1 **Breathlessness amplifies amygdala responses during affective processing**

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3 M. Cornelia Stoeckel¹; Roland W. Esser¹; Matthias Gamer^{1,2}; Andreas von Leupoldt^{1,3}

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5 ¹ Department of Systems Neuroscience, University Medical Center Hamburg-Eppendorf,
6 Martinistraße 52, 20246 Hamburg, Germany

7 ² Department of Psychology 1, University of Würzburg, Marcusstraße 9-11, 97070
8 Würzburg, Germany

9 ³ Health Psychology, University of Leuven, Tiensestraat 102, 3000 Leuven, Belgium

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11 **Running title:** Breathlessness and affect processing

12 **Correspondence to:**

13 Andreas von Leupoldt, PhD

14 Health Psychology, University of Leuven

15 Tiensestraat 102, 3000 Leuven (Belgium)

16 Telephone: +32 (0)16 326006, Telefax: +32 (0)16 326144

17 Email: andreas.vonleupoldt@kuleuven.be

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1 **Abstract**

2

3 Breathlessness is an aversive symptom in many prevalent somatic and psychiatric diseases
4 and is usually experienced as highly threatening. It is strongly associated with negative
5 affect, but the underlying neural processes remain poorly understood. Therefore, using
6 functional magnetic resonance imaging (fMRI), the present study examined the effects of
7 breathlessness on the neural processing of affective visual stimuli within candidate brain
8 areas including the amygdala, insula, and anterior cingulate cortex (ACC). During scanning,
9 42 healthy volunteers (mean (SD) age: 29.0 (6.0) years; 14 female) were presented with
10 affective picture series of negative, neutral and positive valence while experiencing either no
11 breathlessness (baseline conditions) or resistive load induced breathlessness (breathlessness
12 conditions). Respiratory measures and self-reports suggested successful induction of
13 breathlessness and affective experiences. Self-reports of breathlessness intensity and
14 unpleasantness were significantly higher during breathlessness conditions (mean (SD): 45.0
15 (16.6) and 32.3 (19.8)) as compared to baseline conditions (mean (SD): 1.9 (3.0) and 2.9
16 (5.5)). Compared to baseline conditions, stronger amygdala activations were observed during
17 breathlessness conditions for both negative and positive affective picture series relative to
18 neutral picture series, while no such effects were observed in insula and ACC. The present
19 findings demonstrate that breathlessness amplifies amygdala responses during affective
20 processing, suggesting an important role of the amygdala for mediating the interactions
21 between breathlessness and affective states.

22

23 **Keywords:** affect, amygdala, breathlessness, dyspnea, threat

1 **1. Introduction**

2

3 Breathlessness (dyspnea) is a prominent symptom in many prevalent diseases including
4 respiratory conditions such as asthma and chronic obstructive pulmonary disease (COPD),
5 but also in psychiatric conditions such as anxiety disorders (American Thoracic Society,
6 1999; Laviolette et al., 2014). Breathlessness is usually experienced as highly threatening and
7 significantly contributes to patients’ individual burden (Parshall et al., 2012). Not
8 surprisingly, high levels of negative affect such as depression and anxiety are frequently
9 observed in patients suffering from repeated or chronic breathlessness (Maurer et al., 2008;
10 Ritz et al., 2013; Scott et al., 2007; von Leupoldt et al., 2012; Yohannes & Alexopoulos,
11 2014).

12

13 Previous studies have shown that affective states and traits can impact the perception of
14 breathlessness, even in the absence of parallel changes in ventilation (Hayen et al., 2013;
15 Janssens et al., 2009; von Leupoldt et al., 2014). Moreover, higher levels of breathlessness
16 were observed in everyday life as well as in experimental studies in those patients with
17 asthma and COPD, who demonstrated higher trait levels of depression and anxiety (Janson et
18 al., 1994; Livermore et al., 2008; Spinhoven et al., 1997; von Leupoldt et al., 2011; Xu et al.,
19 2008). Furthermore, experimentally induced, short lasting negative affective states have
20 been shown to increase perceived breathlessness in healthy individuals and in patients with
21 asthma and COPD, whereas positive affective states decrease perceived breathlessness
22 (Rietveld & van Beest, 2007; Sharma et al., 2016; von Leupoldt & Dahme, 2013; von
23 Leupoldt et al., 2006; von Leupoldt, Riedel, & Dahme, 2006; von Leupoldt et al., 2010).

24

1 Several studies using respiratory-related evoked potentials (RREP) - a non-invasive
2 electroencephalographic measure of cortical activity elicited by short respiratory stimuli such
3 as inspiratory occlusions - demonstrated that affective states can also impact the neural
4 processing of respiratory sensations (Chan et al., 2016; Chan et al., 2012; Chenivesse et al.,
5 2014; von Leupoldt, Chan, et al., 2011; von Leupoldt et al., 2013). However, it is still unclear
6 which brain networks underlie this impact of affective states on the processing of respiratory
7 sensations. Activity changes in limbic and paralimbic brain areas such as amygdala, insula
8 and anterior cingulate cortex (ACC) are frequently observed in neuroimaging studies on
9 breathlessness perception as well as affective processing and so it has been hypothesized that
10 especially these areas are involved in this interaction (Davenport & Vovk, 2009; Evans, 2010;
11 Guz, 1997; Herigstad et al., 2011; O'Donnell et al., 2009; Paulus, 2013; von Leupoldt &
12 Dahme, 2005). Indirect support for this assumption comes from some studies demonstrating
13 that anticipatory fear of breathlessness as well as subjective ratings of the affective
14 unpleasantness of breathlessness are related to activations in amygdala, insula and ACC
15 (Faull & Pattinson, 2017; Hayen et al., 2017; Paulus et al., 2012; Stoeckel et al., 2015;
16 Stoeckel, Esser, Gamer, Büchel, & von Leupoldt, 2015, 2016; von Leupoldt et al., 2008).

