

# The slow breathing reduces anxiety: An EEG study

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## Abstract

Anxiety is an interactive disorder of the mind and body, characterized by excessive worry about uncertain future events and a dysfunction of the autonomic nervous system. Previous studies have shown that slow, deep breathing can affect the body's internal organs by increasing the activity levels of the vagus nerve, reducing physical tension, and anxiety. Although we know that slow and deep breathing techniques can effectively regulate anxiety and other emotions, the psychological and neurophysiological mechanisms of slow breathing on anxiety have not been systematically explored. In the study, we combined the paced breathing task with the threat uncertainty task for the first time to investigate the role of slow breathing in regulating anxiety. Here we investigated this question, using Spectral analysis of EEG to assess brain activity relating respiratory rate and the mechanism of respiratory rate impact on the anxious. Twenty-seven individuals participated in the experiment, which followed a 2 (respiratory rate: fast breathing, slow breathing)  $\times$  2 (certainty: certain, uncertain). The results showed that: (1) Slow breathing effectively reduced anxiety, the valence and arousal are lower under the slow breathing. (2) The EEG of fast and slow breathing showed different characteristics. The delta, theta and alpha EEG power are increased during the slow-paced breathing. (3) The EEG of Respiratory rate and certainty had a significant effect on the theta power. When individuals are faced with uncertain information the theta EEG power decreased during the slow-paced breathing, however, the theta EEG power increased during the fast-paced breathing.

## The slow breathing reduces anxiety: An EEG study

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## **Abstract:**

Anxiety is an interactive disorder of the mind and body, characterized by excessive worry about uncertain future events and a dysfunction of the autonomic nervous system. Previous studies have shown that slow, deep breathing can affect the body's internal organs by increasing the activity levels of the vagus nerve, reducing physical tension, and anxiety. Although we know that slow and deep breathing techniques can effectively regulate anxiety and other emotions, the psychological and neurophysiological mechanisms of slow breathing on anxiety have not been systematically explored. In the study, we combined the paced breathing task with the threat uncertainty task for the first time to investigate the role of slow breathing in regulating anxiety. Here we investigated this question, using Spectral analysis of EEG to assess brain activity relating respiratory rate and the mechanism of respiratory rate impact on the anxious. Twenty-seven individuals participated in the experiment, which followed a 2 (respiratory rate: fast breathing, slow breathing)  $\times$  2 (certainty: certain, uncertain). The results showed that: (1) Slow breathing effectively reduced anxiety, the valence and arousal are lower under the slow breathing. (2) The EEG of fast and slow breathing showed different characteristics. The delta, theta and alpha EEG power are increased during the slow-paced breathing. (3) The EEG of Respiratory rate and certainty had a significant effect on the theta power. When individuals are faced with uncertain information the theta EEG power decreased during the slow-paced breathing, however, the theta EEG power increased during the fast-paced breathing.

**Key words:** respiration rate; slow breathing; uncertainty; anxiety; EEG;

## **Introduction**

Anxiety disorders are the most common mental illness in children and adolescents, with prevalence rates as high as 20% (Costello et al. 2011). Anxiety disorders are typically characterized by persistent and excessive worry, often accompanied by physical and cognitive symptoms that can lead to dysfunction (Connolly and Bernstein 2007). Anxiety in moderation is useful. However, when anxiety is severe, it can become chaotic and therefore counterproductive (Hoehn-Saric and McLeod 1990). Knowing the impending adverse situation can

