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ScienceDirect
Biological Psychology 78 (2008) 87–92
BIOLOGICAL PSYCHOLOGY
www.elsevier.com/locate/biopsycho

# To inhale or not to inhale: Conditioned avoidance in breathing behavior in an odor—20% $CO_2$ paradigm

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Received 22 June 2007; accepted 20 January 2008
Available online 31 January 2008

**Abstract**

This study investigated breathing behavior in an odor—$CO_2$-inhalation fear conditioning paradigm. A differential conditioning paradigm was applied in 55 participants. Both acquisition and extinction consisted of three CS+ and three CS− trials. Diluted ammonia and butyric acid served as conditional odor cues (CSs); inhalation of 20% $CO_2$-enriched as US. The US was presented 10 s after CS+ onset and both stimuli co-terminated 30 s later. Subjective anxiety and US-expectancy were measured online upon presentation of the CSs. Respiratory behavior showed a biphasic pattern during CS+ acquisition trials. Participants paradoxically lowered their ventilation first; an increased ventilation was observed only towards the end of the trial. Extinction of this breathing inhibition was found. Participants avoiding the $CO_2$ during acquisition did not show a reduction in fear from acquisition to extinction, whereas Non-avoiders did. We conclude that paradoxical decreases in ventilation constitute a relevant behavioral index of fear in $CO_2$-inhalation paradigms.


*Keywords:* Respiration; Avoidance; Conditioning; $CO_2$-inhalation; Odors; Fear

Inhalation of $CO_2$-enriched air has been used repeatedly as an unconditional stimulus (US) in human conditioning fear paradigms (e.g., Acheson et al., 2007; Devriese et al., 2006; Forsyth and Eifert, 1998; Forsyth et al., 1996). The rationale to use $CO_2$ rather than more traditional, exteroceptive USs, such as electrocutaneous stimuli or white noise, is that $CO_2$ is more suitable as an analogue of the response patterns characteristic for patients diagnosed with anxiety disorders, especially panic (Forsyth et al., 1996; Griez et al., 2007). Depending on the concentration and the duration, inhalation of $CO_2$-enriched air causes escalating symptoms of arousal, mimicking to a certain extent the topography of human fear responses: increased breathing, dizziness, a sense of dyspnea (breathlessness), increased heart rate, reactive hyperemia, sweaty palms, feeling of unreality, etc. As such, $CO_2$-inhalation can be applied to experimentally induce a ''false alarm'', i.c., an abrupt autonomic activation in the absence of real threat or harm (Barlow, 1988; Forsyth and Eifert, 1996). Pairing such a false alarm ($CO_2$-inhalation) with internal/external neutral cues (conditional stimuli, CSs) in the laboratory may produce so-called ''learned alarms'', i.c., autonomic activation and subjective fear in response to these originally neutral cues.

Much of the work with this paradigm has been done by Forsyth and colleagues (Forsyth and Eifert, 1998; Forsyth et al., 1996) who studied traditional measures of fear conditioning, such as electrodermal responses, heart rate, subjective units of distress, and panic symptoms. In one of those studies (Forsyth and Eifert, 1998), video fragments varying in fear-relevance (snake, heart beating, and flowers) were paired with 20 s inhalations of 20% or 13% $CO_2$-enriched air. Evidence for stronger fear conditioning to the fear-relevant compared to the fear-irrelevant video fragments was found, both in the autonomic indices and the subjective reports.

Former studies from our laboratory typically applied odors or mental imagery as CSs and 2 min inhalations of 7.5% or 5.5% $CO_2$-enriched air as the US in a differential conditioning paradigm (for reviews, see: Van den Bergh et al., 2001, 2002). Results showed that participants easily learn to report bodily symptoms in response to an unpleasant CS+ odor after only three pairings of the respective odor with the $CO_2$-inhalation. Importantly, these effects tended to be more pronounced in participants scoring high on Negative Affectivity (NA)

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doi:10.1016/j.biopsycho.2008.01.003

(Devriese et al., 2000; Van den Bergh et al., 1998) and in psychosomatic patients (Van den Bergh et al., 1997). In some studies, also breathing behavior was conditioned, but the conditioning effect on respiration lacked consistency across studies (Van den Bergh et al., 1995, 1997). A first reason for this may be that relatively broad time windows (2 min) were used for the averaging of the breathing parameters, potentially masking transient, short-lived conditioned changes in breathing behavior. In addition, participants may differ in the way they show primarily instrumental or respondent conditioning of their breathing behavior. Whereas some may try to actively inhibit inhaling the $CO_2$ (leading to a conditioned instrumental decrease in breathing), others may show a respiratory CR similar to the unconditional effects of $CO_2$ (increased breathing). It can be expected that the avoidant pattern (decreased ventilation) is most prominent at the onset of a $CO_2$-inhalation, whereas the unconditional effects of $CO_2$ (increased ventilation) are more likely to occur towards the end of a trial.

Attempts to avoid $CO_2$-inhalation have been described both in humans (Lejeuz et al., 1998) and animals (Raj and Gregory, 1995). Nonetheless, most human fear conditioning studies using $CO_2$-inhalation have focused on respondent classical conditioning and have overlooked instrumental avoidance of the $CO_2$ as a potential behavioral index of fear. Particularly during inhalations of short durations (e.g., 20 s), trying not to inhale the $CO_2$ is likely an active way to cope with the aversive event.