17

18 Notably, little is known about the reverse effect, i.e., the influence of breathlessness on
19 affective processing. This is particularly surprising, inasmuch the strong interactions between
20 breathlessness and negative affect were regularly highlighted in the literature (American
21 Thoracic Society, 1999; Laviolette et al., 2014; Parshall et al., 2012) and chronic experiences
22 of breathlessness were repeatedly suggested as a source for the development of depression
23 and anxiety in affected patients (Atlantis et al., 2013; Hasler et al., 2005; Livermore et al.,
24 2010; von Leupoldt & Kenn, 2013). To our knowledge, only three previous experimental
25 studies are currently available which examined this research topic. Vinckier and colleagues

1 (Vinckier, Morélot-Panzini, & Similowski, 2018) recently demonstrated that breathlessness
2 as elicited by inspiratory threshold loading as well as carbon dioxide inhalation changed the
3 performance to detect fearful faces in a fear recognition task. Two further recent studies using
4 electroencephalography suggested that resistive-load induced breathlessness as well as its
5 threatening anticipation might also impact the neural processing of affective pictures, as
6 quantified by changes in event-related potentials elicited by picture onsets (Juravle et al.,
7 2014; Juravle et al., 2017). However, the brain areas underlying these findings remain
8 untested, but presumably include the aforementioned (para-)limbic areas, i.e. amygdala,
9 insula and ACC, which have not only most consistently been linked to the interactions
10 between affect and breathlessness (Davenport & Vovk, 2009; Evans, 2010; Guz, 1997;
11 Herigstad et al., 2011; O'Donnell et al., 2009; Paulus, 2013; von Leupoldt & Dahme, 2005),
12 but also play a key role in affective processing (Adolphs, 2013; Morris et al., 1998; Paulus
13 and Stein, 2006; Vogt, 2014). Especially for the amygdala, a large body of literature suggests
14 that it might not only be a key region for the processing of affective states (Adolphs, 2013;
15 Davis & Whalen, 2001; LeDoux, 2000), but also for linking affective processing with
16 somatosensory input (Legrain et al., 2011; Pourtois et al., 2013; Veinante et al., 2013;
17 Vuilleumier, 2005).

18

19 Using functional magnetic resonance imaging (fMRI), the present study aimed to contribute
20 to the investigation of neural interactions between breathlessness and affective processing.
21 Specifically, we tested the hypothesis that resistive load induced breathlessness would
22 amplify the neural processing of affective pictures in the amygdala, but also in the insula and
23 ACC.

24

2. Materials and Method

2.1 Participants

Participants were recruited from an institutional data base of volunteers (mostly students) and invited to participate in a fMRI study on brain processes related to breathlessness. We tested 42 healthy individuals who self-reported the absence of: history of respiratory disease, breathing difficulties during simple physical activities (e.g., climbing stairs), acute respiratory symptoms (e.g., cough, flu) within two weeks prior to testing, neurological/cardiac/internal diseases, psychological disorders, use of psychotropic medications, pregnancy, metallic implants, and large tattoos. Normal lung function (forced expiratory volume in one second in % predicted > 80%) was confirmed by standard spirometry on the day of the experiment (Miller et al., 2005), based on established normative reference values (Quanjer et al., 1993). Written informed consent was obtained prior to the study. The study protocol was approved by the ethics committee of the Medical Association Hamburg (PV3662). An overview on participants' baseline characteristics is given in Table 1.

INSERT TABLE 1 ABOUT HERE

2.2 Apparatus and Respiratory Measurements

Volunteers breathed through a face mask connected with an MRI compatible pneumotachograph (ZAN 600 unit, ZAN Messgeräte GmbH, Oberhulba, Germany). The pneumotachograph contained ports for recording of end-tidal CO₂ pressure (P_{ET}CO₂) and peak inspiratory mouth pressure (P_I), which was followed by a two-way non-rebreathing valve (Hans Rudolph Inc., Shawnee, USA). The inspiratory port of the valve was connected to a 2.6 m tube for the easy introduction and removal of MR-compatible resistive loads in the scanner environment. The free expiratory port prevented re-breathing of CO₂. This breathing circuit allowed continuous measurements of respiratory parameters including P_{ET}CO₂, P_I, as

1 well as tidal volume (V_T), breathing frequency (f), minute ventilation (V_E), and inspiratory
2 time (T_I), which were derived from the airflow signal.

