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Anxiety reduction through biofeedback from pupil size

Experimental research on the effect of pupil size biofeedback on anxiety reduction through breathing

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Abstract

In this thesis, the effects of biofeedback from audible pupil size on the effectiveness of respiratory anxiety reduction were studied. Previous studies report proof for the effectiveness of passive mindfulness methods on self-regulation of emotions, but this effectiveness is measured subjectively with self-reports. Biofeedback can be used to add objectivity to anxiety reduction. Biofeedback is a real time representation of physiological data, and it has been proven to increase effectiveness of anxiety reduction compared to passive anxiety reduction methods. Since pupil size it not commonly used for biofeedback research, but correlates with anxiety, it is interesting to use pupil size biofeedback during an anxiety reduction task to find new insights to add to the existing literature and to find new methods to self-regulate anxiety.

Forty-two participants were randomly assigned to a biofeedback condition or a control condition. They completed self-report anxiety questionnaires, a timed math task and a five-minute breathing exercise. Participants in the biofeedback condition heard their real time pupil size during the experiment updating them on their anxiety level, whereas participants in the control condition did not hear any sound. Independent samples T-tests and a Repeated Measures analysis were performed with the self-reported anxiety scores and measured pupil size values. The results showed that there were differences between the self-reported anxiety levels and the measured anxiety levels over the course of the experiment, but no differences were found between the conditions for both self-reported anxiety and anxiety measured through pupil size. These results show that there is no effect of biofeedback from audible pupil size on the effectiveness of respiratory anxiety reduction.

1. Introduction

Anxiety is a brain state that elicits defensive behavioral responses to avoid or reduce harm to an individual (Tovote, Fadok & Lüthi, 2015). It puts large burdens on affected individuals and society in general, resulting in high odds of psychiatric disorder hospitalizations (ADAA, 2019). Anxiety often comes from stress and fear, which are feelings of emotional tension (O'leary, 1990). To self-regulate anxiety, the process of making self-corrective adjustments to one's thoughts and feelings (Carver & Scheier, 2004), mindfulness methods such as meditation, relaxation and breathing exercises are used (Jerath, Crawford, Barnes & Harden, 2015). These methods, for example using a breathing exercise application that presents a breathing rhythm, help the user calm down. While passive mindfulness methods have been proven to have positive effects on self-regulation of emotions (Brown & Ryan, 2003), they do not measure anxiety reduction during the process. Measuring and presenting anxiety levels during self-regulation methods can possibly support and improve these methods. A technology that does have the features to measure and present physiological cues and that can be used during anxiety reduction methods, is biofeedback.

Biofeedback is the perception of physiological cues: a real time representation of physiological data derived from unconscious biological processes (Wheat & Larkin, 2010). The sensory feedback provided by this data can be visualized to discover the existence of normally invisible physiological processes (Miller, 1978), which makes it possible to utilize this real time presentation of physiological data to be used for self-regulation methods (Gruzelier & Egner, 2004). These self-regulatory methods can be used to control one's thoughts, behaviors and feelings, making it possible to self-regulate one's anxiety (Carver & Scheier, 2000). Biofeedback can be perceived by sensory technologies tracking, translating and displaying physiological information such as heart rate, skin conductance, brain waves (Christopher deCharms et al., 2005) and pupil size (De Rooij, Schraffenberger & Bontje, 2018). Because biofeedback makes these physiological processes accessible, for example through vision or sound, they can be used to understand more about one's cognitive and emotional state in certain situations (Carver & Scheier, 2000). Sherlin, Gevritz, Wyckoff and Muench (2009) for example showed that breathing based on a rhythm created from one's heart rate biofeedback has greater effects on anxiety reduction than passive relaxation methods. Breathing in a rhythm based on one's heart rate stimulates the baroreceptors which strengthens the body's homeostatic function. It increases vagal activity and stimulates relaxation (Lehrer et al., 2003). This incentivizes to look into biofeedback technology to support and improve passive mindfulness methods.

