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Dyspnea and Anxiety:
An inquiry into their relationship

Proefschrift aangeboden tot het verkrijgen van de graad van Doctor in de Psychologie

door
Wan Li, MD

Promotor: Prof. Dr. O. Van den Bergh
Copromotor: Prof. Dr. K. P. Van de Woestijne
Prof. Dr. J. Gallego (INSERM)

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Wan Li, MD Dyspnea and Anxiety: An inquiry into their relationship?
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The term "dyspnea" is generally applied to describe the subjective experience of breathing discomfort that consists of qualitatively distinct sensations varying in intensity such as air hunger, effort to breathe and chest tightness. As a subjective sensation, dyspnea may be influenced by past experience, perceptual-cognitive processes, emotional state, and various personal characteristics that interact with the underlying physiological mechanisms. Dyspnea, particularly its air hunger component, is not only experienced in congestive heart failure or states of intense exercise, it is also a typical symptom during anxiety and panic attacks in subjects with normal pulmonary status. This doctoral projects aims to investigate how psychophysiological mechanisms interact to produce air hunger is association with anxiety. Understanding these mechanisms may provide further insights into the etiology of behavioral dyspnea related to panic and stress disorders and may improve clinical diagnosis and management of a wide variety of somatic (pulmonary, cardiac and terminal cancer) patients with subjective dyspnea in excess of their physical status.

In Part I of this dissertation, the definition and mechanisms of dyspnea and air hunger are described. As for the mechanisms of air hunger, several hypotheses with respect to physiological and psychological factors involved in etiology of air hunger associated with anxiety are elaborated in details. A new paradigm combining an online subjective rating and a rebreathing test is utilized in the present project. The relevant parameters and measurements are illustrated as well.

Part II consists of two studies investigating the perception of air hunger and ventilatory behavior during repeated exposure to hypercapnia. Both studies employ a rebreathing paradigm to induce dyspnea, particularly its air hunger component. The participant in this test rebreathes into a bag filled with a hyperoxic mixture to avoid hypoxia, while gradually augmenting the inspired CO_2-level, and, as a result, increasing levels of air hunger and ventilation up to a level that is felt like suffocating. In these studies habituation versus sensitization of both the ventilatory and the subjective air hunger response to repeated hypercapnic stimulation is tested in persons varying in type and intensity of anxiety: high and

low trait anxiety in normals. This will allow disentangling (1) sensitization and/or habituation effects in both the subjective and the ventilatory responses to hypercapnia, (2) how both subjective and ventilatory measures relate to one another, and (3) how trait anxiety affects air hunger and ventilatory response. The results may be relevant to improve both the comfort of somatic patients with dyspnea and the treatment of anxiety patients.

Since this test has never been used in combination with detailed measures of the subjective component, the three studies in Part III aim to investigate the role of specific procedural details. Specifically, we tested the importance of (1) inserting a hyperventilation phase prior to hypercapnic stimulation in order to lower the body stores of carbon dioxide below the central chemoreflex threshold. This is typically done to allow a more accurate detection of the threshold for the ventilatory response when PCO2 subsequently increases, but at the same time it can prime (and confound) air hunger perception; (2) a continuous rating of air hunger versus a rating in discrete steps. (3) At the data-analysis level, we compared different curve fitting procedures on the function relating time and/or $PetCO_2$ to both air hunger ratings and several ventilatory parameters.

In Part IV, the results of all studies are summarized and demonstrate the effects of trait anxiety and repetition on the perception of air hunger in terms of the dynamics of air hunger and its association with ventilatory behavior. The influence of the different methods applied in the paradigm is discussed as well.

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List of Abbreviations

AH	Air hunger
ATS	American Thoracic Society
BOLD	Blood oxygen level dependent
CHF	Congestive heart failure
CO_2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
$ETCO_2$	The partial pressure or maximal concentration of carbon dioxide at the end of an exhaled breath
$FetCO_2$	The partial pressure or maximal concentration of carbon dioxide at the end of an exhaled breath
fMRI	Functional magnetic resonance imaging
HV	Hyperventilation
ITI	Intertrial interval
MRT	The modified Reads rebreathing test
NA	Negative affectivity
$PACO_2$	The partial alveolar pressure of carbon dioxide
$PaCO_2$	The partial pressure of carbon dioxide of arterial blood
PCO_2	The partial pressure of carbon dioxide
$PetCO_2$	The fractional end-tidal CO2-concentration expressed in partial pressure

PET	Positron emission tomography
PLR	Piecewise linear regression in STATISTICA 7.0
PaO_2	The partial pressure of oxygen of arterial blood
pH	The concentration of hydrogen
RR	Respiratory rate or frequency
RT	The original Reads rebreathing test
SA	State anxiety
SD	Standard deviation
Segcurve	A software program estimating segmented linear regression and breakpoints
Sigmaplot	Segmented linear regression in Sigmaplot 9.0
STAI-T	The trait version of the State-Trait-Anxiety-Inventory
Te	Expiratory time
Ti	Inspiratory time
Ttot	A total cycle time
V_E	Minute ventilation
Ve	Expiratory volume
Vi	Inspiratory volume
VH	Voluntary hyperventilation
VQ ration	Ventilation/perfusion relationship
V_T	Tidal volume

Part I

General Introduction

What is dyspnea? What is air hunger? How are these respiratory sensations produced? Why does air hunger appear without pulmonary or other dysfunction when it's related to anxiety and panic? In this section the background information will help us to understand the definitions and mechanisms of dyspnea and air hunger. The relevant measurements and major aims of studies will be elaborated subsequently.

Chapter 1

Dyspnea

Respiratory sensations can be divided into two broad (and not entirely separable) categories: respiratory proprioception and respiratory discomfort (Shea et al., 1996). The former is the sense of the mechanical motion, displacements, position, and forces, which arise from somatic receptors or receptors in the lungs and other viscera. The latter is often referred to with the term "dyspnea", which is thought to be a generic term for qualitatively different kinds of unpleasant respiratory sensations. The definition and mechanism of dyspnea will be described in this chapter. To understand the mechanism of dyspnea better, information concerning the respiratory control system will be represented. Afterwards, the physiological mechanisms and psychological effects will be elaborated based on the qualities of dyspnea.

1.1 Definition of Dyspnea

Dyspnea is a medical term used to describe a variety of symptoms related to unpleasant or uncomfortable respiratory sensations. Respiratory sensations are neural activations caused by stimulation of peripheral receptors. Dyspnea, however, refers to the perception of discomfortable respiratory sensations, which is the subjective reaction to the respiratory sensations (Guz, 1997). The American Thoracic Society (ATS, 1999) defined dyspnea in a broad way as "a subjective experience of breathing discomfort that consists of qualitatively distinct sensations that vary in intensity". This definition gives us better understanding of the involvement of both

psychological effects and physiological mechanisms in the mechanism of dyspnea.

Based on descriptions of patients with cardiopulmonary diseases, multiple types of dyspnea have been addressed in a series of studies (See Table 1.1, Simon et al., 1990; Elliott et al., 1991; Manning & Schwartzstein, 1995). Recently, "air hunger", "work effort", and "chest tightness" are frequently applied to describe the sensations subsumed under the term dyspnea (ATS, 1999; Evans et al., 2002; Scano et al., 2005; Ambrosino and Serradori, 2006).

Table 1.1: Respiratory Sensations Associated with Various Conditions (Manning & Schwartzstein, 1995)

Sensation	COPD	Congestive Heart Failure	Interstitial Lung Disease	Asthma	Neuromuscular & Chest Wall Disease	Pregnancy	Pulmonary Vascular Diease
Rapid breathing		×					×
Incomplete exhalation				×			
Shallow breathing				×	×		
Increased work/effort	×		×	×	×		
Feeling of suffocation	×	×					
Air hunger	×	×				×	
Chest tightness				×			
Heavy breathing				×			

1.2 Mechanisms of Dyspnea

The sensation of dyspnea is provoked by the activation of sensory systems related to respiratory control. The sensory information may be relayed to higher brain centers such as the sensory cortex so that the final expression of dyspnea may be shaped by effects of context, cognition, and behavior (Guz, 1997; ATS 1999).

1.2.1 Respiratory Control System

The sensations of respiration are very important in the control of breathing, which functions to regulate ventilation to match metabolic requirements of the body. When the respiratory controller attempts to regulate blood gas tensions via an appropriate change in ventilation, this demand to breathe may lead to the sensation of respiratory discomfort. Fig1.1 illustrates the homeostatic systems involved in the regulation of respiration, which may provide a framework for understanding the mechanisms of dyspnea.

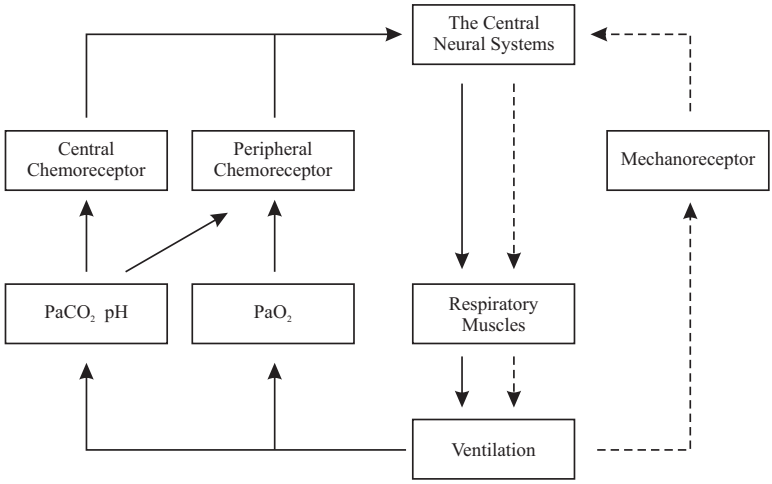


Figure 1.1: Schematic view of the metabolic control of breathing

The left cycle of Fig1.1 displays that the central and peripheral chemoreceptors send the information of the partial pressure of carbon dioxide of arterial blood ($PaCO_2$), the concentration of hydrogen (pH), the partial pressure of oxygen of arterial blood (PaO_2) to the central neural systems. The respiratory muscles perform the motor commands from the central neural systems to achieve ventilation. In terms of system control, this component of breathing is a feedback controller, using afferent information from chemoreceptors (the "error signal") to increase or decrease the ventilatory motor output until $PaCO_2$ and PaO_2 have returned to their respective set-point levels (Gallego et al, 2001).

The right cycle of Fig1.1 illustrates another feedback system. The af-

ferent discharge from bronchial or pulmonary mechanoreceptor modulates the central command of respiratory muscles (Gallego et al, 2001).

1.2.2 Qualities of Dyspnea and Physiological Mechanisms

Dyspnea includes several qualitatively distinct sensations that probably arise from different pathophysiological mechanisms as Table 1.2 shows (Manning and Schwartzstein, 1995). It is well known that the respiratory control system receives the afferent information from chest wall receptors, pulmonary vagal receptors, chemoreceptors and then sends efferent respiratory discharges to activate the ventilatory muscles to satisfy the metabolic requirements of the body. The afferent feedback from peripheral sensory receptors may allow the brain to assess the effectiveness of the motor commands. Any derangement of the respiratory network will result in a dissociation between the motor command and the mechanical response of the respiratory system.

Table 1.2: Qualities of Dyspnea and Physiologic Mechanisms

Qualities of Dyspnea	Physiologic Mechanisms	Cardiopulmonary Disorder
The sensation of air hunger/suffocating	The stimulation of chemoreceptors	Patients with congestive heart failure
The sense of increased effort or work of breathing	Central respiratory motor command	Patients with abnormal mechanical loads (e.g., COPD, interstitial lung disease) and neuromuscular weakness
Chest tightness	Induction of bronchoconstriction	Asthma

1.2.3 Qualities of Dyspnea and Psychological Effects

Because dyspnea depends for its identification on subjective judgments, patient experience, expectation, behavioral pattern, and emotional state may all be involved in shaping the quality and intensity of dyspnea at a given level of respiratory activity as Table 1.3 shows (Dudley et al., 1968; Guenard H et al., 1995). Meanwhile, tolerance thresholds and intensity ratings of breathing discomfort are also influenced by the same personal characteristics that are known to influence the response to pain: cultural differences, past experience, level of anxiety, perceived duration of the discomfort, perceived consequences of giving high or low ratings, and the

ability to personally control the duration and intensity of the stimulus (Shea et al., 1996).

Table 1.3: Qualities of Dyspnea and Psychological effects (Dudley et al., 1968)

Qualities of Dyspnea	Emotional State
Dyspnea in association with decreased ventilation and hypoventilation	Depression
Dyspnea in association with hyperpnea or hyperventilation (e.g., air hunger)	Anger and Anxiety

The physiological mechanisms can not fully account for the sensation of dyspnea experienced by subjects with minimal or no structural change in their cardiopulmonary system, nor for the lack of dyspnea experienced by some subjects with severe structural change. The subsequent chapters will explore the mechanism underlying one type of dyspnea, air hunger, associated with anxiety.

Chapter 2

Air Hunger

Air hunger is a common symptom of cardiopulmonary diseases or intense exercise. It is proposed to be caused by the disarrangement between central respiratory motor activity and incoming afferent information from chemoreceptors (Manning and Schwartzstein, 1995). However, air hunger is often experienced also during intense emotional states or by patients with anxiety, panic or psychosomatic disorders. In the latter cases, air hunger is typically not related to any impairment in cardio-respiratory functioning. Fig 2.1 illustrates that multiple effects, besides the physiological mechanisms, may be involved in shaping air hunger. Understanding how each stage is affected by multiple factors may improve the effectivity of treatment of air hunger particularly in patients with anxiety, panic disorder, and other psychosomatic disorders. In this chapter the definition of air hunger and relevant hypotheses dealing with both physiological and psychological mechanisms will be elaborated in more detail.

2.1 Definition of Air Hunger

As a discomfortable respiratory sensation produced by hypercapnia, air hunger is similar to the perception at the end of a long breath hold (Banzett et al., 1990; Banzett et al., 1996). It suggests that air hunger may be considered part of the warning system for humans to recognize when they are at risk of receiving inadequate ventilation such as in states of intense exercise or congestive heart failure (CHF).

As a subjective experience, air hunger may also be greatly affected

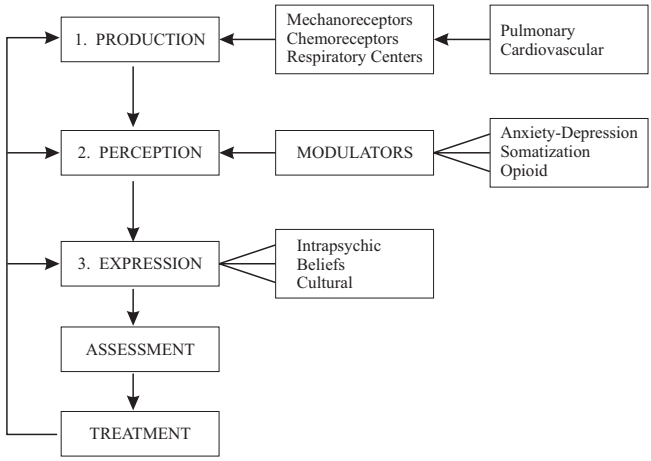


Figure 2.1: Different stages in the production of air hunger (Adapted from Ripamonti et al., 1997)

by experience, expectation, attention, behavioral pattern, and emotional state - especially anxiety (ATS, 1999). For example, air hunger is a typical symptom in panic disorder without any cardiopulmonary disease; asthmatics, on the other hand, may present a blunted perception of dyspnea leading to a greater risk of fatal attacks (Kikuchi et al., 1994); anxiety and/or depressive symptoms in patients with chronic lung diseases can increase symptoms of breathlessness out of proportion to the impairment in cardio-respiratory function (ATS, 1999). It is difficult to explain air hunger associated with anxiety only by a physiological mechanism. In the following sections, background information of physiological mechanisms and psychological effects will be described to explain the genesis of air hunger associated with anxiety.

2.2 Physiologically-oriented Explanations

2.2.1 How is air hunger produced?

Air hunger typically occurs during CO_2-inhalation or during intense exercise. The sensation is produced by the projection of the afferent information from central and peripheral chemoreceptors, which monitor the

pH, PCO_2, and PO_2 of body fluid, to the sensory cortex (Shea et al., 1995). Meanwhile, the respiratory center is stimulated and increases the ventilatory drive, which also relays to the sensory cortex to provoke the sensation of air hunger (Manning et al., 1992; Moosavi et al., 2004). Findings in recent human brain-imaging work with positron emission tomography (PET) suggested that the limbic system, a main nervous system involved in emotions, is also involved in the unpleasant sensation of air hunger (Guz, 1997). Corfield et al. (1995) identified areas of neuronal activation including the upper brainstem, the midbrain, hypothalamus and thalamus in healthy humans reporting dyspnea as a result of hypercapnia ($FetCO_2$ = 50 mmHg). Another PET study revealed that air hunger, which was evoked by restraining ventilation below spontaneous levels in healthy humans, activated the insular cortex (Banzett et al., 2000). An important observation from a fMRI study on healthy subjects performing a CO_2 challenge, is that air hunger was associated with increased activity in the limbic, paralimbic and cerebella, and especially the insula (Evans et al., 2002).

2.2.2 Hypotheses dealing with the production of air hunger associated with anxiety – hypersensitivity of chemoreflex

Several studies found that hypercapnic chemosensitivity can be influenced by a number of factors. Munakata et al. (1993) found an augmentation of hypercapnic chemosensitivity during the luteal phase in female asthmatic patients and Garcia-Rio et al. (1996) found a higher sensitivity to CO_2 in some pregnant women. Osanai et al. (1999) concluded that abnormalities in the dopaminergic mechanisms might result in the decline of peripheral chemosensitivity in patients with obstructive sleep apnoea syndrome, suggesting a relationship between chemosensitivity of CO_2 and the internal humoral system.

To explain the respiratory abnormalities during panic attacks, hypersensitivity of the brainstem areas has been assumed (e.g., the noradrenergic locus coeruleus, Gorman et al., 1989). Papp et al. (1993) suggested that respiratory abnormalities in panic patients might adaptively aim at coping with a hypersensitive CO_2 chemoreceptor and that antipanic medications may reset the receptor threshold. Klein (1993) hypothesized a "suffocation false alarm" mechanism: CO_2 hypersensitivity would be due to a suffocation monitor misinterpreting normal physiologic states and

triggering a suffocation alarm.

Hypersensitivity of chemoreflex to CO_2 may play an important role in the genesis of air hunger associated with anxiety, particularly as a symptom of panic disorder. Higher chemosensitivity and lower threshold for response to CO_2 stimulus was reported in several studies (Carr et al., 1987; Lousberg et al., 1988; Battaglia, 2002). In addition, trait anxiety is associated with elevated (and unfounded) symptom reporting and has been found to be an important vulnerability factor for panic disorder (Hayward et al., 2000). However, there are few studies to investigate the relationship between trait anxiety and chemosensitivity.

2.3 Psychologically-oriented Explanations

Because air hunger depends for its identification on subjective judgments of the patients and is not definable in terms of blood gas or ventilatory abnormalities, some experience it without any organic causes, while others experience no air hunger or mild air hunger in present of severe physiological change. Mood or other psychological states, especially anxiety, may significantly affect the experience of dyspnea. It has been documented that with strong action-oriented emotions such as anger and anxiety the experienced dyspnea is associated with changes in respiratory drive (Dudley et al., 1968).

2.3.1 Modulation at cortical level

The cerebral cortex is responsible for interpreting physiological distress, for instance air hunger, as a dangerous, life-threatening event (Craig, 2003). Because of the multiple connections between cerebral cortex with both the limbic lobe and the brain stem, cognitive processes such as misattribution and catastrophic interpretation of somatic symptoms or the sense of loss of control may in turn trigger full-blown panic attacks by stimulating autonomic control centers including those involved in respiration (Papp et al., 1993). In a 2000 article, Gorman et al. reviewed recent literature on the phenomenology, neurobiology, and treatment of panic disorder and hypothesized a hypersensitive fear network, which is situated at the level of the amygdala through its projections to the hypothalamus and the brainstem. They also assumed that effective psychosocial treatments may reduce contextual fear and cognitive misattributions generated at the level of the prefrontal cortex and hippocampus.

2.3.2 Modulation of the perception of air hunger

As Fig2.1 shows, the evaluation of air hunger is based on how the subject expresses the air hunger that he perceives and the influence of a number of factors such as perceptual-cognitive, behavioral, attentional and emotional processes and large individual differences occur (Ripamonti and Bruera, 1997; ATS, 1999; De Peuter et al., 2004). In a study using a path analysis model to evaluate the interrelationships among dyspnea, disease severity, mastery, depression, functional status, and quality of life, dyspnea was more related to the psychological variable of mastery than to disease severity (Moody et al., 1990). In panic disorder, dyspnea is a prominent symptom that may be related to increased work of breathing and hyperventilation (Roth et al., 1992), but interoceptive hypervigilance and negative interpretation biases are also involved (Clark et al., 1997).

Several studies (Howell, 1990; Han et al., 1998; Han et al., 2004) observed dyspnea without identifiable pathology in psychiatric disorders and in persons with a high level of anxiety or negative affectivity (NA), a "diffuse non-specific personality measure of subjective distress and dissatisfaction" (Watson and Pennebaker, 1989). These studies strongly suggest that anxiety or NA might influence the perception of discomfortable respiratory sensations. Breath holding, excessive crying, congestive heart failure, and allergic and infectious events, etc., may lead to the association of physiologic changes with threatening experiences in which air hunger is produced. People with high levels of NA are hypersensitive to bodily sensations and consequently report more somatic symptoms whereas other people are unaware of those sensations or they do not experience them as unpleasant symptoms (Watson and Pennebaker, 1989). However, there is no direct evidence for high trait-anxiety people being hypersensitive to perceive air hunger.

2.4 Association between Psychological influences and Physiological Mechanisms

Several hypotheses concerning psychological influences and physiological mechanisms of air hunger associated with anxiety were described in the previous sections. However, the association between them may also play an important role in the etiology of air hunger. Besides a biologically based hypersensitivity of the CO_2 chemoreceptor system, high trait anxiety or anxiety sensitivity may also result in exaggerating the significance

of physiological changes, which may produce some of the anxiogenic reaction to CO_2, air hunger, or hyperventilation. These reactions may be sensitized or habituated by repeated exposure, emotional state, or attention. In the following section the background information and relevant models will be elaborated in detail.

2.4.1 The association of air hunger and respiratory behavior

The interrelationship among CO_{2max} stimulus, consequent air hunger and ventilatory response plays an important role in the mechanism of air hunger. The traditional, physiologically-oriented account for air hunger implies three important associations (Banzett et al., 1989; Banzett et al., 1990). First, afferent information from the chemoreceptors during CO_2 inhalation projects to the sensory cortex, triggering the perception of air hunger. Secondly, at the same time, this afferent information triggers ventilatory adaptive changes throughout projections to the brainstem respiratory centers (see Fig 2.2). Third, ventilatory behavior alleviates the air hunger sensation via the modification of the concentration of CO_2 in blood and maybe also via voluntary changes in breathing pattern controlled by the higher brain centers.

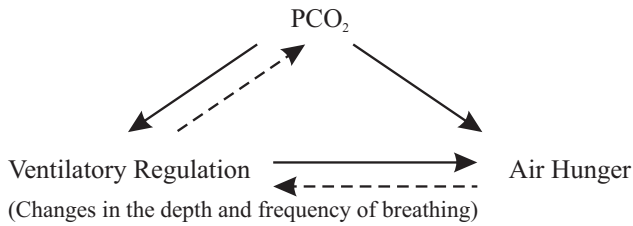


Figure 2.2: Schematic representation of the association among CO_2 stimulus, ventilatory behavior and air hunger

2.4.2 The association among anxiety, air hunger and respiratory behavior

The effect of anxiety on air hunger and respiratory behavior has been addressed in a number of studies. Anxiety and fear generated in the limbic

system cause physiological changes in heart rate, perspiration, and respiration (Davis, 1992). Anxiety (trait and state) increases respiratory rate rather than tidal volume and decreases expiratory time with no change in metabolism (Boiten et al., 1994; Masaoka and Homma, 1999; Van Diest et al, 2001a; 2001b; 2005; Gallego et al., 2001). During period of increased state anxiety, an excessive increase in respiratory rate in subjects with idiopathic hyperventilation may due to the interaction of trait anxiety and perception of breathlessness (Masaoka et al., 2004). Another study observed a faster but shallower breathing pattern and greater levels of suffocation sensations during rebreathing challenge in panic disorder patients who experienced a panic attack comparing to other panic disorder patients (Rassovsky et al., 2006). Three models have been proposed to explain the interrelationship between air hunger, panic and hyperventilation (Fig 2.3, Smoller et al., 1996).

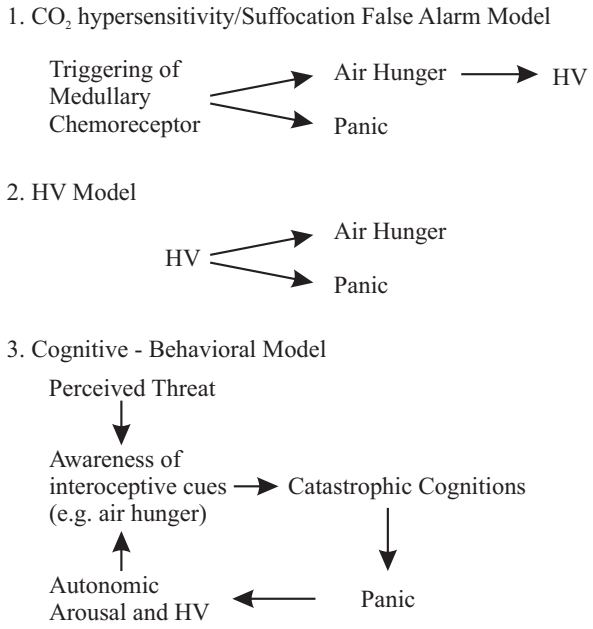


Figure 2.3: Schematic representation of three models of the association between air hunger, panic and hyperventilation (HV) (adapted from Smoller et al., 1996)

The first model proposes that a sense of air hunger and anxiety/panic may be caused by hypersensitivity in chemoreceptors, which may lead to an excessive response of the respiratory controller as well (Klein, 1993; Papp et al., 1993). Based on the "CO_2 hypersensitivity" theory of panic, Papp et al. (1995) assumed that the subjective sense of dyspnea triggered a series of typical autonomic symptoms at the time that hypersensitive respiratory control nuclei were stimulated during CO_2 inhalation. However, the results of studies on the chemosensitivity to CO_2 in panic disorder patients are mixed. By use of Read's rebreathing test Carr et al. (1987) and Lousberg et al. (1988) reported higher CO_2 sensitivity in panic patients than controls, whereas Pain et al. (1988) found only an increased respiratory rate, but no tidal volume response. Furthermore, Woods et al. (1986) and Papp et al. (1995) found no difference in CO_2 sensitivity in panic patients compared to controls. Katzman et al. (2002) did not find significant differences in sensitivities or thresholds between panic disorder patients and healthy controls using the modified rebreathing test.

The hyperventilation model postulates that breathing discomfort and anxiety may be a result of increased ventilation (Ley, 1985). Patients with anxiety or panic disorder frequently experience air hunger and hyperventilation. This model implies that hyperventilation may be the cause of the somatic symptom of panic because the respiratory sensations associated with it are interpreted as catastrophic events (Garssen et al., 1996). Hyperventilation is the state of breathing faster or deeper than necessary, and thereby reducing the carbon dioxide concentration of the blood below normal (Nardi et al., 2001). It is breathing in excess of what the body needs, which differs from the normal regulation of ventilatory response because the latter may be necessary for a sufficient gas-exchange of the body, for example after exercise. Voluntary hyperventilation (VH) has been applied to investigate breath pattern and respiratory discomfort associated with panic and anxiety (Meuret et al., 2005).

However, another cognitive-behavioral model assumes panic is a direct response to the misinterpretation of the perception of air hunger, which is contradictive to the theory of hyperventilation (Clark, 1986; 1988; 1997). It suggests that individuals misinterpret physical sensations as dangerous, threatening signals. Accordingly a vicious cycle of panic – more symptoms- panic is induced. Irregular breathing behavior such as hyperventilation is the consequence instead of the cause in this cycle, which is different from the hyperventilation model. Two studies on panic attacks during sleep found that sufferers were mostly awaked by sensations (dyspnea and tachycardia) and then experienced fear (Hauri et al., 1985; Craske

et al., 1986). Another classical conditioning analysis of panic disorder provided further evidence that the misattribution of sensations produced by hyperventilation occurred after the experience of fear during panic attack (Wolpe & Rowan, 1988).