3

4 *2.3 Induction and Measurement of Breathlessness*

5 Transient states of breathlessness were induced by breathing through inspiratory resistive
6 loads. We explained breathlessness to our participants as a sensation of difficult and
7 uncomfortable breathing. In addition, we explained in detail the two distinct dimensions of
8 breathlessness intensity and breathlessness unpleasantness prior to testing with standardized
9 examples (von Leupoldt, Taube, Schubert-Heukeshoven, Magnussen, & Dahme, 2007)
10 including the commonly used radio analogy (Banzett et al., 2008). Shortly, participants were
11 told that breathlessness intensity is analogous to how loud a sound on the radio is and that
12 breathlessness unpleasantness is analogous to how unpleasant this sound is, which is
13 depending for each individual on what sound is played. In this regard, a sound can be
14 unpleasant even if it is not played loud and be pleasant even if it is played very loud. In a pre-
15 test outside the scanner, participants were placed in a supine position and presented with
16 inspiratory resistive loads of increasing magnitude using the same breathing circuit as during
17 subsequent scanning. During the pre-test, each load was presented for 24 s and breathlessness
18 intensity subsequently rated on a Borg-scale (0 = 'not noticeable' to 10 = 'maximally
19 imaginable') (Borg, 1982). Load magnitude was increased in repeated trials until participants
20 reported a sensation of 'severe' breathlessness (Borg score ≥ 5) in more than 50% of the trials.
21 The respective load was then used to induce breathlessness during scanning (mean load
22 resistance = 2.28 kPa/l/s, $SD = 1.20$), while no-load conditions served as baseline conditions.

23

24 During scanning, a computerized horizontal visual analog scale (VAS) was used for the

evaluation of perceived breathlessness intensity and unpleasantness. Participants delivered ratings via a button-box operated by their right hand. For intensity ratings one end of the scale was verbally anchored as “not noticeable” (corresponding to a numeric value of 0), the other end was anchored as “maximally imaginable” (corresponding to a numeric value of 100). For breathlessness unpleasantness, the verbal anchors were “not unpleasant” and “maximally imaginable unpleasant”, respectively. As in previous studies, the VAS was used during scanning in order to prevent potential memory biases such that participants would simply provide the same numeric rating score they provided during the pre-test on the Borg-scale (von Leupoldt et al., 2008).

2.4 Affective Picture Series

During the experiment, negative, neutral and positive affective states were induced by presenting respective picture series depicting pictures from the International Affective Picture System (IAPS, Lang et al., 2008). Pictures were chosen on the basis of normative data on each picture’s valence and arousal as provided by the technical manual of the IAPS. For each affective category, eight series of four pictures each were composed with either negative, neutral or positive affective content.¹ These were grouped such that the average normative valence (p ’s > 0.834) and arousal (p ’s > 0.956) ratings were similar between the eight series of the same affective category with comparable normative arousal ratings for the negative and the positive series ($p > 0.303$). The mean normative ratings for valence and arousal, respectively, were 2.1/6.1 for the negative series, 5.0/3.3 for the neutral series, and 7.5/5.9 for the positive series. In addition, the picture series were paralleled with regard to attributes such as visual complexity, luminance, and content (human, animal, landscape). Four additional neutral picture series of four neutral pictures each were composed, which were

1 used during adaptation and practice trials within the scanner.

2 ##### INSERT FOOTNOTE 1 ABOUT HERE #####

3 *2.5 fMRI Data Acquisition*

4 Imaging was performed on a 3-Tesla TRIO-Magnetom Scanner (Siemens, Medical Solutions,
5 Erlangen, Germany) using a standard 32-channel head-coil. For each data volume we
6 acquired 48 continuous axial-slices in descending order with 2×2 mm in-plane resolution, 2
7 mm slice thickness and a 1 mm gap using T2*-weighted parallel echoplanar imaging (TR =
8 2870 ms, TE = 25 ms, flip angle = 80°, field of view = 208×208 mm) with GRAPPA
9 acceleration (R = 2). Prior to fMRI, we acquired a high-resolution T1-weighted structural
10 brain scan using a standard MP-RAGE sequence (1×1×1 mm spatial resolution, TR = 2300
11 ms, TE = 2.98 ms, flip angle = 9°, field of view = 256×256, 240 slices).

13 *2.6 Experimental Protocol*

14 Prior to scanning, participants followed computer-based standardized instructions outside the
15 scanner, introducing the experimental protocol. Immediately after instructions and pre-test
16 for the determination of individual load magnitudes, participants entered the scanner with the
17 face mask tightly fitted. Affective pictures and VAS-scales were projected into the scanner
18 bore via a mirror using Presentation software (Neurobehavioral Systems, Inc., Albany, CA).
19 Presentation was also used to send condition markers to the ZAN-system.