help prepare for and potentially avoid such incidents. However, there is often uncertainty as to whether the upcoming aversion event will actually happen, and how dangerous or negative it will be. Uncertainty about a possible future threat disrupts our ability to avoid it or to mitigate its negative impact and thus results in anxiety (Dan W. Grupe and Nitschke 2013), that has historical roots in animal studies of stress response and fear learning, as well as in previous influential models of anxiety pathology. Scholars provided evidence that anticipation about potential future threats induce anxiety in multiple levels, which range from affective and cognitive to behavioral manifestations (Mobini, Reynolds, and Mackintosh 2013; Simmons et al. 2011; Dan W. Grupe and Nitschke 2013). Anticipatory anxiety is defined as the time between warning and stimulus (Masaoka and Homma 2001). In real life, it is frequently characterized by potential unfavorable clues that signify future threats, such that people with anticipatory anxiety remain in a state of stress, including physiological arousal, cortical activation, and cognitive bias (Acheson et al. 2012; Carlson et al. 2011; Onoda et al. 2008). Constant stress can take a toll on one's body and mind. Uncertainty always exists in daily life, and solving the negative expectations associated with uncertainty has become the main motivation of people's life. New evidence has demonstrated that respiratory rhythms exert surprising, substantive influences on perception, emotion, and cognition (Allen, Varga, and Heck 2022). Breathing is an integral component of interoceptive processing, that is, the sensing of the physiological condition of the body (Craig 2002). Respiration is unique compared with other systems (e.g. gastrointestinal) insofar as conscious regulation can immediately impact respiratory processes. Instructed breathing patterns are widely applied to treat a variety of complaints and conditions, eg, pain, stress, post-traumatic stress disorder. (Zautra et al. 2010; Kim et al. 2013; Laborde et al. 2017). Most often, these patterns involve a voluntary reduction of breathing frequency, which is assumed to increase parasympathetic activity. Slow breathing at 4.5 to 6.5 breaths per minute (coherent or resonance breathing) has been shown to optimally balance sympatho-vagal stress response for most adults (Bernardi et al. 2001; Lehrer et al. 2010; Song and Lehrer 2003; Vaschillo and Lehrer 2006). Heart rate variability (HRV), the beat-to-beat variation in either heart rate or the duration of the R-R interval—the period, has become a popular clinical and investigational tool. HRV is an index of cardiac autonomic activation as well as an outcome variable in breathing training or HRV biofeedback research (Del Pozo et al. 2004; Sakakibara et al. 2013). The relationship between breathing and electroencephalogram (EEG) activity was first mentioned by Hobson. The study found that with the change of frog's state from rest to activity, EEG also changed from low frequency fast wave mode to high frequency slow wave mode, and the degree of synchronization of EEG was proportional to the respiratory frequency (Hobson, 1967). Faber et al. (1970) described the oscillatory activity of the human EEG during the breathing cycle, and found that the amplitude of the EEG curve increased during inhalation, while the EEG curve showed the opposite trend during exhalation. A cyclic pattern of electrical activity in the brain can be observed during sleep as breathing changes (Evans, 1992). Later, it was found that different modes of nasal breathing (including left nostril inhalation and right nostril exhalation, and vice versa) increased power in the beta and alpha bands (Stančák and Kuna 1994). The results of electroencephalogram (EEG) studies on breathing focused meditation activities indicate an increase in alpha and theta power in the posterior brain region and the frontal region (Tang, 2017; Tang et al., 2019). An increase in frontal theta may indicate a need for cognitive control and call on other brain regions (Cavanagh and Frank 2014). Recently, one study compared Heartbeat Evoked Potential (HEP) activity for heartbeats occurred during inhalation and exhalation at rest. The found higher HEP amplitude during exhalation, compared to inhalation, over fronto-centro-parietal areas. This suggests increased brain-heart interactions and improved cortical processing of the heartbeats during exhalation (Zaccaro et al. 2022). There is growing evidence that breathing directly effects on neural oscillations in various brain regions (Kluger and Gross 2020). Previous studies have primarily focused on the characteristics of brain activity during exhalation and inspiration. However, given the benefits of slow breathing for emotional regulation, it is essential to also consider the characteristics of brain activity during slow breathing. Anxiety are the psychological symptoms typically associated with autonomic overactivity, subjectively, the individual feels nervous and flushed, heart palpitations, shortness of breath, increased sweating (Goddard and Charney 1997). Changes in breathing can be both the consequence of an increased level of anxiety as well as the source of threat experienced by the individual, which, in turn, leads to increased anxiety. Thus, assessing breathing might be a useful physiological marker of the level of anxiety but can also serve as an experimental tool to influence anxiety levels (Paulus 2013).