2.4.3 The modification of respiratory sensation and behavior with the effect of anxiety

Yet an additional, more dynamical approach to the association between air hunger and anxiety may involve the contribution of learning effects in the perception of air hunger and in respiratory behavior. Anxiety may importantly shape both the initial responses to a CO_2-challenge and the responses to subsequent challenges. Exploring how the air hunger and breathing responses evolve over time across repeated challenges and how it is affected by anxiety may shed light on the etiology of the air hunger associated with anxiety.

2.4.3.1 Concepts of learning

Learning is aprocess of adjusting, adapting, or improving the individual behavior to a situation (Skinner 1950). In the following paragraphs two important concepts reflecting the shaping of a response througout repeated experiences will be introduced.

Classical Conditioning (Pavlov 1927) Classical conditioning occurs when a natural reflex responds to a stimulus. The most popular example is Pavlov's observation that dogs salivate in response to cues (e.g., a bell) announcing food. A typical classical conditioning procedure involves the pairing of an unconditioned stimulus, which elicits responding on its own, with a neutral stimulus that does not evoke the response from its own. "Unconditioned" means that the stimulus and the response are naturally connected. "Conditioning" means that we are trying to associate, connect, or bond something new with the old relationship, which is just opposite to the concept of "Unconditioned". Therefore, as the result of a classical conditioning procedure in which the presentation of a neutral stimulus is repeatedly paired with the subsequent presentation of an unconditioned stimulus, this initial neutral stimulus starts to elicit responding and thus becomes a conditioned stimulus.

Habituation and Sensitization Stimulus repetition typically results in habituation or sensitization in many response systems, both physiological and psychological (Domjan, 1993; Bradley et al., 1996; Figueiredo et al., 2003). Habituation is the decrease in response strength with repeated exposure to a particular eliciting stimulus. Sensitization is the increase in response strength with repeated exposure to a particular stimulus. The dual-process theory of Groves and Thompson (1970) assumes that habituation occurs in the "Stimulus (S)-Response (R) system" and sensitization occurs in the "State system", which is assumed to respond only to strong, arousing, or emotional stimulus. Therefore, sensitization is more likely to occur when a person is in an emotional state. Other authors clarified that sensitization is likely to overrule habituation when the stimulus is aversive, especially when it is unpredictable in time and space (Catania, 1998), and in persons with high levels of neuroticism or trait anxiety (Eysenck, 1979).

2.4.3.2 Learning and feedforward process

Somjen et al. (1992) hypothesized that the cardiorespiratory response is guided by past experience, which means that autonomic responses are learned at an early age. He defined such a learned response as feedforward control. He hypothesized that the ventilation could be driven without chemoreceptor feedback signals because the brain has learned to anticipate these errors in blood gases over a period of many years. Some studies regarding the increase in ventilation at the onset of exercise suggested that the ventilatory response was elicited by feedforward central processes rather than by chemosensitivity feedback (Gallego et al., 2001) although this was not confirmed by other studies (Moosavi et al., 2002). A number of studies addressed that the process of feedforward may play an important role in the mechanisms of air hunger associated with anxiety. Some condition studies found ventilatory behavior can be modified by the psychological factors of emotion (anxiety) with cognition (the anticipation of a threatening event) (Stegen et al., 1995; Van den Bergh, et al., 1995; van Diest, 2001a). Other studies (Van den Bergh et al., 1997; Van den Bergh et al., 2001; De Peuter et al., 2005) have shown that a few repeated experiences of dyspnea, induced by CO_2-inhalation in association with an odor may result in reporting increased dyspnea to the odor alone, independent of the actual breathing behavior. The 3rd model of Fig 2.3 assumes that the experience of fear during panic attack is caused by a contextual environment, which increases the expectancy of

strained breathing with uncontrollable dyspnea (Mongeluzi et al., 2003). Subsequently, the elicited fear results in changes in ventilatory behavior. A clinical study also found that exercise training, by repeatedly exposing the patient to breathing discomfort under safe, monitored conditions, may also "desensitize" the patient to the fear associated with the sensations of dyspnea (Carrieri-Kohlman et al., 1996).

Chapter 3

Methodology – Rebreathing Test and Online Subjective Rating

In this project a new paradigm combining a rebreathing test and a subjective air hunger rating was designed to investigate the physiological and psychological mechanisms of air hunger. The rebreathing test was performed to provide a progressive increasing stimulus of CO_2 to induce air hunger and ventilatory responses in participants. Air hunger was rated by participants simultaneously during the whole rebreathing test. Physiological and psychological parameters were recorded online to register the dynamic changes in air hunger and ventilation. Four response characteristics (threshold, maximum, slope, and Beta coefficient) were measured for each of the test responses (air hunger, tidal volume and respiratory rate).

3.1 Respiratory Parameters and Measurements

3.1.1 $ETCO_2$

As Fig 3.1 shows, $ETCO_2$ is the partial pressure or maximal concentration of carbon dioxide (CO_2) at the end of an exhaled breath, which is expressed as CO_2 in percentage or mmHg (LaValle & Perry, 1995; Santos

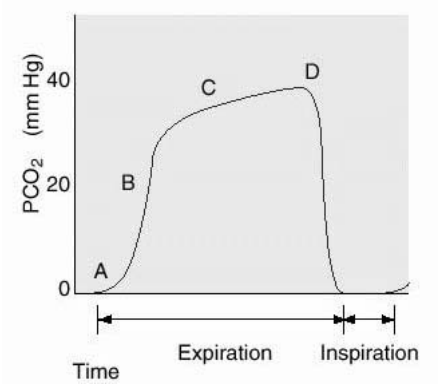


Figure 3.1: Normal capnogram, (A) Carbon dioxide cleared from the anatomic dead space; (B) dead space and alveolar carbon dioxide; (C) alveolar plateau; (D) end-tidal carbon dioxide tension ($ETCO_2$). (`http://medical-dictionary.thefreedictionary.com/_/viewer.aspx?path=dorland&name=capnogram.jpg`)

et al., 1993; Trillo et al., 1994). The normal values are 5% to 6% CO_2, which is equivalent to 35-45 mmHg (Trillo et al., 1994).

CO_2 reflects cellular metabolism (Sanders, 1989; Idris et al., 1994). There are four main stages of normal physiology of CO_2: production, transport, buffering and elimination. The partial pressure of produced CO_2 of venous blood entering pulmonary capillaries is normally 45 mmHg while the partial alveolar pressure of CO_2 ($PACO_2$) is normally 40 mmHg (LaValle & Perry, 1995; Trillo et al., 1994). The pressure difference of 5 mmHg results in the diffusion of all the produced CO_2 out of pulmonary capillaries into the alveoli (LaValle & Perry, 1995). The second stage is CO_2 transport from the lungs to the rest of the body, which is a way of maintaining the CO_2 tension of arterial blood at approximately 35-45 mmHg despite high CO_2 production (Trillo et al., 1994). The third stage is where the buffer action of hemoglobin blood flow maintaining the normal level of CO_2 tension by eliminating the excess CO_2 (Trillo et al., 1994). The fourth stage involves CO_2 elimination by alveolar ventilation under the control of the respiratory center (LaValle & Perry, 1995; Trillo et al., 1994). This process allows the diffusion of CO_2 from blood to the alveoli where the partial alveolar pressure of CO_2 is lower than the tissue

pressure (Trillo et al., 1994).

When CO_2 diffuses out of the lungs into the exhaled air, a device called capnometer measures the partial pressure or maximal concentration of CO_2 at the end of exhalation (Sanders, 1989; Santos et al., 1993). $ETCO_2$ monitoring is a noninvasive method to evaluate adequacy of ventilation and pulmonary blood flow. During normal circulatory conditions with equal ventilation/perfusion (VQ ratio) relationships, $PACO_2$ is closely comparable to $PaCO_2$ and $ETCO_2$ (Sanders, 1989; Santos et al., 1993). The normal $ETCO_2$ is about 38 mmHg at 760 mmHg of atmospheric pressure with less then 6 mmHg gradient between $PaCO_2$ and $ETCO_2$ (Sanders, 1989; Santos et al., 1993). $ETCO_2$ can be used as a quantitative index of evaluating adequacy of ventilation and pulmonary blood flow (Falk et al., 1988).

3.1.2 Time and volume parameters

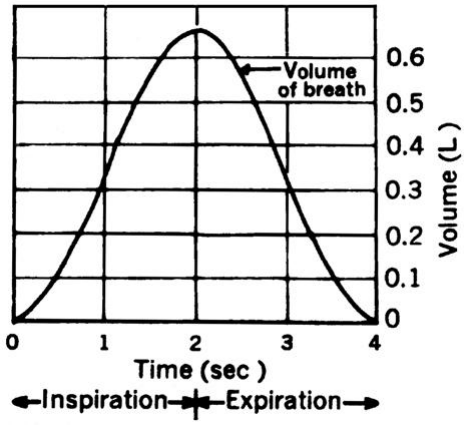


Figure 3.2: The Ventilatory Cycle, showing Tidal Volume. (`http://www.coheadquarters.com/PennLibr/MyPhysiology/lect1p/lect1.00.htm`)

Ventilation is the movement of air in and out of the lungs, which is composed of inspiration and expiration. One inspiration followed by one expiration is called a respiratory cycle (see Fig 3.2). The respiratory

cycle can be described by its frequency and amplitude, or, by its time and volume components, respectively. A total cycle time (Ttot, sec) includes inspiratory time (Ti, sec) and expiratory time (Te, sec). The amount of air that enters or leaves the lungs during one respiratory cycle is inspiratory volume (Vi, L) or expiratory volume (Ve, L) respectively. Vi will be used as measurement of tidal volume (V_T, L) in this project, which is the volume that is displaced during each breathing cycle. Moreover, two other parameters will be calculated from Ti, Te and V_T: (1) respiratory rate (RR) or frequency is the number of respiratory cycles per minute: RR = (Ti +Te)/60; (2) minute volume (V_E, L/min) is the product of RR and mean V_T over one minute, which reflects the total amount of air that is being displaced during one minute: $V_E = V_T$ RR.

Dead space is the portion of each tidal volume that does not take part in gas exchange. There are two different ways to define dead space– anatomic and physiologic. Anatomic dead space is the total volume of the conducting airways from the nose or mouth down to the level of the terminal bronchioles. The anatomic dead space fills with inspired air at the end of each inspiration, which is exhaled without being exchanged. Physiologic dead space includes all the non-respiratory parts of the bronchial tree included in anatomic dead space. In healthy individuals, the anatomic and physiologic dead spaces are roughly equivalent. However, in this project the dead space was enlarged because the length of the tube between the mouthpiece and the rebreathing bag had to be long enough to keep the equipment out of participants' sight. The possible effect of increased dead space on the investigation will be discussed in the middle chapters.

3.1.3 Measurement techniques

In this project airflow was measured by use of a pneumotachograph (Fleisch N.2, Epalinges, Switzerland). Tidal volume was derived from integration of the flow signals. The pneumotachgraph was calibrated daily with a 1 liter calibration syringe. This technique provides a reliable and valid assessment of time and volume measurements. However, an important disadvantage is that it may modify the breathing pattern because participants have to breathe through a mouthpiece or in a face-mask to which the pneumotachograph is connected.

Two types of infrared CO_2 monitors (Capnograph, Poet RC, Criticare, USA; CAPNOGARDTM $ETCO_2$ Monitor, Novametrix Medical Systems Inc., Wallingford, Connecticut) were used in different studies of this project to measure the fractional concentration of $ETCO_2$ ($FetCO_2$).

The monitor was connected through a sample tube to the mouthpiece, monitoring $FetCO_2$ continuously. Prior to each experimental session the monitors were calibrated using calibration gas containing 7.5% CO_2 and 5% CO_2 respectively.

3.2 The Rebreathing Test

The steady-state canopy procedure (Kinney et al., 1964) and the rebreathing method (Read, 1967) are two commonly used methods for determining the CO_2 sensitivity of chemoreceptors. With both methods, minute volume is measured at different concentrations of inhaled CO_2, and the ratio of change in minute volume to change in expired or arterial PCO_2 is labeled as the ventilatory response or CO_2 sensitivity (Papp et al., 1989).

The steady state test is a dynamic end-tidal forcing method to measure steady state ventilation during the inhalation of several different concentrations of CO_2. After 10 to 20 minutes of each inhalation a steady state is attained and a PCO_2-equilibrum exists between arterial blood and chemoreceptor tissues. Then the CO_2 stimulus can be estimated from the changes of $PaCO_2$.

The original Read's rebreathing test is a simple method for estimating the ventilatory response to the progressive increase of PCO_2 by rebreathing from a bag in which the initial PCO_2 is close to the PCO_2 of mixed venous blood (Read, 1967). With this technique, a PCO_2-equilibrium is rapidly established between mixed venous blood, arterial blood and end-expiratory gas. Then the relationship between the CO_2 stimulus and ventilation can be estimated by measuring the parallel changes between end-expiratory PCO_2 and ventilation.

The modified Read's rebreathing test is similar to the original one but maintains iso-oxia during rebreathing by providing a flow of oxygen to the rebreathing bag under computer control and includes a prior voluntary hyperventilation phase to lower CO_2 below the central-chemoreflex threshold (Mohan et al., 1999). Therefore, it allows measuring the ventilatory response to CO_2 in terms of threshold and sensitivity as well as the basal ventilation below the threshold.

Compared with the steady-state test the rebreathing test is rapid, tolerable and easily repeated and is particularly suitable for clinical investigations (Read, 1967).

Both the original and modified Read's rebreathing test were applied in this project to investigate the associations among air hunger, ventila-

tory behavior, and increases in CO_2. Increased blood CO_2 levels induce the perception of air hunger and trigger the regulation of a ventilatory response to eliminate more CO_2. During the process of regulation, both blood CO_2 levels and the intensity of air hunger are modified.

3.2.1 The original Read's rebreathing test (Read, 1967)

In the present project participants sat in a comfortable chair, wore noseclips and breathed through a mouthpiece connected to a rebreathing bag containing 5 litre of 100% oxygen. Each trial last 7 minutes or is terminated when subjects rated 100 on the air hunger scale (see below). As Fig 3.3 shows, the original Read's rebreathing test included two phases during which participants were instructed to breathe spontaneously: 1. Room-air-breathing phase (60 sec); 2. Rebreathing phase ($\leq$ 360 sec) in which participants were switched to the rebreathing bag unwittingly. In the first part the ventilation kept constant and in the second part it started to rise when $FetCO_2$ was higher than the threshold.

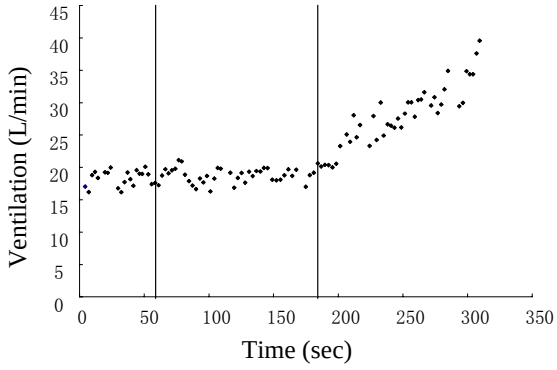


Figure 3.3: Procedure of the original Read's rebreathing test. The panel shows breath-by-breath data of ventilation vs. time of the standard rebreathing test. The first segment shows the room-air-breathing period of respiration. The second and third segments show the bag-air-rebreathing period.

3.2.2 The modified Read's rebreathing test (Mohan and Duffin, 1997)

As Fig 3.4 shows, a rebreathing trial in the modified rebreathing test consists of the following phases: 1. Hyperventilation phase: participants were instructed to hyperventilate room air until $FetCO_2$ was reduced to 3.3%, a degree of hyperventilation which is on the edge of becoming dizzy (Mohan, 1997). 2. Rebreathing phase-1: subsequently, participants were switched to the rebreathing bag, which was initially filled with 5% CO_2 and 95% oxygen, and asked to take three deep breaths so that alveolar air, the rebreathing gas and the partial pressure of CO_2 in the arterial blood equilibrated rapidly so that $FetCO_2$ provided an accurate indication of CO_2 levels at ventral medullary (central) and carotid body (peripheral) chemoreceptor regions; 3. Rebreathing phase-2: from then on, participants were asked to breathe spontaneously. Adequate equilibration was ensured by the observation of a plateau in $FetCO_2$. At the beginning of the rebreathing phase the air hunger rating started. Rebreathing continued until $FetCO_2$ reached 7.9% or participants rated 100 for air hunger intensity (see below).

Before the trials in which the actual data are collected, there was a training trial in which participants received instructions on the rebreathing apparatus, hyperventilation phase, and the air hunger rating technique. The training trial started with a 1 minute spontaneous room-air breathing during which $FetCO_2$ (%), V_E (L/min), V_T (L) and RR (breaths/min) were measured at rest. Subsequently, the training trial included the hyperventilation phase, the rebreathing phase-1, and the rebreathing phase-2 until the air hunger rating reached 30 (see Fig3.5).

3.3 Online Air Hunger Rating

There are two measurements of the perception of sensations. "Detection" studies assess the smallest stimulus subjects can perceive ("absolute threshold") or the least perceptible stimulus change (difference threshold). "Magnitude" studies require the subject to judge the intensity of a range of stimuli (Shea et al., 1995). The standard way to measure air hunger during exercise is to ask the subjects at specific time intervals (e.g., each minute of an incremental or ramp exercise test) to select a rating on a visual analogue scale (VAS) or on the 0-10 category ratio (CR-10) scale that matches the subjects' perception of breathlessness. A disadvantage

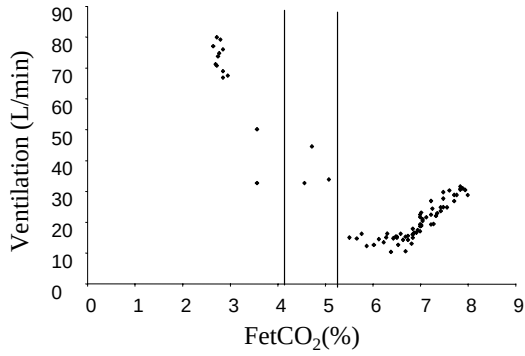


Figure 3.4: Procedure of an entire modified Read's rebreathing trial. The panel shows breath-by-breath ventilation in L/min vs. $FetCO_2$. The first segment shows the period of hyperventilation and the second segment shows the three deep breaths while switching to the rebreathing bag. The third segment shows the spontaneous rebreathing period which the data were analyzed.

of this method is that valuable information about the progressive nature of dyspnea during exercise may be lost (Mahler et al., 2001). In 1993, Harty et al. firstly described the methodology and results of a continuous method (using a potentiometer to record the ratings on a VAS displayed on a monitor) in six healthy subjects. Moreover, studies on comparing the continuous and the discrete measurement of breathlessness have demonstrated the continuous measurement allowed the experimenter to calculate both an absolute threshold and just-noticeable differences (Mahler et al., 2001; Fierro-Carrion et al., 2004).

3.3.1 Measurement techniques

During a trial, participants were asked to rate the intensity of their sensations of air hunger on a 0-100 computerized Borg type scale by means of a mouse click. The scale was a vertical bar positioned in the middle of the screen (0 = bottom of the screen). On its right, verbal descriptors were positioned at every tenth step describing different severity levels of air hunger (none, very slight, slight, moderate very, very severe, maximal-intolerable). The nature and spacing of the numbers and descriptive cat-

egories (see Fig 3.5) were identical to a modified version of the CR-10 scale developed by Borg (Borg, 1982). Each rating score (i.e., level of air hunger) and the time of each rating were recorded by the computer program.

3.3.2 Discrete rating with cues

Participants were instructed (Appendix A) to rate the intensity of their sensation of air hunger (AH) on a scale on a PC screen. Ratings were auditorily cued at 12 s intervals that signaled participants to rate air hunger by moving the mouse and clicking on the scale, filling up the bar up to the indicated level.

3.3.3 Continuous rating without cues (Mahler et al., 2001)

Using the same rating scale as the discrete rating participants were instructed (Appendix B) to rate air hunger at any time during the experiment when they experienced a change in their air hunger by moving the mouse and clicking on the scale, filling up the bar up to the indicated level.

3.4 Other Self-report Measures

3.4.1 Trait version of the State-Trait-Anxiety-Inventory (STAI, Appendix C)

Participants completed a validated Dutch version (Van der Ploeg, 1980) of the trait version of the STAI. The STAI is composed of 40 self-reported measures of state and trait anxiety. The state anxiety scale (state) consists of 20 questions that determine how the respondents "feel right now." The trait anxiety scale (trait) consists of 20 questions that assess how the respondents "generally feel."

3.4.2 State anxiety and fatigue (Appendix D)

Participants were instructed (Appendix E) to rate the maximal intensity of the anxiety they had felt during rebreathing trials and how fatigued they had been during rebreathing, using separate 7-point ordinal scales (Appendix D, Lansing et al., 2000).

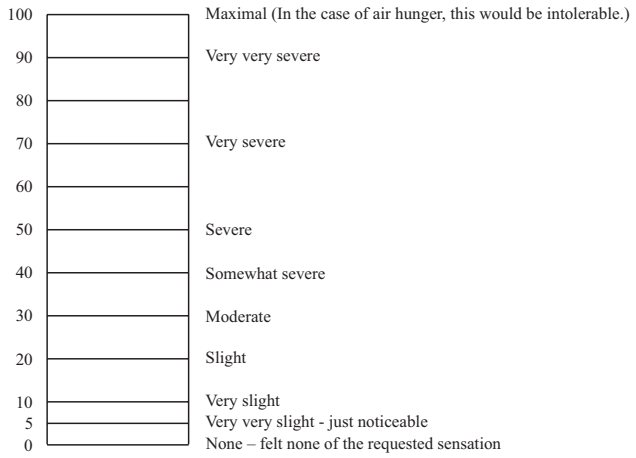


Figure 3.5: A 0-100 computerized Borg type scale for the subjective air hunger rating with verbal descriptors positioned on the right at every tenth step describing different severity levels of air hunger

3.5 Data Analyses

The data processing of this project focused on the characteristics of air hunger and the ventilatory response in reaction to increases in CO_2, more specifically the thresholds of these characteristics, and their interrelationship.

3.5.1 Threshold for air hunger and ventilatory response to hypercapnia

The thresholds for air hunger and the ventilatory response in reaction to the stimulus of CO_2 reflected the timing of the reaction and the intensity of the stimulus when the perception of air hunger and the increase of ventilatory response were triggered, respectively. The time threshold was indicated as the time point when ventilation (V_T and RR) increased above the basal ventilation (respectively, V_T and RR) , or the participants started perceiving air hunger. The CO_2 threshold was indicated as the $FetCO_2$ level at the time breakpoint (see Figure 3.6).

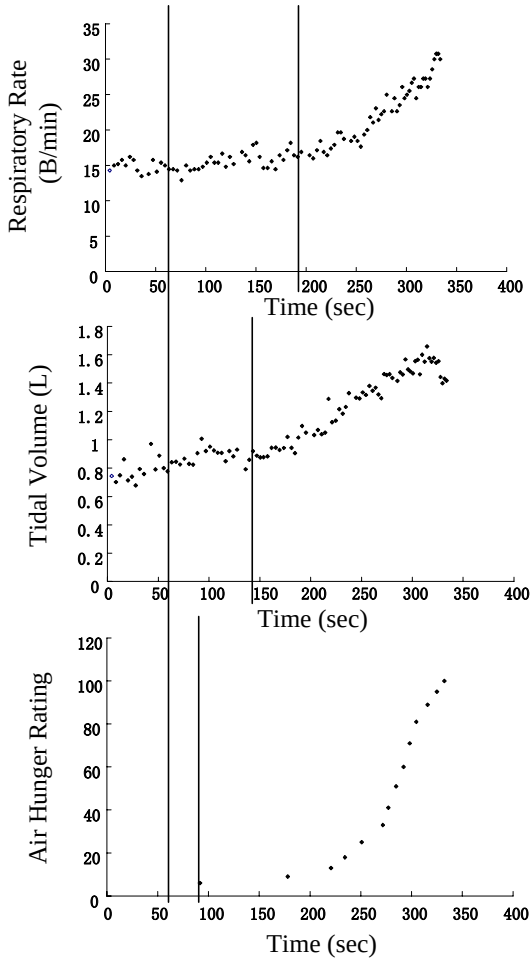


Figure 3.6: Breath-by-breath respiratory rate in B/min, tidal volume in L, and air hunger rating vs. time in Sec of a representative subject, which illustrates time breakpoints of RR, V_T and AH response to hypercapnia in one trial. The first segment of each panel on the left side of the first vertical line includes the data points in the first minute of the trial (room air breathing period). The remaining data (rebreathing period) are at the right. The second line in each panel indicates the time threshold.

3.5.2 Sensitivity of air hunger and ventilatory response to hypercapnia

The sensitivity of air hunger and the ventilatory response to the stimulus of CO_2 reflected how much air hunger and the ventilatory response, respectively, increased in parallel with the increase of the CO_2 stimulus. As PCO_2 continued to rise above the breakpoint, ventilation started to increase proportionately with elevations in PCO_2, which led to sloped ventilation PCO_2 relationship (see the upper and middle panel of Fig 3.7). The air hunger PCO_2 relationship presented the similar linear regression as well (see the lower panel of Fig 3.7). The slopes of tidal volume PCO_2, respiratory rate PCO_2, and air hunger PCO_2 regression were interpreted as the sensitivities of V_T, RR, and air hunger response to the stimulus of CO_2, respectively.

3.5.3 Association between air hunger and ventilatory behavior

The association between air hunger and respiratory behavior was investigated using the slopes and Beta coefficients. The associations between air hunger and ventilatory behavior (V_T and RR) during the process of ventilatory regulation were fitted linear regressions, which presented some information concerning the physiological mechanism of air hunger. The slope, which was indicated by B coefficient, showed how much the intensity of air hunger varied against the regulation of respiratory behavior. The within-subject comparison of slopes indicated the across-trial changes in the association between air hunger and each aspect of ventilatory behavior. However, the difference of the association between air hunger and different aspects of ventilatory behavior could not be indicated by the comparison of slopes because the independent variables have different measurement scals. Therefore, the B coefficient was standardized to the Beta coefficient, which shows how much the dependent variable increases (in standard deviations) as the independent variable increases by one standard deviation. In the present project, the within-subject comparison of Beta coefficient indicated the difference in the strength of the association between air hunger and different aspects of ventilatory behavior.

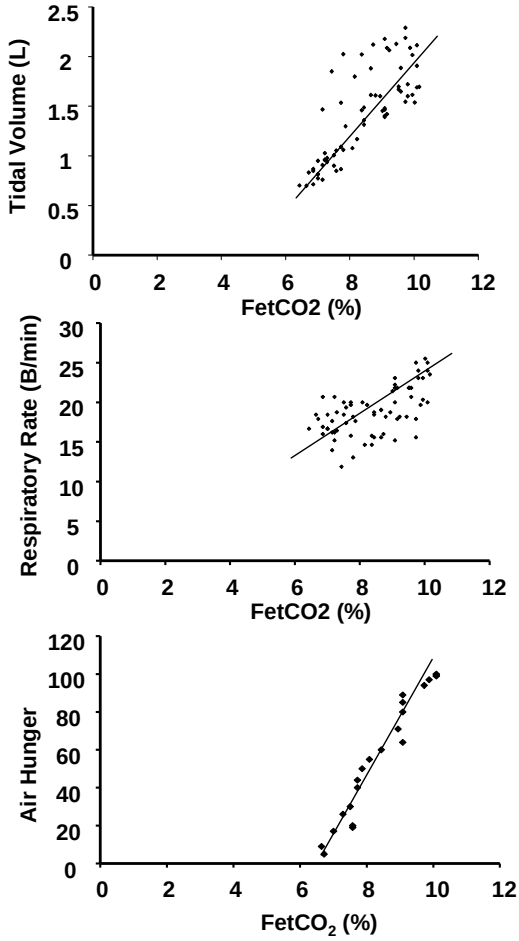


Figure 3.7: Sloped regression of breathe-by-breath V_T, RR and air hunger rating against $FetCO_2$ of a representative subject

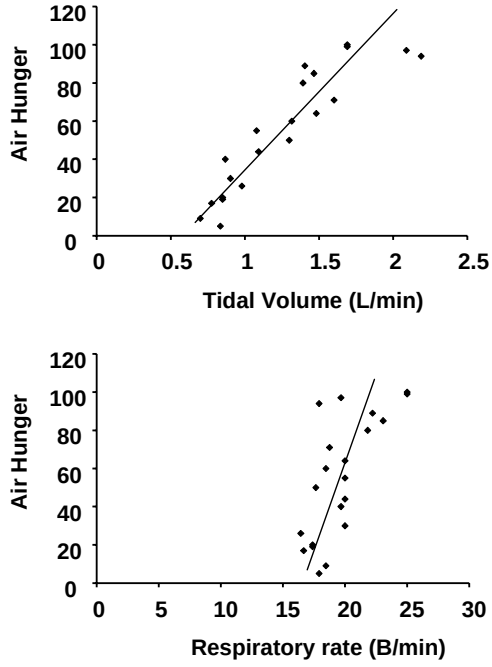


Figure 3.8: The association between Air hunger rating and ventilatory behavior (V_T, and RR) of a representative subject

Chapter 4

Aim

The purpose of this project was to investigate:

1. Air hunger response to hypercapnia (Threshold and sensitivity)
2. Ventilatory response to hypercapnia (Threshold and sensitivity)
3. Association between air hunger and ventilatory behavior
4. The effects of trait anxiety and repetition on air hunger and ventilatory response
5. Methodology investigation

4.1 The Association of Psychological influences and Physiological Mechanisms of Air Hunger

The major aim of this thesis was to study the psychological influences and physiological mechanisms of air hunger associated with anxiety. The investigation focused on the effects of repetition and trait anxiety on air hunger and ventilatory response to hypercapnia. Fig 4.1 displays the underlying mechanism of air hunger investigated in the thesis in terms of the different stages in the production of air hunger. We expected that trait anxiety and repetition might both affect the air hunger and ventilatory response to hypercapnia. The perception of air hunger might be influenced

by the effect of trait anxiety and repetition on the association between air hunger and ventilatory behavior.