21 Four neutral picture series, two without and two with accompanying resistive load and
22 followed by VAS-ratings, served the adaptation towards the scanner environment and the use

1 of VAS-scales within the scanner. These trials did not enter the analyses. The subsequent 24
2 series of negative, neutral and positive pictures were presented in pseudorandomized order so
3 that no more than two series of the same valence were presented consecutively. Each picture
4 within each picture series was presented in randomized order for 6 s, resulting in a total
5 duration of 24 s per series. Each second series was combined with resistive loaded breathing
6 during the whole duration of the series (breathlessness conditions). The remaining series
7 were presented without resistive loads (baseline conditions). After each picture series,
8 participants rated the perceived intensity and unpleasantness of breathlessness in randomized
9 order using the VAS. In total, this resulted in four picture series for each of the six
10 experimental conditions: negative-baseline (NEG-BASE), neutral-baseline (NEU-BASE),
11 positive-baseline (POS-BASE), negative-breathlessness (NEG-BREATH), neutral-
12 breathlessness (NEU-BREATH), and positive-breathlessness (POS-BREATH). Depending on
13 the time spent on ratings, participants needed 14-18 min to complete the experimental
14 protocol.

15

16 Outside the scanner, participants were again presented with all affective picture series on a
17 computer monitor using the same order as in the scanner, but without parallel induction of
18 breathlessness. After each picture series, participants indicated their level of affective valence
19 and arousal using a computer-based version of the self-assessment manikin (SAM, Bradley
20 & Lang, 1994). SAMs are 9-step rating scales supported by visualizations (“manikins”) of
21 different degrees of either arousal (1 = calm, 9 = aroused) and valence (1 = unpleasant, 9 =
22 pleasant), respectively.

23

24 2.7 Data Analysis

1 Means of respiratory parameters and ratings of breathlessness intensity and breathlessness
2 unpleasantness were analyzed using separate repeated measures ANOVAs with the factors
3 AFFECT (negative vs. neutral vs. positive) and BREATHLESSNESS (baseline vs.
4 breathlessness), followed by paired *t*-tests. Similar analyses were performed for post-
5 scanning ratings of valence and arousal for which the factor BREATHLESSNESS was
6 defined as picture series during preceding scanning were presented during baseline vs.
7 breathlessness conditions. Including GENDER as an additional factor in post-hoc explorative
8 analyses did not result in changes in findings. Analyses were calculated with SPSS 20.0
9 software (SPSS Inc., Chicago, IL) using a significance level of $p < 0.05$.

10

11 All steps of fMRI data preprocessing and statistical analysis were carried out using SPM8
12 (www.fil.ion.ucl.ac.uk/spm), with the exception of noise-correction, which was carried out
13 using fsl-MELODIC 3.0 (Beckmann & Smith, 2004). Functional data were unwarped and
14 realigned using 6-parameter rigid-body transformations. After normalization to the standard
15 MNI-T1-template using linear and nonlinear transformations, noise was identified following
16 a two-step probabilistic independent component analysis: Preprocessed data were first
17 whitened and projected into a 40-dimensional subspace using a principal component analysis
18 and further decomposed into sets of vectors that describe signal variation across the temporal
19 domain (time-courses) and across the spatial domain (spatial maps) by optimizing for non-
20 Gaussian spatial source distributions using an independent component analysis (Beckmann
21 & Smith, 2004). Each component was then categorized as either function-related (resting-
22 state networks or paradigm related) or noise-related (e.g. noise due to respiration, cardiac
23 activity, motion or scanner drifts) by considering the spatial pattern, the time-course and the
24 power distribution following a heuristic described by Kelly et al. (Kelly et al., 2010). This
25 procedure also controlled for potential effects of $P_{ET}CO_2$ fluctuations on the blood-oxygen-

level dependent (BOLD) measure of brain activation. Two independent raters showed high inter-rater agreement (97.5 %, Cohens's Kappa = 0.8). Components consistently identified as noise were filtered out. Noise-corrected functional data were smoothed using an 8×8×8 mm full-width at half-maximum Gaussian filter. For statistical analysis, functional data were high-pass filtered with a 128 s cut-off, while serial correlations were accounted for by using an autoregressive model.

Data modeling on the first level involved separate regressors for each of the six experimental conditions (NEG-BASE, NEU-BASE, POS-BASE, NEG-BREATH, NEU-BREATH, POS-BREATH) based on the canonical hemodynamic response function implemented in SPM8. Mean BOLD signal intensity of each volume and VAS ratings periods were included as regressors-of-no-interest. Within individual first-level models, contrasts for the negative and positive affective picture series versus the neutral series were calculated separately for baseline ([NEG-BASE > NEU-BASE] and [POS-BASE > NEU-BASE]) and breathlessness conditions ([NEG-BREATH > NEU-BREATH] and [POS-BREATH > NEU-BREATH]). In addition, respective interactions of affective picture series x breathlessness ([NEG-BREATH – NEU-BREATH > NEG-BASE – NEU-BASE] and [POS-BREATH – NEU-BREATH > POS-BASE – NEU-BASE]) were calculated and then subjected to across-participants second-level analyses.

The second-level analyses focused on the predefined regions of interest (ROIs) amygdala, insula and ACC, based on previous findings suggesting affective aspects of breathlessness to be linked most consistently to these areas. Respective bilateral masks were generated from the automated anatomical labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002). Activation

1 was considered significant at a family-wise error of $p < 0.05$ after small volume correction
2 (SVC) within the bilateral masks, which is statistically more conservative than testing in
3 unilateral masks separately. Interaction-effects of affective picture series x breathlessness on
4 amygdala activation were investigated based on target coordinates from the initial contrasts.