A review by Weng examined studies on the manipulation of breathing (Weng et al. 2021). It suggests that slow breathing may activate cardiopulmonary pressure receptors, resulting in reduced reflexive sympathetic nerve activity and subsequently lower anxiety levels. However, the exact process remains unclear. This study combined paced breathing with the threat uncertainty task (Morriss, 2019) to create a new experimental paradigm. The aim was to induce anticipatory anxiety and investigate whether slow breathing is beneficial in reducing responses (valence, arousal) associated with anticipatory anxiety. After participants performed breathing exercises, we investigated the genesis and development of biased estimates of emotional pictures under conditions of uncertainty. We continuously monitored participants' self-reported scores of emotional pictures (de Jong, Merckelbach, and Arntz 1995) and EEG measurements of neuronal activity during the experiment. On the basis of conceptualizing uncertainty as an anxiety state, we expected to observe biases related to uncertainty in post-experiment estimates, where uncertain cues would be overly associated with negative pictures. Across species, physiological responses to threats are heightened when there is uncertainty about their nature, probability, or timing (Susan Mineka and Kihlstrom, 1978; S Mineka, 1985; Shankman et al. 2011; Sarinopoulos et al. 2010; Grillon et al. 2004; Dunsmoor, Bandettini, and Knight 2008; Daniel W. Grupe and Nitschke 2011). Furthermore, aversive events that are not fully predictable have a greater negative impact on mood, state anxiety and physiological indices of reactivity than those that are fully predictable (Dunsmoor, Bandettini, and Knight 2008; Sarinopoulos et al. 2010; Daniel W. Grupe and Nitschke 2011). We hypothesized that: (1) Slow breathing has a significant regulating effect on anticipatory anxiety. Compared with fast breathing, individuals under slow breathing conditions exhibit lower valence and arousal in response to negative images; (2) Uncertainty can increase anticipatory anxiety, and individuals show more valence and arousal to pictures presented after uncertain cues; (3) The electrical activity of the brain varies significantly at different breathing rates, exhibiting distinct characteristics between fast and slow breathing patterns.

## Materials and methods

### Participants

Twenty-seven college students were recruited for this study, but two participants lacked complete EEG data. The final sample included 25 healthy female participants from Southwest University who took part in the study. The participants were 18-26 years old, with a mean age of 20.72 years ( $SD = 2.324$ ). All individuals signed a written informed consent form. According to self-reports, all participants were right-handed, had no psychiatric or neurological diseases, and had normal or corrected-to-normal eyesight. This study was approved by the Ethics Committee of the Department of Psychology, Southwest University.

### The Paced breathing-Threat uncertainty task and procedure

The experiment was conducted in a quiet room at the appropriate temperature. After the participants arrived at the laboratory, they first took a rest, then were briefed on the tasks they needed to complete, and practiced the experimental procedures. After the participants had familiarized themselves with the task, the experiment was conducted in 8 blocks, each containing 15 trials. After completing each block, participants can rest for 1-2 minutes. The individuals completed all the trials at the end of the study. Participants performed the Paced Breathing-Emotional Anticipation Task. This experiment mainly consists of two phases: the breathing exercise and emotional anticipation. During the breathing phase, individuals were instructed to complete the breathing exercises guided by the audio. The audio was recorded by a teacher with 9 years of experience teaching yoga. The fast breathing conditions were defined as 15 breaths per minute, and slow breathing conditions were defined as 5 breaths per minute. The two breathing conditions were presented alternately, and a blank screen was displayed for 1 second immediately after each breathing exercise. During the threat uncertainty task phase, a 3-second cue was presented to indicate the type of picture that would appear later. The cue "O" represented negative pictures, while "?" represented uncertainty about the type