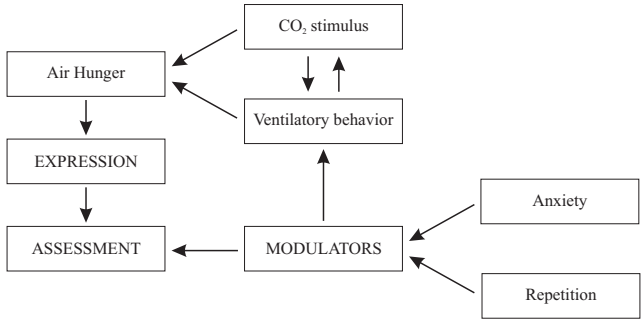


Figure 4.1: Different factors investigated in the thesis, which may play an important role in shaping air hunger (Adapted from Ripamonti et al., 1997)

4.1.1 Study 1: effects of trait anxiety and repetition

According to the hypothesis of "Suffocation Alarm System", the symptom of air hunger in panic disorder patients may be due to the hypersensitivity of chemoreflex to carbon dioxide. The first aim of this study is to test whether the sensitivity of ventilatory response to hypercapnia could be influenced by trait anxiety.

Apart from the physiological mechanism, the aim of this study was extended to the comparison of threshold between air hunger and ventilatory response and the effect of trait anxiety on the sensitivity of air hunger response to hypercapnia. Moreover, air hunger and ventilatory response were measured in repeated hypercapnic challenge trials to assess the effect of repetition, which had not been investigated yet.

4.1.2 Study 2: association between air hunger and respiratory behavior

In the second study the paradigm was adapted by employing several changes concerning the procedure and data processing to extend and improve the accuracy of the investigation. The first aim of this study was to

replicate the findings of the first study with respect to the prior threshold for air hunger perception to ventilatory response and the effects of trait anxiety and repetition on air hunger response to hypercapnia. Another aim was to investigate the association between air hunger and respiratory behavior. The effects of trait anxiety and repetition on the association were also investigated to explore the association of psychological and physiological mechanism of air hunger.

4.2 Methodology Investigation

The combination of the rebreathing test and online subjective air hunger rating, which is rarely applied in studies of respiratory psychophysiology, is an effective technique to investigate the association between air hunger, respiratory behavior, and CO_2 stimulus by measuring dynamic changes in air hunger and ventilatory response to hypercapnia. The second part of this thesis aimed to replicate the basic findings of the first part with respect to the effects of trait anxiety and repetition on air hunger and ventilatory response to hypercapnia. Furthermore, the methodology was tested in order to improve the accuracy of the new paradigm, which includes the application of two different rebreathing tests, two different air hunger ratings, and three different computer programs in data processing.

4.2.1 Study 3: comparing the original and modified Read's rebreathing test

Read's rebreathing test is a simple technique to monitor the dynamic changes in ventilatory response to the progressive increase of carbon dioxide stimulus. In some studies the original Read's rebreathing test was modified by adding a preceding voluntary hyperventilation, which lowers carbon dioxide pressure below the central-chemoreflex threshold, in order to estimate the threshold for ventilatory response to hypercapnia. However, the prior voluntary hyperventilation may influence the perception of air hunger during rebreathing. The aim of this study was to investigate whether and how the voluntary hyperventilation affected air hunger and ventilatory response to hypercapnia, whether it affected the association between air hunger and respiratory behavior, and whether it had significant interaction with trait anxiety and repetition in the mechanism of air hunger.

4.2.2 Study 4: comparing the online subjective rating with and without auditory cues

Measuring the perception of air hunger using online rating during a rebreathing test is a new paradigm to explore the mechanism of air hunger. Air hunger can be rated by subjects with (rating is discretely cued at specific time intervals) or without (subjects rate their sensation continuously when they feel changes) cues. However, there was no study comparing both methods in measuring air hunger response to repeated hypercapnic challenge. The aim of this study was to investigate the influences of different rating methods on air hunger and ventilatory response to hypercapnia and to investigate whether different rating methods had significant interaction with trait anxiety and repetition in the mechanism of air hunger.

4.2.3 Study 5: comparing the breakpoint estimation of different computer programs

The determination of the threshold for ventilatory response to hypercapnia is crucial to the investigation of the association between air hunger and ventilatory behavior. The result may be influenced by the application of techniques in estimating the threshold. There are several methods processed by different computer programs in recent studies employing the rebreathing test to measure the threshold for ventilatory response. However, no study compared the results by using of different techniques. Piecewise linear regression in STATISTICA, Segcurve, and Sigmaplot are three different methods to estimate segmental linear regression and breakpoints. The aim of this study is to evaluate the application of these methods in determining the threshold for ventilatory response in a rebreathing test.

Part II

Air Hunger and Ventilatory Response to Repeated Hypercapnic Challenge

The interaction of both physiological and psychological processes related to the mechanisms of air hunger was investigated in this project. In the first experiment using the modified rebreathing test and the discrete rating, we measured the subjective air hunger and ventilatory response to hypercapnia simultaneously. We haven't found any other studies using the same paradigm, which could be used to compare the physiological and psychological response to stimulus effectively. In the second experiment the paradigm was improved by replacing the modified rebreathing test with the standard one in order to avoid the effect of hyperventilation on the perception of air hunger. We also replaced the discrete rating with the continuous one so that the absolute threshold of air hunger perception could be measured.

Chapter 5

Air Hunger and Ventilation in Response to Hypercapnia: Effects of Repetition and Anxiety

Wan, L., Daems, E., Van de Woestijne, K.P., Van Diest, I., Gallego, J., De Peuter, S., Bogaerts, K., Van den Bergh, O., 2006. Air hunger and ventilation in response to hypercapnia: effects of repetition and anxiety. Physiology & Behavior 88, 47-54.

5.1 Abstract

We investigated the effects of anxiety on the intensity of air hunger during gradually increasing levels of CO_2 until the end-tidal fractional concentration of CO_2 was 7.9% or air hunger was intolerable. Normal high and low (trait) anxious participants (N = 23) went through three rebreathing trials (15 min interval). Breathing behaviour was continuously monitored and air hunger was rated every 12 sec. The threshold for responding to the increased CO_2 was always lower for the subjective rating than for the ventilatory response. Habituation across trials was observed for both the ventilatory response and the air hunger rating regardless of anxiety. However, beyond the threshold, the slope in air hunger ratings decreased

across trials in low anxious persons and tended to increase in high anxious persons (interaction p¡.05). No differences occurred in the slopes of the breathing responses. The findings documented uncoupling of the physiological and subjective responses during CO_2-induced air hunger.

5.2 Introduction

Dyspnea – a subjective experience of breathing discomfort – is a common symptom in many pulmonary, cardiovascular and neuromuscular diseases. Dyspnea can occur in different intensities, but in clinical cases it generally causes severe suffering. It is also common in panic and anxiety, depression and somatization disorders and it occurs in normal as well as during intense emotional states and heavy labor or exercise (American Thoracic Society, 1999). Dyspnea is not a unitary concept and different aspects, sensory as well as affective, can be distinguished (Simon et al., 1990; Banzett and Moosavi, 2001; Han et al., 2005). Air hunger feels like "not getting enough air" or as an "uncomfortable urge to breathe" and is comparable to the feeling at the end of a long breath hold (Banzett et al., 1996). Tightness is typically reported by asthmatics and refers to an uncomfortable symptom localized to the chest (Simon et al., 1990). The feeling of effort or work of breathing is evoked by high ventilation or ventilation against external loads (Lansing et al., 2000). Air hunger in particular may be considered part of an alarm system warning the organism for inadequate ventilation, such as in states of intense exercise, congestive heart failure or ill ventilated environments.

There is no single dyspnea receptor and different physiologic mechanisms related to respiratory muscle activity, blood gas levels and their interactions may underlie the many facets of dyspnea sensations. Gas exchange status is signalled by central (ventral medulla cells) and peripheral (carotid and aortic bodies) chemoreceptors, monitoring the pH, PCO_2, and PO_2 of body fluids (Shea et al., 1995). Very slight increases in arterial PCO_2 ($PaCO_2$, hypercapnia) and more substantial decreases in arterial PO_2 (PaO_2, hypoxia) stimulate the respiratory centre and increase the ventilatory drive. Both the central and peripheral chemoreceptors respond to hypercapnia, whereas the peripheral receptors respond mainly to hypoxia. Increased ventilation is registered by mechanoreceptors in the chest wall and lungs, whereas restored arterial PO_2 and PCO_2 is fed back by the chemoreceptors. In general, dyspnea is thought to result from disturbances in the balance between the afferent signals, respiratory drives,

actual ventilation, and how well the cardiorespiratory apparatus responds to the physiologic needs of the body. Air hunger, in particular, seems to arise when the ventilatory drive increases and ventilation, as reported by the mechanoreceptors, is inadequate to meet the increased needs (Banzett et al., 1989).

As a subjective experience, dyspnea and air hunger may be greatly affected by perceptual-cognitive, attentional and emotional processes and large individual differences occur (American Thoracic Society, 1999; De Peuter et al., 2004). Often, dyspnea is presented as a disabling medically unexplained complaint, occurring in the absence of obvious pulmonary insufficiency, abnormal blood gas changes, and/or disproportionate work of breathing (Howell, 1990; Dudley et al., 1968). Medically unexplained dyspnea is typically accompanied by elevated levels of negative affectivity and/or psychopathological disorder (Howell, 1990; Han et al., 1998; 2005). In some panic disorder patients, dyspnea is a prominent symptom that may be related to increased work of breathing and hyperventilation (Roth et al., 1992), but interoceptive hypervigilance and negative interpretation biases are also involved (Clark et al., 1997).

Several studies of our group (Van den Bergh et al., 1997; Van den Bergh et al., 2001; De Peuter et al., 2005) have shown that a few repeated experiences of dyspnea, induced by CO_2-inhalation in association with a specific cue (e.g. an odor) may result in reporting increased dyspnea to the cue alone, independent of the actual breathing behaviour. In other words, dyspnea (and other respiratory symptoms) can be learned in response to harmless cues. Interoceptive accuracy, assessed as the within-subject correlation between subjective respiratory sensations and their physiological referent, was particularly low in high trait anxious persons when they were in distressing situations (Van den Bergh et al., 2004; Bogaerts et al., 2005). This suggests that processes governing symptom perception in dyspnea may be different from and in certain conditions become dissociated from processes governing the physiological mechanisms underlying dyspnea.

In the present study, dyspnea – particularly its air hunger component – was induced using the modified rebreathing test (Mohan and Duffin, 1997), to investigate how repeated experience would influence both the subjective experience and the respiratory behaviour, and whether the effect of repetition was moderated by personality, i.c. trait anxiety. The participant in this test rebreathes into a bag filled with a hyperoxic mixture keeping the person in a normoxic state, but gradually augments the inspired CO_2-level, and, as a result, induces increasing levels of air hunger

and ventilation up to a level that is felt like suffocating.

Stimulus repetition typically results in habituation or sensitization in many response systems, both physiological and psychological (Domjan, 1993; Bradley et al., 1996; Figueiredo et al., 2003). Habituation refers to a decrease in response amplitude across repeated experiences that cannot be attributed to sensory adaptation. Sensitization refers to an increase in the response amplitude across trials. Sensitization is likely to overrule habituation when the stimulus is aversive, especially when it is unpredictable in time and space (Catania, 1998), and in persons with high levels of neuroticism or trait anxiety (Eysenck, 1979). In addition, trait anxiety is associated with elevated (and unfounded) symptom reporting and has been found to be an important vulnerability factor for panic disorder (Hayward et al., 2000). We expected sensitization of subjective air hunger in high and habituation in the low trait anxious participants. Ventilatory responses were assessed to test whether subjective sensitization or habituation co-occurs with, respectively, sensitization or habituation of breathing behavior.

5.3 Method

5.3.1 Participants

Twenty-three healthy women, all undergraduate psychology or medical students (age 18-20 yrs) participated in return for course credit or 10 Euro. Because ventilation differs importantly between men and women, only women were included in order to reduce error variance in the design. They were selected on the basis of their scores on the State-Trait-Anxiety-Inventory (STAI, Spielberger et al., 1970) (see procedure). Exclusion criteria were a history of significant pulmonary, cardiovascular or neuromuscular disease, or other medical conditions that likely affect exercise capacity, such as acute illnesses, fever or headache. Also participants with depression, panic, or any other psychiatric condition were excluded. All participants took oral contraceptives and were not menstruating. They were instructed to abstain from coffee, tea or alcoholic beverages after midnight before participating. The experiment was approved by the Ethical Committee of the Department of Psychology and an informed consent was obtained from all participants.

5.3.2 Measures and apparatuses

5.3.2.1 Self-report measures

– **Trait anxiety.** The trait version of the State-Trait-Anxiety-Inventory (STAI-T, Spielberger et al., 1970; validated Dutch version, Van der Ploeg, 1980) was used. It consists of 20 questions that assess how the respondents "generally feel."

– **Measurements of air hunger.** During a trial, participants were asked to rate the intensity of their sensations of air hunger on a 0-100 computerized Borg type scale by means of a mouse click. The scale was a vertical bar positioned in the middle of the screen (0 = bottom of the screen). On the right, verbal descriptors were positioned at every tenth step describing different severity levels of air hunger (none, very slight, slight, moderate very severe, maximal-intolerable). The nature and spacing of the numbers and descriptive categories were identical to a modified version of the CR-10 scale developed by Borg (Borg, 1982). This method is often used to measure perception of air hunger induced by steady-state stimulus of hypoxia or hypercapnia (Banzett et al., 1996; Mahler et al., 2001). In the present case, ratings were auditorily cued at 12 sec intervals that signalled participants to rate air hunger by moving the mouse and click on the scale, filling up the bar up to the indicated level.

– **Anxiety and fatigue.** After each trial participants were asked to rate the maximal intensity of the anxiety they had felt during the trial and how fatigued they had been during rebreathing, using a 7-point ordinal scale with indications "non, none plus, slight, slight plus, moderately strong, moderately strong plus, extreme"(see Lansing, et al., 2000).

5.3.2.2 Apparatuses and physiological recordings

Participants wore noseclips and breathed through a mouthpiece connected to a Y-valve via a wide vinyl tube ending on a pneumotachograph (Fleisch N.2, Epalinges, Switzerland) measuring airflow. The valve enabled the experimenter to switch the participants breathing between room air and a rebreathing bag. This was a plastic bag initially filled with a 5-liter gas mixture of 5% carbon dioxide and 95% oxygen in order to prevent hypoxia during rebreathing. A PC screen was put in front of the participant to collect the online ratings. A paper screen placed behind the PC kept

the manipulations of the valves out of sight (which could have induced expectancies). The wide bore vinyl tube was 60 cm long and acted as an extension of the bag by 650 ml, which may have slightly diluted the 95% oxygen and slightly increased the 5% carbon dioxide (from exhaled air being kept in the tube).

A small tube, connected directly to the mouthpiece, allowed a continuous sampling of respiratory gases. The fractional end-tidal concentration of CO_2 ($FetCO_2$) was determined using an infrared CO_2 monitor (Capnograph, Poet RC, Criticare, USA). Alveolar PCO_2 ($PACO_2$) is considered a valid approximation of arterial PCO_2 ($PaCO_2$) and can, in turn, be reliably estimated by measuring the end-tidal concentration of CO_2, expressed in percentage, $FetCO_2$, or in pressure, $PetCO_2$. The signals from the pneumotachograph and the CO_2 monitor were sent to a Labmaster card in a personal computer. The airflow and CO_2 signals were sampled at 20 Hz. All signals were plotted as a function of time and visually inspected to eliminate technical abnormalities and artifacts. Then, respiratory signals were treated breath-by-breath to extract pauses and irregularities and to determine the following parameters: inspiratory (Ti) and expiratory time (Te) in s, inspiratory (Vi) and expiratory volume (Ve) in ml and fractional concentration of end-tidal CO_2. Only inspiratory volume was used as a measure of tidal volume, V_T. Minute ventilation, V_E, was calculated from the product of Vi*RR, with RR (respiratory rate) = 60/(Te+Ti). The measurement system was calibrated prior to each experimental session using a 1-L calibrated syringe (Calibration Pump 1L, Viasys Healthcare GmbH, Germany) for the pneumotachograph, and calibration gas containing 7.5% CO_2 for the gas monitor.

5.3.3 Procedure

The participants were selected from a large pool of students (N = 403) that had completed the STAI-T questionnaire a few weeks before the experiment. Two groups were created that were highly distinct with respect to trait anxiety. The low trait anxiety (LTA) group (11 women) scored in the lower quartile of the STAI-T, the high trait anxiety (HTA) group (12 women) scored in the upper quartile. The participants were invited to participate in an experiment announced to investigate psychophysiological responses during breathing. Upon arrival, they were asked to fill out the questionnaire again before the experiment. The score of the first and second measurement was averaged. Thereafter, they were led to sit in a comfortable chair in a room, adjacent to the equipment room

with a monitor in front of them, which was connected to the computer in the equipment room. After completing the STAI-T, the rebreathing test started with a training trial in order to introduce the rebreathing apparatus, the hyperventilation and air hunger rating technique. The following instruction was given (see Lansing et al., 2000): Please start rating the intensity of your breathing sensation following the auditory cues. During this period, please pay attention to your respiratory sensation, not your respiratory behaviour. In particular, we want you to notice the sense of air hunger. The sense of air hunger is the discomfort caused by your urge to breathe, a feeling of being starved for air. Have you ever held your breath for a long time? How did that feel? (The similarity was discussed.) The sensation of air hunger may come and go throughout the experiment. Sometimes it is possible that you will not feel any air hunger sensation at all. As soon as you hear the auditory cues, please click on the square next to the verbal label that most closely describes your actual sensation at that moment. If you choose 100, it means air hunger is intolerable and we will immediately stop the trial. You can select this any time you want.

The training trial started after 1 min spontaneous breathing in rest during which $FetCO_2$ (%), ventilation (V_E, L/min), tidal volume (V_T, L) and respiratory rate (RR, breaths/min) was measured. Next three rebreathing trials started, each one consisting of the following phases (Fig. 5.1): 1. Hyperventilation phase: participants were instructed to hyperventilate room air through the Y-valve until fractional end-tidal CO_2-concentration ($FetCO_2$) was reduced to 3.3%, a degree of hyperventilation, which is on the edge of becoming dizzy. This phase is standardly inserted in the modified rebreathing test in order to lower body stores of carbon dioxide below the central chemoreflex threshold to allow a more accurate detection of the threshold for the ventilatory response when PCO_2 subsequently increases (Mohan and Duffin, 1997); 2. Rebreathing phase 1: subsequently, participants were switched to the rebreathing bag and asked to take three deep breaths so that alveolar air, rebreathing gas and arterial blood partial pressures of CO_2 equilibrated rapidly to provide an accurate indicator of $FetCO_2$ at central and peripheral chemoreceptor regions. Adequate equilibration was ensured by the observation of a plateau in $FetCO_2$; 3. Rebreathing phase 2: from then on, participants were asked to breathe spontaneously. At the beginning of the rebreathing phase 2, air hunger rating started. Rebreathing continued until $FetCO_2$ rose to 7.9% or participants rated 100 for air hunger intensity. The training trial, however, stopped when the air hunger rating reached 30.

After each trial every participant rated the maximum state anxiety

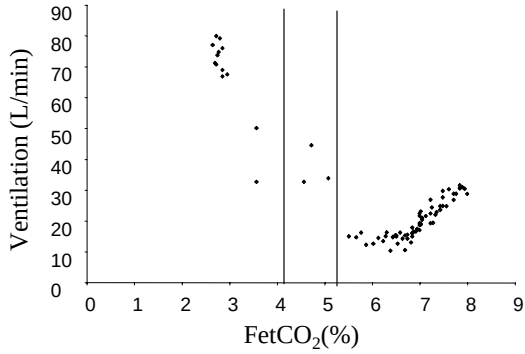


Figure 5.1: Breath-by-breath ventilation in L/min vs. PCO_2 in % of a representative subject. The first segment shows the period of hyperventilation and the second segment shows the three deep breaths while switching to the rebreathing bag. The third segment shows the spontaneous rebreathing period that was analyzed in this study.

and fatigue they had felt during the trial. The following instruction was given before the training trial: We will be asking you to rate the maximal intensity of your felt anxiety and fatigue during the trial. In particular, we want you to notice the following two kinds of sensations related to the breathing test. The sense of anxiety "right now" is defined as feelings of nervousness, worry, and apprehension associated with arousal of the body, which change from moment to moment. Fatigue is a feeling of weariness, tiredness, or lack of energy. The different sensations may change together at times and go in opposite directions at other times. It is possible that you may not feel any sensation of this kind throughout the experiment. A 15 min break was inserted after each trial in order to ensure full recovery from the trial. Compared to Read's standardized rebreathing test (Read, 1967), this version was modified by including a hyperventilation phase prior to rebreathing in order to lower PCO_2 below the central chemoreflex threshold and by maintaining normoxia during the test.

5.3.4 Data analysis and design

5.3.4.1 Ventilatory response to hypercapnia.

Analysis of the breath-by-breath data was accomplished using a spreadsheet designed for this purpose (Microsoft, Excel). The analysis included only Rebreathing phase 2 data. Sighs, swallows and other irregular or incomplete breaths were removed. Then, breath-by-breath ventilation (V_E, L/min), tidal volume (V_T, L) and respiratory rate (RR, breaths/min) were each plotted against $FetCO_2$. Each rebreathing test was analyzed by fitting a model made up of the sum of two segments divided by a breakpoint to each of V_E, V_T and RR. The breakpoint between the two segments was determined by a Piecewise Linear Regression (Statistica 6.0) and represented the $FetCO_2$-value or threshold at which ventilation starts reacting to the increase in $FetCO_2$. The segment before the threshold is called basal ventilation and was analyzed by taking the mean of the ventilatory parameters. Mahamed et al. (2001) showed that the mean adequately describes this segment and that short-term elevation and subsequent waning of ventilation as a result of prior hyperventilation is minimal. The segment above the threshold was fitted with a linear regression and its slope was interpreted as the sensitivity of ventilatory response to CO_2. We used the standardized Beta coefficient in order to allow a direct comparison between dependent variables. V_E is the primary parameter for this segment. Slopes for V_T and RR will be calculated to help interpreting the findings for V_E.

5.3.4.2 Air hunger response to hypercapnia

Using the time indications, every air hunger rating was plotted against the nearest $FetCO_2$-value or the mean of two adjacent values if the rating fell in-between. Air hunger ratings of each trial were analyzed in the same way as V_E data, that is, by fitting a model made up of the sum of two segments and divided by a breakpoint (threshold) that was determined by Piecewise Linear Regression. The first segment below the breakpoint showed the sensation of air hunger before being triggered by hypercapnia. The second segment was fitted with a linear model, paralleling the method to describe the ventilatory responses to hypercapnia (see above). Slopes were also calculated, standardized to a Beta coefficient, and interpreted as the sensitivity of the subjective response to hypercapnia.

From both of these fitting procedures, we used the estimates of the basal values, the breakpoint, and the slope for the ventilatory response,

and the breakpoint and the slope for the air hunger response. Mean values for each of these parameters, and for state anxiety (SA), fatigue (F) were calculated for all subjects in each group and each trial. Also the $FetCO_2$, V_E, V_T, and RR of the rest state measured before the training trial were analyzed.

T-test for independent samples was performed to compare differences in trait anxiety, anxiety sensitivity and rest state respiration between groups. Repeated measures ANOVA (Statistica 6.0) were performed on the dependent measures using 2 by 3 design with Trait Anxiety (high, low) as a between-subject variable and Trial (3 levels) as a repeated measures variable. Pearson correlations were calculated to identify significant relations between the parameters and state anxiety or fatigue. Data are presented as meansSD. Statistical significance was accepted at the $P < 0.05$ level (2-tailed). Greenhouse-Geisser corrections of the degrees of freedom and associated P-values will be reported where appropriate.

5.4 Results

5.4.1 Manipulation check

Most subjects reached the 7.9 % $FetCO_2$ criterium. Only two HTA subjects rated 100 in the first trial ($FetCO_2$ was 7.39% and 7.85%) and one LTA subject rated 100 in the first, second, and third trial ($FetCO_2$ was 7.33%, 7.47%, and 7.85 respectively).

5.4.2 Rest state (before the training trial)

The score of STAI of HTA group was 46.21 6.49 which is significantly higher than of LTA group, 27.09 3.27, $P < 0.01$ (see Table 5.1). There were no significant between group differences for $FetCO_2$, V_E, V_T, nor RR.

5.4.3 Ventilatory response to hypercapnia

Means SD per trial of the basal ventilation, thresholds and sensitivities of ventilatory response to hypercapnia for the HTA and LTA groups are presented in Table 5.2. The threshold for the ventilatory response increased across trials ($F(1.57, 33.06) = 12.90$, $P < 0.001$). No significant repetition (trials), anxiety (group) or interaction effects were found for any of

Table 5.1: Means (and SD's) of trait anxiety, anxiety sensitivity and rest state respiratory characteristics of the High (HTA) and Low Trait Anxious (LTA) groups

	HTA (n = 12) Mean (S.D.)	LTA (n = 11) Mean (S.D.)	t-value
Age (yrs)	19.33(1.23)	18.64(1.21)	1.38
STAI	46.21(6.49)	27.09(3.27)	8.79**
Rs $FetCO_2$ (%)	5.54(0.54)	5.65(0.38)	-0.54
Rs V_E (L/min)	13.44(3.52)	13.17(0.22)	0.20
Rs V_T (L)	0.87(0.19)	0.84(0.22)	0.30
Rs RR (b/min)	15.27(2.40)	16.50(4.28)	-0.86

Notes: Rs = rest state; ** p <.001

the ventilatory parameters during the segment before (basal ventilation), nor after the threshold (sensitivities).

In the first trial, HTA had a higher sensitivity in their RR than LTA participants ($F(1,\ 21) = 5.80,\ P < 0.05$), which is likely due to anxiety: their state anxiety and fatigue ratings were also higher than in LTA (respectively, $F(1,\ 21) = 17.23,\ P < 0.001$; $F(1,\ 21) = 6.61,\ P < 0.02$).

5.4.4 Air hunger response to hypercapnia

Table 5.3 displays the means SD per trial of the thresholds and slopes of air hunger response to hypercapnia in both the HTA and LTA groups. A main trial effect showed increasing thresholds across trials ($F(1.39,\ 29.29) = 14.73,\ P < 0.01$). The sensitivities of the air hunger ratings showed a significant trial by group interaction ($F(1.80,\ 37.81) = 3.83,\ P < 0.05$). Follow up analyses showed a nonsignificant tendency ($F(1,\ 21) = 1.14,\ P = 0.29$, n.s.) to increasing sensitivities in HTA participants, and a significant linear decreasing trend for the LTA group ($F(1,\ 21) = 4.89,\ P < 0.05$) (see the lower panel of Fig. 5.2).

