Fear-conditioned respiration and its association to cardiac reactivity

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1. Introduction

Both clinical phenomenology and theory highlight the centrality of respiratory behavior and respiratory distress in states of fear (e.g., Ley, 1985; Gorman et al., 2000; Sinha et al., 2000; Wilhelm et al., 2001). Despite this, ventilatory correlates of conditioned fear responses in humans are not well documented, perhaps because, compared to other psychophysiological measures of learning (e.g., heart rate), changes in the mechanics of respiration are slower and more variable. A more tractable respiratory parameter, however, may be carbon dioxide pressure, since, in many circumstances, healthy subjects are able to regulate arterial carbon dioxide pressure, maintaining it within relatively narrow limits. As a consequence, normal breathing is characterized by little variability in carbon dioxide pressure, maintaining it within relatively narrow limits. As a consequence, normal breathing is characterized by little variability in carbon dioxide pressure (Sinha, 1997), compared to the variability in respiratory timing and volume parameters (Bruce and Daubenspeck, 1995). In the current study, we assessed end-tidal carbon dioxide pressure (PetCO$_2$, a valid approximation of arterial CO$_2$ pressure; Gardner, 1996; Pahn et al., 1987) during affective conditioning. PetCO$_2$ is thought to be less variable than timing and volume parameters of breathing. Moreover, it is the only relevant outcome measure in the context of hyperventilation, which reflects a breathing pattern in excess of metabolic needs that occurs during emotional arousal, particularly fear (Van Diest et al., 2001a,b, 2005).

The control of breathing is a complex interplay that relies on many factors, including the bulbopontine respiratory network, central and peripheral chemoreceptor control, modulation of respiratory muscles by mechanoreceptors, and numerous suprapontine networks located in the limbic, cerebellar, and cortical areas (Gallego et al., 2001; Shea, 1996). It is not currently known how these different networks interact during an emotional event and in which measures they are reflected. Addressing this concern, Boiten (1993, 1998) suggested that, in addition to assessing traditional respiratory parameters of depth (tidal volume) and rate (total cycle time, inspiratory time, expiratory time and post-expiratory pause) of breathing, compound measures should also be pursued. One such parameter is the ratio of inspiratory time to the total breathing cycle time (inspiratory duty cycle), which reflects the cyclical on/off switching of the central inspiratory drive mechanism (Gautier, 1980). Several authors suggest that, in contrast to volume parameters, this timing parameter is weakly controlled by the chemical drive to breathe, allowing for its modulation by non-metabolic factors (Gallego et al., 1996; Rafferty and Gardner, 1996).

Thus, to the extent that respiratory changes in anticipation of an aversive event are not driven by changes in metabolism, one would expect to see effects of affective learning on timing, compared to volume parameters. This is exactly what has been observed in studies of humans using a standard aversive conditioning paradigm (e.g., tone CS and shock US; Ley, 1999). Obrist (1968)
described important interindividual differences in the direction of the conditioned breathing responses: whereas most participants showed a slightly increased breathing frequency following conditioning, a subgroup of participants showed a marked decrease in respiratory activity in response to the CS. The latter also showed a more sustained conditioned cardiac deceleration.

Similar to respiration, fear-conditioned heart rate responses also show different patterns among individuals (Hamm and Vaitl, 1996; Hodes et al., 1985; Moratti and Keil, 2005). Heart rate decreases or increases have been interpreted as distinguishing between defensive attention and fear in the context of the defense cascade model (Lang et al., 1997). This model describes an aversive motivational circuit that, with increasing arousal, triggers reactions ranging from orienting to flight/flight. The associated autonomic and somatic responses can be functionally organized into two broad output classes of defensive immobility and attention (i.e., freezing and hypervigilance in which the organism is passive, but primed to respond) and defensive action (contextual variations in flight/flight that are more or less direct responses to nociception or imminent attack).

Whereas defensive attention is associated with cardiac deceleration, cardiac acceleration prepares the organism to actively escape from an imminent threat, and previous studies have found that different individuals show different cardiac reactions following aversive conditioning (Hamm and Vaitl, 1996; Hodes et al., 1985). For instance, when a picture was paired with a loud noise, participants could be clustered based on their conditioned heart rate responses, which consisted of acceleration or deceleration (Hodes et al., 1985). Compared to individuals showing cardiac deceleration, individuals showing a conditioned cardiovascular acceleration to the CS+, also reported greater fear to the CS+ than the CS− (Hodes et al., 1985), showed more resistance to extinction in electrodermal responses (Hodes et al., 1985) and displayed potentiated startle responses to the CS+ (Hamm and Vaitl, 1996). This pattern of results is interpreted as indicating that, whereas all participants learned an anticipatory orienting response (late interval heart rate deceleration), only accelerators showed a conditioned preparation for defensive action.