of picture to be presented, which could be neutral, positive, or negative (emotional anticipation: After ”O” or ”?”, before the picture.). Subsequently, the picture was shown, and participants rated the negativity of the picture on a scale from 0 (not negative at all) to 6 (very negative), as well as their level of arousal on a scale from 0 (no feeling at all) to 6 (very strong) after viewing the picture for 4 seconds. In fact, only negative images appeared after ”?”, which the subjects did not know. The response time for each question is 5 seconds, and the process is repeated until the experiment is complete, totaling 60 attempts. ECG and EEG were recorded simultaneously throughout the entire experiment, and the experimental procedure was presented using E-prime 2.0(see Fig. 1). The 60 negative images from the International Affective Image System had a valence range of 1.26-3.07 (mean  $\pm$  SD,  $2.31\pm 0.41$ ) and an arousal range of 2.50-6.89 (mean  $\pm$  SD,  $5.60\pm 0.63$ ). The pictures were randomly assigned to each experimental condition. In order to ensure the equitable distribution of images, we conducted a 2 (respiratory rate: fast breathing, slow breathing)  $\times$  2 (certainty: certain, uncertain) repeated-measures ANOVA, with valence and arousal as dependent variables. The results show that whether valence or arousal is the dependent variable, the main effect of respiratory rate ( $p=0.161$ ) or ( $p=0.121$ ) was not significant, and the main effect of certainty ( $p=0.500$ ) or ( $p=0.771$ ) was not significant. The interaction between respiratory rate and determinism ( $p = 0.746$ ) or ( $p = 0.941$ ) was not significant. The results indicate that the distribution of images in each experimental condition does not affect the study outcomes. Therefore, the random allocation of images in this study is deemed reasonable and effective

## Electroencephalography data acquisition, preprocessing and analysis

Acqknowledge 5.0 was used for Heart rate and heart rate variability (HRV) analysis; time and frequency domain parameters were calculated. The time domain indicator used was RMSSD (ms), namely the square root of the mean squared differences between successive RR intervals. The frequency domain index was HF (ms<sup>2</sup>), namely, the absolute power of the high-frequency band (0.15–0.4HZ). Specifically, RMSSD represents short-term variation in NN cycles and high-frequency oscillations caused by parasympathetic activity. HF is a marker of parasympathetic tone. In this study, to ensure the stability of each indicator in each experimental condition, we calculated the average value of each indicator under the same conditions for subsequent data analysis. EEG data were collected with a 64-channel Brain Products system (Brain Products GmbH, Munich, Germany), according to the international 10-20 system. The midfrontal electrode (FCz) was used as the reference and the inion electrode (AFz) as the ground. Electrode impedance was kept below 10 k $\Omega$  for all channels. All signals were recorded with a sampling rate of 500 Hz; EEG data were pre-processed offline using EEGLAB (v2021.1; Delorme and Makeig 2004) toolbox algorithms running on a MATLAB environment (R2021a, MathWorks Inc.). Two electrodes, HEO and VEO, were excluded. Then, the EEG signal was re-referenced to the average reference (i.e., calculated across all electrodes)(Dien 1998). An introduction to the event-related potential technique. MIT press.). Data were filtered by applying a high-pass filter of 0.1 Hz (forward-phase Butter-worth filter, 6-dB/octave roll-off) and a low-pass filter of 30 Hz (zero-phase Butterworth filter, 24-dB/octave roll-off) with an additional notch filter of 50 Hz (2 Hz width). For all participants, EEG epochs were extracted with a time window of 18s (breathing period: 15s; emotional anticipation period: 3s). The signal were visually inspected for the removal of artefacts and the detection of noisy channels. Bad segments were manually rejected. Noisy EEG channels were then removed and interpolated using their neighbouring channels(Al et al. 2021; Junghöfer et al. 2000). Rejected channels were generally few (~5%, depending on the EEG recording). Retained signal was submitted to Independent Component Analysis to visualize and manually remove sources of heartbeat, ocular, and muscle artifacts (Fast ICA algorithm; Hyvärinen 1999). EEG data for the Spectral analysis employed the EEGLAB toolbox (41, <http://scn.ucsd.edu/eeglab>). Spectral analysis of absolute power for 1-30Hz was conducted using the multitaper spectral estimation with Hanning taper and 0.5 frequency resolution in EEGLAB. The spectral power was decomposed into the following spectral components: delta (0.5-3.5 Hz), theta (4.0-7.5 Hz), alpha (8.0-12.0 Hz) and beta (12.5-30.3 Hz) (Motamedi-Fakhr et al. 2013). This spectral analysis was performed on data recorded during the breathing and emotional anticipation conditions in the whole brain.

