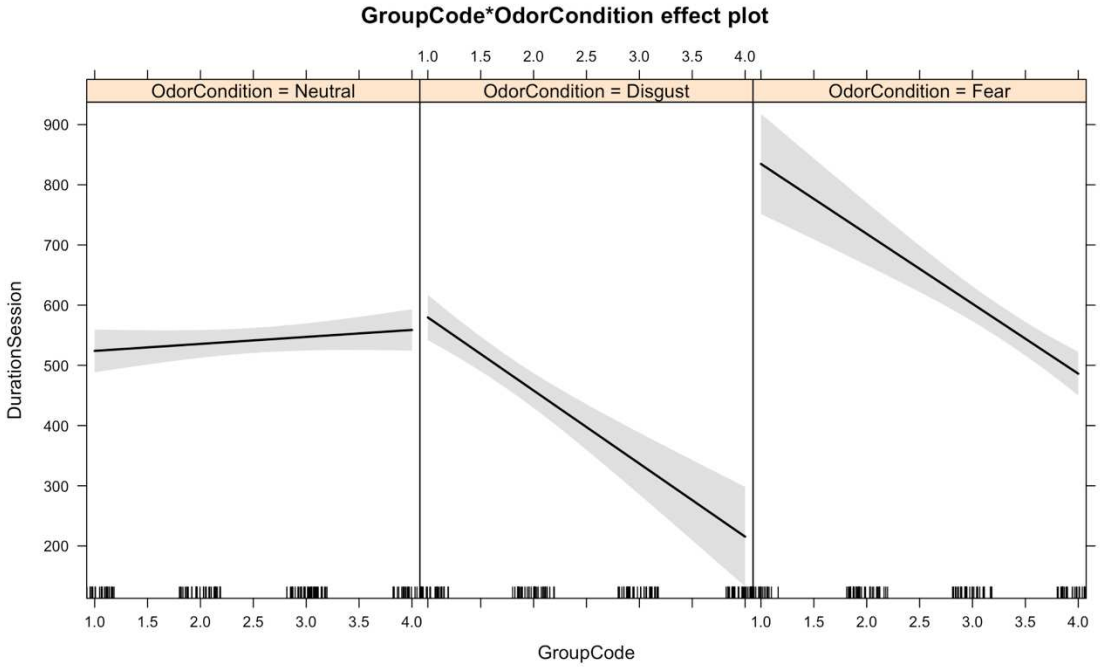

Figure 13. Graphical representation of Linear mixed-effects model fit by REML: Duration~ Group*Odor

Table 18. Linear mixed-effects model fit by REML: HRV ~ Group*Order of presentation of odor 1 and odor 2. The first three models in the first page of Table S4 - [(1), (2), (3)] - refer to mean RR interval values. Models (4), (5), (6) in such page are performed on Standard Deviation of Normal-to-Normal Intervals (SDNN). Models (1), (2), (3) in the second page of Table S4 are performed on Root-Mean Square Differences of Successive R-R intervals (RMSSD). Models (4), (5), (6) in such page are performed on the high frequency of HRV (HF). Finally, in the third page of Table S4, Models (1), (2), (3) are performed on the percentage of high frequency of HRV (HF%). Models (4), (5), (6) in such page are performed on the total power of the spectrum (Total power). From the top to the bottom line for each variable in the model, it is reported: beta value, confidence interval, t value, p value. P values are rounded automatically by the stargazer package and hereby reported for consistency in that way.