5
6 **3. Results**

7 *3.1 Respiratory Parameters*

8 Respiratory parameters showed respiratory changes typically associated with breathlessness
9 with significant main effects for the factor BREATHLESSNESS for V_E ($F(1,41) = 14.20, p =$
10 0.001), T_I ($F(1,41) = 29.39, p < 0.001$), f ($F(1,41) = 17.54, p < 0.001$) and P_I ($F(1,41) =$
11 $211.93, p < 0.001$). V_E (9.61 ± 2.08 vs. $10.47 \pm 2.10, p = 0.001$) and f (12.42 ± 2.66 vs. 13.17
12 $\pm 2.84, p < 0.001$) were significantly lower, while P_I (7.91 ± 3.10 vs. $1.10 \pm 0.25, p < 0.001$)
13 and T_I (2.40 ± 0.66 vs. $2.07 \pm 0.56, p < 0.001$) were significantly higher during
14 breathlessness conditions as compared to baseline conditions. Means and standard deviations
15 for all conditions are given in Table 2.

16 ##### INSERT TABLE 2 ABOUT HERE #####

17 *3.2 Ratings of breathlessness experience*

18 Similarly, ratings of breathlessness experience confirmed the successful induction of
19 breathlessness with significant main effects for the factor BREATHLESSNESS for
20 breathlessness intensity and breathlessness unpleasantness (intensity: $F(1,41) = 309.82, p <$
21 0.001 ; unpleasantness: $F(1,41) = 113.17, p < 0.001$). Both, breathlessness intensity ($45.00 \pm$
22 16.57 vs. 1.86 ± 3.01) and breathlessness unpleasantness ratings (32.32 ± 19.77 vs. $2.89 \pm$
23 5.49) were significantly higher during breathlessness conditions as compared to baseline

conditions. Means and standard deviations for all conditions are given in Table 3.

INSERT TABLE 3 ABOUT HERE

3.3 Self-reports of Affective Experiences

Analyses of the post-scanning self-reports of the affective experiences induced by picture viewing confirmed the expected effects. There was a significant effect for the factor AFFECT for ratings of both, arousal ($F(2,82) = 78.14, p < 0.001$) and valence ($F(2,82) = 336.5, p < 0.001$). Post-hoc tests revealed significantly higher arousal for negative picture series (5.85 ± 1.56) as compared to either positive (4.49 ± 1.54) ($t(41) = 5.93, p < 0.001$) or neutral picture series (2.84 ± 1.01) ($t(41) = 11.58, p < 0.001$). Arousal for positive picture series was significantly higher compared to neutral picture series ($t(41) = 7.09, p < 0.001$). Valence ratings for negative picture series (2.54 ± 0.81) were significantly lower than for neutral (5.02 ± 0.39) ($t(41) = -18.06, p < 0.001$) and positive picture series (6.77 ± 0.88) ($t(41) = -20.98, p < 0.001$). Valence ratings for neutral picture series were significantly lower compared to positive picture series ($t(41) = -12.09, p < 0.001$). Although no breathlessness was induced during this post-scanning manipulation-check, we tested for potential differences between picture series that were presented during baseline conditions and picture series presented during breathlessness conditions. There was no significant effect of “BREATHLESSNESS” on ratings of either arousal ($F(1,41) = 0.24, p = 0.624$) or valence ($F(1,41) = 0.61, p = 0.44$). Means and standard deviations for all conditions are given in Table 3.

3.4 Functional Imaging Data

3.4.1 Negative Affect

1 Under unloaded baseline conditions ([NEG-BASE > NEU-BASE]), the right amygdala was
2 significantly activated during negative picture series ($x = 26, y = 0, z = -16$, SVC $p = 0.018$,
3 Fig 1 A). When accompanied by breathlessness ([NEG-BREATH > NEU-BREATH]),
4 activation within the amygdala was bilaterally significant during negative picture series ($x = -$
5 $22, y = -2, z = -16$, SVC $p = 0.002$ and $x = 22, y = -2, z = -12$, SVC $p = 0.004$, respectively,
6 Fig 1 B). When using the coordinates from the contrast [NEG-BREATH > NEU-BREATH]
7 as targets (4 mm sphere around peak voxels) to examine potential interaction effects of
8 negative affect and breathlessness ([NEG-BREATH vs. NEU-BREATH > NEG-BASE vs.
9 NEU-BASE]), we found a significant interaction within the left amygdala ($x = -22, y = -2, z$
10 $= -20$, SVC $p = 0.047$, Fig 1 C). No significant effects were observed in insula and ACC.

11 ##### INSERT FIGURE 1 ABOUT HERE #####

12 *3.4.2 Positive Affect*

13 Under unloaded baseline conditions ([POS-BASE > NEU-BASE]), there was no significant
14 activation within the amygdala during positive picture series. However, when accompanied
15 by breathlessness ([POS-BREATH > NEU-BREATH]), there was a significant activation
16 within the left amygdala during positive picture series ($x = -20, y = -2, z = -18$, SVC $p =$
17 0.028 , Fig 2 A). When using the coordinates from the contrast [POS-BREATH > NEU-
18 BREATH] as target (4 mm sphere around peak voxel) to examine potential interaction effects
19 of positive affect and breathlessness ([POS-BREATH vs. NEU-BREATH > POS-BASE vs.
20 NEU-BASE]), we found a significant interaction within the left amygdala ($x = -22, y = -4, z$
21 $= -20$, SVC $p = 0.009$, Fig 2 B). Again, no significant effects were observed in insula and
22 ACC.