## Result

### The result of self-report scores

In order to test the effects of breathing rate and certainty on anxiety, we performed ANOVA with breathing rate (fast breathing or slow breathing) and certainty (certain or uncertain) as the independent variables, and the scores of valence and arousal as the dependent variables. The analysis was corrected by Tukey. The descriptive statistics of self-report scores are presented in Table 1 and Figure 2. The valence, as the dependent variable, showed a main effect of respiratory rate ( $F = 5.46, p = 0.028, \eta^2 = 0.023$ ). The scores for valence were lower during slow breathing compared to fast breathing. The main effect of certainty ( $F = 20.98, p = 0.001, \eta^2 = 0.073$ ) was significant, with higher valence scores found in uncertain situations compared to certain ones. The interaction between respiratory rate and certainty was marginally significant ( $F = 4.16, p = 0.052, \eta^2 = 0.013$ ). Post hoc comparisons indicated that the main effect of respiratory rate was significant ( $p = 0.028$ ), with valence scores being lower during slow breathing than during fast breathing. The main effect of certainty was significant ( $p = 0.001$ ), the scores for valence were lower in certainty than in uncertainty. The arousal as dependent variable, showed a main effect of respiratory rate ( $F = 5.473, p = 0.028, \eta^2 = 0.018$ ), the scores of arousal were lower during slow breathing compared to fast breathing. The main effect of certainty ( $F = 5.940, p = 0.023, \eta^2 = 0.017$ ) was significant, with higher arousal scores found in uncertain situations compared to certain ones. The interaction between respiratory rate and certainty was not significant ( $F = 0.607, p = 0.443, \eta^2 = 0.001$ ). Analysis of self-reported valence and arousal showed that certainty of success induced anxiety, and slow breathing can reduce the anxiety experienced by the participants.

**Table1: Descriptive statistics of self-report scores**

Respiration rate	certainty	Valence ( <b>m, <math>\sigma</math></b> )	Arousal ( <b>m, <math>\sigma</math></b> )
Fast breathing	certain	<b>4.36(0.68)</b>	<b>4.16(0.94)</b>
	uncertain	<b>4.85(0.64)</b>	<b>4.35(0.97)</b>
Slow breathing	certain	<b>4.31(0.58)</b>	<b>3.85(0.83)</b>
	uncertain	<b>4.52(0.59)</b>	<b>4.16(0.78)</b>

m = mean,  $\sigma$  = standard deviation

### The result of HR and HRV

To investigate the effect of breathing rates on cardiac activity, paired sample t-tests were conducted between slow and fast-paced conditions to detect group differences in heart rate (HR), the time domain (RMSSD), and frequency domain index (HF). The descriptive statistics and test comparison between two breathing rates are presented in Table 2. The results showed that in both RMSSD and HF, slow breathing resulted in higher values compared to fast breathing, but the differences did not reach statistical significance. The heart rate was significantly different between the two breathing rates, with a higher heart rate observed at faster breathing rates.

**Table2: Descriptive statistics and test comparisons between two breathing rates**

	Fast-paced breathing ( <b>m, <math>\sigma</math></b> )	Slow-paced breathing ( <b>m, <math>\sigma</math></b> )	t-test fast versus slow ( <b>t, df, p</b> )
<b>HR bpm</b>	73.6(9.66)	71.4(7.57)	(3.77, 24, <0.001)
<b>RMSSD (ms)</b>	77.3(39.16)	84.9(59.44)	(-1.07, 24, 0.297)
<b>HF power (ms<sup>2</sup>)</b>	14409(28978)	22467(74608)	(-0.76, 24, 0.454)

## The result of spectral analysis during in breathing phase

The absolute power of the four spectral bands in the breathing phase is obtained through spectral analysis (Table 3). In order to explore the characteristics of the EEG related to respiratory rate, a paired-sample t-test was conducted. The analysis was based on the power of the entire brain during the respiratory phase to examine the variances in the power of the four frequency bands: delta, theta, alpha, and beta under conditions of fast and slow breathing (see Fig. 3). Within each frequency band, there was a significant effect by respiration rate (delta ( $p = 0.008$ ), theta ( $p = 0.001$ ), alpha ( $p = 0.006$ )), but no significant difference was found in the beta band ( $p = 0.192$ ). There is an overall increase in power during slow breathing in the delta, theta, and alpha bands, while this increase is less pronounced for beta.