	Dependent variable:					
	mean RR			SDNN		
	(1)	(2)	(3)	(4)	(5)	(6)
Constant	751.793***	748.333***	784.495***	54.663***	50.299***	52.555***
	(696.371, 807.215)	(694.566, 802.100)	(732.243, 836.748)	(46.919, 62.408)	(42.330, 58.268)	(44.341, 60.770)
	t = 26.587	t = 27.279	t = 29.426	t = 13.834	t = 12.371	t = 12.540
	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.000	p = 0.000
Group DN	−3.460		−36.162	−4.364		−2.256
	(−80.677, 73.757)		(−111.137, 38.812)	(−15.477, 6.748)		(−13.701, 9.188)
	t = −0.088		t = −0.945	t = −0.770		t = −0.386
	p = 0.931		p = 0.348	p = 0.445		p = 0.701
Group ND		3.460	−32.702	−2.108	2.256	
		(−73.757, 80.677)	(−108.872, 43.468)	(−13.397, 9.181)	(−9.188, 13.701)	
		t = 0.088	t = −0.841	t = −0.366	t = 0.386	
		p = 0.931	p = 0.404	p = 0.716	p = 0.701	
Group FN	32.702	36.162			4.364	2.108
	(−43.468, 108.872)	(−38.812, 111.137)			(−6.748, 15.477)	(−9.181, 13.397)
	t = 0.841	t = 0.945			t = 0.770	t = 0.366
	p = 0.404	p = 0.348			p = 0.445	p = 0.716
Group NF	44.526	47.986	11.824	0.349	4.714	2.457
	(−31.644, 120.696)	(−26.988, 122.961)	(−62.072, 85.720)	(−10.603, 11.302)	(−6.399, 15.826)	(−8.832, 13.747)
	t = 1.146	t = 1.254	t = 0.314	t = 0.063	t = 0.831	t = 0.427
	p = 0.257	p = 0.215	p = 0.755	p = 0.951	p = 0.409	p = 0.672
Observations	138	138	138	138	138	138
Log Likelihood	−721.772	−721.772	−721.772	−568.910	−568.910	−568.910
Akaike Inf. Crit.	1,455.544	1,455.544	1,455.544	1,149.820	1,149.820	1,149.820
Bayesian Inf. Crit.	1,472.931	1,472.931	1,472.931	1,167.207	1,167.207	1,167.207

Note: *p<0.05; **p<0.01; ***p<0.001

Table 18. Continued

	Dependent variable:					
	RMSSD			HF		
	(1)	(2)	(3)	(4)	(5)	(6)
Constant	26.895***	26.663***	32.890***	675.125***	381.943**	346.454*
	(20.368, 33.422)	(20.331, 32.995)	(26.737, 39.043)	(430.995, 919.256)	(130.735, 633.151)	(87.515, 605.393)
	t = 8.077	t = 8.253	t = 10.476	t = 5.420	t = 2.980	t = 2.622
	p = 0.000	p = 0.000	p = 0.000	p = 0.00000	p = 0.004	p = 0.011
Group DN	−0.232		−6.227	−293.183		35.489
	(−9.325, 8.861)		(−15.056, 2.602)	(−643.475, 57.110)		(−325.281, 396.259)
	t = −0.050		t = −1.382	t = −1.640		t = 0.193
	p = 0.961		p = 0.172	p = 0.106		p = 0.848
Group ND		0.232	−5.995	−328.671	−35.489	
		(−8.861, 9.325)	(−14.965, 2.975)	(−684.549, 27.206)	(−396.259, 325.281)	
		t = 0.050	t = −1.310	t = −1.810	t = −0.193	
		p = 0.961	p = 0.195	p = 0.075	p = 0.848	
Group FN	5.995	6.227			293.183	328.671
	(−2.975, 14.965)	(−2.602, 15.056)			(−57.110, 643.475)	(−27.206, 684.549)
	t = 1.310	t = 1.382			t = 1.640	t = 1.810
	p = 0.195	p = 0.172			p = 0.106	p = 0.075
Group NF	5.462	5.694	−0.533	−53.879	239.303	274.792
	(−3.508, 14.432)	(−3.135, 14.523)	(−9.235, 8.169)	(−399.131, 291.373)	(−110.989, 589.596)	(−81.086, 630.670)
	t = 1.193	t = 1.264	t = −0.120	t = −0.306	t = 1.339	t = 1.513
	p = 0.238	p = 0.211	p = 0.905	p = 0.761	p = 0.186	p = 0.136
Observations	138	138	138	138	138	138
Log Likelihood	−549.552	−549.552	−549.552	−1,047.343	−1,047.343	−1,047.343
Akaike Inf. Crit.	1,111.103	1,111.103	1,111.103	2,106.686	2,106.686	2,106.686
Bayesian Inf. Crit.	1,128.490	1,128.490	1,128.490	2,124.073	2,124.073	2,124.073