Besides heart rate variability, another physiological cue that is tied with anxiety is pupil size (White & Depue, 1999). Pupil size is not commonly researched as a biofeedback method (Ehlers, Strauch, Georgi & Huckauf, 2016), but interestingly, there are correlations between pupil size and anxiety (White & Depue, 1999). The eye and its pupil are highly sensitive to light, causing the dilator pupillae muscle to dilate with little light and to contract with bright light. However, arousal also correlates with pupil dilation (Eckstein et al, 2017). The noradrenergic locus coeruleus modulates pupil dilation by releasing noradrenaline, a neuromodulator that influences the central nervous system activity. Noradrenaline is essential for brain development and influences cortical processing globally, having a crucial influence on cognitive processes being a neuromodulator of brain activity. Allocation of cognitive resources is modulated by noradrenaline, thus correlating with associated cognitive processes. Therefore, aroused and cognitive brain states such as anxiety correlate with pupil size through the noradrenergic locus coeruleus (Eckstein et al, 2017), opening up possibilities to self-regulate anxiety with biofeedback through pupil size. It should be stated that not everyone is capable of cognitively controlling their pupil size (Ehlers, Strauch & Huckauf, 2018). However, since the majority of people is capable of cognitively controlling their pupil size, it is an interesting field of research. But, so far there is little research done on respiratory anxiety reduction methods through biofeedback from pupil size.

Passive respiratory anxiety reduction methods can perceivably reduce a user's anxiety level. A user can subjectively self-report that anxiety levels have been reduced even when this is objectively not the case. Using biofeedback from pupil size, a sensory cue correlated with anxiety (White & Depue, 1999), these subjective self-report errors can be prevented by providing the users with their real time anxiety level based on their physiological data. This can for example be done by making users' pupil size audible, using a sound that represents their real time pupil size. This results in more accurate anxiety reduction by transitioning from solely subjective measurements by adding objective measurements. It excludes the possibility for subjectively but not objectively reducing anxiety, which does not aid the users but leaves them with increased risk of experiencing physical health problems (ADAA, 2019). Using pupil-size biofeedback could improve the effectiveness of traditional passive respiratory anxiety reduction methods, for example by increasing the amount of anxiety reduced, accelerating the anxiety reduction process and by helping users reach lower anxiety levels. Since the scarceness of biofeedback from pupil size research on respiratory anxiety reduction in combination with the potential improvement of traditional passive anxiety reduction methods incentivizes to do research in this area, the following research question will be answered in this thesis: *What is* *the effect of biofeedback from audible pupil size on the effectiveness of respiratory anxiety reduction?*

In the next chapter, containing a theoretical framework, the literature on anxiety reduction and biofeedback will be introduced. In the third chapter, the research question will be explained based on the literature. The fourth and fifth chapters will describe the study and the results, and the sixth chapter will contain the conclusion and discussion of the study.

2. Theoretical framework

2.1 Anxiety

Emotions play an important role in the variety of human experience (Dolan, 2002). They are key to human relationships and can enhance or decrease human performance (Ashkanasy, 2004). Emotions are manifestations of components of arousal and valence (Russell, 1980). Arousal indicates the excitement level of an emotion, where valence indicates the degree of pleasure and displeasure (Cederholm, Hilborn, Lindley, Sennersten & Eriksson, 2011). Anger and happiness are typical unpleasurable and pleasurable aroused emotions, while sadness and satisfaction are typical unpleasurable and pleasurable non-aroused emotions. An aroused and unpleasurable emotional state that has clear affinities with stress, a feeling of emotional tension, is anxiety (Lovibond & Lovibond, 1995).

Anxiety is a brain state caused by both external and internal stimuli. It can be identified with measurable behavioral, physiological, hormonal and autonomic reactions (Tovote, Fadok & Lüthi, 2015). Symptoms of anxiety include excessive worry, tension, restlessness and irritability (Archer et al., 2012). Anxiety disorders are one of the most common mental disorders. According to Baxter, Scott, Vos and Whiteford (2013), globally, one in thirteen people is affected by anxiety. People with anxiety experience poorer quality of life, have greater disability and use more health care services than people without anxiety (Porensky et al., 2009). They also suffer greater functional and economic burden (Hoffman, Dukes & Wittchen, 2008). As functional methods to combat anxiety, mindfulness methods have raised an increased interest and are integrated into therapeutic work (Allen, Chambers & Knight, 2006). With these techniques, anxiety can be regulated and the negative effects of anxiety can be diminished (Goldin & Gross, 2010).

2.2 *Anxiety reduction*

There are many methods to reduce one's anxiety. Some of the most popular and effective methods are mindfulness methods (Jerath, Crawford, Barnes & Harden, 2015). Mindfulness has been described as paying attention in a present experience (Bishop et al., 2004). It originates from cultural and religious traditions, such as Budhism, but in the current day, mindfulness practice and techniques can effectively be used by anyone without any prior knowledge about the cultural or religious traditions (Allen, Chambers & Knight, 2006). In psychology, mindfulness has been adopted as an approach of increasing awareness and handling cognitive processes, for example emotion regulation, and specifically as treatment for emotional and behavior disorders such as chronic stress and anxiety (Bishop et al., 2004). There are multiple different mindfulness methods that can be used to reduce one's anxiety. The most common methods are relaxation and meditation.