**Table3: EEG power in breathing phase**

	delta	theta	alpha	beta
	(m, $\sigma$ )	(m, $\sigma$ )	(m, $\sigma$ )	(m, $\sigma$ )
Fast breathing	<b>8.95(2.06)</b>	<b>0.83(1.66)</b>	<b>-0.20(3.28)</b>	<b>-5.48(1.76)</b>
slow breathing	<b>9.32(2.04)</b>	<b>1.15(1.73)</b>	<b>0.03(3.31)</b>	<b>-5.38(1.78)</b>

EEG power for delta, theta, alpha and beta frequency bands ( $10 \cdot \log_{10}(\mu V^2 / \text{HZ})$ ) (m = mean,  $\sigma$  = standard deviation) for breathing phase.

## The result of spectral analysis during in emotional anticipatory phase

Using the same method mentioned above, the absolute power of the four spectral bands during the emotional anticipation phase is obtained through spectral analysis (Table 4). Next, we performed ANOVA with the absolute power of delta, theta, alpha, and beta as the dependent variables, and respiration rate (fast breathing or slow breathing) and certainty (certain or uncertain) as the independent variables. The analysis was corrected by Tukey.

For the absolute power of delta, the ANOVA shows that the main and interaction effects of respiration rate and certainty were not significant. For the absolute power of theta (see Fig. 4), the ANOVA shows a main effect of respiratory rate ( $\Phi = 2.7516$ ,  $\pi = 0.006$ ,  $\eta^2 = 0.014$ ), the power under fast breathing is higher than that under slow breathing. The main effect of certainty ( $\Phi = 0.107$ ,  $\pi = 0.747$ ,  $\eta^2 = 0.001$ ) was not significant. The interaction of respiration rate and certainty was found to be significant ( $\Phi = 7.767$ ,  $\pi = 0.10$ ,  $\eta^2 = 0.006$ ). To be specific, during fast breathing, the absolute power was higher under uncertain conditions than under certain conditions. Conversely, during slow breathing, the absolute power was higher under certain conditions than under uncertain conditions. Moreover, the absolute power of theta was highest when participants were engaged in fast breathing and faced uncertainty.

For the absolute power of alpha, the ANOVA shows a main effect of respiratory rate ( $\Phi = 8.862$ ,  $\pi = 0.007$ ,  $\eta^2 = 0.005$ ), the power under fast breathing is higher than that under slow breathing. The main effect of certainty ( $\Phi = 0.736$ ,  $\pi = 0.399$ ,  $\eta^2 = 0.000$ ) was not significant. The interaction between respiration rate and certainty was not significant ( $\Phi = 0.181$ ,  $\pi = 0.675$ ,  $\eta^2 = 0.000$ ). Finally, for the absolute power of beta, the ANOVA shows a main effect of respiratory rate ( $F = 12.020$ ,  $p = 0.002$ ,  $\eta^2 = 0.022$ ), the power under the fast breathing is higher than that under slow breathing. The main effect of certainty ( $F = 0.914$ ,  $p = 0.349$ ,  $\eta^2 = 0.001$ ) was not significant. The interaction between respiration rate and certainty was not significant ( $F = 3.736$ ,  $p = 0.065$ ,  $\eta^2 = 0.004$ ). In summary, only theta, respiratory rate, and certainty had a significant effect on brain activity during emotional anticipation tasks. When breathing slowly, the absolute power of theta decreases when facing uncertain tasks. Conversely, when breathing quickly, the absolute power of theta increases in response to uncertain tasks.

**Table4: EEG power in anticipatory phase**

Respiration rate	certainty	delta	theta	alpha	beta
		( <b>m</b> , $\sigma$ )	( <b>m</b> , $\sigma$ )	( <b>m</b> , $\sigma$ )	( <b>m</b> , $\sigma$ )
Fast breathing	certain	<b>9.05(1.62)</b>	<b>1.11(1.45)</b>	<b>-0.61(2.55)</b>	<b>-5.47(1.79)</b>
	uncertain	<b>8.93(1.75)</b>	<b>1.31(1.46)</b>	<b>-0.56(2.51)</b>	<b>-5.16(1.78)</b>
slow breathing	certain	<b>8.80(1.84)</b>	<b>1.00(1.42)</b>	<b>-0.97(2.16)</b>	<b>-5.61(1.53)</b>
	uncertain	<b>8.42(1.68)</b>	<b>0.75(1.38)</b>	<b>-0.86(2.37)</b>	<b>-5.73(1.66)</b>

EEG power for delta, theta, alpha and beta frequency bands ( $10 \cdot \log_{10}(\mu V^2/HZ)$ ) ( $m = \text{mean}$ ,  $\sigma = \text{standard deviation}$ ) for **anticipatory phase**.