A first aim of the current study was to provide a detailed description of changes in ventilation and fractional end-tidal $CO_2$ across time in a differential fear conditioning paradigm pairing an odor (CS) with $CO_2$-inhalation (US). A relative strong US (20% during 30 s) was opted for to maximize the chance to observe fear conditioning. We expected that avoidance in breathing behavior as indicated by a decrease in minute ventilation would develop across acquisition trials in response to the odor paired with the $CO_2$ (CS+), but not in response to the control odor (CS−).

A second aim was to explore the relation of avoidance behavior in respiration with subjective anxiety and US-expectancy. We expected more subjective anxiety and higher US-expectancy ratings in participants showing conditioned avoidance in breathing behavior compared to participants not avoiding the $CO_2$.

## 1. Method

### 1.1. Participants

Fifty-eight healthy participants (29 men and 29 women, 55 undergraduate students/3 community members, 53 Caucasian/5 Asian, mean age 22, age range 18–55 years) volunteered in return for course credit or 10€. Participants were only allowed to participate if they confirmed not to (have) suffer(ed) from any major respiratory or cardiac disease, epilepsy, or psychiatric disorder. Data from three participants were excluded from analyses because of technical problems.

### 1.2. Materials

#### 1.2.1. Subjective measures

Participants completed the Checklist of Psychosomatic Symptoms (CPS, Wientjes and Grossman, 1994) measuring the occurrence of 35 symptoms in daily life, the Anxiety Sensitivity Index (Reiss et al., 1986) and the Dutch version of the Positive and Negative Affect Schedule (Engelen et al., 2006) before the start of the experiment. Following each breathing trial, they completed a state version of the CPS, but the latter results are beyond the scope of this paper and will not be discussed. Upon presentation of the CS, participants rated their online anxiety (“How anxious are you now?”) and US-expectancy (“To what extent do you expect to experience bodily complaints in the present trial?”) on a 7-point bipolar scale [ranging from −3 (not at all) to +3 (very much)].

#### 1.2.2. Apparatus and software

Participants wore a $CO_2$ nasal sampling cannula and a face mask (8900 Series, Hans Rudolph™) connected to a flow meter (Fleish no. 2, Epalinges, Switzerland). Upstream from the latter device, a non-rebreathing valve ensured the separation of inspiratory and expiratory air. A vinyl tube (inner diameter: 3.5 cm; length 100 cm) connected the inspiratory side of the non-rebreathing valve with a three-way Y-valve (stopcock type). The latter enabled easy switching between room air and air from a meteorological balloon containing a decompressed mixture of 20% $CO_2$, 17% $O_2$, and 63% $N_2$.

The odors were being vaporized using a DevilBiss 646 nebulizer at a constant airflow of 2 L/min. Small vinyl tubes connected the nebulizer to the side of the mask, allowing the mixing of the odor with the inspiratory gas. Two foul-smelling odors were used: diluted ammonia (0.8%) and butyric acid (100%).

The signals from the infrared $CO_2$-monitor (Poet II, Criticare, Waukesha, WI), and the pressure transducer (Sine Wave Carier Demodulator CD15, Valydine Engineering™) were sampled at 20 Hz and were daily calibrated using a 7.5% $CO_2$ mixture and a 1 L syringe, respectively.

Both the $CO_2$ and the flow signal were treated off-line with PSPHA (De Clerck et al., 2006), a modular script-based program which we further developed to generate and apply calibration factors for each signal and to extract the following parameters for each breath: end-tidal $CO_2$-pressure ($FetCO_2$, in %) and minute ventilation (in ml/min). All waveforms were visually inspected off-line and technical abnormalities and movement artifacts were eliminated using the PSPHA software.

### 1.3. Procedure

Participants first received written information about the purpose and possible adverse effects of the experimental manipulation, then signed the informed consent form and completed the questionnaires. They were told (a) that the study was designed to monitor breathing behavior during the inhalation of several odorous gases; (b) that two innocuous mixtures would be administered, and that one of them could temporarily cause harmless symptoms, such as shortness of breath, a little dizziness and headache which would disappear quickly after the trial, while the other mixture would not cause such symptoms; and (c) that they were allowed to stop the experiment at any time.

All participants started with a context exposure trial (breathing room air through the system for 2 min in absence of any odor) to get habituated to the breathing circuit. The acquisition phase consisted of six semi-randomized trials: three CS+ trials (odor presented together with $CO_2$-enriched air) and three CS− trials (odor presented together with room air). Half of the participants received ammonia as the CS+ and butyric acid as the CS−, whereas this was reversed for the other half. Breathing trials lasted for 40 s. The US ($CO_2$-enriched air) was presented 10 s after CS+ onset. Both co-terminated 30 s later. After the administration of the CS+/US compound, participants continued to breathe through the mask for another 10 s, to assure that all $CO_2$ was being eliminated from the tubing system after CS+ trials. During the CS− trials, regular room air was administered instead of $CO_2$-enriched air. Intertrial intervals lasted 4 min after CS+ trials and 2 min after CS− trials. A pause of 5 min was inserted between acquisition and extinction.

The extinction phase was identical to the acquisition phase, with the exception that (a) no $CO_2$-enriched air was used in any trial (CS+ only and CS− only trials); and (b) all intertrial intervals lasted 2 min.

Participants were seated in a small room next to the experimenter’s room and were unable to see the apparatuses. The experimenter gave instructions through a microphone, manipulated the switches and carefully watched the participant on a monitor to ensure that the mask remained in place during the breathing trials.