Note: *p<0.05; **p<0.01; ***p<0.001

Table 18. Continued

	Dependent variable:					
	HF(%)			Total power		
	(1)	(2)	(3)	(4)	(5)	(6)
Constant	11.189***	13.522***	18.351***	3,291.268***	2,667.242***	3,001.134***
	(7.762, 14.616)	(10.197, 16.847)	(15.120, 21.582)	(2,358.938, 4,223.597)	(1,707.883, 3,626.601)	(2,012.250, 3,990.019)
	t = 6.399	t = 7.971	t = 11.131	t = 6.919	t = 5.449	t = 5.948
	p = 0.00000	p = 0.000	p = 0.000	p = 0.000	p = 0.00000	p = 0.00000
Group DN	2.333		−4.828*	−624.025		−333.892
	(−2.442, 7.108)		(−9.465, −0.192)	(−1,961.788, 713.737)		(−1,711.667, 1,043.883)
	t = 0.958		t = −2.041	t = −0.914		t = −0.475
	p = 0.342		p = 0.046	p = 0.364		p = 0.637
Group ND		−2.333	−7.162**	−290.133	333.892	
		(−7.108, 2.442)	(−11.872, −2.452)	(−1,649.226, 1,068.959)	(−1,043.883, 1,711.667)	
		t = −0.958	t = −2.980	t = −0.418	t = 0.475	
		p = 0.342	p = 0.005	p = 0.678	p = 0.637	
Group FN	7.162**	4.828*			624.025	290.133
	(2.452, 11.872)	(0.192, 9.465)			(−713.737, 1,961.788)	(−1,068.959, 1,649.226)
	t = 2.980	t = 2.041			t = 0.914	t = 0.418
	p = 0.005	p = 0.046			p = 0.364	p = 0.678
Group NF	4.735	2.401	−2.427	−8.601	615.424	281.532
	(0.024, 9.445)	(−2.235, 7.037)	(−6.997, 2.142)	(−1,327.115, 1,309.912)	(−722.338, 1,953.187)	(−1,077.560, 1,640.624)
	t = 1.970	t = 1.015	t = −1.041	t = −0.013	t = 0.902	t = 0.406
	p = 0.054	p = 0.314	p = 0.302	p = 0.990	p = 0.371	p = 0.687
Observations	138	138	138	138	138	138
Log Likelihood	−459.807	−459.807	−459.807	−1,212.959	−1,212.959	−1,212.959
Akaike Inf. Crit.	931.614	931.614	931.614	2,437.918	2,437.918	2,437.918
Bayesian Inf. Crit.	949.002	949.002	949.002	2,455.305	2,455.305	2,455.305

Note: *p<0.05; **p<0.01; ***p<0.001

Table 19. Bayes factor analysis of intensity ratings.

	Intensity Odor ~ ID
Group + Subject ID	0.2151536 ±0.48%
Odor Condition + Subject ID	27.77361 ±1.64%
Group + Odor Conditon + Subject ID	5.569247 ±0.97%
Group+ Odor Condition + Group:Oodor	5.120602 ±1.06%

Table 20. Bayes factor analysis of pleasantness ratings.

	Pleasantness Odor ~ ID
Group + Subject ID	1.040173 ±0.62%
Odor Condition + Subject ID	0.008628253 ±1.03%
Group + Odor Conditon + Subject ID	0.009134411 ±0.98%
Group+ Odor Condition + Group: Odor	0.0001485325 ±0.92%

Table 21: Excerpts of the movies used in the experiments I, II and in the donation study from study IV.

	Pink Flamingos	The Shining	Easter Island-Solar Eclipse
Length of film clip (hour: min: sec)			
	08:42-11:11	57:24-1:03:23	00:59-02:27
	17:03-18:54	1:20:32-1:21:19	06:05-20:09
	19:45-20:14	1:39:31-1:49:30	21:21-24:54
	25:34-26:04	2:00:40-2:05:36	27:46-20:18
	30:19-32:20	2:08:00-2:09:18	36:11-37:20
	40:30-47:00	2:08:20-2:09:49	38:00-42:20
	54:08-54:37	2:10:09-2:12:04	
	55:02-56:51	2:17:11-2:17:25	
	58:58-59:48		
	1:00:35-1:02:00		
	1:03:32-1:07:11		
	1:13:32-1:14:10		
	1:15:04-1:15:45		
	1:18:56-1:19:08		
	1:28:32-1:28:34		
	1:28:54-1:28:56		
	1:31:02-1:32:08		

Note: Since the speed of some excerpts were changed, please contact the author for such information- jacquelineferreira@ua.pt.