5.4.5 Comparison between physiological and subjective response to hypercapnia

Because the units for the ventilatory and the subjective response were the same for the threshold ($FetCO_2$ level) and the sensitivities (Beta's), we compared both parameters in a 2 (group) × 2 measure (ventilation, air hunger) × 3 (trial) repeated measures ANOVA. The threshold for air

Table 5.2: Mean basal ventilation (and SD's), mean threshold (and SD's) and mean sensitivity (Beta's, and SD's) of ventilatory response to hypercapnia per trial in the high (HTA) and low trait anxiety (LTA) groups with the significant main effect indicated

	HTA group			LTA group		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Basal ventilation						
b-V_E (L/min)	19.05(4.34)	17.54(4.38)	18.38(4.89)	17.32(5.88)	16.12(4.47)	16.63(5.78)
b-V_T (L)	1.05(0.23)	1.05(0.24)	1.08(0.27)	1.02(0.29)	0.98(0.32)	0.97(0.27)
b-RR (b/min)	18.96(5.00)	17.42(4.60)	17.88(5.13)	17.34(3.46)	17.13(2.91)	17.40(2.83)
Thresholds						
T-V_E (%)	6.68(0.18)	6.79(0.16)	6.83(0.16)	6.75(0.19)	6.80(0.20)	6.81(0.19)
T-V_T (%)	6.68(0.18)	6.79(0.16)	6.83(0.16)	6.75(0.19)	6.80(0.20)	6.81(0.19)
T-RR (%)	6.68(0.19)	6.79(0.16)	6.83(0.16)	6.75(0.19)	6.80(0.20)	6.81(0.19)
Sensitivities						
S-V_E	0.76(0.13)	0.70(0.19)	0.74(0.16)	0.68(0.19)	0.72(0.16)	0.71(0.18)
S-V_T	0.48(0.24)	0.40(0.21)	0.47(0.27)	0.48(0.22)	0.30(0.36)	0.32(0.39)
S-RR	0.52(0.21)	0.55(0.30)	0.50(0.29)	0.29(0.24)	0.43(0.33)	0.34(0.45)

Notes: b-V_E: basal minute ventilation of ventilatory response to CO_2; b-V_T: basal tidal volume of ventilatory response to CO_2; b-RR: basal respiratory rate of ventilatory response to CO_2; T-V_E: threshold for minute ventilation of ventilatory response to CO_2; T-V_T: threshold for tidal volume of ventilatory response to CO_2; T-RR: threshold for respiratory rate of ventilatory response to CO_2; S-V_E: slope of minute ventilation versus $FetCO_2$; S-V_T: slope of tidal volume versus $FetCO_2$; S-RR: slope of respiratory rate versus $FetCO_2$.

Table 5.3: Mean thresholds (and SD's) and mean sensitivities (Beta's, and SD's) of subjective air hunger to hypercapnia per trial in the high (HTA) and low trait anxiety (LTA) groups with significant main effects indicated

	HTA group			LTA group		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
T-AH (%)	6.60(0.20)	6.70(0.16)	6.75(0.18)	6.68(0.22)	6.73(0.20)	6.76(0.19)
S-AH	0.94(0.09)	0.97(0.03)	0.97(0.02)	0.96(0.03)	0.93(0.06)	0.91(0.08)

Notes: T-AH: thresholds for air hunger response to CO_2; S-AH: slope of air hunger response versus $FetCO_2$

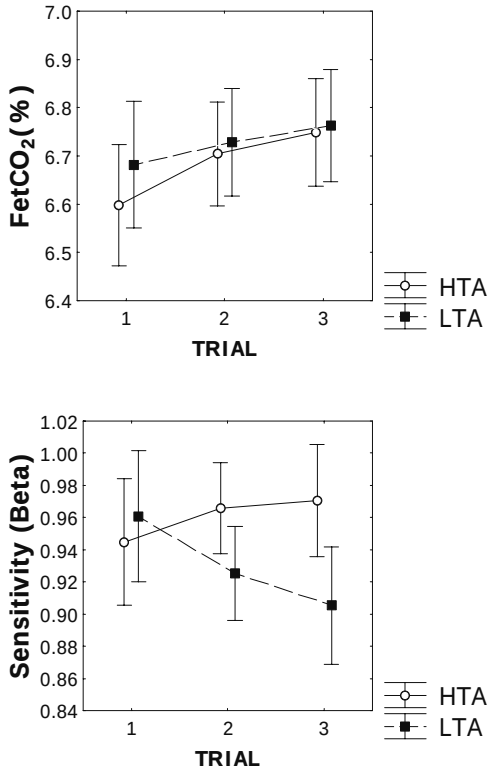


Figure 5.2: Mean threshold (upper panel) and mean sensitivity Beta (lower panel) and 0.95 confidence intervals for the air hunger reponse to hypercapnia in high (HTA) and low trait anxious (LTA) participants in three trials.

hunger was significantly lower than for the ventilatory response, $F(1, 21) = 26.83$, $P < 0.01$. Also the sensitivities were greater (steeper slopes) for the air hunger response than for the ventilation, V_E, $F(1, 21) = 57.94$, $P < 0.01$. This effect was also found for the comparison between air hunger and V_T, $F(1, 21) = 117.82$, $P < 0.01$, and for air hunger and RR, $F(1, 21) = 120.25$, $P < 0.01$.

5.4.6 State anxiety and fatigue

State anxiety was overall greater in the HTA than in the LTA group, $F(1, 21) = 65.10$, $P < 0.01$, and it decreased over trials, $F(1.70, 35.67) = 5.67$, $P < 0.01$). The decrease was stronger for HTA than for LTA (interaction: $F(1.70, 35.67) = 3.46$, $P < 0.05$), which is likely related to a floor effect in the LTA group. HTA participants reported overall more fatigue, $F(1, 21) = 5.33$, $P < 0.05$, and fatigue increased over trials, $F(1.82, 38.31) = 5.89$, $P < 0.05$. Within each trial, neither state anxiety nor fatigue correlated with any of the variables of air hunger and the ventilatory response.

Only two HTA subjects rated 100 in the first trial ($FetCO_2$ was 7.39% and 7.85%) and one LTA subject rated 100 in the first, second, and third trial ($FetCO_2$ was 7.33%, 7.47%, and 7.85 respectively).

5.5 Discussion

This study used the modified rebreathing test to investigate the effects of repetition and trait anxiety on both the threshold and the sensitivity (slope) of the ventilatory and subjective air hunger response to hypercapnia. A novel aspect was the introduction of an online rating of air hunger that was prompted every 12 sec to provide between 20 and 30 ratings per breathing trial. This allowed to calculate the threshold ($FetCO_2$ value) and sensitivity (Beta) in the same way as for ventilation and to directly compare the effects of the independent variables on both sets of measures. Overall, this comparison showed no effects of anxiety on the ventilatory parameters. However, the threshold for ventilation in response to hypercapnia raised across trials, which is, as far as we know, the first demonstration of this kind. Also the threshold for subjective air hunger raised regardless of the trait anxiety level, whereas the sensitivity for air hunger (slope of the response within trial) evolved differently for both anxiety groups: It levelled off across trials in the LTA group, whereas it tended to become greater in the HTA group. This effect was unparalleled

by comparable effects on ventilatory behaviours.

This pattern of results shows that the repeated experience of CO_2 induced respiratory challenges caused habituation of the threshold for the ventilatory and subjective air hunger response. Differences in trait anxiety level did not influence this level. Once the challenge did take effect, however, the air hunger sensation increased steeper for every change in end-tidal PCO_2 in high than in low anxious persons, whereas the increase in ventilation was not different. It is likely, then, that symptom perception processes, such as attentional direction and interpretation, account for our finding. Particularly interpretative processes are into play rather than interoceptive hypervigilance, which might have affected the perceptual threshold as well. Also the respiratory findings may be influenced by psychological variables, such as voluntary changes or detection biases. Future studies should sort that out.

Habituation and sensitization of anxiety and panic symptoms to repeated CO_2 inhalations have been observed in panic patients and in (anxious) normals (Beck and Shipherd, 1997; Beck et al., 1999; Forsyth et al., 2000), but reliable predictors for either response type did not emerge in those studies. Also dissociations among response systems (anxiety, heart rate, skin conductance) did occur. However, these studies applied brief (20 sec of 20% CO_2) or single breath inhalations (35% CO_2) and did not assess breathing behaviour in a detailed way. In addition, the focus in those studies was on subjective anxiety and panic symptoms, and not on the air hunger sensation itself. Further research with panic patients using the present paradigm may reveal how breathing behaviour and subjective air hunger become dissociated across trials. Indeed, the weight of the evidence suggests no differences between panic patients and normals in the respiratory threshold to respond to hypercapnia (Sinha et al., 2000; Katzman et al., 2002), but their air hunger responses may evolve differently. Anxiety and panic symptoms in response to CO_2 may be mediated by interpretational processes related to the hunger sensation.

As regards the differential air hunger response in both anxiety groups, the potential role of hyperventilation prior to each rebreathing trial should be considered. This aspect of the modified rebreathing test has obvious pro's and con's. On the one hand, lowering the CO_2 buffers in the body well below the normal levels has obvious advantages when one wants to measure the ventilatory threshold, while the costs in terms of a potential short term elevation of the ventilation is minimal. Indeed, several studies have shown that the effect of inserting three deep breaths after the hyperventilation phase on the basal ventilation segment is negligible (Mohan

and Duffin, 1997). On the other hand, the hyperventilation phase may have different effects on the subjective air hunger ratings in HTA and LTA. Sorting out these effects would imply a comparison of the present results with a condition without prior hyperventilation, which has never been done. However, one important argument against the interpretation that prior hyperventilation affected air hunger ratings through some differential sensitization of anxiety in the two trait anxiety groups across trials is found in the state anxiety results. State anxiety decreased across trials and relatively more so in the HTA group. In addition, no correlations were found between air hunger ratings and state anxiety scores.

Interestingly, both the threshold and the sensitivity for the subjective air hunger ratings were always lower and greater, respectively, than for the ventilatory responses. Further research should determine whether this is the result of less variance in the subjective ratings compared to breathing responses, which may influence the estimation of both the threshold and sensitivity parameters, or whether it should be interpreted that air hunger is not a direct reflection of the perception of one's own actual behaviour nor of feedback by the mechano- and/or chemoreceptors (Banzett et al., 1989). Also, the thresholds for the ventilatory response to hypercapnia were overall rather high ($FetCO_2 > 6.5\%$) compared to other studies using the rebreathing method. Several reasons may account for this. First, our participants rebreathed a hyperoxic mixture of 95% O_2 and 5% CO_2 in order to exclude additive effects on breathing behaviour caused by hypoxia (which would have occurred when rebreathing in a 5L bag filled with a normoxic mixture). Several other studies also found the threshold of the chemoreflex with hyperoxia higher than with hypo- or iso-oxia (Mohan and Duffin, 1997; Duffin et al., 2000) Second, the breakpoints were determined using Piecewise Linear Regression (Statistica 6.0), whereas other studies determined the breakpoint by visual inspection (Duffin and McAvoy, 1988) or by another curve fitting program (SPSS, Sigmaplot, Duffin et al., 2000). Differences in sensitivity to determine the threshold may exist among methods (Luwel et al., 2001). Another issue that deserves further testing is whether the present findings can be generalized beyond this young and healthy female student sample.

In summary, the present findings showed habituation of both the ventilatory and air hunger thresholds across trials. However, once passed the threshold, subjective air hunger sensitivity tended to sensitize in high trait anxious persons and habituated in low anxious persons. The latter findings were not paralleled by comparable findings in the physiological responses, documenting that processes underlying subjective air hunger

and dyspnea may become dissociated from the physiological processes they are supposed to be closely linked to.

Chapter 6

Repeated Experiences of Perceived Air Hunger and Ventilatory Behavior in Response to Hypercapnia in The Standardized Rebreathing Test: Effects of Anxiety

Wan, L., Van Diest, I., De Peuter, S., Bogaerts, K., Oyen, N., Hombroux, N., Van de Woestijne, K., Gallego, J., Van den Bergh, O. Submitted (a). Repeated experiences of subjective air hunger and ventilatory behavior in response to hypercapnia in the standard rebreathing test: effects of anxiety. Biological Psychology

6.1 Abstract

In this study, we assessed air hunger (AH) and ventilatory responses to repeated CO_2 exposures in healthy women (N = 31), scoring high or low for trait anxiety. A standardized rebreathing test, implying a gradually increasing CO_2 stimulus, was administered three times with 15-min intervals. Respiratory behavior and the intensity of AH perception were measured continuously. Across repeated exposures, maximal tolerance for AH habituated and the slope of AH (increase in AH per unit increase in CO_2) diminished. Also the dynamics of the breathing response changed across trials. The thresholds for AH and tidal volume (V_T) moved closer to each other, whereas the threshold for the respiratory rate (RR) was generally postponed. In addition, the association between AH and V_T was stronger than between AH and RR, and the latter association became weaker over trials, particularly in high anxious persons. This suggests that AH perception became increasingly influenced by psychological factors, especially in high anxious persons. The results suggest that habituation of perceived air hunger is depending on a complex interplay between both changes in respiratory behavior and in perceptual-cognitive processes as related to trait anxiety.

6.2 Introduction

Dyspnea or breathlessness is a subjective experience of respiratory discomfort, which occurs as a result of a mismatch between actual ventilation and ventilation required to satisfy the metabolic needs. It is a common symptom, not only in cardiopulmonary and neuromuscular diseases but also in anxiety, panic, and psychosomatic disorders. It occurs in healthy subjects as well during intense emotional states and heavy labor or exercise (American Thoracic Society, 1999). One particular aspect of dyspnea, air hunger (AH), feels like an "unpleasant urge to breathe". It is similar to the perception at the end of a long breath hold (Banzett et al., 1996) and it is particularly sensitive to increased levels of PCO_2 in the blood. AH seems to arise from both the projection of afferent information from the central and peripheral chemoreceptors and from corollary discharge of the brain stem respiratory motor drive to the cortical areas involved in interoception (Manning et al., 1992; Moosavi et al., 2004). In addition, AH may be shaped by past experience, expectations and emotional states (American Thoracic Society, 1999).

Evidence suggests an intricate relationship between anxiety, breathing and dyspnea. Dyspnea is more likely in psychiatric disorders and in persons with a high level of anxiety or negative affectivity (NA) without identifiable physical pathology (Howell, 1990; Papp et al., 1993; Han et al., 1998). For example, AH is often experienced during panic attacks in panic disorder patients without cardiopulmonary diseases (American Thoracic Society, 1999), although some respiratory abnormalities have been documented in panic disorder patients (Abelson et al., 2001). Conversely, the prevalence of anxiety disorders is elevated in pulmonary diseases (Van Peski-Oosterbaan et al., 1996) and asthma has been shown to be a predictor for the development of panic in a longitudinal study (Carr, 1998). The relationship between anxiety, breathing and dyspnea is poorly understood. In addition, in most cases such as in asthma and panic, episodes of dyspnea occur repeatedly. Also the effects of repeated episodes upon subsequent perceived dyspnea, breathing behavior and their relationship have not been systematically investigated. In the present study, we aim to investigate the effects of repeated episodes of on subsequent perceived dyspnea, breathing behavior and their association in high and low trait anxious persons.

When stimuli are administered repeatedly, habituation or sensitization is often observed in various response systems. Habituation refers to a decrease in response amplitude across repeated experiences that cannot be attributed to sensory adaptation, whereas sensitization refers to an increase in the response amplitude across trials. Habituation has been shown in CO_2 chemosensitivity after repeated exercise trainings (McMahon et al., 2002; Tomital et al., 2003). Exercise training also reduced dyspnea and anxiety in patients with chronic obstructive pulmonary disease (Carrieri-Kohlman et al., 1996; Gigliotti et al., 2003). Sensitization is more likely to occur when the stimulus is unpredictable, aversive and in high trait anxious persons (Bradley et al., 1996; Figueiredo et al., 2003). However, only a few studies have addressed this issue. Sensitization of fear has not been systematically found during repeated episodes of dyspnea (Beck & Shipherd, 1997; Beck et al., 1999; Forsyth at al., 2000), but neither dyspnea itself, nor breathing behavior and their association was extensively measured. Because respiratory behavior is sensitive to anticipation and feedforward regulation (Somjen, 1992; Van den Bergh et al., 1995; Gallego et al., 1996; Gallego et al., 2001), it is not clear whether habituation or sensitization in perceived dyspnea across repeated episodes depends on changes in breathing responses.

In a previous study (Wan et al., 2006), we applied the Modified Re-

breathing Test (MRT) three times (15 min intertrial interval, ITI) in a group of low and high trait anxious participants and we assessed both respiratory behavior and perceived AH continuously. In the MRT, participants first hyperventilated to lower the carbon dioxide stores in the body. Next, they rebreathed into a large bag filled with 95% O_2 and 5% CO_2 until the fractional end-tidal concentration of CO_2 ($FetCO_2$) reached 7.9% or perceived AH was rated intolerable. Across trials, the CO_2 threshold (the level at which the response to increasing CO_2 starts) for both perceived AH and the respiratory response increased. However, once past the threshold, the sensitivity of the AH response (the rate of increase with increasing CO_2) habituated in low anxious persons and tended to sensitize in high anxious ones. This means that a dissociation between the sensitivity of subjective and respiratory responses occurred: Anxiety moderated perceived AH sensitivity, but not sensitivity of respiratory behavior. These findings were the first to show the effects of repetition on the thresholds for subjective and respiratory responses to CO_2. Also another intriguing finding emerged: The CO_2 threshold for perceived AH was consistently lower than for respiratory behavior.

These findings may have particular relevance for both physiological studies on chemosensitivity and for studies investigating respiration in panic disorder. in this patient group, CO_2 hypersensitivity of the brainstem areas has been assumed to trigger a "suffocation alarm" (Gorman et al., 1989; Klein, 1993), not much evidence exists in support of it (Papp et al., 1995; Katzman et al., 2002; Rassovsky et al., 2006). In contrast, sensitization of the sensitivity to perceived AH in high anxious persons may help to explain respiratory abnormalities in panic disorder.

In order to corroborate and extend our findings, we replicated our study and added several improvements in order to make a more fine-grained analysis of the effects of repeated AH. Firstly, we used the Standardized Rebreathing Test (SRT) instead of the MRT, the difference being a hyperventilation (HV) phase inserted in the latter test prior to each rebreathing trial. HV is useful to lower the body stores of carbon dioxide, which may contribute to a more accurate measurement of the chemoreceptor threshold. However, HV may impact upon the perceived AH sensation differently in high and low anxious persons. In the present study, no prior HV was applied, leaving the perceived AH sensation unbiased by a preceding hypocapnic respiratory challenge. Secondly, in the previous study AH ratings were prompted by a tone every 12 sec. In this study, participants were instructed to indicate the very first sensation of AH and, subsequently, to rate their AH each time they felt a change of the sensation.

Studies comparing continuous and discrete (prompted) measurement of breathlessness during exercise have demonstrated better validity, reliability, and responsiveness of the continuous method (Mahler et al., 2001; Fierro-Carrion et al., 2004). Thirdly, in the previous study threshold determination of the curves representing ventilatory behavior or AH plotted against end-tidal fractional concentration of CO_2 ($FetCO_2$) was based on Piecewise Linear Regression software (Statistica 6.0), which may be more conservative than the more widely used Sigmaplot 9.0, STAT software (Duffin et al., 2000; Mateika et al., 2004). Although no study provides evidence to prefer one over the other, we opted for the more widely used method in order to allow comparisons with other studies. Fourthly, in the present study, AH ratings were plotted both against $FetCO_2$ and time in order to compare the relative thresholds of both response channels (subjective and respiratory). Finally, we explored the association between AH perception and respiratory behavior more in detail. Other research from our group (Bogaerts, et al., 2005; Van den Bergh et al., 2004) has repeatedly demonstrated more dissociation between respiratory behavior and subjective respiratory sensations in high compared to low anxious persons, especially in a distressing context.

Across trials, we expected to (1) replicate the finding that perceived AH precedes the ventilatory response to increasing CO_2; (2) replicate habituation of the threshold for both the perceived AH and the ventilatory response; (3) replicate the moderating effect of trait anxiety on the sensitivity (rate of increase) of the perceived AH across trials (i.e. habituation in low and sensitization in high anxious subjects); (4) find a lower correlation between AH and respiratory behavior in high anxious than in low anxious persons.

6.3 Method

6.3.1 Participants

Thirty-one healthy women, all undergraduate psychology students, age 18-23 years, participated in return for course credit or 6 Euro. The participants were selected from a large pool of students (N = 288) that had completed the STAI-T questionnaire (see further) a few weeks before the experiment. Sixteen women scored in the lower quartile of the STAI-T (the low trait anxiety group) and fifteen women scored in the upper quartile (the high trait anxiety group). The participants were invited to

participate in an experiment announced to investigate psychophysiological responses during breathing.

Exclusion criteria were a history of significant pulmonary diseases, cardiovascular or neuromuscular disease, or other medical conditions that likely affect exercise capacity, such as acute illnesses, fever or headache. Participants with depression, panic, or any other psychiatric condition were also excluded. None of the participants was menstruating at the time of participation. They were instructed to abstain from coffee, tea or alcoholic beverages after midnight before participating. The experiment was approved by the multidisciplinary Ethical Committee of the Department of Psychology and an informed consent was obtained from all participants.

6.3.2 Self-report Measures

- Trait and state anxiety, fatigue: The trait version of the State-Trait-Anxiety-Inventory (STAI-T, Spielberger et al., 1970; validated Dutch version: Van der Ploeg, 1980) was administered at the start of the experiment. As a measure of state anxiety, participants were asked to rate the maximal intensity of anxiety they had felt during each trial. They also indicated how fatigued they had been during rebreathing. The latter two questions were answered on two separate 7-point ordinal scales (Wan et al., 2006).

- Continuous rating of air hunger: Air hunger was measured by clicking with a computer mouse on a scale on a PC screen, filling up the bar up to the indicated level (Wan et al., 2006). Unlike in the previous study, where the participant was prompted to give a rating every 12 sec, the participant was asked to rate air hunger each time she experienced a change in her air hunger sensation. Each rating was recorded and time-stamped by the computer.

6.3.3 Apparatuses and physiological recordings

Participants wore noseclips and breathed through a mouthpiece connected to a Y-valve via a wide vinyl tube ending on a pneumotachograph (Fleisch N.2, Epalinges, Switzerland) measuring airflow. The valve enabled the experimenter to switch the participants' breathing between room air and a rebreathing bag. This was a plastic bag initially filled with 5 liter of 100% oxygen in order to prevent hypoxia during rebreathing. A PC screen

was put in front of the participants to collect the online ratings. The wide bore vinyl tube was 60 cm long and acted as an extension of the bag by 650 ml, which may have slightly increased the dead space (from exhaled air being kept in the tube). A paper screen placed behind the PC screen kept the manipulations of the valves out of sight of the participant.

A small tube continuously sampled respiratory gases from the mouthpiece. $FetCO_2$ was determined using an infrared CO_2 monitor (CAPNOGARDTM $ETCO_2$ Monitor, Novametrix Medical Systems Inc., Wallingford, Connecticut, USA, 06492). The signals from the pneumotachograph and the CO_2 monitor were sent to a Labmaster card in a personal computer. The airflow and CO_2 signals were sampled at 20 Hz. All signals were plotted as a function of time and visually inspected offline to eliminate (technical) abnormalities and artifacts. Then, respiratory signals were treated breath-by-breath by software specifically designed to extract pauses and irregularities and to determine the following parameters: inspiratory (Ti) and expiratory time (Te) in sec, inspiratory (Vi) and expiratory volume (Ve) in ml and $FetCO_2$ in percentage. Only inspiratory volume was used as a measure of tidal volume (V_T). Respiratory rate (RR) was calculated as RR = 60/(Te+Ti). The measurement system was calibrated prior to each experimental session using a 1-L calibrated syringe (Calibration Pump 1L, Viasys Healthcare GmbH, Germany) for the pneumotachograph, and calibration gas containing 5% CO_2 in 95% O_2 for the CO_2 monitor.

6.3.4 Procedure

Upon arrival, the participants were asked to complete the STAI-T questionnaire. The scores of the first measurement (a few weeks earlier, used for selection purposes) and this second measurement were averaged. Next, each participant was asked to perform three breathing trials during which she was instructed to breathe spontaneously through the mouthpiece and rate the perceived intensity of air hunger. Each trial took seven minutes. The intertrial interval was 15 min in order to ensure full recovery from the previous trial. Each trial included the SRT and the continuous air hunger rating, both starting at the same time. The SRT comprised two phases during which participants were instructed to breathe spontaneously: 1. Room air breathing phase (60 sec); 2. Rebreathing phase ($\leq$ 360 sec): participants were switched to the rebreathing bag unwittingly. The air hunger rating and rebreathing were stopped at the end of the seventh minute or when the participants' rating reached 100. In the latter case,

participants were switched back to room air breathing until the end of the seventh minute. The switching back and forth between room air breathing and rebreathing was unknown by the participants.

We used the following instructions to help participants identify the quality and intensity of air hunger (Lansing et al., 2000; Mahler et al, 2001): "Please start rating the intensity of your breathing sensation when you see the rating square on the screen. During this period, please pay attention to your respiratory sensation, not your respiratory behavior. In particular, we want you to notice this kind of sensation related to breathing: the sense of air hunger. We define the sense of air hunger as the discomfort caused by your urge to breathe, a feeling of being starved for air. Have you ever held your breath for a long time? How did that feel? (Similarity was discussed.) The sensation of air hunger may come and go throughout the experiment. Sometimes it is possible that you will not feel any air hunger sensation at all. At any time when you experience a change in your air hunger please click on the square next to the verbal label that most closely describes your actual sensation at that moment. As you may see, once you start to feel air hunger, you rate 5. If you choose 100 it means air hunger is intolerable and we will immediately make a change to make you more comfortable. You can select this any time you want."

6.3.5 Data analysis and design

6.3.5.1 Maximal CO_2 stimulus and air hunger rating

The maximal air hunger rating (AH_{max}) during the rebreathing period was recorded, together with the maximal $FetCO_2$ (maximal CO_2 stimulus). Because the maximal rating was always rated by participants at the end of each rebreathing period, $FetCO_{2max}$ was also the maximal CO_2 stimulus subjects received in each trial.

6.3.5.2 Thresholds and sensitivities for air hunger and ventilatory response to hypercapnia

Air hunger response

An air hunger rating of 5 was defined by instruction as the just noticeable level ("absolute threshold") (Shea et al., 1995). Therefore, the time at which participants rated 5 was interpreted as the time threshold for subjective air hunger, as the lower panel of Fig.6.1 shows. The $FetCO_2$ at

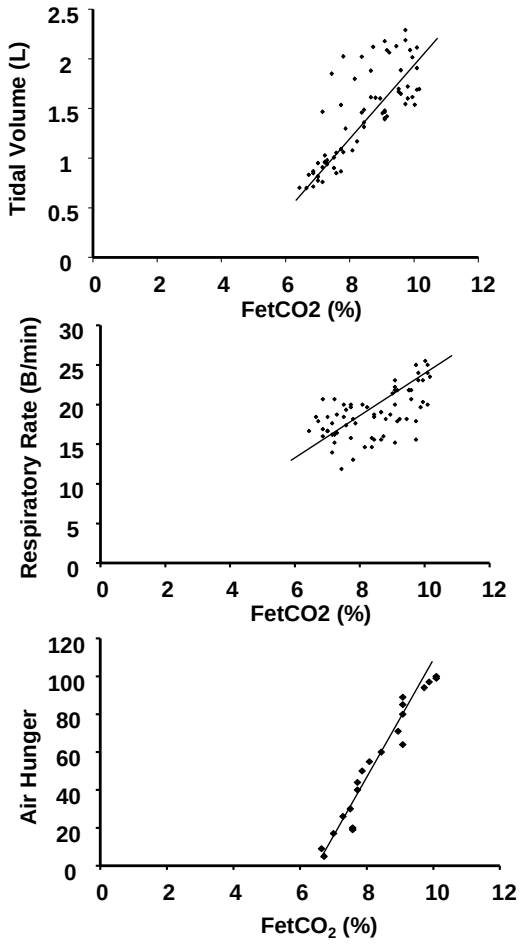


Figure 6.1: Breath-by-breath tidal volume in L, respiratory rate in B/min and air hunger rating vs. time in Sec of a representative subject, which illustrates time breakpoints of RR, V_T and AH response to hypercapnia in one trial. The first segment of each panel on the left side of the first vertical line includes the data points in the first minute of the trial (room air breathing period). The remaining data (rebreathing period) are at the right. The second line in each panel indicates the time threshold.

the time breakpoint was interpreted as the relevant $FetCO_2$, representing the CO_2 concentration in the blood when air hunger was triggered.