2.2.1 *Anxiety reduction through relaxation*

In relaxation, there is an intentional focus to relax during the practice, either through exercises or imaginary techniques (Jain et al., 2007). There are lots of studies that researched anxiety reduction with relaxation techniques. Smith, Hancock, Blake-Mortimer and Eckert (2007) studied whether there were differences between yoga and relaxation in stress, anxiety and quality of live scores over time. Participants with mild to moderate levels of stress attended ten weekly one-hour sessions of yoga or relaxation. The self-reported stress, anxiety and quality of life scores had significantly improved after six weeks. Both yoga and relaxation were effective in reducing stress and anxiety.

Manzoni, Pagnini, Castelnuovo and Molinari (2008) conducted a meta-analysis with 27 studies about the efficacy of relaxation training for anxiety problems and disorders. Most of the studies in the meta-analysis used the State Trait Anxiety Index - State (STAI-S) subscale (Spielberger, 2010), a self-report scale, as a measurement for anxiety. The relaxation trainings they included were Jacobson's progressive relaxation and applied relaxation, which are relaxation methods where one tightens and then relaxes bodily muscles, and autogenic training, which is a form of self-hypnosis. The studies they used in the analysis showed a medium-large effect size in the treatment of anxiety. Consistent and significant efficacy of relaxation training in reducing anxiety was found.

Davis and Thaut (1989) studied physiological and psychological responses to participant's preferred relaxing piece of music. Physiological data that was collected included vascular constriction, heart rate, muscle tension and finger skin temperature. Participants listened to a piece of relaxing music of their choice and completed the STAI-S to report their anxiety levels before and after listening to the relaxing music. The results from the physiological data showed that the music aroused and excited the participants more than it soothed muscular activity. However, the STAI-S self-reported scores showed that anxiety decreased, and relaxation increased after listening to the piece of music.

2.2.2 Anxiety reduction through meditation

Meditation is defined as "the practice to self-regulate the body and mind, thereby affecting mental events by engaging a specific attentional set." (Cahn & Polich, 2006, p.180). Just like relaxation, meditation is also a popular mindfulness method to reduce one's anxiety and it has been researched extensively. Tacón, McComb, Caldera and Randolph (2003) studied the effectiveness of mindfulness meditation in reducing anxiety in women with heart disease. The participants followed weekly two-hour sessions for eight weeks where they practiced mindfulness practices such as body scans, sitting meditation and yoga. Anxiety was measured with the STAI-S. The self-reported anxiety scores for the participants that followed the mindfulness sessions were significantly higher before the intervention compared to after the assessment. No differences were found in anxiety scores for participants in the control group.

Goldin and Gross (2010) studied effects of mindfulness-based stress reduction (MSBR) on emotion regulation in social anxiety disorder. Mindfulness-based stress reduction is a program of mindfulness training shown to reduce symptoms of stress, anxiety and depression and it consists of multiple forms of mindfulness practice, including forms of mediation and yoga. MSBR consisted of weekly 2.5-hour group sessions for 8 weeks and a half-day mediation retreat. Multiple self-report anxiety scales were used to measure anxiety, including the Liebowitz Social Anxiety scale and STAI-S. Results indicated that participants attending MSBR sessions showed improvement in anxiety and depression symptoms.

Kabat-Zinn et al. (1992) studied the effectiveness of a group stress reduction program based on mindfulness meditation for participants with anxiety disorders. Participants attended weekly two-hour meditation classes for eight weeks and attended a 7.5-hour intensive meditation retreat session in the sixth week. Anxiety was measured through self-reports with the Hamilton anxiety and depression scales and by a trained interviewer. The results showed significant reductions in anxiety and depression scores after treatment, which means that group mindfulness meditation can effectively reduce the symptoms of anxiety.

2.2.3 *Anxiety reduction through breathing exercises*

Respiratory anxiety reduction, which is anxiety reduction through the respiratory organs, is also an effective method to reduce anxiety. Breathing exercises are essentially stepping into both relaxation (Gillani & Smith, 2001) and meditation (Paul, Elam & Verhulst) domains and they have been proven to effectively aid a user in reducing anxiety. Valenza et al. (2014) conducted a study in which they researched the effectiveness of controlled breathing techniques on anxiety and depression in hospitalized patients. Participants were instructed of the controlled breathing program through a trained physiotherapist twice a day for a duration of 30 minutes. Anxiety was measured through the hospital anxiety and depression scale. The results showed that controlled breathing significantly improved anxiety scores.