Several findings from other studies suggest that respiration also distinguishes between defensive attention and action. Decreases in breathing frequency and/or a tendency towards expiration (i.e., decreased inspiratory duty cycle time) have been reported as part of the orienting response and during sustained attention in humans (Barry, 1982; Boiten et al., 1994; Denot-Ledunois et al., 1998; Obrist et al., 1969; Stekelenburg and Van Boxtel, 2001). This may either be a direct consequence of a decreased somatic activity during defensive immobility (Obrist et al., 1969), or, it may be functional to inhibit breathing under threat for two reasons: (1) prompting an increase in blood flow to the brain, due to the cerebrovascular dilating effect of CO2 (Giardino et al., 2007; Kastrup et al., 1998), or (2) suppressing the noise associated with breathing in the presence of a predator (Fokkema, 1999). Defensive action, on the other hand, seems to prompt an increased cardiorespiratory activation, as well as a tendency towards hyperventilation (decreased PetCO2; Van Diest et al., 2001a,b).

The present study aimed to investigate fear conditioning of breathing behavior. To this end, respiratory and PetCO2, as well as heart rate responses, were studied during a prototypical affective learning paradigm. Pictures of human faces served as the conditioned stimulus (CS), a loud human scream as the unconditioned stimulus (US). We expected that differential conditioning of respiratory timing parameters, PetCO2 and heart rate would be established during acquisition and dissipate during a subsequent extinction phase. In addition, we expected that heart rate responses would closely follow respiratory timing and PetCO2 responses.

### 2. Method

#### 2.1. Participants

Forty-two students aged 18−31 years old (mean age 20 years; 24 women) from the University of Florida volunteered to participate in return for course credit in their General Psychology class.

#### 2.2. Design and materials

A differential delay-conditioning paradigm was used. Coloured pictures of neutral or angry male faces (“Karolinska Directed Emotional Faces”; Lundqvist et al., 1998) presented for 8 s served as conditioned stimuli. A 95−dB human scream (6 s duration) presented at picture offset was the unconditioned stimulus. The intertrial interval (ITI) was 80 s. For half of the participants, the US was preceded by an angry face (CS+), while the neutral face remained unpaired with the US (CS−). This was reversed for the other half. The expression of the CS faces was primarily manipulated for exploratory reasons. The expression of the CS+/CS− had no effect on the establishment of conditioning in any of the cardio-respiratory measures, and thus the rationale and interpretation of this variable are not central to the primary aims of this study.

The experiment consisted of an acquisition (six trials of CS+/CS−, with CS+ followed by the US) and an extinction (four CS+/4CS−, no US) phase. Trial types (CS+/CS−) were presented in a semi-randomized order. Extinction always started with a CS+ trial.

#### 2.3. Measures

VPM software (Cook et al., 1987) was used for physiological recordings. The ECG was obtained using standard Ag/AgCl electrodes placed on each forearm. The signal was sampled at 1000 Hz and transduced, amplified and filtered through a Coulbourn S75-05 Isolated Bioamplifier. A Dual Comparator/Window Discriminator (Coulbourn S21-10) detected the R-waves in the ECG.

Respiration was measured using an aneroid chest bellows (Coulbourn V94-19) strapped around the participant’s chest wall and connected to a differential aneroid pressure transducer (Coulbourn V94-05) and a DC coupler with a 7.5-V excitation voltage (Coulbourn V72-25). Carbon dioxide pressure in the inhaled and exhaled air was continuously measured using a nasal CO2-sampling cannula connected to a non-dispersive infrared CO2-monitor (Datex 223 CO2 monitor, Pruiton-Bennet Worp., Wilmington, MA). The CO2 signal was digitized at 25 Hz. A calibration procedure preceded each experimental session: a gas mixture containing 55-mmHg CO2 was sent to the capnograph and the corresponding A/D units were recorded, as well as the A/D corresponding to regular room air. This allowed for an off-line linear transformation yielding calibrated PetCO2 values.

Two types of custom-made pen and paper tools were employed. In the first tool, participants rated the CS pictures on pleasantness and arousal. To this end, a bipolar scale was presented below each picture, consisting of a horizontal line of 150 mm with at the extreme points the labels ‘completely happy’ and ‘completely unhappy’, or ‘completely calm’ and ‘completely excited’. Participants could indicate their feeling by putting a vertical bar on the scale. The distance in mm from the left extreme was used as a score of unpleasantness or arousal.

The second tool was a post-experimental questionnaire that asked whether participants at times had voluntarily controlled their breathing pattern during the presentation of the CS pictures. Participants could answer this question on a five-point Likert type scale ranging from ‘never’ to ‘all the time’.

#### 2.4. Procedure

After providing informed consent, participants rated the two pictures on pleasantness and arousal. Following this, the sensors were attached, as well as the respiratory belt and nose cannula. Participants were then instructed that a series of pictures would be presented and that they occasionally would hear an unpleasant loud noise that could be ignored. After this, the lights were dimmed and the experimenter left the room. Following the experiment, participants completed the post-experimental questionnaire and rated both CSs again on pleasantness and arousal.

#### 2.5. Data reduction and analyses

##### 2.5.1. Heart rate

Recorded R–R intervals were edited with an off-line program (VPM; Cook et al., 1987) and transformed to average beats/min for each half second. Heart rates per half second bins were averaged for each CS type (CS+ and CS−) within each block of two trials. Heart rate in the 1 s before picture onset was subtracted from the average heart rate for every 0.5 s after picture onset. Initial and secondary deceleration (D1 and D2) and midinterval acceleration (A) were scored according to Hodes et al. (1985):
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