## Discussion

Respiratory rhythm is one of the main oscillating rhythms of the body, which is the main source of interoceptive information for the brain. Anxiety is a disease of physical and mental interaction, which is characterized by excessive worry about uncertain events in the future and disturbance of autonomic nervous system. Previous studies have explored the relationship between breathing and anxiety from low-level perceptual sensitivity and higher level metacognition (Harrison et al. 2021), but have not explored the regulating effect of interoception on anxiety at the regulatory level. This study explored the relationship between anxiety and interoception from the perspective of operable interoception-breathing, and explored the role and mechanism of slow breathing in regulating anxiety. This study combined paced breathing with the threat uncertainty task to create a new experimental paradigm for the first time. The self-reported results found that slow-paced breathing was associated with lower valence and arousal, which means that compared with fast-paced breathing rate, slow-paced breathing is a beneficial intervention response to uncertain threat information. Analysis of heart rate variability found that the heart rate was lower during slow breathing conditions. Some interesting results were found by the spectral analysis of EEG. In the breathing phase, the delta, theta, and alpha EEG power are increased during the slow-paced breathing in emotion anticipatory phase, only in theta, respiratory rate and certainty had a significant effect on the brain activity. When participants are faced with uncertain information, the theta EEG power decreased during slow-paced breathing, however, the theta EEG power increased during fast-paced breathing. In the self-reported results, slow breathing rate was found to be more effective in reducing anxiety compared to fast breathing. We found higher levels of valence and arousal in the uncertain condition compared to the certain condition. Through slow breathing exercises, participants' responses to the emotional stimuli of an impending uncertain threat were diminished. It may be that slow breathing prepares the individual physically and psychologically for future anxious events. Across disorders, uncertainty is thought to provoke anticipatory anxiety and to result in behaviors that are maladaptive attempts to reduce uncertainty, such as worry, reassurance seeking, checking, and hypervigilance (Meisler 2006). In previous studies, uncertainty has been defined as a lack of information about an event and has been characterized as an aversive state that people are motivated to reduce. The researcher propose an uncertainty intensification hypothesis, whereby uncertainty during an emotional event makes unpleasant events more unpleasant (Bar-Anan, Wilson, and Gilbert, 2009). This study supports the hypothesis. Why might online uncertainty amplify reactions to an ongoing event? One possibility is that uncertainty heightens people's attention. That is, just as uncertainty keeps an event accessible after it occurs (Wilson et al. 2005), it might also maintain people's attention on an ongoing event, intensifying their reactions to it. Another possibility is that uncertainty increases people's curiosity about an emotional event, leading them to become more emotionally engaged with it. That is, people may pay equal attention to an emotional event, but those who are uncertain may be more curious about what is happening, which makes them more engaged in the event. In this study, it is confirmed that slow breathing can better regulate emotional experiences in anxious situations and expand the application of slow breathing as a convenient mode of regulation. A higher HRV is an indicator of adequate adaptation to the new environment and effective functioning of the autonomic nervous system (ANS). The yoga practicing