Beyond the threshold, each air hunger rating was plotted against the $FetCO_2$ of the previous breath using time indications. The curves were fitted with a linear regression (Moosavi et al., 2003). Their slopes (B) were calculated and interpreted as the sensitivity of the air hunger response to CO_2.

Ventilatory response

Analysis of the breath-by-breath data was accomplished using a spreadsheet (Microsoft, Excel) especially designed for this purpose. The analysis only contained the rebreathing phase data (no room air breathing). Swallows, sighs, and other irregular or incomplete breaths were removed. Then, tidal volume (V_T, L) and respiratory rate (RR, breaths/min) were each plotted against time (T, sec). As the upper and middle panels of Fig.6.1 show, each rebreathing test was analyzed by fitting a model made up of the sum of two segments divided by a calculated breakpoint using a commercial fitting program (Sigmaplot 9.0, STAT) (Mohan et al., 1999; Duffin et al., 2000). The first segment before the time breakpoint was interpreted as constant ventilation and the second segment after the time breakpoint was interpreted as the linearly (increasing) ventilatory response to hypercapnia. $FetCO_2$ at the time breakpoint was interpreted as the relevant CO_2, representing the CO_2 concentration in the blood at which the ventilatory response started increasing. Beyond the time breakpoints, each V_T and RR were plotted against end tidal CO_2 ($FetCO_2$, %). The curves were fitted with a linear regression and their slopes were interpreted as the sensitivity of, respectively, the tidal volume and the respiratory rate response to CO_2 (Papp et al., 1995).

Anchoring the breakpoints for AH, V_T and RR to the time base have some advantages over the method used in the previous study (Wan et al., 2006). In that study, AH (measured every 12 sec) and minute volume (V_E) were both plotted against $FetCO_2$ and a software program calculated the breakpoints. No distinction was made between the relative contribution of RR and V_T to V_E. In the present method, the threshold for AH was measured as the first sensation of AH a subject reported (absolute threshold) and the $FetCO_2$ at that moment was registered. The time thresholds for V_T and RR were defined by the Sigma-plot program and the $FetCO_2$ at that moment was also registered. This allowed investigating whether the air hunger sensation occurred earlier (and how much) as well as whether

it occurred at a lower $FetCO_2$ (and how much). In addition, the relative change of the time thresholds in V_T and RR in relation to the threshold for the air hunger sensation across consecutive trials can be measured more accurately, allowing a more detailed description of the dynamics of the ventilatory response across repeated experiences of air hunger.

6.3.5.3 Association between Air Hunger Rating and Respiratory Behavior

Using time indications, every air hunger (AH) rating after the breakpoint was plotted against V_T and RR values of the previous breath. Each curve was fitted a linear model (Moosavi et al., 2004). Slopes (B) were calculated and standardized to a Beta coefficient. B shows how much air hunger increases with each unit increase of V_T, and RR. Beta weights can be interpreted as a partial r (correlation coefficients), and can be used to compare the association strength between air hunger and other variables (V_T, and RR) with different measurement scales.

6.3.6 Statistical analysis

Four response characteristics (threshold, maximum, slope and Beta coefficient) were measured for each of the test responses (AH, V_T and RR). The threshold for the test responses was determined in two ways: (1) in plots of the variable with time on the X-axis (time based) and (2) in plots with $FetCO_2$ on the X-axis (CO_2 based). Besides state anxiety (SA) and fatigue (F) in each trial, fifteen response characteristics were generated: time threshold, the relevant CO_2 and slope values for AH, V_T and RR; AH_{max} and maximal CO_2 stimulus; slope and Beta coefficient values for air hunger response to V_T and RR (respectively, B_{AH-V_T}, B_{AH-RR}, $Beta_{AH-V_T}$, $Beta_{AH-RR}$).

Mean values for each parameter were calculated for each subject from the repeated rebreathing tests and air hunger ratings. T-tests for independent samples were performed to compare age and trait anxiety between groups. The differences in SA, F, and response characteristics were compared using repeated measures ANOVA (Statistica 6.0) with trait anxiety (high, low) as a between subject variable and trial (1,2,3) as within subject variable. Because the ventilatory and the subjective air hunger responses were expressed in the same units for the threshold (time and $FetCO_2$ level) and for the Beta coefficients, we compared both parameters in a 2 (group) $\times$ 3 (measure: AH, V_T, RR response to hypercapnia) $\times$ 3 (trial) or

2 (group) × 2 (measure: V_T, RR) × 3 (trial) repeated measures ANOVA. The differences in SA, F, and slopes were compared using 2 × 3 repeated measures ANOVA with trait anxiety and trial as between- and within-subject effects respectively. Data are presented as means SD. Statistical significance was accepted at the $p < .05$ level (2-tailed). Greenhouse-Geisser corrections of the degrees of freedom and associated p-values are reported where appropriate.

6.4 Results

6.4.1 Anxiety and fatigue

The HTA group had more trait anxiety than the LTA group (t (29) = 11.57, $p < .01$; 45.80 5.60 in the HTA vs 28.75 1.81 in the LTA group). SA was high in both groups during each trial and increased across trials in the HTA group, but not in the LTA group (interaction of trial by group: $F(2, 58) = 3.69$, $p < .05$, $\epsilon = .80$; $f^2 = .13$; mean SD was, respectively for the subsequent trials, 5.27 1.03, 5.93 0.88, 6.00 1.25 in the HTA group and 5.38 0.96, 5.19 1.17, 5.38 1.26 in LTA group).

Fatigue increased significantly across trials in both groups (F (2, 58) = 4.32, $p < .05$, $\epsilon = .84$; $f^2 = .15$; mean SD of fatigue was, respectively for the subsequent trials, 4.33 1.59, 4.67 1.84, 5.00 1.69 in the HTA group and 3.44 1.75, 3.94 1.53, 4.19 1.56 in the LTA group).

6.4.2 Maximal CO_2 stimulus and air hunger rating

The maximal CO_2 stimulus increased across trials in both groups (F (2, 58) = 12.87, $p < .01$, $\epsilon = .77$; $f^2 = .45$; mean SD values of $FetCO_{2max}$ were, respectively for the subsequent trials, 8.46 1.18%, 8.93 .93%, 9.04 1.08% in HTA group and 8.90 1.16%, 9.20 1.02%, 9.51 .84% in LTA group).

Conversely, the maximal perceived AH rating decreased across trials in both groups (F (2, 58) = 4.94, $p < .05$, $\epsilon = .73$; $f^2 = .17$; mean SD values of AH_{max} were 91.40 15.78, 87.27 20.08, 86.80 22.94 in the HTA group and 97.44 7.42, 83.81 25.50, 88.13 17.78 in the LTA group). Neither the group effect, nor the trial by group interaction was significant in any of these comparisons. These data clearly show habituation of the maximal tolerance for perceived AH.

The number of ratings given did not differ according to groups, trials

or their interaction (the mean SD number in trial 1, 2, and 3 were, respectively, 12.67 5.58, 14.67 7.34, 17.33 9.50 in the HTA group and 13.56 7.61, 17.31 17.71, 18.94 19.80 in the LTA group).

Table 6.1: Mean values (SD) per trial of time and CO_2 thresholds for the AH and ventilatory response to hypercapnia; slopes and Beta coefficients of AH response vs. tidal volume and respiratory rate in the high trait anxiety group (HTA) and the low trait anxiety group (LTA).

	HTA (n = 15)			LTA (n = 16)		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
T_{AH} (s)	73.43(71.44)	72.43(67.06)	76.26(62.84)	74.44(56.06)	93.98(50.82)	101.98(44.50)
T_{VT} (s)	148.08(69.20)	81.09(13.24)	89.96(20.70)	110.40(44.90)	112.36(6.75)	109.15(32.08)
T_{RR} (s)	163.03(53.82)	160.98(67.43)	202.61(80.75)	171.39(61.60)	214.66(52.58)	226.97(58.35)
CO_{2AH} (%)	6.47(0.73)	6.50(0.67)	6.58(0.70)	6.71(0.81)	6.68(0.73)	6.84(0.69)
CO_{2VT} (%)	6.88(0.70)	6.51(0.56)	6.59(0.54)	6.85(0.73)	6.94(0.76)	6.88(0.59)
CO_{2RR} (%)	6.94(0.62)	7.13(0.94)	7.62(1.11)	7.48(0.91)	7.66(0.74)	7.97(0.89)
B_{AH}	44.46(23.20)	38.01(16.56)	36.52(18.06)	47.47(22.62)	39.12(18.43)	33.86(14.26)
B_{RR}	1.73(2.04)	2.05(1.98)	3.57(2.54)	3.09(1.47)	3.20(2.08)	3.86(2.23)
$Beta_{AH-V_T}$	0.70(0.28)	0.73(0.20)	0.78(0.15)	0.82(0.13)	0.83(0.14)	0.80(0.19)
$Beta_{AH-RR}$	0.35(0.54)	0.36(0.55)	0.48(0.34)	0.66(0.26)	0.64(0.31)	0.74(0.16)
B_{AH-RR}	3.41(6.34)	3.22(7.42)	5.25(5.38)	10.66(6.11)	7.32(5.00)	7.22(3.26)

Notes: T_{AH}, T_{VT}, T_{RR}: Time threshold for AH, tidal volume, and frequency responses respectively; CO_{2AH}, CO_{2VT}, CO_{2RR}: Relevant CO_2 at the time threshold for AH, tidal volume, and frequency responses respectively; B_{AH}, B_{RR}: Sensitivity of AH and respiratory rate response to hypercapnia; $Beta_{AH-V_T}$, $Beta_{AH-RR}$: Beta coefficients between AH and tidal volume, respiratory rate respectively; B_{AH-V_T}, B_{AH-RR}: Slope of AH response to tidal volume, respiratory rate respectively. (See text for statistical analyses)

6.4.3 Thresholds for air hunger and ventilatory responses to hypercapnia

No differences related to group, trial or their interactions were found for any breathing parameter during room air breathing prior to rebreathing.

The mean values of the time thresholds and relevant CO_2 values of both groups in each trial are displayed in Table 6.1. In both groups and across trials, the time thresholds and relevant CO_2 for the first AH sensation and for the V_T response were lower than for RR response (Measure

effect respectively, $F(2, 58) = 72.58$, $p < .001$, $\epsilon = .92$; $f^2 = 3.16$; $F(2, 58) = 49.24$, $p < .001$, $\epsilon = .81$; $f^2 = 1.99$). Moreover, the time thresholds and relevant CO_2 for AH were smaller than the V_T response in the first trial (interaction of Measure × Trial respectively, $F(4, 116) = 7.93$, $p < .001$, $\epsilon = .81$; $f^2 = .27$; $F(4, 116) = 6.30$, $p < .001$, $\epsilon = .69$; $f^2 = .22$). In a further contrast analysis, AH was perceived before the V_T response (F (1, 29) = 29.24, $p < .001$). Also the relevant CO_2 for AH was lower than that for the V_T response (F (1, 29) = 14.88, $p < .001$).

No changes across trials of the time threshold and associated $FetCO_2$ for AH were observed. For the RR response, the time threshold and relevant CO_2 increased across trials in both HTA and LTA groups (respectively, $F(2, 58) = 7.11$, $p < .01$, $\epsilon = .89$; $f^2 = .25$; $F(2, 58) = 8.35$, $p < .001$, $\epsilon = .91$; $f^2 = .29$). However, the time threshold and relevant CO_2 for the V_T response in HTA participants decreased across trials, but remained stable in LTA participants (for V_T : trial effect for time threshold $F(2, 58) = 6.02$, $p < .01$, $\epsilon = .76$; $f^2 = .21$; interaction of Trial × Group $F(2, 58) = 6.29$, $p < .01$, $\epsilon = .76$; $f^2 = .22$; for relevant CO_2 : interaction of Trial × Group $F(2, 58) = 5.09$, $p < .01$, $\epsilon = .70$; $f^2 = .18$).

The differences of time threshold (Fig. 6.2) and relevant CO_2 (Fig. 6.3) between V_T and RR became larger across trials in both groups (interaction of Measure × Trial respectively, $F(2, 58) = 12.85$, $p < .001$, $\epsilon = .90$; $f^2 = .44$; $F(2, 58) = 10.67$, $p < .001$, $\epsilon = .94$; $f^2 = .37$). However, both for the time thresholds and the relevant CO_2, the difference between AH and V_T tended to become smaller across trials in both groups (interaction of Measure × Trial for the time threshold $F(2, 58) = 8.77$, $p < .001$, $\epsilon = .91$; $f^2 = .30$; interaction of Measure × Trial × Group for the relevant CO_2 $F(2, 58) = 3.20$, p = .07, $\epsilon = .73$; $f^2 = .11$). In a further contrast analysis, a significant difference between the time threshold for AH and V_T was found in the HTA and LTA groups in trial 1 (respectively, $F(1, 29) = 25.80$, $p < .001$; $F(1, 29) = 6.38$, $p < .05$) whereas the difference of the relevant CO_2 between AH and V_T was only significant in the HTA group in trial 1 (F (1, 29) = 15.78, $p < .001$).

6.4.4 Sensitivities of air hunger and ventilatory response to hypercapnia

As Table 6.1 shows, the sensitivity of AH decreased (F (2, 58) = 6.03, $p < .01$, $\epsilon = .63$; $f^2 = .21$), whereas the sensitivity of the RR increased significantly across trials (F (2, 58) = 8.79, $p < .001$, $\epsilon = .998$; $f^2 = .30$). There were no significant repetition (trials), anxiety (group), or

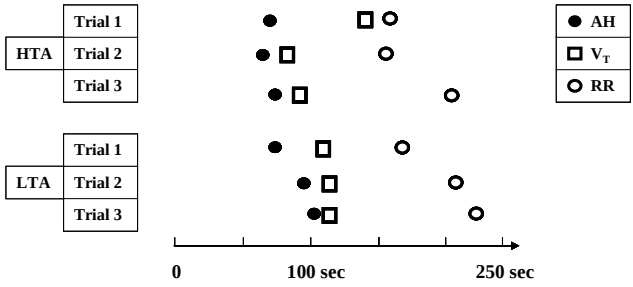


Figure 6.2: Time threshold for Air Hunger (AH), tidal volume (V_T) and respiratory rate (RR) during repeated hypercapnia. High Trait Anxiety (HTA, N = 15) and Low Trait Anxiety (LTA, N = 16) subjects were subjected to three hypercapnic tests (Standardized Rebreathing Test).

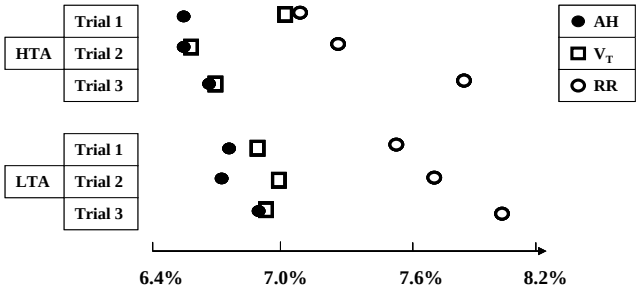


Figure 6.3: Relevant CO_2 at time threshold for Air Hunger (AH), tidal volume (V_T) and respiratory rate (RR) during repeated hypercapnia. High Trait Anxiety (HTA, N = 15) and Low Trait Anxiety (LTA, N = 16) subjects were subjected to three hypercapnic tests (Standardized Rebreathing Test).

interaction effects for sensitivities of V_T response.

6.4.5 Associations between air hunger and respiratory behavior

As Table 6.1 shows, the correlation (Beta coefficient) between AH and V_T was higher than between AH and RR (Measure effect $F(1, 29) = 54.09$, $p < .001$, $\epsilon = 1.00$; $f^2 = .31$). In addition, AH correlated stronger with V_T and RR in the LTA group than in the HTA group (Group effect $F(1, 29) = 8.08$, $p < .01$; $f^2 = .34$). Further analyses using contrasts revealed that $Beta_{AH-RR}$ was significantly lower than $Beta_{AH-V_T}$ for the HTA group (in trial 1, 2, and 3, respectively, $F(1, 29) = 7.21$, $p < .01$; $F(1, 29) = 7.90$, $p < .01$; $F(1, 29) = 14.55$, $p < .01$), but not for the LTA group.

As Table 6.1 shows, B_{AH-RR} in the LTA group was significantly higher than that in the HTA group (Group effect $F(1, 58) = 7.97$, $p < .01$; $f^2 = .28$). There was no trial or interaction effect. Also the group effect, trial effect and interaction of B_{AH-V_T} were not significant in the comparison between HTA and LTA groups.

6.5 Discussion

In the present study, CO_2-induced AH and ventilatory behavior were assessed continuously and simultaneously in three consecutive trials with the Standardized Rebreathing Test (SRT), administered to high and low trait anxious young women. Compared to the Modified Rebreathing Test (MRT, Wan et al., 2006), the SRT does not include a voluntary hyperventilation phase prior to a rebreathing trial. Such phase may have both influenced subsequent breathing behavior and primed interoceptive attention underlying perceived AH perception in the MRT.

In general, both groups reported equal increases in fatigue levels across trials. State anxiety was overall high in both groups and increased across trials among high anxious persons only. The groups did not breathe differently during baseline room air breathing, and also the number of AH ratings given was not different.

Effects of repetition

Across the repeated exposures to hypercapnia, participants endured a higher maximal CO_2 stimulus, while at the same time rating lower levels of AH. This means that the maximal tolerance for perceived AH habituated

in both HTA and LTA groups. This finding is consistent with prolonged durations of voluntary breath holding with successive trials (Nishino et al., 1996) and with decreased subjective anxiety in panic patients across repeated 35% CO_2 challenges (Van den Hout et al., 1987). In both groups, the thresholds for perceived AH remained stable, whereas the sensitivity of AH decreased. The latter means that per unit increase in $FetCO_2$, there was less increase in AH.

Also the dynamics of the breathing response changed across trials. The threshold for the RR response was gradually postponed, whereas the V_T response advanced in time to come closer to the perceived AH threshold. This earlier threshold for V_T appeared in high anxious participants only and was mainly due to a particularly late V_T response in the first trial in the HTA group being advanced in subsequent trials. Possibly, HTA participants tried to avoid deep breathing as a result of anxious anticipation induced by the instructions, but this is inconsistent with the fact that HTA were not more anxious than LTA in the first trial. So, at present, it is not clear how to interpret this finding. More importantly, it was also found that the sensitivity of the RR increased across trials, that is, the increase in RR per unit $FetCO_2$ increase augmented. It is likely that the faster V_T response in cooperation with a steeper increase in RR contributed to the increased maximal tolerance for hypercapnia. In well-trained persons put on a mechanical ventilator, it was found that tidal expansion reduced the sensation of air hunger (Banzett et al., 1996). Also in other studies, it was found that changes in ventilation reduced the intensity of air hunger (Manning et al., 1992; Bloch-Salisbury et al., 1998).

In sum, repeated experiences with AH seem to alter both the AH response and the dynamics of the ventilatory behavior in order to cope with the hypercapnic challenge. We found little effect of trait anxiety, except for a particularly late V_T threshold in the first trial, and we found no evidence for sensitization of perceived dyspnea.

Association between subjective and ventilatory responses to repeated hypercapnia

AH was overall more strongly related to V_T than to RR, which is compatible with the fact that hypercapnia triggers V_T first and RR only in a second stage. In addition, it is known that chemical mechanisms are dominating V_T regulation, whereas RR is more open to behavioral influences (Papp et al., 1995; Masaoka & Homma, 1997). Therefore, it is not

surprising that the relationships of AH with RR were influenced by the anxiety level of the participants: They were overall weaker in the HTA than in the LTA group. This is consistent with previous research of our group on interoceptive accuracy. Typically, the within-subject correlation between a subjective sensation and a clear physiological referent is lower in persons with high trait anxiety, especially when they are put in a distressing context (Bogaerts et al, 2005; Van den Bergh et al., 2004). The present data suggest that the AH rating in HTA is, more than in LTA, influenced by emotional cues that weaken the relationship with behavioral responses such as RR.

The difference in threshold between AH perception and ventilatory response

We replicated the finding of consistently lower thresholds for AH than for ventilation. This was true for both the time and relevant CO_2 levels and, in addition, it applied to both V_T and RR, the latter showing the highest threshold. This is consistent with the finding of a phasic response to hypercapnia, implying a first response in volume, followed by a second response in frequency (Duffin et al., 2000; Mahamed & Duffin, 2001). The temporal priority of the subjective response compared to the breathing response is consistent with the assumption (1) that the AH sensation is dependent on projections from signals in the respiratory centers of brain stem to cortical levels sustaining the subjective experience (American Thoracic Society, 1999), and (2) that these projections arrive before outgoing motor signals are put into operation in order to cope with the hypercapnic challenge. This functional organization increases the response repertoire to cope with unsatisfactory air supply as it allows both physiological and behavioral adaptive responses (Banzett & Moosavi, 2001).

Comparison with the previous study

Comparing the results of the present and previous studies shows that some phenomena appear robust and other findings seem to depend on specific details of the procedure. For instance, we replicated (1) the earlier threshold for AH perception than for the ventilatory response, and (2) the habituation of AH tolerance to maximal CO_2. However, we did not replicate habituation of the threshold for perceived AH across trials as AH thresholds remained stable. It should be noted that in the previous study, each AH rating was triggered by a tone cue (every 12 sec)

and the threshold was determined by a curve fitting program. In this study, the threshold was determined as the first AH sensation the subject reported, which was a priori given a rating of 5. Also, the participant was instructed to provide a rating anytime a change in AH was felt. It is likely that these instructions led the participants to more closely and accurately monitor their internal sensations, while the threshold itself was more clearly defined. This means that, in this regard, the conclusion of the previous study should be modified.

Also, we did not replicate the moderating effect of trait anxiety on the change in sensitivity of the AH response to CO_2 across trials. In our previous study, the sensitivity of AH (calculated by Beta) habituated in low anxiety persons, whereas it tended to sensitize in high anxious participants. In the present study, the sensitivity of AH (calculated by B) habituated in both groups. This lack of moderation of AH sensitivity by trait anxiety may be related to the absence of the hyperventilation phase in the SRT, as this is a quite demanding respiratory challenge that may have increased the difference in perceived AH experience between LTA and HTA in the former study. In the present study, this phase was absent and all participants remained at a relatively high anxiety level. However, a direct comparison between a MRT and SRT would be needed in order to test this interpretation.

Overall, more studies are needed with larger samples and with both men and women to determine the robustness and generalizability of our findings. If confirmed, they may have important implications for future research and clinical practice. First, the present findings suggest that studies to determine chemosensitivity thresholds in physiology should take into account behavioral variables such as anxiety level and previous experience of the participants with AH, because both these variables influence respiratory behavior (RR and V_T) during rebreathing. Second, in physiological studies on respiration and in clinical studies on patients suffering from AH and dyspnea, one should take into account that the relationship between perceived AH and respiratory (dys)function may change over time, due to changes in both perceptual-cognitive and respiratory systems. As it appeared particularly low in HTA in this study (replicating earlier findings), it is likely that acting only upon respiratory variables may not profoundly change perceived dyspnea. Third, despite a long standing interest in panic, fear of suffocation and respiration, a detailed analysis of the change of both the perceived AH sensation and the dynamics of respiratory behavior as a result of repeated experiences of dyspnea and AH has not been carried out (Papp et al., 1993; Papp et al., 1995; Rassovsky

et al., 2006). The present study documents its necessity to improve our understanding of respiratory abnormalities in panic disorder.

Conclusion

The standardized rebreathing test was used to investigate the dynamic changes of AH and ventilatory responses to repeated hypercapnia. AH was always triggered before an increase in ventilation was observed. Furthermore, participants reported lower maximal AH while they endured higher maximal CO_2 levels during repeated exposures to hypercapnia. Also the sensitivity of the AH response habituated and. shifts in the dynamics of the breathing response occurred : The thresholds for AH and V_T moved closer to each other, particularly in high anxious persons, the threshold for RR was generally postponed across trials and the sensitivity of the RR increased across trials. It is likely that these ventilatory changes helped to tolerate AH better. The association between AH and RR became lower in HTA persons, suggesting that AH perception became increasingly influenced by psychological factors. These results suggest that habituation of perceived air hunger is depending on a complex interplay between both changes in respiratory behavior and in perceptual-cognitive processes as related to trait anxiety.

Part III

Methodology Investigation

The paradigm applied in this project was firstly designed and performed. There were few literatures on the application of this paradigm. Therefore, the methodology was tested. In the 3rd-5th experiment we want to investigate the effects of different rebreathing tests and different ratings on the air hunger and ventilatory response to CO_2-inhalation. We also want to compare the results of different curve fitting processes.

Chapter 7

Air Hunger Perception and Ventilatory Response to Repeated Hypercapnic Challenges: Comparing Read's (1967) Original and Modified Rebreathing Test

Wan, L., Van Diest, I., De Peuter, S., Bogaerts, K., Vlemincx, E., Fannes, S., Bresseleers, J., Gallego, J., Van de Woestijne, K.P., Van den Bergh, O., Submitted (b) . Air hunger perception and ventilatory response to repeated hypercapnic challenges: Comparing Read's (1967) original and modified rebreathing test. Journal of Applied Physiology

7.1 Abstract

The aim of this study was to compare air hunger and ventilatory behavior in two standardized rebreathing methods developed to investigate chemosensitivity of the breathing response to hypercapnia, namely Read's original and modified rebreathing test (RT and MRT, respectively). Unlike the RT, the MRT comprises a voluntary hyperventilation (VH) phase prior to rebreathing in order to lower the carbon dioxide stores in the body below the chemosensitivity threshold. Perceived air hunger and the ventilatory response (end tidal PCO_2, tidal volume, respiratory rate) to hypercapnia were continuously assessed during three subsequent rebreathing trials (intertrial interval, ITI = 15 min) in healthy women divided in to the RT (N = 30) and MRT (N = 23) group according to the applied rebreathing test. In both tests, perceived air hunger habituated: the maximal tolerated end-tidal PCO_2 increased across trials with air hunger remaining stable. Also the sensitivity of the respiratory response to CO_2 did not differ among the tests. However, in the MRT, both state anxiety and air hunger were substantially lower and air hunger was more weakly related to respiratory measures compared to the RT. The results show that VH prior to the rebreathing test has important effects on air hunger perception and the aversiveness of the test, but not on ventilatory behavior.

7.2 Introduction

Rebreathing in a bag gradually increases the level of inhaled CO_2, changing breathing (deeper and faster) and inducing a discomfortable respiratory sensation, termed air hunger. This is largely the result of an increasing partial pressure of CO_2 in the blood ($PaCO_2$), sending afferent information from the chemoreceptors to receptors in the respiratory centers of the brainstem that trigger ventilation. The chemoreceptor information also projects to the limbic system and the sensory cortex, underlying the perception and affective appreciation of air hunger (Manning et al., 1992; Moosavi et al., 2004). Because the breathing response acts to reduce the increase of $PaCO_2$ in the blood, it may subsequently influence the intensity and quality of the perceived air hunger.

However, behavioral requirements related to cognitive and affective-motivational processes also influence the respiratory system, both voluntary and involuntary. The final output of air hunger and ventilatory

behavior is therefore the result of a complex interaction between both physiological and psychological processes (Wan et al., 2006; in press). For example, anxiety influences breathing (Boiten, 1994; Van Diest et al., 2006) and, in addition, influences cognitive processes involved in subjective symptom perception which may feed back to anxiety. Significant correlations between breathing pattern, subjective air hunger intensity, and (trait) anxiety have been found in several studies (Rietveld et al., 1998; Han et al., 1998; Masaoka and Homma, 1999; Masaoka et al., 2004).

The rebreathing technique (RT; Read, 1967) appears to be an interesting and powerful tool to analyze the complex interactions between physiological and psychological processes involved in breathing regulation and air hunger perception. Read's rebreathing test has originally been developed to estimate the sensitivity of the ventilatory response to a progressive increase of CO_2 pressure (PCO_2). The modified rebreathing test (MRT, Katzman et al., 2002; Mahamed et al., 2003; Jensen et al., 2004) is similar to the original rebreathing test, but it maintains iso-oxia during rebreathing by providing a flow of oxygen to the rebreathing bag under computer control and it includes a prior phase of voluntary hyperventilation (VH) to lower CO_2 below the central chemoreflex threshold (Mohan et al., 1999). Therefore, it allows measuring the ventilatory response to CO_2 in terms of threshold and sensitivity as well as the basal ventilation below the threshold.