23 ##### INSERT FIGURE 2 ABOUT HERE #####

1 **4. Discussion**

2 The present study investigated the interactions between breathlessness and affective
3 processing by testing the impact of resistive load induced breathlessness on the neural
4 processing of affective pictures. In line with previous studies (Hayen et al., 2017; Peiffer et
5 al., 2001; Stoeckel et al., 2016; von Leupoldt et al., 2008), respiratory parameters and ratings
6 of breathlessness intensity and breathlessness unpleasantness suggested successful induction
7 of breathlessness. Similarly, ratings of affective valence and arousal confirmed the expected
8 effects of viewing negative, neutral and positive affective picture series on affective
9 experience, which corresponds with typical results obtained in previous studies using
10 respective IAPS picture series (Cuthbert et al., 2000; Lang et al., 2008; von Leupoldt et al.,
11 2006). Most importantly, we observed stronger amygdala activations when negative and
12 positive affective picture viewing was accompanied by breathlessness as compared to picture
13 viewing under unloaded baseline conditions. These findings suggest that breathlessness
14 amplifies amygdala responses during affective processing, thus, highlighting an important
15 role of the amygdala for mediating the interactions between breathlessness and affective
16 states.

17

18 The present results support earlier hypotheses suggesting a significant role of (para-)limbic
19 system structures for the interactions between breathlessness and affective processing
20 (Davenport & Vovk, 2009; Evans, 2010; Guz, 1997; Herigstad et al., 2011; O'Donnell et al.,
21 2009; Paulus, 2013; von Leupoldt & Dahme, 2005). Furthermore, the present findings
22 converge with previous neuroimaging studies linking both, breathlessness and negative
23 affect, to an activation of the amygdala (Esser et al., 2017). For example, Stoeckel and
24 colleagues demonstrated that anticipatory fear of upcoming breathlessness is associated with

1 increased amygdala activation (Stoeckel et al., 2015). Similarly, experimental manipulations
2 of the affective unpleasantness of perceived breathlessness by parallel opioid administration
3 or affective picture viewing, respectively, were related to amygdala activations (Hayen et al.,
4 2017; von Leupoldt et al., 2008). However, we did not observe a significant modulation of
5 activity in other (para-)limbic areas such as insula and ACC, which have previously been
6 shown to be linked to affective aspects of breathlessness (Hayen et al., 2017; Paulus et al.,
7 2012; Stoeckel, Esser, Gamer, Büchel, et al., 2015; Stoeckel et al., 2016; von Leupoldt et al.,
8 2008), breathlessness-related personality traits such as breathlessness catastrophizing
9 (Stöckel et al. 2017) as well as to the processing of various other affective stimuli including
10 pain and aversive pictures (Craig, 2002; Morris et al., 1998; Vogt, 2014; von Leupoldt et al.,
11 2009; Wiech and Tracey, 2013). These discrepancies might be related to several differences
12 in the experimental designs between studies. For example, we presented in a blocked passive
13 viewing design only four affective pictures of the same valence within each picture series.
14 This resulted in shorter affective stimulation periods than other fMRI studies using affective
15 pictures, which partly included additional active responses and/or used event-related instead
16 of blocked designs (Killgore and Yurgelun-Todd, 2004; Morris et al., 1998; Phan et al., 2004;
17 Schienle et al., 2002). In addition, the present ratings for affective arousal were somewhat
18 lower than the normative ratings of the IAPS (Lang et al., 2008) as well as those observed in
19 some previous studies using affective pictures, which demonstrated activations of insula
20 and/or ACC (Phan et al., 2004; Schienle et al., 2002; Wright et al., 2004). Therefore, it might
21 be speculated that stronger, more arousing affective stimulation would be needed in order to
22 detect additional activations of these areas, which deserves future exploration.

23

24 Furthermore, the present findings demonstrate that breathlessness does not exclusively
25 amplify amygdala responses to negative affective stimuli, but also to positive affective

1 stimuli. This converges with the known role of the amygdala as a ‘salience detector’, related
2 to the preferential processing of significant and intensive stimuli of both, positive and
3 negative affective valence (Bonnet et al., 2015; Costa et al., 2010; Sabatinelli et al., 2011;
4 van Marle et al., 2009; Weymar & Schwabe, 2016). Following this lead, breathlessness might
5 prime the amygdala and facilitate the processing of other salient stimuli presented in parallel.
6 This might also help to explain recent findings in high compared to low anxious individuals,
7 which showed increased early neural processing of affective pictures across different valence
8 categories during breathlessness (Juravle et al., 2017). In general, the present results are in
9 line with previous notions that the amygdala is a central structure for linking affective
10 processing with the parallel processing of somatosensory input (Legrain et al., 2011; Pourtois
11 et al., 2013; Veinante et al., 2013; Vuilleumier, 2005).