group showed a significant increase in HRV ( $< 0.0304$ ) and a reduction in resting heart rate ( $p < 0.0389$ ) in a study (Iii et al. 2007.). The escalation in the heart rate is due to increased sympathetic and decreased parasympathetic activity. A significant improvement in HRV may be due to an increase in parasympathetic activity or a decrease in sympathetic activity. These factors indirectly contribute to reducing psychological parameters such as distress, anxiety, and depression in young healthy subjects (Gaur, Panjwani, and Kumar 2020). In this study, the short, slow-paced breathing exercises reduced participants' heart rate. The high spectral energy of the HRV power spectrum component reflects the activity of the parasympathetic nervous system. According to the results of Heart Rate Variability (HRV) analysis, we found that the RMSSD and HF values were higher during slow-paced breathing. This suggests that parasympathetic activity could be activated through slow breathing, effectively reducing participants' negative emotional experiences. No significant effect of respiratory rate on RMSSD and HF was found, which may be attributed to the short duration of the breathing exercises. Previous studies have shown that the breathing exercises lasted longer than one minute. It is also worth exploring the influence of breathing on HRV from the perspective of time effect. In this study, we pay close attention to the EEG activity in participants during the breathing phase. This study found that slow breathing induced stronger neural oscillatory activity. During slow-paced breathing, we observed an increase in the amplitudes of delta, theta, and alpha bands in specific brain regions. However, small and insignificant differences were observed in the beta band. It is generally believed that the main waveform when the cerebral cortex is excited is alpha, which reflects the excitability of brain neurons. They are more common when normal individuals wake up, and generally, normal EEG fast wave bands and alpha band waves appear simultaneously. The beta band belongs to the fast-wave band, which is different from the wave in the alpha band. Some scholars (Petsche, Etlinger, and Filz 1993) believe that beta waves are characteristic waves of cognitive function. They are more common when normal individuals wake up, and generally, normal EEG fast wave bands and alpha band waves appear simultaneously. Slow breathing exercises can elevate the level of arousal in the cerebral cortex. The results presented in the beta band may be related to the breathing task of the experiment itself. During the breathing exercises, there is more of a change in physiological activity, specifically in the respiratory rate, rather than being related to cognitive activity. There are fewer studies on EEG recordings at various respiratory rates. The influence of the respiratory cycle on the EEG is also observed in the literature. During spontaneous breathing and bradypnea, there was an increase in delta power (Bušek and Kemlink 2005). EEG exploration of the electrophysiological evidence of slow breathing can offer a deeper understanding of the neurophysiological mechanisms involved in slow breathing. It can also lead to the development of more effective strategies for managing anxiety and stress. Over the past six years, a rapidly growing number of studies have shown that respiration exerts a significant influence on sensory, affective, and cognitive processes. At the same time, an increasing amount of experimental evidence indicates that this influence occurs via the modulation of neural oscillations and their synchronization between brain areas (Heck et al. 2022). This study also examined the impact of slow breathing on the cognitive processing of anxiety-related cues at the neural activity level. During the emotional anticipation phase, participants experience a decrease in theta EEG power when presented with uncertain information while engaging in slow-paced breathing. Slow breathing induces a calm and relaxed state, enhancing the brain's focus on deterministic information while reducing its attention to uncertain information. However, when participants are faced with uncertain information, the theta EEG power increased during fast-paced breathing. Fast breathing creates a mild state of alertness or anxiety that causes the brain to pay more attention to uncertain information. At the same time, increased theta band activity in response to uncertain tasks may also be associated with mental effort level (Smit et al. 2005). The elucidation of the physiological mechanisms and neural pathways regulating breathing can help to better understand how an emotional state emerges from the interaction between the body and the brain. Much work needs to be done to better delineate the direction of the relationship between breathing and anxiety, as well as to evaluate how brain systems respond to the modulation of breathing as a powerful intervention to reduce levels of anxiety. The results of this study are encouraging. However, researchers must consider many potential shortcomings when interpreting these research results. In this experiment, we focused solely on anxiety, specifically state anxiety. Further research is needed to explore the impact of slow breathing on trait anxiety. Finally, this study only explores the regulation effect of slow breathing on anxiety, and other

measures can be combined mindfulness, meditation to regulate anxiety in the future.

## Conclusion

In conclusion, the present study we combined the paced breathing task with the threat uncertainty task for the first time to investigate the role of slow breathing in regulating anxiety, and using Spectral analysis of EEG to assess brain activity relating respiratory rate and the mechanism of respiratory rate impact on the anxious. In the study, including self-report scores, heart rate, and EEG provided evidence for the mood-regulating effects of slow breathing. These findings provide direct insight into slow breathing between anxiety, providing direct evidence that slow breathing reduces anxious or other negative emotions.

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