Both methods have been used in studies to compare the chemosensitivity to CO_2 between panic disorder patients and healthy controls (Carr et al., 1987; Papp et al., 1995; Katzman et al., 2002). Occasionally, also subjective air hunger has been closely monitored during rebreathing. For example, Rassovsky et al. (2006) showed that panic patients experiencing a panic attack during rebreathing breathed faster and shallower and reported greater levels of suffocation sensations.

In two previous rebreathing studies (Wan et al., 2006; in press), we investigated the effect of trait anxiety on perceived air hunger and ventilatory behavior during three repeated rebreathing challenges. In the first study (Wan et al., 2006), the MRT was used, whereas in the second study the RT (Wan et al., in press) was administered. In both studies, we found habituation across trials of air hunger and respiratory rate to repeated hypercapnia. However, the results of the two studies also diverged in several aspects: in the MRT study the slope of the air hunger response to hypercapnia (rate of increase in air hunger per unit increase in PCO_2) became steeper across trials in high anxious persons and it tended to become flatter in low anxious persons. In the RT study, no interactions with

anxiety emerged. In addition, in the latter study we observed a weaker relationship between the perceived air hunger and breathing behavior, especially respiratory rate, in high compared to low anxious persons.

The differences between the results of the two studies may be due to the VH episode in MRT prior to rebreathing. Several mechanisms triggered by the additional hypocapnic challenges in a series of hypercapnic challenges may operate to induce different results between the two paradigms, especially when applied repeatedly. For example, both sensitization and habituation processes at the subjective level and at the level of breathing responses may become affected. However, direct systematic comparisons between the MRT and the RT have not been made and clear predictions as to the effects of prior VH are hard to make.

In the present study, we directly compared the RT and the MRT, allowing testing the effects of additional VH episodes on air hunger and ventilatory behavior. We also compared the strength of the relationship between the changes in both the subjective and objective measures.

7.3 Method

Data of the MRT were recovered from Wan et al., (2006). The data of the RT group were newly collected from the different samples.

7.3.1 Participants

Healthy women, all undergraduate psychology or medical students (age 18-23 yrs), volunteered to participate in the study in return for course credit or money and were divided into two groups according to the type of the rebreathing test they participated (MRT: N = 23; RT: 30). Participants were selected from different pools of students (MRT, N = 403 and RT, N = 288) based on their scores on the State-Trait-Anxiety-Inventory (STAI, Spielberger et al., 1970). Students from the high and low quartiles of each distribution were invited to participate in the study to form two subgroups of high and low trait anxious participants (HTA and LTA respectively) in each group. The mean STAI scores did not differ for the two HTA subgroups (RT group = 47.27 7.70; MRT group = 46.21 6.49) or the LTA subgroups (RT group = 27.40 2.13; MRT group = 27.09 3.27).

Exclusion criteria were a history of significant pulmonary, cardiovascular or neuromuscular disease, or other medical conditions that likely affect exercise capacity, such as acute illnesses, fever or headache. Also partici-

pants with depression, panic, or any other psychiatric condition were excluded. All participants were not menstruating. They were instructed to abstain from coffee, tea or alcoholic beverages after midnight before participating. The experiment was approved by the Multidisciplinary Ethical Committee of the Department of Psychology and an informed consent was obtained from all participants.

7.3.2 Measures and apparatuses

7.3.2.1 Self-report Measures

Trait anxiety was measured using the trait version of the State-Trait-Anxiety-Inventory (STAI-T, Spielberger et al., 1970; validated Dutch version, Van der Ploeg, 1980).

State anxiety and fatigue was rated separately by the participant after each trial on 7-point ordinal scales with indications "none, none plus, slight, slight plus, moderately strong, moderately strong plus, extreme" (Lansing, et al., 2000; Wan et al., 2006). Participants were asked to rate the maximal intensity of the anxiety and fatigue they had felt during the trial.

Air hunger was measured as in Wan et al. (2006). Ratings were auditorily cued at 12 sec intervals that signaled participants to rate air hunger by moving the mouse and click on the 0-100 scale, filling up a bar up to the indicated level. Each rating was time stamped and recorded by the computer program.

7.3.2.2 Apparatuses and physiological recordings

Participants wore noseclips and breathed through a mouthpiece connected to a Y-valve via a wide vinyl tube ending on a pneumotachograph (Fleisch N.2, Epalinges, Switzerland) measuring airflow. The valve enabled the experimenter to switch the participants' breathing between room air and a rebreathing bag. This was a plastic bag initially filled with 5-liter of 5% CO_2 in 95% O_2 (MRT group) or 100% O_2 (RT group) in order to prevent hypoxia during rebreathing. Because the initial CO_2-level in the bag may have influenced the duration before the partial pressure of CO_2 in blood increased to the threshold of hypercapnia for air hunger and ventilatory response, the present study did not analyse the thresholds and only focused on the linear regressions (sensitivity) when participants started hypercapnic rebreathing.

When rebreathing started, the exhaled CO_2 gradually increased the concentration of CO_2 in the rebreathing bag and then evoked air hunger and a ventilatory response. A PC screen was put in front of the participant to collect the online ratings. A paper screen placed behind the PC kept the manipulations of the valve out of sight. The wide bore vinyl tube was 60 cm long and acted as an extension of the bag by 650 ml, which may have slightly increased the carbon dioxide (from exhaled air being kept in the tube). A small tube, connected directly to the mouthpiece, permitted a continuous sampling of respiratory gases. The fractional end-tidal concentration of CO_2 ($FetCO_2$) was determined using an infrared CO_2 monitor (Capnograph, Poet RC, Criticare, USA for MRT group; CAPNOGARDTM $ETCO_2$ Monitor, Novametrix Medical Systems Inc., Wallingford, Connecticut, USA for RT group). Before the experiment both monitors were compared using CO_2 samples of the same concentration. The results were identical. The signals from the pneumotachograph and the CO_2 monitor were sent to a Labmaster data-acquisition card in a personal computer. The airflow and CO_2 signals were sampled at 20 Hz. All signals were plotted as a function of time and visually inspected to eliminate technical abnormalities and artifacts. Then, respiratory signals were treated breath-by-breath by software specifically designed to extract pauses and irregularities and to determine the following parameters: inspiratory (Ti) and expiratory time (Te) in sec, inspiratory (Vi) and expiratory volume (Ve) in ml and fractional concentration of end-tidal CO_2 ($FetCO_2$). Only inspiratory volume was used as a measure of tidal volume (V_T). Respiratory rate was calculated with RR (respiratory rate) = 60/(Te+Ti). The measurement system was calibrated prior to each experimental session using a 1-L calibrated syringe (Calibration Pump 1L, Viasys Healthcare GmbH, Germany) for the pneumotachograph, and calibration gas containing 7.5% CO_2 (MRT group) or 5% CO_2 (RT group) for the CO_2 monitor.

7.3.3 Procedure

The participants were invited to participate in an experiment announced to investigate psychophysiological responses during breathing. Upon arrival after filling informed consent, they were asked to fill out the STAI-T. Thereafter, three trials were administered, during which they were instructed to breathe through the mouthpiece spontaneously and rate the perceived intensity of air hunger following auditory cues every 12 sec.

After each trial, participants were asked to rate the maximum anxi-

ety and fatigue they had felt during the trial. The instructions given to participants to identify these sensations were the same as used previously (Wan et al., 2006). A 15 min break was inserted after each trial in order to ensure full recovery from the trial.

All participants in the MRT group performed one training trial and three experimental trials. In the training trial participants were taught how to hyperventilate to reduce their $FetCO_2$ to 3.3%. The training trial was terminated earlier than the experimental trial, that is, when the air hunger rating reached 30 (the moderate level of intensity). The next three trials were identical. Each of them consisted of the following phases: (1) Hyperventilation phase: participants were instructed to hyperventilate room air until $FetCO_2$ was reduced to 3.3%; (2) Rebreathing phase 1: participants were switched to the rebreathing bag and asked to take three deep breaths; (3) Rebreathing phase 2: from then on, participants were asked to breathe spontaneously. Air hunger rating started when subjects were switched to rebreathing bag.

In the RT group, participants were administered three identical rebreathing trials. Each trial comprised two phases during which participants were instructed to breathe spontaneously: (1) Room-air-breathing phase (60 sec) during which air hunger rating was started; (2) Rebreathing phase: participants were switched to the rebreathing bag unwittingly.

In both studies, after the participants were switched to the rebreathing bag, the rebreathing and rating stopped within 6 minutes. All participants were instructed that the trial would be terminated whenever they rated 100 for air hunger (which was labeled intolerable'). In addition, they were told that they could discontinue the trials or the whole experiment at any time. The duration of the whole experiment was approximately 90 min and 60 min in MRT and RT groups, respectively.

7.3.4 Data analysis and design

7.3.4.1 The characteristics of CO_2 stimulus

The duration and maximal $FetCO_2$ (CO_{2max}) that was reached by a participant was recorded in each trial. The duration and maximal $FetCO_2$ indicated the extent and intensity of the gradually increasing stimulus of CO_2, which participants received during rebreathing.

7.3.4.2 The sensitivity of the ventilatory response to hypercapnia

Analysis of the breath-by-breath data was accomplished using a spreadsheet (Microsoft, Excel) specially designed for this purpose. The analysis only contained data of the rebreathing period (for MRT group, the data of the first three deep breaths in each rebreathing period was excluded). Sighs, swallows and other irregular or incomplete breaths were removed. Then, tidal volume (V_T, L) and respiratory rate (RR, breaths/min) were each plotted against time (T, sec). Each rebreathing test was analyzed by fitting a model made up of the sum of two segments divided by a breakpoint using a commercial fitting program (Sigmaplot 9.0, STAT) that simultaneously calculated the breakpoints (Duffin et al., 2000). The second segment after the time breakpoint was interpreted as the increasing ventilatory response to hypercapnia (ventilatory regulation). Each breath-by-breath data of V_T and RR of the second segment were also plotted against $FetCO_2$ (%). The curves were fitted with a linear regression and their slopes were interpreted as the sensitivity of tidal volume and respiratory rate response to hypercapnia respectively (Papp et al., 1995).

7.3.4.3 Air hunger response

The analysis only contained the ratings during the rebreathing period. The data of the last air hunger rating during rebreathing (AH_{max}) was collected, which was also the maximal air hunger rating during rebreathing. Using the time indications, every air hunger rating (AH) was plotted against $FetCO_2$, V_T, and RR values of the previous breath. Each curve was fitted a linear model (Moosavi et al., 2004). Slopes (B) show how much air hunger increases against the increase of $FetCO_2$, V_T, and RR. B's were also standardized to a Beta coefficient. Beta weights can be interpreted like partial r's (correlation coefficients), which is useful to compare the relative strength of the correlations between parameters having different measurement scales ($FetCO_2$, V_T, and RR).

7.3.5 Statistical analysis

B's and Beta coefficients were calculated for AH, V_T, and RR. Therefore, besides state anxiety (SA), fatigue, CO_{2max}, AH_{max} and duration time of rebreathing in each trial, eight response characteristics were generated: B

values for V_T and RR responses to $FetCO_2$; B and Beta coefficient values for air hunger response to $FetCO_2$, V_T, and RR (respectively, B_{AH-CO_2}, B_{AH-V_T}, B_{AH-RR}, $Beta_{AH-CO_2}$, $Beta_{AH-V_T}$, $Beta_{AH-RR}$).

Mean values per participant for each parameter were calculated. T-Tests for independent samples were performed to compare differences in age, and trait anxiety between groups. The differences in SA, fatigue, CO_{2max}, AH_{max}, duration time of rebreathing and response characteristics were compared between and within participants, using repeated measures ANOVA (Statistica 7.1) with both Test (MRT and RT) and trait anxiety (TA, High and Low) as between-subject variables and trial as within-subject variable. Statistical significance was accepted at the $P < 0.05$ level (2-tailed). Greenhouse-Geisser corrected p-values and ϵ-values are reported where appropriate. Not to overload the result section, we reported only significant effects. Effect sizes of significant effects were calculated using f^2 by estimating the proportions of variability in the criterion variable as additional sources of variance are taken into account. Values of 0.02, 0.15, and 0.35 were considered as small, medium, and large, respectively (Cohen, 1988; Bakeman and McArthur, 1999).

7.4 Results

7.4.1 Maximal CO_2 stimulus

CO_{2max} of each group in each trial is shown in table 1. CO_{2max} and the duration of rebreathing increased significantly across trials in both groups to the same extent (respectively $F(2, 98) = 10.55$, $p < .01$, $\epsilon = .84$; $f^2 = .22$; $F(2, 98) = 3.52$, $p < .05$, $\epsilon = .87$; $f^2 = .06$). The duration of rebreathing in each trial was 269.31 (74.22) sec, 286.05 (60.48) sec, 295.92 (54.34) sec in RT group and 279.14 (45.76) sec, 286.18 (41.14) sec, 279.65 (41.75) sec in MRT group. Type of Test (RT, MRT) or TA (HTA, LTA) had no significant effect.

7.4.2 Maximal air hunger during rebreathing

The mean values per trial of the last air hunger rating of high and low trait anxious participants in the RT and MRT groups are presented in Table 7.1. Overall, the maximal rating of the MRT group was significantly lower than that of the RT group ($F(1, 49) = 38.38$, $p < .01$; $f^2 = .98$). The effects of TA, repetition or the interaction of Test TA were only

marginally significant (respectively, $F(1, 49) = 3.05$, p = .09; $f^2 = .04$; $F(2, 98) = 3.08$, p = .07, $\epsilon = .69$; $f^2 = .11$; $F(2, 98) = 2.89$, p = .10, $\epsilon = .69$; $f^2 = .06$). The lower panel of Fig 1 shows that only in the MRT AH tended to decrease across trials in both TA subgroups.

Table 7.1: Mean values (and SD's) of duration of rebreathing, maximal $FetCO_2$, maximal intensity of state anxiety and the last air hunger rating during rebreathing of the high and low anxious subjects (HTA and LTA) in the RT and MRT group in each trial.

	RT (n = 30) Mean (S.D.)		MRT (n = 23) Mean (S.D.)	
	HTA	LTA	HTA	LTA
Maximal $FetCO_2$ during rebreathing (%)				
Trial 1	8.57(1.14)	8.26(1.14)	8.30(0.36)	8.34(0.35)
Trial 2	8.60(0.97)	8.62(1.26)	8.46(0.18)	8.47(0.40)
Trial 3	8.86(0.99)	8.88(1.12)	8.48(0.19)	8.54(0.37)
Maximal intensity of state anxiety during rebreathing				
Trial 1	5.40(1.06)	5.40(0.91)	4.42(1.72)	2.09(0.70)
Trial 2	6.00(1.07)	5.53(1.13)	4.25(1.54)	2.00(0.77)
Trial 3	6.20(1.32)	5.60(1.18)	3.17(1.40)	1.91(0.70)
The last air hunger rating during rebreathing				
Trial 1	90.33(20.86)	91.33(15.61)	68.75(22.68)	47.82(28.99)
Trial 2	94.53(11.59)	91.80(17.68)	67.75(21.30)	45.82(30.11)
Trial 3	88.67(19.38)	89.60(18.90)	60.92(22.96)	45.45(28.63)

(See text for statistical analyses)

7.4.3 Sensitivities for ventilatory response to hypercapnia

No significant repetition (trial), type of test, TA or interaction effects was found for sensitivities of V_T and RR response to hypercapnia.

7.4.4 Relationship between air hunger perception and respiratory behaviors

Table 7.2 displays B and Beta coefficients of air hunger vs. $FetCO_2$, V_T, and RR. Because TA was not involved in significant main nor interaction effects, we focus only on the comparison between MRT and RT test.

Table 7.2: Mean values (and SD's) of Beta and B coefficients of air hunger response vs. $FetCO_2$, V_T, and RR per trial in the RT and MRT group.

	RT (n = 30) Mean (S.D.)			MRT (n = 23) Mean (S.D.)		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
$Beta_{AH-CO_2}$	0.84(0.25)	0.92(0.10)	0.92(0.10)	0.88(0.08)	0.87(0.10)	0.88(0.07)
$Beta_{AH-V_T}$	0.64(0.31)	0.73(0.29)	0.79(0.18)	0.46(0.32)	0.38(0.28)	0.49(0.28)
$Beta_{AH-RR}$	0.60(0.37)	0.60(0.35)	0.49(0.39)	0.44(0.32)	0.45(0.38)	0.33(0.39)
B_{AH-CO_2}	33.11(18.50)	37.03(20.71)	33.70(18.19)	16.75(11.12)	16.64(11.94)	15.00(10.41)
B_{AH-V_T}	76.73(48.10)	81.66(38.00)	76.07(33.46)	17.55(14.53)	16.61(22.87)	20.65(18.57)
B_{AH-RR}	6.24(4.54)	6.36(4.24)	5.06(4.41)	2.61(2.60)	2.60(2.58)	1.46(2.01)

Notes: $Beta_{AH-CO_2}$, $Beta_{AH-V_T}$, $Beta_{AH-RR}$: Beta coefficients of air hunger vs. $FetCO_2$, V_T, and RR; B_{AH-CO_2}, B_{AH-V_T}, B_{AH-RR}: Slopes (B) of air hunger vs. $FetCO_2$, V_T, and RR
(See text for statistical analyses)

7.4.4.1 Beta coefficients of air hunger vs. $FetCO_2$, V_T, and RR

Because Beta units for AH vs $FetCO_2$, V_T, and RR can be directly compared, we carried out a 2 (Test) × 3 (Measure: $FetCO_2$, V_T, and RR) × 3 (Trial) repeated measures ANOVA. We found a significant test effect, measure effect, interaction of Measure Test and Measure Trial (respectively, $F(1, 102) = 14.97$, $p < .01$, $f^2 = .30$; $F(2, 98) = 50.63$, $p < .01$, $\epsilon = .76$; $f^2 = 2.52$; $F(2, 98) = 4.97$, $p < .01$, $\epsilon = .76$; $f^2 = .24$; $F(4, 196) = 4.28$, $p < .01$, $\epsilon = .66$; $f^2 = .14$). Follow up analyses showed that $Beta_{AH-V_T}$ was significantly higher in the RT than in the MRT test and increased significantly across trial in both tests (Test effect $F(1, 51) = 18.63$, $p < .001$; $f^2 = .37$; Trial effect $F(2, 102) = 3.73$, p = .03, $\epsilon = .97$; $f^2 = .07$). No significant trial, test or interaction effects were found for $Beta_{AH-CO_2}$ and $Beta_{AH-RR}$.

7.4.4.2 Slopes (B) of air hunger vs. $FetCO_2$, V_T, and RR

B_{AH-CO_2}, B_{AH-V_T}, and B_{AH-RR} were all significantly higher in the RT group than in the MRT group (respectively, $F(1, 51) = 26.94$, $p < .001$; $f^2 = .54$; $F(1, 51) = 70.28$, $p < .001$; $f^2 = 1.40$; $F(1, 51) = 20.72$, $p < .001$; $f^2 = .39$). However, only B_{AH-RR} increased significantly across trials in both groups (F (2, 102) = 3.32, p = .04, $\epsilon = .83$; $f^2 = .07$). None of the interactions was significant.

7.4.5 State anxiety and fatigue

Table 1 shows the mean values of SA of high and low anxious participants of the RT and MRT groups in each trial. SA of the RT group was significantly higher than that of the MRT group (F (1, 49) = 94.69, $p < .001$; $f^2 = 1.93$). High anxious participants reported higher SA than low anxious participants (F (1, 49) = 16.97, $p < .001$; $f^2 = .35$). The difference of SA between high and low anxious participants was larger in the MRT group than that in the RT group (interaction of Test TA $F(1, 49) = 8.10$, $p < .01$; $f^2 = .17$). Moreover, the changes of SA in high and low anxious participants were also different in both MRT and RT groups (interaction of Trial Test TA $F(2, 98) = 4.39$, p = .01, $\epsilon = .75$; $f^2 = .09$). The middle panel of Fig 1 shows the different trends of SA across trials of high and low anxious participants in both RT and MRT groups. In a follow up analysis of RT group (TA × Trial repeated measures ANOVA), SA increased significantly across trials in high and low anxious participants (Trial effect $F(2, 56) = 4.05$, p = .02, $\epsilon = .66$; $f^2 = .15$) while the group effects and interaction were not significant. However, in the MRT group SA of high anxious participants was overall greater than low anxious subjects and it decreased over trials in both groups (respectively, $F(1, 21) = 19.02$, $p < .001$; $f^2 = .91$; $F(2, 42) = 5.67$, $p < .01$, $\epsilon = .85$; $f^2 = .27$). Moreover, the decrease was stronger for high anxious participants than for low anxious participants (interaction $F(2, 42) = 3.46$, $p < .05$, $\epsilon = .85$; $f^2 = .16$), which likely represents a floor effect in the low anxious group.

As for fatigue, a significant increase was found, but the effects of test and TA and the interactions were not significant (F (2, 98) = 16.89, $p <.001$; $f^2 = .35$; fatigue of each trial was 3.40 (1.57), 4.03 (1.71), 4.46 (1.83) in the RT group and 2.96 (1.19), 3.30 (1.22), 3.57 (1.27) in the MRT group).

7.5 Discussion

In the present study, Read's original (RT) and the modified rebreathing tests (MRT) were compared to investigate the effects of repetition and TA on air hunger and ventilatory responses. Firstly, a habituation effect of the maximal CO_2 stimulus was replicated in the RT group as well as the MRT group (Wan et al., 2006) as a result of repetition: across trials, the CO_{2max} reached a higher level (see the upper panel of Fig.1) while the maximal air hunger rating did not change significantly or in the MRT tended

to decrease (see the lower panel of Fig.1). This means that participants tolerated a higher maximal level of CO_2 for the same or a lower level of subjective air hunger. We also replicated (see Wan et al., in press) that the slope linking air hunger and respiratory rate became overall steeper across trials: per unit increase in air hunger, the increase in RR became bigger. Because faster breathing is a natural homeostatic response to remove the excess of CO_2 in the blood, this suggests that the participants learned to respond more efficiently to the respiratory challenge over trials. It is as yet unclear whether this increase in sensitivity of the RR helped to keep the self-reported air hunger stable across trials while the maximal CO_2 increased.

Secondly, the most striking difference between the two tests was that the maximal air hunger was overall lower in the MRT than in the RT with roughly equal levels of CO_2. TA and its interaction with the type of test were only marginally significant. The data pattern of air hunger was strikingly similar to the pattern observed for state anxiety, and the differences as regards the latter variable were more reliable: In the MRT, state anxiety was overall lower, it further decreased across trials and HTA participants reported higher state anxiety than LTA participants. In the RT, on the other hand, state anxiety was not different for HTA and LTA participants and it increased across trials. Obviously, a prior hypocapnic challenge overall reduced anxiety and self-reported air hunger during a subsequent hypercapnic challenge, and - for state anxiety - it did so relatively more in low than in high anxious persons.

Several reasons may account for this finding, such as (1) more habituation of state anxiety produced by the addition of three VH challenges, (2) reduced aversiveness of a hypercapnic challenge immediately after a hypocapnic one, or (3) an anchoring effect of one aversive subjective experience on a subsequent one. We consider the first two explanations as less likely than the third. Indeed, an effect of additional habituation would explain why state anxiety decreased more across trials in the MRT than in the RT, but not why there is no reduction of state anxiety in the RT. Also the second explanation less aversiveness of a hypercapnic stimulus after a hypocapnic one is unlikely, because the rebreathing only started after recovery from VH and the same level of hypercapnic stimulation was achieved in both types of rebreathing tests. The most likely explanation is an anchoring effect on the subjective rating (Wilson & Jones, 1990): a strong forced hypocapnic challenge (VH in the MRT) may make a subsequent slow and gradually increasing challenge, such as hypercapnic overbreathing, comparatively less aversive. This may have reduced

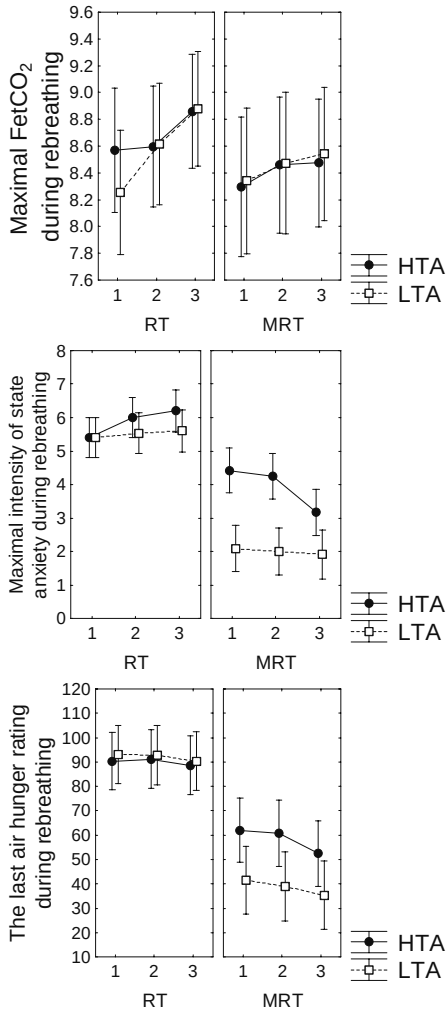


Figure 7.1: The changes of maximal $FetCO_2$, maximal intensity of state anxiety and air hunger during rebreathing across trials in HTA and LTA participants of the RT and MRT group. With the same stimulus of CO_2, SA and air hunger presented different trends across trials between high and low anxious participants in the RT and MRT group.

the subjective anxiety in response to hypercapnic rebreathing, compared to the same experience that was not preceded by VH. It may also explain why a difference between HTA and LTA persons appeared in the MRT and not in the RT: Mogg and Bradley's (1998) cognitive-motivational analysis of information processing in anxiety suggests that HTA and LTA will not differ in subjective appreciation and attentional processing of stimuli that are either neutral or highly anxiogenic. Only stimuli of medium intensity will be processed differently by both groups: HTA persons would be biased to and/or have difficulties to disengage from the stimuli compared to LTA. Previously, we reported several studies with respiratory stimuli, fitting nicely within this framework (Stegen et al., 1998), both as regards symptom ratings and attentional performance measures.

This analysis suggests that a rebreathing trial is experienced as more aversive, if it is not preceded by a challenge that anchors the perceived aversiveness at a higher level, and that it depends on the level of the experienced aversiveness whether sensitization or habituation of the state anxiety occurs over trials. Overall, the similar pattern of air hunger and state anxiety suggests that both subjective ratings are closely linked, although the causality and its direction is not clear.

A third finding is that the relationship between air hunger and respiratory behavior was overall much weaker in the MRT than in the RT (B, Beta). Consistent with the analysis above, we speculate that the higher state anxiety in the RT induced closer monitoring of the effects of the respiratory challenge, resulting in better correspondence between air hunger and respiration. Conversely, the lower anxiety level in MRT may have led to relatively more disengagement from it, resulting in a weaker link. Obviously, the observed differences in state anxiety between HTA and LTA in the MRT-test were not large enough to result in reliable differences between groups in the strength of the relationship between air hunger and respiration.

Read's rebreathing test is a simple method for testing the ventilatory response to hypercapnia for not only experimental studies but also clinical studies. The present study demonstrated that the application of RT and MRT may affect state anxiety and the association between respiratory sensation and behavior, which migh shed light on improving the methodology of relevant studies, especially on panic disorder patients. Firstly, the influence of preceding VH of MRT should be taken into account when the rebreathing test is employed to determine the breathing behavior related to emotional state or dyspnea. Secondly, the combination of the rebreathing test and online subjective rating extends the investigation

from chemosensitivity (Papp et al., 1995; Mohan et al., 1999; Katzman et al., 2002; Mahamed et al., 2003) to the association between subjective and physiological response (Wan et al., in press). It may help us understand the etiology of air hunger better by exploring the irregular response pattern related to air hunger and anxiety of panic disorder patients (Rassovsky et al., 2006). Considering the small group size and the single gender of the samples of the present study, the comparison of the RT and MRT would be replicated on the larger population and patient samples.