12

13 In line with previous studies examining interactions between affective states and
14 breathlessness (e.g., von Leupoldt et al., 2008; 2006), the present study did not observe
15 significant differences in respiratory parameters between the negative, neutral and positive
16 affective conditions during loaded breathing, which have been reported in previous studies
17 using IAPS picture stimuli (Gomez et al., 2008; Ritz et al., 2002). This is not unexpected
18 given that these previous studies were testing unloaded breathing in comfortably seated
19 individuals, whereas the present study tested individuals lying still in a narrow scanner bore
20 with the head tightly fixed in a head coil. These restraints on breathing mechanics, with the
21 intermittent presentation of strong resistive loads as additional restraint, might well
22 overwhelm affect-induced changes in respiratory parameters.

23

24 As outlined above, high levels of negative affectivity (e.g. depression and anxiety) are

1 prevalent in patients suffering from breathlessness, but the underlying causes are not well
2 understood (Maurer et al., 2008; Ritz et al., 2013; Scott et al., 2007; von Leupoldt et al.,
3 2012; Yohannes & Alexopoulos, 2014). The present results might support one potential
4 causal pathway that has been suggested for these comorbidities, namely that frequent
5 threatening experiences of breathlessness over the course of the disease might lead to
6 subsequent depression and anxiety (Atlantis et al., 2013; Hasler et al., 2005; Livermore et al.,
7 2010; von Leupoldt & Kenn, 2013). Based on the present study, it might be speculated that
8 threatening and especially chronic experiences of breathlessness (for example in patients
9 with COPD) are associated with upregulated amygdala responses to negative affective
10 stimulation, which might facilitate the development of chronically increased negative affect
11 over time. Some support for this speculation comes from a previous fMRI study in patients
12 with COPD, which demonstrated stronger amygdala activation during the fearful anticipation
13 of breathlessness in patients relative to controls, which was further related to higher trait
14 anxiety levels (Esser et al., 2017). In addition, longer disease duration in these patients
15 correlated with stronger amygdala activation during breathlessness perception. Moreover,
16 this speculation would be in line with respective findings in patients suffering persistent pain
17 where functional plasticity of amygdala activity has been shown to contribute to enhanced
18 negative affect (Veinante et al., 2013). However, future studies in respective patient groups
19 are clearly required to test this hypothesis.

20

21 When interpreting the present findings, several limitations need to be acknowledged. We
22 exclusively investigated the effects of resistive-load breathing for the experimental induction
23 of short-lasting states of breathlessness, which accounted for the technical limitations
24 inherent to a scanner environment. Albeit effective, this technique only mirrors some aspects
25 of breathlessness, i.e. the sense of increased work and effort of breathing, and might not fully

1 be comparable to sustained breathlessness experiences outside a well-controlled laboratory
2 context. In addition, it might not fully mirror the breathlessness experienced by
3 cardiopulmonary patients during daily physical activities both in terms of magnitude as well
4 as quality. Therefore, future studies are needed to extend the present findings to other
5 magnitudes as well as qualities of breathlessness, for example air hunger, which is inherently
6 more unpleasant than work and effort of breathing at the same level of perceived intensity
7 (Banzett et al., 2008). Furthermore, breathlessness was evoked predictably every second
8 picture series, which might have created states of anticipation and related brain activity, thus
9 potentially impacting the present findings. Future studies might, therefore, benefit from a
10 more unpredictable or randomized pattern of breathlessness induction. In addition, adding
11 conditions of breathlessness without parallel picture viewing could be beneficial to further
12 disentangle effects of affective processing and effects of breathlessness. Moreover, our
13 findings in predominantly young and healthy individuals cannot be generalized to patients
14 suffering from frequent or chronic states of breathlessness. Therefore, future research is
15 required to investigate whether breathlessness is related to comparable brain activations
16 during affective processing in patients with breathlessness and how this is related to clinical
17 outcomes. In particular, future longitudinal studies seem promising that directly address
18 whether neural interactions between breathlessness and affective processing relate to the
19 subsequent development of chronic levels of negative affectivity such as depression and
20 anxiety. Respective findings might open new avenues to interventions aimed at reducing
21 negative affect in patients suffering from breathlessness.

22

1 **Conflict of Interest Statement**

2 The authors declare that there is no conflict of interest regarding the publication of this paper.

3

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8

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12

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1 **Table 1** Mean (*SD*) baseline characteristics of participants

Age (yr)	29.0 (6.0)
Sex (female/male), n	14/28
Weight (kg)	76.6 (12.7)
Height (cm)	179.7 (9.5)
Body Mass Index (kg/m ²)	23.6 (2.3)
FEV ₁ (L)	4.8 (1.0)
FEV ₁ (% predicted)	115 (12)
FVC (L)	5.9 (1.2)
FVC (% predicted)	118 (13)

2

3 FEV₁ = forced expiratory volume in 1s,

4 FVC = forced vital capacity

5

6

Table 2 Group mean (*SD*) respiratory parameters for affective picture series under unloaded (baseline) and loaded (breathlessness) conditions