In summary, the present study illustrates the complexity and plasticity of air hunger perception by comparing an identical respiratory challenge in two standardized rebreathing protocols that are widely used to assess chemosensitivity of the respiratory system. A hyperventilation phase that is inserted prior to rebreathing in order to lower carbon dioxide below the chemosensitivity threshold did not alter ventilatry sensitivity, but it reduced the level of perceived air hunger substantially. In addition, it was shown that in that case air hunger was more loosely coupled with respiratory behavior. Overall, it was shown that during repeated challenges perceived air hunger habituates and that the participants obviously learned to increase their RR. Probably this can be seen as a way to cope with the challenge.

Chapter 8

The Type of Online Rating of Perceived Breathlessness Influences Respiratory Behavior During Hypercapnia in The Rebreathing Test

Wan, L., Van Diest, I., De Peuter, S., Bogaerts, K., Van de Woestijne, K.P., Gallego, J., Van den Bergh, O. Submitted. The type of online rating of perceived breathlessness influences respiratory behavior during hypercapnia in the rebreathing test. Chest.

8.1 Abstract

Online rating of perceived breathlessness (air hunger) in the rebreathing test is a new and powerful technique to analyze the psychological and physiological process related to the mechanisms of air hunger. The aim of the present study was to assess the influence of rating type on respiratory sensation and behavior during repeated hypercapnic exposures. Sixty-one

healthy women performed three rebreathing trials. Part of them was auditorily cued to rate air hunger intensity every 12 sec (CR,, N = 30), the other part was instructed to rate air hunger each time a change in air hunger intensity was perceived (UCR, N = 31). The ventilatory response was assessed simultaneously. The CR group yielded an earlier V_T threshold than the UCR group. Only in the latter group, changes occurred across rebreathing trials: the V_T threshold decreased, the RR threshold increased and the sensitivity of the RR increased. Different types of air hunger ratings affect attentional and emotional processes that influence respiratory behavior in the rebreathing test.

8.2 Introduction

Read's rebreathing test (RT, Read, 1967) was developed to investigate the chemosensitivity and the ventilatory response to a progressive increase of CO_2 pressure (PCO_2) in experimental and clinical pneumology. A new extension comprised an online rating of subjective air hunger. In two recent studies of our group, this extension was used to assess the dynamic changes in both subjective and physiological responses across repeated episodes of hypercapnia (Wan et al., 2006; Wan et al., accepted). Also in studies on panic disorder, online ratings in the RT have added to our understanding of the relationship between chemosensitivity, breathing behavior and subjective panic (Rassovsky et al. 2006).

Two different rating methods are typically used to assess perceived air hunger. In the standard one, e.g. during an incremental or ramp exercise test, the subject is prompted at specific time intervals (e.g., each minute) to select a rating on a visual analogue scale (VAS) or on a 0-10 category ratio scale that matches the subjects' perception of breathlessness. In 1993, Harty et al. described the methodology and results of an uncued method, in which subjects were instructed to rate air hunger (on a VAS displayed on a monitor, using a potentiometer) each time a change in air hunger was perceived. High reliability was observed in both approaches, but the uncued rating yielded more ratings compared with the rating cued each minute (Mahler et al., 2001; Fierro-Carrion et al., 2004). As a result, the uncued rating was better at estimating the linear regression between breathlessness ratings and physiological variables than the other one (Mahler et al., 2001).

In order to augment the number of cued ratings, we employed a shorter interval and cued a rating every 12 secs (Wan et al., 2006). Interestingly,

we observed habituation of the ventilatory threshold across trials and, in addition, that the sensitivity of air hunger response to hypercapnia habituated in low anxious but tended to sensitize in high anxious persons (Wan et al., 2006). In another study, an uncued rating was administered in which the participants were instructed to rate their sensation whenever they felt changes. We largely replicated the findings in the first study, but the results were also partly different. In addition, we found a lower association between air hunger and respiratory rate in high than in low anxious participants (Wan et al., accepted). The extent to which the type of rating could have played a role is not known, as a direct comparison between cued and uncued ratings in the RT has never been made.

Because cued versus uncued ratings may differentially influence both attentional processes involved in the perception and apprehension of respiratory discomfort (Stegen et al., 1998; Meek 2000; Bauldoff et al., 2002), and breathing behavior itself (Han et al., 1997), the purpose of the present study was to test the effect of rating type (cued versus uncued) on perceived breathlessness and the ventilatory response during the RT. Because cues were given every 12 sec, we expected air hunger being rated more frequently in the cued than in the uncued group. No a priori directional hypotheses concerning the effect of the rating type on the level of air hunger or on respiratory behavior are advanced.

8.3 Method

Sixty-one healthy female undergraduate students (age 18-23 yrs) received course credit or 6 Euro for participating. Exclusion criteria were a history of significant pulmonary diseases, cardiovascular or neuromuscular disease, or other medical conditions that may affect the breathing response, such as acute illnesses, fever or headache. Also participants with depression, panic, or any other psychiatric condition were excluded. The participants were not menstruating during the experiment. They were instructed to abstain from coffee, tea or alcoholic beverages after midnight before participating. An informed consent was obtained from all participants. The experiment was approved by the multidisciplinary Ethical Committee of the Department of Psychology and considered in accordance with the Declaration of Helsinki (2000) of the World Medical Association.

Upon arrival, participants were asked to sit comfortably in front of a PC screen. Each participant received three 7-min rebreathing trials with 15 min inter-trial intervals (Wan et al., accepted). During each trial

they wore a noseclip and breathed through a mouthpiece connected to a Y-valve via a wide vinyl tube ending on a pneumotachograph (Fleisch N.2, Epalinges, Switzerland) measuring airflow. The valve enabled the experimenter to switch the participants' breathing between room air and a rebreathing bag. This was a plastic bag initially filled with 5 liter of 100% O_2 in order to prevent hypoxia during rebreathing. The fractional end-tidal concentration of CO_2 ($FetCO_2$) was determined using an infrared CO_2 monitor (CAPNOGARDTM $ETCO_2$ Monitor, Novametrix Medical Systems Inc., Wallingford, Connecticut, USA). The respiratory signals were processed and the parameters ($FetCO_2$, tidal volume (V_T), and respiratory rate (RR)) were determined as in the previous study (Wan et al., 2006).

Air hunger and the rating scale were explained as in Wan, et al., 2006. Ratings in both groups were delivered on a Borg type scale by moving the mouse on a vertical bar labeled from 1 to 100 (Wan et al., 2006). Each rating score and the time of each rating were recorded by the computer program. The amount of ratings (Nr) during rebreathing of each trial was counted. The participants of the uncued rating group (UCR, N = 31) were asked to rate AH each time they experienced a change in their AH sensation. In the cued rating group (CR, N = 30), ratings were auditorily cued at 12 sec intervals signaling the participants to rate air hunger.

The analysis of breath-by-breath data and subjective rating data only contained data of the rebreathing period. Using a commercial fitting program (Sigmaplot 9.0, STAT) tidal volume (V_T, L) and respiratory rate (RR, breaths/min) were each plotted against time (T, sec) in order to estimate the time breakpoints at which V_T and RR started to increase (Duffin et al., 2000; Wan et al., 2006). The breath-by-breath data before the breakpoints were excluded from the further analysis. Least squares linear regressions were calculated using $FetCO_2$ (%) as the independent variable with V_T and RR as the dependent variables. Their slopes were interpreted as the sensitivity to hypercapnia of tidal volume and respiratory rate, respectively (Papp et al., 1995). The duration time of the rebreathing phase in each trial was also measured. Using the time indications, every air hunger rating (AH) was plotted against $FetCO_2$, V_T, and RR values of the previous breath. Each curve was fitted a linear model (Moosavi et al., 2004). Slopes (B) were calculated and standardized to a Beta coefficient. B (respectively, B_{AH-CO_2}, B_{AH-V_T}, B_{AH-RR}) shows how much AH increases with each unit increase of $FetCO_2$, V_T, and RR. Beta weights (respectively, $Beta_{AH-CO_2}$, $Beta_{AH-V_T}$, $Beta_{AH-RR}$) can be interpreted as a partial r (correlation coefficients), and can be used

to compare the association strength of AH with other variables having different measurement scales.

Repeated measures ANOVA (Statistica 7.1) with Rating (UCR vs. CR) as between-subject variables and Trial as within-subject variable were performed, with P set at the .05 level. Data were presented as means standard deviation (SD). Greenhouse-Geisser corrected p-values and ϵ-values are reported where appropriate. Effect sizes of significant effects were calculated and values of 0.02, 0.15, and 0.35 were considered as small, medium, and large, respectively (Cohen, 1988; Bakeman and McArthur, 1999).

8.4 Results (see Table 8.1)

The CR group had a larger Nr than the UCR group (F (1, 59) = 12.36, p = .001; f^2 = .21). No significant group nor interaction effects were observed as regards the duration of rebreathing.

The time breakpoint for V_T response was significantly earlier in the CR group compared to the UCR group (F(1, 59) = 9.82, $p < .01$; f^2 = .17). Also, the time breakpoint for V_T of the UCR group decreased across trials, whereas it remained unchanged in the CR group (the interaction of Trial Rating, F(2, 118) = 3.65, p = .03, ϵ = .71; f^2 = .06). In addition, the time breakpoint for RR response increased significantly in the UCR group, whereas it remained stable in the CR group (the interaction of Trial Rating, F(2, 118) = 3.24, p = .04, ϵ = .998; f^2 = .06).

Also the sensitivity of RR increased across trials, but in the UCR group only (the interaction of Trial Rating, F(2, 118) = 5.48, $p < .01$, ϵ = .82; f^2 = .09). As for the association between AH and respiratory behavior, no significant main effect nor interaction was observed.

8.5 Discussion

In the present study, we investigated air hunger and the ventilatory response to repeated hypercapnia using two different subjective rating methods. With an equal duration of rebreathing, fewer ratings were provided by the UCR than by the CR group. Whereas Mahler et al., 2001 and Fierro-Carrion et al., 2004 found the reverse, this is obviously due to fewer cues being given in their studies (one per min). Interestingly, the rating type influenced ventilatory behavior during the RT. First, the CR

Table 8.1: Mean values (and SDs) of Nr, the time breakpoint for ventilatory response (V_T and RR), and the sensitivity of RR response of the UCR and CR group in each trial

	UCR (n = 31) Mean (S.D.)			CR (n = 30) Mean (S.D.)		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Nr	13.13(6.35)	16.03(15.56)	18.16(15.45)	21.73(6.37)	23.47(5.08)	24.13(4.56)
T_{VT}	128.63(60.07)	97.23(30.43)	99.87(28.45)	93.88(30.66)	93.08(18.59)	93.84(17.53)
T_{RR}	167.34(57.16)	188.69(65.18)	215.18(69.99)	168.99(59.44)	194.11(68.49)	180.31(65.22)
B_{RR}	2.43(1.87)	2.64(2.08)	3.72(2.35)	3.40(4.36)	2.56(2.82)	2.14(1.76)

Notes: Nr indicates the amount of rating during the rebreathing period; T_{VT} and T_{RR} indicate the time breakpoint for V_T and RR response; B_{RR} indicates the sensitivity of RR response to hypercapnia.

group yielded an earlier V_T threshold than the UCR group. Second, only in the latter group changes occurred across rebreathing trials: the V_T threshold decreased, the RR threshold increased and the sensitivity of the RR increased. This means that when no auditory cues to rate air hunger were given, participants were more sensitive to the effect of repetition of the same rebreathing challenge in that their gradually responded earlier with a volume and later with frequency response, but once the frequency started to increase, this increase became steeper over trials. It is also remarkable that the rating type affected breathing behavior more importantly that perceived air hunger itself or than the association between air hunger and respiratory parameters.

The findings are important because they emphasize the importance of a strict and complete standardization of the rebreathing method. Conversely, they document how sensitive an objective measure may be to contextual and behavioral influences. It is currently unclear why and how these effects on respiratory behavior come about. Obviously, attentional and emotional processes are differently affected by the rating type. One can imagine that an auditorily cued rating induces a cognitive task set in the participants: they have to remain continuously vigilant to the sounds and emit a response every 12 sec. This cognitive task set, which is kept constant throughout the experiment, may stabilize breathing behavior in response to the hypercapnic stimulus. Conversely, an uncued rating and fewer ratings as a result - may leave more room for attentional distraction and/or free floating emotional concerns about the effects of the respiratory challenge. This may make the breathing response to repeated hypercap-

nia more versatile and flexible. Earlier research has shown that cognitive load manipulations affect ventilation (Boiten 1998; Denot-Ledunois et al., 1998) and a strained breathing pattern has been described in situations of high vigilance (Fokkema et al., 1999). Further research in which also measures of attentional and emotional processes are collected is needed to sort out the explanations for these effects.

Two practical conclusions also emerge from this study. The short-interval cued rating might be better than the every-one-minute cued rating to assess perceived air hunger, firstly because more frequent ratings provide more data points to calculate the change in air hunger and its relationship to the change in breathing behavior, and secondly, because it induces a consistent cognitive set that stabilizes breathing behavior.

Chapter 9

Thresholds Estimation of The Ventilatory Response to Hypercapnia in Read's Rebreathing Test: A Test of Different Software Programs

9.1 Abstract

Fitting a segment model in order to determine the threshold of a physiological response to a homeostatic disturbance is a frequently occurring exercise in physiology. In the present study, the ventilatory response (tidal volume, V_T, respiratory rate, RR, and minute ventilation, V_E) to three subsequent hypercapnic challenges in Read's Rebreathing test was investigated (N = 31). Breath-by-breath data were plotted against time. The responses (V_T, RR and V_E) were fitted a two-segment model and the time breakpoints were estimated by three different software programs: Piecewise Linear Regression (STATISTICA), SegcurvN, and Sigmaplot 9.0. Overall, the time breakpoint of Sigmaplot was significantly earlier

than of the other programs. The time breakpoints for V_T and V_E were similar for STATISTICA and SegcurvN and did not change across trials, whereas Sigmaplot suggested different thresholds for V_T and RR and also found changes across trials. We conclude that Sigmaplot has a higher responsiveness to detect changes than the other programs, and may be considered a more suitable program to estimate the time breakpoint for the ventilatory response during repeated exposure to hypercapnia.

9.2 Introduction

Read's rebreathing test (RT; Read, 1967) is a simple and widely used method to investigate breathing control in both clinical and experimental situations. It implies rebreathing from a bag filled with a gas mixture, in which the initial PCO_2 is close to the PCO_2 of mixed venous blood (Read, 1967). With this technique, a PCO_2-equilibrium is rapidly established between mixed venous blood, arterial blood and end-expiratory gas. The RT has been modified to maintain iso-oxia during rebreathing by providing a flow of oxygen to the rebreathing bag under computer control and by including a prior voluntary hyperventilation to lower CO_2 below the central chemoreflex threshold (Casey et al., 1987; Duffin and McAvoy, 1988; Mohan et al., 1999).

The control of breathing involves chemoreflex drives to breathe, which has been described in the "Oxford model" (Cunningham et al., 1986). Chemoreflex responses to PCO_2 are represented in this model by a segmented linear relation between ventilation and PCO_2 divided by a breakpoint (Duffin et al., 2000). The breakpoint is determined as the minimal PCO_2 level triggering a ventilatory increase above the basal ventilation (Duffin et al., 2000; Wan et al., accepted). As PCO_2 continues to rise, ventilation increases proportionally to elevations in PCO_2, which leads to a sloped ventilation PCO_2 relationship. Plotting ventilation to time produces a similar segmented linear relationship (Wan et al., accepted). The time breakpoint is indicated as the moment in time, at which ventilation increases above the basal level. Fig9.1 illustrates the segmented linear regression line.

Three analytic methods have been used to identify the breakpoint and the sloped portion of the ventilation - PCO_2 or ventilation time relationship : (1) plotting ventilation data (tidal volume, respiratory rate, or minute volume) against the fractional end-tidal concentration of CO_2 ($FetCO_2$) in order to calculate the breakpoint of CO_2 (Papp et al., 1995;

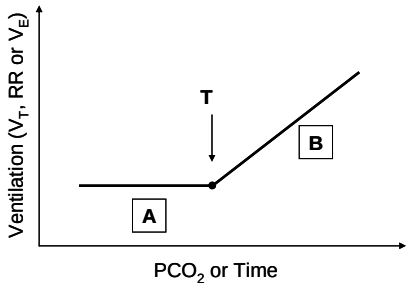


Figure 9.1: Theoretic plot of breath-by-breath data of ventilation (V_T, RR, and V_E) versus PCO_2 or Time illustrating two distinct regions of ventilatory response to hypercapnia (constant-ventilation segment A and increasing-ventilation segment B) divided by a breakpoint (T) as predicted by the "Oxford" model.

Mohan & Duffin, 1997; Wan et al., 2006); (2) fitting the $FetCO_2$ vs. time plot firstly with a least-squares regression line of which the equation provided a predicted value of $FetCO_2$ vs. time; thereby, plotting ventilation data against the predicted $FetCO_2$ and then calculating the breakpoint of CO_2 (Duffin et al., 2000; Mohamed et al., 2001); (3) plotting ventilation data against time to calculate the breakpoint of time firstly and then select the $FetCO_2$ at the time breakpoint (Mohan et al., 1999; Wan et al., submitted).

In the present study, breath-by-breath data were plotted against time to estimate the time breakpoint of the ventilatory response, as in the third method. In studies using the rebreathing test, the initial method to estimate the breakpoint was visual inspection (Duffin and McAvoy, 1988; Mahan & Duffin, 1997). In recent studies, statistical approaches were reported using software programs such as Piecewise Linear Regression in STATISTICA (StatSoft, Inc. 1995; Wan et al., 2006) and the curve fitting routine in the Sigmaplot statistical and graphing package (Sigmaplot 9.0, Systat Software, Inc., Duffin et al., 2000; Mohamed et al., 2001; Wan et al., accepted). Segcurve is another software program estimating segmented linear regression and breakpoints (Luwel, 2001). It has not been employed in studies using the rebreathing test. The aim of the present study was to compare these different statistical methods to estimate the threshold for ventilatory response to hypercapnia in RT.

9.3 Method

The participants and methods were those of Wan et al., (submitted (a)) and will only be briefly summarized. The different data analytic methods will be described more elaborately.

9.3.1 Participants

Healthy female undergraduate students (N = 31, age 18-23 years,) participated in return for course credit or 6 Euro. The inclusion/exclusion criteria were identical to the previous study (Wan et al., accepted). The experiment was approved by the Ethical Committee of the Department of Psychology and an informed consent was obtained from all participants.

9.3.2 Protocol

Participants sat comfortably in front of a PC screen. Each participant received three 7-min rebreathing trials with 15 min inter-trial intervals (Wan et al., accepted). During each trial they wore a noseclip and breathed through a mouthpiece connected to a Y-valve via a wide vinyl tube ending on a pneumotachograph (Fleisch N.2, Epalinges, Switzerland) measuring airflow. The valve enabled the experimenter to switch the participants' breathing between room air and a rebreathing bag. This was a plastic bag initially filled with 5-liter of 100% O_2 in order to prevent hypoxia during rebreathing. The fractional end-tidal concentration of CO_2 ($FetCO_2$) was determined using an infrared CO_2 monitor (CAPNOGARDTM $ETCO_2$ Monitor, Novametrix Medical Systems Inc., Wallingford, Connecticut, USA). The respiratory signals were processed and the parameters ($FetCO_2$, tidal volume (V_T), and respiratory rate (RR)) were determined in the same way as in the previous study (Wan et al., 2006).

9.3.3 Breakpoint estimation

Breath-by-breath data were first treated in a spreadsheet (Microsoft, Excel) specially designed for this purpose. Sighs, swallows and other irregular or incomplete breaths were removed. Then, tidal volume (V_T, L), respiratory rate (RR, breaths/min), and minute ventilation (V_E, L/min) were each plotted against time (T, sec), at which each inspiration started. Then, the curves relating ventilation (V_T, RR, and V_E) to time were inputed into the computer programs.

9.3.3.1 PLR (STATISTICA 7.0)

PLR is one of the functions of STATISTICA 7.0 (StatSoft, Inc. 1995) to estimate two separate linear regressions with a breakpoint. Individual curves were processed one by one, using least squares, with the model:

$$y = (b_{01}+b_{11}x_1+\cdots+b_{m1}x_m)(y \leq b_0)+(b_{02}+b_{12}x_1+\cdots+b_{m2}x_m)(y > b_0)$$

Least squares is a criterion that says the best curve is the one for which the sum of the squares of the vertical distances of each point from the fitted curve is minimized. Thus, PLR estimates two separate linear regression equations; one for the y values that are less than or equal to the breakpoint (b0) and one for the y values that are greater than the breakpoint.

9.3.3.2 SegcurvN

SegcurveN is a computer program fitting segmented linear regression models to individuals' response-time or error-rate patterns to investigate strategy use and strategy shift in cognitive tasks (Beem, 1999; Luwel, 2001). The program estimates two regression equations consisting of different ranges of the independent variable. Meanwhile, the unknown breakpoint separating the two segments was estimated. The following equations describe the model fitted by the program:

$$\begin{aligned} y &= a_1 + b_1 x + e \qquad \text{for } x \leq s \\ y &= a_2 + b_2 x + e \qquad \text{for } x > s \end{aligned}$$

In the equations x is the indepentent variable and y is the dependent variable. The parameter a_i and b_i (i = 1, 2) indicate the intercept and the slope of the regression lines, respectively, while e is the error term. For each individual data pattern, the first regression equation is fitted for values of the indepentent variable less than s while the rest larger than s is fitted to the second equation. The parameters and breakpoint are estimated simultaneously using a least squares algorithm. The data of all participants can be fitted in one run of the program.

9.3.3.3 Sigmaplot 9.0 (Systat Software, Inc)

Most recent studies using the modified rebreathing test employed Sigmaplot statistical and graphing package to analyze the data pattern of the ventilatory response to hypercapnia. As follows, the model we chose contains

two simple linear regressions fitted to different nonoverlapping data ranges that meet at a breakpoint (Kovac et al., 1999):

$$y = f(x - p) + h \qquad \text{for } x < h$$
$$y = g(x - p) + h \qquad \text{for } x \geq h$$

where f and g are slope parameters for the two limbs of the regression. Respectively, h and p are the Y-axis and X-axis coordinates of the breakpoint. The parameters for the equation are estimated by least squares using the curve fitting routine (Motulsky & Christopoulos, 2003). The curve fitting is an iterative procedure performed on the data of each individual separately. Firstly, we defined initial values for each parameter. f was fixed to a constant value of zero because the ventilatory response keeps constant in the first segment before the breakpoint (Duffin et al., 2000; Mohamed et al., 2001). We also fixed p to a constant value equaling to the mean value of ventilation during the first minute of each trial. The initial values of h and g we provided were based on the result we found in the previous study (Wan et al., 2006). Secondly, we evaluated the outputs producing by the program to find the best-fit value of h and g. The initial values were altered with respect to the R2 value and how close the resulting curve comes to the data after each evaluation. R2 is a fraction between 0.0 and 1.0, which has no units. The more R2 is closer to 1.0, the closer the curve comes to the data. The best-fit h value was considered the time breakpoint of interest.

9.3.4 Data analysis

Mean values of time breakpoint for V_T, RR, and V_E response to hypercapnia were calculated for each subject from each trial. Repeated measures ANOVA (STATISTICA 7.0) were performed on the dependent measures using 3 by 3 by 3 design with Software (PLR, SegcurveN, and Sigmaplot), Measure (V_T, RR, and V_E response) and Trial (3 levels) as within-subject variables. Data are presented as means SD. Statistical significance was accepted at the $p < 0.05$ level (2-tailed). Greenhouse-Geisser corrections of the degrees of freedom and associated P-values will be reported where appropriate. Effect sizes of significant effects were calculated and values of 0.02, 0.15, and 0.35 were considered as small, medium, and large, respectively (Cohen, 1988; Bakeman and McArthur, 1999).

9.4 Results

Table 1 displays the mean values of time breakpoints for V_T, RR, and V_E response to hypercapnia in each trial.

Table 9.1: Mean values (SD) per trial of time breakpoints for tidal volume (V_T), respiratory rate (RR), and minute ventilation (V_E) response to hypercapnia estimated by use of Piecewise Linear regression in STATISTICA 7.0, Segcurve in SegcurvN, and Segmented linear regression in Sigmaplot 9.0

	Trial 1	Trial 2	Trial 3
Time breakpoint for V_T response to hypercapnia			
PLR	178.47(33.55)	191.58(31.58)	193.94(32.22)
Segcurve	206.77(80.86)	185.19(68.54)	219.98(74.42)
Sigmaplot	128.63(60.07)	97.23(30.43)	99.87(28.45)
Time breakpoint for RR response to hypercapnia			
PLR	178.47(33.55)	191.58(31.58)	193.94(32.22)
Segcurve	196.73(83.60)	196.86(55.17)	228.15(69.50)
Sigmaplot	167.34(57.16)	188.69(65.18)	215.18(69.99)
Time breakpoint for V_E response to hypercapnia			
midrule PLR	178.47(33.55)	191.58(31.58)	193.94(32.22)
Segcurve	178.24(65.80)	183.34(48.71)	216.55(58.01)
Sigmaplot	140.31(44.73)	132.31(47.72)	141.86(46.22)

Notes: PLR: Piecewise Linear regression in STATISTICA 7.0; Segcurve: Segcurve in SegcurvN; Sigmaplot: Segmented linear regression in Sigmaplot 9.0 (See text for statistical analyses)

9.4.1 Effect of Software

For each ventilatory response (V_T, RR, and V_E), the time breakpoints differed significantly depending on the computer program (F (2, 60) = 77.58, $p < .001$, $\epsilon = .97$; $f^2 = 1.22$). Contrast analysis showed that Sigmaplot located the breakpoints significantly earlier than the others, while there was no difference between the other two (between PLR and Sigmaplot $F(1, 30) = 122.91$, $p < .001$; between SegcurveN and Sigmaplot $F(1, 30) = 116.77$, $p < .001$; between PLR and SegcurveN $F(1, 30) = 1.63$, n.s.).

9.4.2 Interaction of Software × Measure

As Table 1 shows, the time breakpoints for the V_T, RR, and V_E response were the same for both PLR and SegcurveN, whereas Sigmaplot located the time breakpoint for RR significantly later than that for the V_E response. The latter one was significantly later than that for V_T response (respectively, $F(1, 30) = 29.94$, $p < .001$; $F(1, 30) = 16.71$, $p < .001$; interaction of Software Measure $F(4, 120) = 20.70$, $p < .001$, $\epsilon = .78$; $f^2 = .58$).

9.4.3 Interaction of Software × Trial

The time breakpoints for all responses estimated by PLR increased across trials (F (1, 30) = 13.44, $p < .001$). As for SegcurveN, only the time breakpoint for V_E response increased (F (1, 30) = 7.92, $p < .01$). Using Sigmaplot, no significant changes were observed for V_E, whereas the time breakpoint for the V_T response decreased, while that for RR response increased (respectively, $F(1, 30) = 5.11$, p = .03; $F(1, 30) = 11.60$, $p < .01$; Software Trial $F(4, 120) = 3.09$, p = .04, $\epsilon = .74$; $f^2 = .07$).

9.5 Discussion

In the present study, the time breakpoint of the ventilatory response during the rebreathing test was estimated using three software programs, PLR, SegcurveN, and Sigmaplot, which were employed in recent studies to analyze segmented linear regression and breakpoints. Accurately determining a breakpoint, in other words separating two phases in the ventilatory response to a hypercapnic challenge, is crucial to understand the ventilatory regulation mechanisms. We found that the location of the breakpoint differed depending on the segmented regression approaches as applied by these programs. The time breakpoint using Sigmaplot was significantly earlier than the others. Moreover, PLR and SegcurevN did not distinguish between the breakpoint for V_T and V_E, whereas Sigmaplot did. Also, Sigmaplot identified a difference between the breakpoint of V_T and RR and a relatively different evolution across trials, whereas the other two did not.

Two strategies can be used to determine the relative value of these programs. On the one hand, an analysis of the soundness of the different underlying mathematical assumptions and algorithms can be carried out to arrive at a mathematically based normative judgment' on this relative

value. On the other hand, the findings can be compared with a physiological model of what should be found to arrive at a physiologically based judgment. We will follow the latter strategy.

Based on the "Oxford model", a ventilatory response to a gradually increasing stimulus of CO_2 comprises two phases: a constant phase (basal ventilation) and an increasing ventilation phase (ventilatory regulation). However, recent studies on the breakpoint of the different components of the ventilatory response have demonstrated that two breakpoints may be distinguished: a first response is related to an increasing V_T response, and a second one, somewhat later, implying a response in RR (Duffin et al., 2000; Katzman et al., 2002). In this study, we only focused on the first breakpoint of the V_E response, which separated basal ventilation from the ventilatory regulation in terms of "Oxford model". Therefore, in the present study, V_E and V_T response should theoretically have the same breakpoint at an earlier moment than that for RR response. The estimations of PLR and SegcurveN were consistent with this by not distinguishing between the time breakpoints for V_E and V_T response. However, the breakpoint of V_E response estimated by Sigmaplot was in the middle of the breakpoint of V_T and RR response. This might be the result of the limitation enforced on the program to find only one breakpoint in the rising limb of a curve containing two breakpoints.

Sigmaplot also identified changes over trials, such as an increase in the breakpoint for V_T and a decrease in the breakpoint for RR, whereas the other two methods did not establish these changes over trials. Dynamic changes in the respiratory response over trials (or, in other words, changes as a result of repeated experiences with breathlessness) have not been investigated systematically before, except by Wan et al. 2006 using PLR. As it is theoretically logical to assume that participants - anticipating breathlessness - learn to cope with the challenge by altering their respiratory behavior, we believe that Sigmaplot correctly identifies these changes, whereas the other two approaches miss them.

The fact that Sigmaplot appears more able to detect true changes may not come as a surprise: During the curve fitting procedure of Sigmaplot, each result of estimation is verified by checking the graph and to inspect the R2 value, and p value of the model. This verification helps the experimenter adjust the initial parameters for the next estimation. This allows the experimenter to interact with the program to get the best-fit breakpoint. Obviously this interactive character has benefits and drawbacks: it is more time consuming and it also makes room for experimenter bias to play. In STATISTICA and SegcurveN, all analyses are performed without

involvement of the experimenter.

In summary, different computer programs estimating the time breakpoint for ventilatory response during the rebreathing test produced quite different results. PLR and SegcurveN appears more conservative, whereas Sigmaplot is more sensitive at the cost of being more time consuming to apply and more open to experimenter bias. Additional testing, also as regards the mathematical assumptions, will be required to evaluate the estimation of different techniques in order to improve the accuracy of the breakpoint estimations of ventilatory response to hypercapnia.

Part IV

The present project examined the dynamics of air hunger perception and ventilatory response to hypercapnic challenges by means of a partly new paradigm. In these studies, repeated rebreathing trials were administered to high and low trait-anxious women to provide a gradually increasing CO_2 stimulus. Our modification of the paradigm implied that air hunger was rated online, while ventilatory behavior was measured continuously. Overall, the results provide support for the view that the perception of air hunger as well as ventilatory behavior is influenced by anxiety and psychological processes such as anticipation. In addition, it is interesting to note that some characteristics of air hunger perception, which had not been investigated previously, were revealed in the present project. First, it was consistently found that the threshold for air hunger perception is prior to the ventilatory response. Second, significant effects of repetition and anxiety were found providing further evidence for the role of psychological processes on the mechanisms of air hunger. This modified paradigm appears to be an interesting and powerful tool to analyze the complex interactions between physiological and psychological processes involved in the mechanisms of air hunger. Future studies with more precise design would help to clarify this issue.

Chapter 10

General Discussion and Conclusions

In this chapter, the conclusions of the studies investigating air hunger and the ventilatory response to repeated hypercapnic challenges will be discussed in the first , second and third section. The findings from the methodological investigation, regarding the application of different rebreathing tests, different subjective rating techniques, and different data-analysis methods, will be discussed in the fourth section. The last two sections are related to the limitation and future perspectives.

In the present studies, the rebreathing test was administered to induce the ventilatory and air hunger response to hypercapnia in healthy participants. Air hunger was rated by the participants simultaneously during the whole rebreathing test. Previous studies measured the perception of air hunger at several moments (e.g. every 30 second or 1 minute) during a breathing trial, which does not allow to determine exactly the start and full course of air hunger perception (Banzett et al, 1989; Bijl-Hofland et al., 2000; Moosavi et al, 2003). Other studies measured air hunger perception continuously but did not relate it to the changes in ventilatory behavior, which does not allow assessing the association between air hunger and ventilatory behavior (Flume et al., 1994; Rassovsky et al., 2006). Also, the effect of repetition nor the effect of some personality variables were investigated in previous studies on air hunger perception and ventilatory response (Moosavi et al., 2004).

The present paradigm explores the mechanism of air hunger more com-

prehensively than previous studies did. Generally, we administered three subsequent trials and divided the group in persons with high and low trait anxiety. The parallel and continuous measurement of perceived air hunger and ventilatory behavior allowed investigating the subjective and physiological responses and their relationship, and how these were modified by experience/anticipation in different trait anxiety groups. The project generated a number of new insights which will be summarized here.

10.1 The Threshold for Air Hunger and Ventilatory Response

The present project was the first to apply the rebreathing test in combination with an online air hunger rating to monitor both the subjective and physiological response to a hypercapnic challenge. The gradually rising CO_2 stimulus triggers both a physiological response, which act as the ventilatory regulation including changes of tidal volume and respiratory rate, and a subjective response which is perceived air hunger. The time and $FetCO_2$ level at which air hunger and ventilatory response were triggered were interpreted as the threshold for these responses.

10.1.1 Which one is triggered first, air hunger perception or ventilatory regulation?

Study 1 and 2 compared the thresholds of air hunger perception and ventilatory regulation response. Ventilatory behavior is hypothesized to be involved in the production of air hunger via the projection of respiratory drive from the respiratory centers to the sensory cortex (Banzett et al., 1989; 1990; Shea et al., 1993). This model suggests that perceived air hunger follows in time the triggering of the respiratory drive. However, in our two studies, the threshold for air hunger was lower than that for the initiation of the ventilatory regulation. The lower threshold for the perceived air hunger response implies that air hunger is perceived before the ventilatory regulation was triggered, particularly during the first exposure to hypercapnia. Assuming that perceived air hunger is, as suggested by Banzett et al., (1990), the result of the corrollary activation of the sensory cortex by the activation of the respiratory drive, this might mean that the time course to activate the sensory cortext is shorter than the time course to activate the respiratory muscles. Alternatively, other factors related to

perceptual-cognitive processes involved in symptom perception that are in part independent from the corrollary activation of the sensory cortex by the respiratory centers, may explain this finding. Also methodological reasons for this difference in threshold should be further excluded: it may be that the typically larger error variance in the respiratory measures compared to the air hunger ratings has led to a more conservative estimation of the threshold for the respiratory parameters.

There is no other study comparing the thresholds for subjective and physiological response to compare our results with. Other research techniques (e.g. brain imaging techniques) may be interesting to confirm our observation. As for now, we believe that our finding makes sense. The fact that air hunger is triggered as an alarming signal for a disturbance of blood gases before the occurrence of the ventilatory regulatory response may offer important behavioral advantages: it may signal the organism timely to look out for other behavioral options in addition to changing ventilation, such as leaving the place, looking out for fresh air, etc. Because the breathing system is open to both automatic and voluntary control, it may also have impact upon ventilation itself, such as deliberately gasp for air. Accordingly, the early perception of air hunger might play an important role to increase the behavioral repertoire of the organism to deal with dysregulation of blood gas.

In this respect, it is conceivable that anticipation of air hunger induces ventilatory (and behavioral) responses to an expected challenge before the challenge actually occurs. In addition, anticipatory processes have been shown to be able to induce the dyspneic perceptions themselves, especially in high trait anxious persons : Several studies in our group have shown that the perception of dyspnea could be conditioned to a harmless odor alone (Van den Bergh et al., 1997; Van den Bergh et al., 2001; De Peuter et al., 2005). In this perspective, it is also conceivable that the level of anxiety induced by air hunger which is higher in high trait anxious persons - may have impact upon both the immediate (respondent) and anticipatory breathing regulation. Moreover, air hunger has been shown to be related to anxiety in individuals with normal pulmonary function (ATS, 1999). All this suggests that both trait anxiety and repetition and their interaction may impact upon both breathing regulation and air hunger perception in response to a hypercapnic stimulus.

10.1.2 The effects of repetition and anxiety on the thresholds

The effects of repetition and anxiety on the threshold of air hunger perception and ventilatory response were another interesting part in the present project. Overall, little systematic evidence was found for habituation nor sensitization of the air hunger threshold, neither as a main effect nor in interaction with trait anxiety. Also no systematic evidence was found for habituation nor sensitization in any of the respiratory thresholds. The only evidence we found was for habitutaion of the threshold for the RR in Study 2. However, several dynamic changes occurred as regards the relative distances between the thresholds of respiratory and air hunger thresholds. For example, after repeated exposures to a hypercapnic challenge, the difference in time threshold at which air hunger and tidal volume response were triggered, diminished across trials (see Fig6.2). The threshold for tidal volume across trials became lower to approach the threshold for dyspnea in the high trait anxiety group. Conversely, in the low trait anxiety group, the threshold for dyspnea became higher to approach the threshold for the tidal volume response (Wan et al., submitted (a)).

As mentioned in the previous section, the early perception of air hunger might evoke the changes in ventilatory behavior. With the effect of repetition, the increase in ventilatory behavior occurred sooner in high anxious participants after perceiving air hunger, whereas, the ventilatory regulation in low anxious participants kept stable. This might be because perceived air hunger may have been more interpreted as a dangerous signal by the high anxious participants compared to low anxious participants, which may have sensitized the ventilatory regulation. We just finished an experiment intended to disentangle the aspects of distress and intensity in the air hunger sensation, but cannot report on the data yet.

10.2 The Sensitivity of Ventilatory Response to Hypercapnia

The sensitivities of the tidal volume and respiratory rate responses were not significantly different between high and low anxious participants in study 1, 2, 3, and 4. However, a significant increase in the sensitivity of respiratory rate response across trials was found in study 2, 3 and 4. This result sensitization of the sensitivity - means that per unit increase in end-tidal CO_2, the respiratory rate increased faster across trials.

Typically, in a hypercapnic situation, the regulation of ventilation starts by increasing the depth of breathing, and, subsequently, by increasing respiratory rate. However, the sensitization of sensitivity across trials was only observed in the respiratory. The steeper increase in RR may contribute to the increased maximal tolerance for hypercapnia. A decline of the intensity of air hunger due to the changes in ventilation was observed in some studies (Manning et al., 1992; Bloch-Salisbury et al., 1998), but in those studies, relief of air hunger was mainly realized by altering tidal volume . So, whether the sensitization of RR found in the present studies has caused the habituation of perceived air hunger needs to be tested in more detail.

10.3 The Association between Air Hunger and Respiratory Behavior

10.3.1 The linear regression of air hunger response vs. the stimulus of CO_2

In studies 1 to 4, plotting air hunger rating against $FetCO_2$, we calculated the linear relationship between the air hunger perception and the hypercapnic stimulus. Also, across-trial decreases were observed not only in the sensitivity (slope) of perceived dyspnea to CO_2 stimulus but also in the maximal tolerance for dyspnea (Wan et al., 2006; submitted (a)). The decrease in the slopes across trials /over time indicates that for the same change in the CO_2 stimulus the corresponding change in air hunger declined. This indicates that, as Fig4.1 shows, besides the stimulus of CO_2, the air hunger perception is gradually more modulated by factors such as ventilatory behavior, anxiety, and repetition.

10.3.2 The association between air hunger and ventilatory behavior (V_T and RR)

The repetition effect on the association of air hunger and ventilatory behavior illustrates the interaction of physiological mechanisms and psychological factors of air hunger. While the pressure of CO_2 in the blood increases, air hunger is perceived and the regulation of the ventilatory response is triggered to exhale more CO_2. This process of regulation intends to reduce the blood CO_2 levels and the intensity of air hunger. In our studies, the association between air hunger and ventilatory behavior

(V_T and RR) during the process of regulation was fitted a linear model. The B coefficient shows how much the intensity of air hunger changed per unit change in the regulation of ventilatory behavior. The Beta coefficient is the standardized B and shows how well ventilatory behavior correlates to the perception of air hunger. The Beta coefficient allows a comparison between variables that have different measurement units. Therefore, the change in the B and Beta coefficients represents the modification of the ventilatory regulation in relation to air hunger perception.

An interesting observation is the lower association between air hunger perception and ventilatory behavior in high anxious participants compared to the low anxious participants. These findings are consistent with previous research of our group on interoceptive accuracy. Typically, the within-subject correlation between a subjective sensation and a clear physiological referent is lower in persons with high trait anxiety, especially when they are in a distressing context (Van den Bergh et al., 2004; Bogaerts et al, 2005). This finding suggests that the air hunger rating in high anxious participants is, more than in low anxious participants, influenced by emotional cues that weaken the relationship of air hunger with behavioral responses such as RR.

10.4 Effects of Specific Protocols on Air Hunger and Ventilatory Responses

As a new paradigm to monitor both subjective and physiological responses to hypercapnia simultaneously, several methodological questions emerged. Indeed, we first applied the modified rebreathing test and used a discrete rating. In a second experiment, we wanted to improve our paradigm by replacing the modified rebreathing test with the standard rebreathing test in order to avoid the effect of hyperventilation on the perception of air hunger. We also changed the cued (discrete) rating to the uncued (continuous) one, hoping to more accurately measure the absolute threshold of air hunger. However, none of these methodological issues was ever explicitly investigated before. So, in the course of our project, we wanted to contribute to a better understanding of the implications of some of these methodological features. Firstly, we explicitly compared the rebreating test and its modified version. The latter was intended to improve the methodology solely from the physiological perspective, without considering potential implications for perceived air hunger. Secondly, we explicitly

compared the cued and uncued air hunger rating. The influence of the procedural changes on air hunger and ventilatory response was investigated in study 3 and 4. Finally, in study 5, we compared different software packages that have been (or can be) used to determine the thresholds for the ventilatory response.

10.4.1 The effect of VH

The major difference between the original and the modified rebreathing test is the addition of a prior VH in the latter. This apparently reduced the association between air hunger and respiratory behavior in the MRT group (Study 3/Chapter 7). The lower association between air hunger and respiratory behavior in the MRT group shows that the effect of physiological mechanisms on air hunger is reduced after VH. Participants in the MRT group also perceived lower air hunger than the other group. In addition, in the MRT group compared to the RT group, we observed that the role of the psychological variables, such as trait anxiety and repetition, became significant for state anxiety and air hunger. We assumed that these effects are caused by an anchoring effect of one aversive subjective experience on a subsequent one: a strong forced respiratory challenge such as HV may make a subsequent slow and gradually increasing challenge, such as hypercapnic overbreathing, comparatively less aversive. The MRT group with prior VH experienced less air hunger and anxiety during rebreathing than the RT group without differences in the CO_2 stimulus and the sensitivity of ventilatory response to hypercapnia. This effect was observed in both air hunger and state anxiety in the MRT group regardless of the increase of CO_2 stimulus across trials. So, it appears that, as a result of the anchoring effect, other variables, such as the difference between high and low trait-anxiety participants, gained more importance in determining air hunger in the MRT group compared to the RT group.

10.4.2 The effect of auditory cues

Besides the observation that participants in the cued rating rated their sensation of air hunger more frequently than those without auditory cues, the different rating procedures to assess online air hunger produced some unexpected findings as well. For one thing, the threshold for the V_T response of the cued group was earlier than that of the uncued group. Another finding was that the changes in air hunger and ventilatory behavior across trials were different in the uncued and cued group. Most

changes, especially in respiratory behavior, were only found in the uncued group.

These differences document how sensitive an objective measure may be to contextual and behavioral influences. On the one hand, the cued rating might induce a cognitive task set, which might cause a continuous vigilance of the participants to the sounds and emit a response every 12 sec. Thereby, the breathing behavior might be stabilized because of the constant cognitive task set. On the other hand, an uncued rating may leave more room for attentional distraction and/or free floating emotional concerns about the effects of the respiratory challenge, which might result in more versatile and flexible breathing response to repeated hypercapnia.

10.4.3 Estimating the breakpoint of the ventilatory response: methodological issues

In the last study of the present project, three computer programs offering different segmented regression techniques were applied to identify the breakpoint between two segments. The estimation of the time breakpoint for ventilatory response during the rebreathing test was affected by the type of segmented regression approaches. The time breakpoint estimated by Sigmaplot was earlier than the others. PLR and Segcurve may be more conservative than Sigmaplot as shown by the findings of the time breakpoint of V_T and V_E. Conversely, the time breakpoint of V_T and RR response and their changes across trials illustrated that Sigmaplot may have higher responsiveness than PLR and Segcurve. Therefore, compared to PLR and Segcurve, Sigmaplot may be the more suitable technique to estimate the time breakpoint for ventilatory response during rebreathing in the repeated measures design. Additional testing will be required to evaluate the estimation of different techniques in order to improve the accuracy of determination of breakpoints of the ventilatory response to hypercapnia.

10.5 Limitations

Only in the final stages of the present project, we became aware that the software program used to detect thresholds could have an important impact upon the results. However, not every dataset we collected has been treated with the same program. This makes the comparability of some results problematic. For example, in study 1, curves of air hunger

and ventilatory response against $FetCO_2$ were fitted with a two-segment model and the CO_2 thresholds were estimated by a computer program (PLR). Therefore, the threshold for air hunger response was estimated by software program regardless the intensity of air hunger at the breakpoint. As a result, the threshold for air hunger perception in study 1 was probably not the smallest intensity of perceived air hunger during rebreathing. Further work is needed to arrive at a general standard to analyze such datasets in order to improve comparability of the results.

Another limitation of the present project is the sample. The participants across our studies were only healthy young women. The group size of study 1 and 2 was not particularly large either. So, the present findings need to be tested in larger samples of both men and women of a wider age range.

10.6 Future Perspectives

Based on the present project, more interesting perspectives for future research will be offered with respect to the following aspects.

10.6.1 Dyspnea and ventilatory behavior in panic disorder patients

Dyspnea is a typical symptom in panic disorder without any cardiopulmonary. Several hypotheses of behavioral and physiologic hypersensitivity to inhaled CO_2 were set up to explain the etiology of dyspnea associated with anxiety. The investigation on the dynamics of perceived dyspnea and ventilatory behavior may shed light on the underlying mechanisms. Besides testing potential differences in thresholds for the breathing responses (which has been advanced as a hypothesis to explain breathing abnormalities in panic disorder patients), we expect sensitization of the affective aspect of dyspnea to a much higher degree as compared to anxious healthy controls and a much weaker relationship between breathing variables and perceived dyspnea. This would suggest a particularly strong influence of psychological factors in their dyspnea complaints.

10.6.2 Multiple effects on the perception of dyspnea

Future studies need to focus on identifying which psychological processes may affect perceived dyspnea across repeated trials. For example, up to

now we focused on trait anxiety, but experimentally manipulating state anxiety would be an important extension and approach of particular processes. We expect that the threshold for perceived dyspnea and ventilatory regulation increase while state anxiety decreases across trials. Moreover, dyspnea ratings as we used up to now refer to both a sensory (intensity) aspect and an emotional (distressing) aspect. At present, we have assessed separately both the sensory and affective dimension of perceived dyspnea. At present, we are processing the data and cannot report on the findings yet. We expect little difference in the intensity aspect of the sensation, neither as a result of repetition, nor as a result of different anxiety levels. However, we expect much more pronounced effects in relation to both these factors as regards the affective aspect. Particularly, we expect an interaction of anxiety by repetition, namely sensitization in high anxious persons and habituation in low anxious persons. Further studies could dig more in detail into processes such as catastrophizing, etc. (De Peuter et al., in press).

10.6.3 Brain imaging

Given my prior experience with functional brain imaging techniques (see my publication list), experimental induction of functional dyspnea would allow determining brain structures that subserve its experience. Findings in recent human brain-imaging work with positron emission tomography (PET) or functional magnetic resonance imaging (fMRI) suggested that the limbic system, a main nervous system involved in emotions, is also involved in the unpleasant sensation of air hunger (Guz, 1997; Evans et al., 2002). Future studies on the dyspnea-related activities in central neural system using the technique of brain imaging are another critical aspect to explore the mechanisms of dyspnea.

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Appendices

Appendix A: Instruction for discrete air hunger measurement

Instruction for discrete air hunger measurement

. . . Please start rating the intensity of your breathing sensation following the auditory cues. During this period, please pay attention to your respiratory sensation, not your respiratory behavior. In particular, we want you to notice this kind of sensation related to breathing: the sense of air hunger. We define the sense of air hunger as the discomfort caused by your urge to breathe, a feeling of being starve for air. Have you ever held your breath for a long time? How did that feel? (Similarity was discussed.) The sensation of air hunger may come and go throughout the experiment. Sometimes it is possible that you will not feel an air hunger sensation at all. As soon as you hear the auditory cues, please click on the square next to the verbal label that most closely describes your actual sensation at that moment. If you choose 100 it means air hunger is intolerable and we will immediately stop the trial to make you more comfortable. You can select this any time you want . . .

Instructies voor discrete meting van ademnood

. . . Start met het raten van uw ademhalingsensatie na een auditieve cue (het toontje). Het is de bedoeling dat je gedurende deze periode aandacht schenkt aan je ademhalingsensaties en niet aan je ademhalingsgedrag. In het bijzonder het gevoel van ademnood. We definiren ademnood als een onaangenaam gevoel veroorzaakt door een drang naar ademhaling, het snakken naar lucht. Heb je al ooit je adem ingehouden voor een lange tijd? Hoe voelde dat? (gelijkenissen worden besproken). Het gevoel van ademnood kan komen en gaan gedurende het experiment. Soms is het zelfs mogelijk dat u helemaal geen ademnood ervaart. Het is de bedoeling dat je na het toontje klikt op het vierkantje naast het verbale label dat het meest overeenkomt met je sensaties op dat moment. Wanneer je op 100 duwt betekent dit dat de ademnood onverdraagzaam is en zullen we dus onmiddellijk stoppen met de trail. Je kan 100 aanklikken wanneer je wil . . .

Appendix B: Instruction for continuous air hunger measurement

Instruction for continuous air hunger measurement

. . . Please start rating the intensity of your breathing sensation whe you see the rating square in the screen. During this period, please pay attention to your respiratory sensation, not your respiratory behaviours. In particular, we want you to notice this kind of sensation related to breathing: the sense of air hunger. We define the sense of air hunger as the discomfort caused by your urge to breathe, a feeling of being starve for air. Have you ever held your breath for a long time? How did that feel? (Similarity was discussed.) The sensation of air hunger may come and go throughout the experiment. Sometimes it is possible that you will not feel an air hunger sensation at all. At any time when you experience a change in your air hunger please click on the square next to the verbal label that most closely describes your actual sensation at that moment. If you choose 100 it means air hunger is intolerable and we will immediately make a change to make you more comfortable. You can select this any time you want . . .

Instructies voor de continue meting van ademnood

. . . Start met het raten van uw ademhalinssensaties wanneer je het ratingsvierkant op het scherm ziet. Het is de bedoeling dat je gedurende deze periode aandacht schenkt aan je ademhalingsensaties en niet aan je ademhalingsgedrag. In het bijzonder het gevoel van ademnood. We definiren ademnood als een onaangenaam gevoel veroorzaakt door een drang naar ademhaling., het snakken naar lucht. Heb je al ooit je adem ingehouden voor een lange tijd? Hoe voelde dat? (gelijkenissen worden besproken). Het gevoel van ademnood kan komen en gaan gedurende het experiment. Soms is het zelfs mogelijk dat u helemaal geen ademnood ervaart. Het is de bedoeling dat je wanneer je een verandering in je ademhalingsensaties gewaarwordt op het vierkantje klikt naast het verbale label dat het meest overeenkomt met je sensaties op dat moment. Wanneer je op 100 duwt betekent dit dat de ademnood onverdraagzaam is en zullen we dus onmiddellijk stoppen met de trail. Je kan 100 aanklikken wanneer je wil . . .

Appendix C: Trait version of the State-Trait-Anxiety-Inventory

I. vragenlijst 1: nummers 1 tot en met 20

De eerste vragenlijst bestaat uit 20 uitspraken die door mensen zijn gebruikt om zichzelf te beschrijven. Lees iedere uitspraak en geef aan hoe jij je doorgaans voelt. Er zijn geen goede of slechte antwoorden. Denk niet te lang na en geef je eerste indruk. Het gaat er dus om dat je bij deze vragenlijst weergeeft hoe je je in het algemeen (doorgaans) voelt. Je kan antwoorden door het antwoordalternatief aan te duiden dat het best van toepassing is voor jou.

A = bijna nooit, B = soms, C = vaak, D = bijna altijd. Je kan dus enkel met A, B, C of D antwoorden.

		A bijna nooit	B soms	C vaak	D bijna altijd
1.	Ik voel me prettig				
2.	Ik voel me nerveus en onrustig				
3.	Ik voel me tevreden				
4.	Ik kan een tegenslag maar heel moeilijk verwerken				
5.	Ik voel me in vrijwel alles tekort schieten				
6.	Ik voel me uitgerust				
7.	Ik voel me rustig en beheerst				
8.	Ik voel dat de moeilijkheden zich opstapelen zodat ik er niet meer tegenop kan				
9.	Ik pieker teveel over dingen die niet zo belangrijk zijn				
10.	Ik ben gelukkig				
11.	Ik word geplaagd door storende gedachten				
12.	Ik heb gebrek aan zelfvertrouwen				
13.	Ik voel me veilig				
14.	Ik voel me op mijn gemak				
15.	Ik ben gelijkmatig van stemming				
16.	Ik ben tevreden				
17.	Er zijn gedachten die ik heel moeilijk kan loslaten				
18.	Ik neem teleurstellingen zo zwaar op dat ik ze niet van me af kan zetten				
19.	Ik ben een rustig iemand				
20.	Ik raak helemaal gespannen en in beroering als ik denk aan mijn zorgen van de laatste tijd				

Appendix D: The scale of State Anxiety and Fatigue

II. vragenlijst 3:

Welke mate van angstigheid heb je ervaren tijdens deze trail?

A = Helemaal niet, B = Een klein beetje, C = In enige mate, maar niet sterk (duidelijk gevoeld, maar niet erg sterk; ik zou het zo nog gedurende lange tijd kunnen uithouden), D = Middelmatig, E = Redelijk (zeer duidelijk gevoeld, maar ik zou het toch nog verscheidene minuten kunnen uithouden zo), F = Sterk, G = Heel sterk (De angst heeft een maximum niveau bereikt)

	A Helemaal niet	B Een klein beetje	C In enige mate	D Middelmatig	E Redelijk	F Sterk	G Heel sterk
Trial 1							
Trial 2							
Trial 3							

III. vragenlijst 4:

Welke mate van vermoeidheid heb je ervaren tijdens deze trail?

A = Helemaal niet (Ik voelde niets van vermoeidheid), B = Een klein beetje, C = In enige mate, maar niet sterk (duidelijk gevoeld, maar niet erg sterk; ik zou het zo nog gedurende lange tijd kunnen uithouden, D = Middelmatig, E = Redelijk (zeer duidelijk gevoeld, maar ik zou het toch nog verscheidene minuten kunnen uithouden zo), F = Sterk, G = Heel sterk (De vermoeidheid heeft een maximum niveau bereikt)

	A Helemaal niet	B Een klein beetje	C In enige mate	D Middelmatig	E Redelijk	F Sterk	G Heel sterk
Trial 1							
Trial 2							
Trial 3							

Appendix E: Instruction for State Anxiety and Fatigue measurement

Instruction for State Anxiety and Fatigue measurement

... We will be asking you to rate the maximal intensity of your emotional state and fatigue during the trial. In particular, we want you to notice these two kinds of sensations related to the breathing test. We define the sense of anxiety as "right now" feelings of nervousness, worry, and apprehension associated with arousal of the body, which change from moment to moment. Fatigue is a feeling of weariness, tiredness, or lack of energy. The different sensations may change together at times and go in opposite directions at other times. It is possible that you will not feel a sensation throughout the experiment ...

Instructies voor de toestandsangst en de moeheid meting

... Wij vragen u om de maximale intensiteit van je emotionele status en vermoeidheid dat u gedurende een trail ervaart te noteren. In het bijzonder vragen we u deze twee sensaties die gerelateerd zijn aan de ademhalingstest. We definiren angst als een gevoel van nervositeit, bezorgdheid en vrees die je op het moment van het experiment ervaart en die geassocieerd worden met de opwinding van het lichaam, deze veranderen van moment tot moment. Vermoeidheid is een gevoel van moe worden of een gebrek aan energie. De verschillende sensaties kunnen samen veranderen maar kunnen ook in tegenovergestelde richting veranderen. Het is ook mogelijk dat u geen sensaties ervaart gedurende het experiment ...

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