	Baseline			Breathlessness		
	negative	neutral	positive	negative	neutral	positive
$P_{ET}CO_2$ (mmHg)	34.7 (3.8)	34.5 (3.9)	34.6 (3.7)	34.7 (3.8)	34.5 (3.9)	34.6 (4.0)
f (breaths/min)	13.2 (3.1)	13.2 (2.8)	13.2 (2.9)	12.3 (2.7)	12.5 (2.9)	12.5 (2.6)
P_I (mbar)	1.10 (0.35)	1.06 (0.24)	1.14 (0.38)	7.65 (2.99)	8.13 (3.47)	7.94 (3.02)
T_I (s)	2.09 (0.60)	2.05 (0.55)	2.06 (0.62)	2.43 (0.72)	2.38 (0.67)	2.4 (0.64)
V_E (L/min)	10.2 (2.2)	10.7 (2.3)	10.5 (2.0)	9.3 (2.0)	9.9 (2.3)	9.6 (2.2)
V_T (L)	0.84 (0.27)	0.89 (0.31)	0.87 (0.28)	0.84 (0.30)	0.86 (0.33)	0.86 (0.35)

3

$P_{ET}CO_2$ = end-tidal CO_2 pressure; f = breathing frequency; P_I = peak inspiratory mouth pressure; T_I = inspiratory time; V_E = minute ventilation; V_T = tidal volume.

6

Table 3 Group mean (*SD*) ratings for breathlessness intensity, breathlessness unpleasantness, arousal and valence for affective picture series under unloaded (baseline) and loaded (breathlessness) conditions

	Baseline			Breathlessness		
	negative	neutral	positive	negative	neutral	positive
Breathlessness intensity	2.4 (4.4)	1.9 (3.0)	1.3 (2.2)	45.4 (16.9)	45.3 (17.8)	44.4 (17.4)
Breathlessness unpleasantness	3.8 (6.8)	2.8 (5.7)	2.1 (5.0)	35.2 (20.2)	31.9 (21.8)	29.9 (20.4)
Arousal*	5.8 (1.7)	2.9 (1.1)	4.5 (1.5)	5.9 (1.6)	2.8 (1.1)	4.5 (1.7)
Valence*	2.6 (0.9)	5.0 (0.5)	6.8 (0.9)	2.5 (0.9)	5.0 (0.4)	6.7 (0.9)

*ratings were obtained during a second presentation after scanning

Figure Legends

Figure 1 (A) Amygdala activation during negative compared to neutral picture series in unloaded baseline conditions ([NEG-BASE > NEU-BASE]). (B) Amygdala activation during negative compared to neutral picture series in breathlessness conditions ([NEG-BREATH > NEU-BREATH]). (C) Interaction effect for amygdala activation during negative compared to neutral picture series in breathlessness conditions versus baseline conditions ([NEG-BREATH vs. NEU-BREATH > NEG-BASE vs. NEU-BASE]). Amygdala activations are displayed at a threshold of $p < 0.05$, family-wise error corrected for the specific ROI, and superimposed on the single subject T1-weighted image provided by SPM8.

Figure 2 (A) Amygdala activation during positive compared to neutral picture series in breathlessness conditions ([POS-BREATH > NEU-BREATH]). (B) Interaction effect for amygdala activation during positive compared to neutral picture series in breathlessness conditions vs. baseline conditions ([POS-BREATH vs. NEU-BREATH > POS-BASE vs. NEU-BASE]). Amygdala activations are displayed at a threshold of $p < 0.05$, family-wise error corrected for the specific ROI, and superimposed on the single subject T1-weighted image provided by SPM8.

Footnote 1

The following IAPS pictures were used for the negative, neutral and positive pictures series in the present study: negative 1 (6510, 3350, 3230, 9910), negative 2 (9250, 9040, 9300, 3180), negative 3 (9400, 3100, 9571, 6560), negative 4 (4664, 3170, 2205, 9810), negative 5 (3030, 2800, 9800, 9600), negative 6 (7380, 3530, 9433, 9420), negative 7 (9570, 6260, 9000, 9410), negative 8 (6350, 2730, 9220, 9252); neutral 1 (1122, 2191, 2840, 2440), neutral 2 (7025, 2635, 2393, 2870), neutral 3 (7100, 2516, 2200, 2396), neutral 4 (7590, 2397, 2038, 9070), neutral 5 (7130, 2499, 2850, 2280), neutral 6 (7290, 2102, 2570, 7550), neutral 7 (7050, 2493, 7493, 9700), neutral 8 (5510, 2512, 2595, 2890); positive 1 (4658, 2040, 8080, 7502), positive 2 (4652, 2050, 7330, 5621), positive 3 (4659, 2070, 8370, 4520), positive 4 (4690, 2080, 5480, 8030), positive 5 (4664, 1710, 2340, 8200), positive 6 (4660, 2345, 7270, 8210), positive 7 (4670, 2260, 8400, 4626), positive 8 (4680, 2216, 8490, 1440).

A $y = 0$

NEGATIVE AFFECT
BASELINE

B $y = -2$

NEGATIVE AFFECT
BREATHLESSNESS

C $y = -2$

NEGATIVE AFFECT
BREATHLESSNESS > BASELINE

Psychophysiology

A

Psychophysiology

B $y = -2$ $y = -4$

8

4

2

1

L

POSITIVE AFFECT
BREATHLESSNESS

POSITIVE AFFECT
BREATHLESSNESS > BASELINE