Park, Oh and Kim (2013) studied the effects of relaxation breathing on pain and anxiety during burn care. Anxiety was measured through the Visual Analog Scale – Anxiety, a slider on a scale of 0 to 100. Participants performed relaxation breathing exercises during dressing change procedures. The results showed that both pain and anxiety scores significantly differed right after the intervention and over time, with lower reported pain and anxiety scores after the relaxation breathing exercise. This shows that relaxation breathing exercises help patients manage anxiety.

Chen, Huang, Chien and Cheng (2017) studied the effectiveness of diaphragmatic breathing relaxation training for reducing anxiety. Anxiety was measured using the Beck Anxiety Inventory, a self-report measure for anxiety. Participants attended an 8-week course participating in the diaphragmatic breathing program while also practicing at home at least twice a day, while the control group received no training. The results showed that participants in the diaphragmatic breathing program significantly decreased their anxiety levels between week 0 and week 4, week 0 and week 8 and week 4 and week 8. The control group made no progress in terms of anxiety reduction over the course of the eight weeks.

2.3 *Biofeedback*

Most studies on anxiety reduction use self-reported anxiety scores to measure anxiety levels. To add objectivity to anxiety measurements and to use that information during the anxiety reduction process, biofeedback technology can be used. Biofeedback is the perception of physiological cues, by representing physiological data derived from biological processes in real time (Wheat & Larkin, 2010). The physiological data can be translated into real time feedback to learn more about one’s physiological processes during interventions (Miller, 1978). This feedback can be used to self-regulate emotions (Gruzelier & Egner, 2004) by controlling one's thoughts, behaviors and feelings (Carver & Scheier, 2000).

There are studies reporting proof that using biofeedback improves emotion regulation and anxiety reduction methods. One of these studies, is the study of Henriques, Keffer, Abrahamson and Horst (2011). They explored the effectiveness of a computer-based heart rate variability biofeedback program in reducing anxiety in college students. In the pilot study, participants reported their anxiety levels with the STAI in the week before and in the week after the biofeedback sessions. Participants were introduced to the software and the theory behind it, and they were instructed to use the biofeedback program 20 minutes per day, five days a week for a four-week period. The biofeedback program required participants to smoothen their heart rhythm graph, which was regulating their anxiety through heart rate variability. The results showed a significant decrease in anxiety scores reported with the STAI-S and STAI-T scales.

A second study was conducted studying the effect of immediately beginning the biofeedback program after the orientation and training week compared to beginning the biofeedback program four weeks later. The results of the second study somewhat replicated the results of the pilot study, but the effects were less pronounced. Repeated ANOVAs found significant pre-post differences for four of the six Mood and Anxiety Symptom Questionnaire subscales. This means that some facets of anxiety were significantly reduced over time, but some others were not.

Aritzeta et al. (2017) studied anxiety reduction and improving academic performance through a biofeedback relaxation training program. Anxiety was measured through self-reports with the STAI. Heart rate variability was used as the biofeedback signal. The biofeedback training program consisted of five sessions coupled with three training activities focused on deep breathing, guided imagery and muscle relaxation. The program was applied over an 8-week period. Pre-test and post-test comparisons showed significant differences for state anxiety and trait anxiety. After the program, anxiety levels were reduced, and performance had increased. The results also showed significant differences between the experimental condition and the control condition. Participants following the program showed lower anxiety scores and higher test performance than participants in the control condition.

Morarend, Spector, Dawson, Clark and Holmes (2011) studied the use of respiratory rate biofeedback to reduce dental anxiety. Participants in the experimental group visiting the dental clinic were instructed how to use the respiratory biofeedback device that tracked the user's breathing and played a musical pattern based on the breathing rhythm to synchronize the user's breathing to a calming pace. Anxiety was measured through the Corah Dental Anxiety

Scale, the most widely used measure of dental fear, and the Dental Injection Sensitivity Survey, an instrument used to quantify patient's anxiety level at six stage of the dental encounter. There was a significant difference in anxiety levels pre operation and post operation, but no significant differences were found between the conditions. This shows that even though proof has been found for effectiveness of heart rate biofeedback methods, these effects do not necessarily apply in the respiratory domain.

2.4 Pupil size

An interesting physiological cue for biofeedback is pupil size. So far, pupil size has not commonly been researched as a biofeedback method (Ehlers, Strauch, Georgi & Huckauf, 2016). However, there are correlations between pupil size and anxiety (White & Depue, 1999), making pupil size interesting for anxiety reduction research. In a review about eye-tracking and cognition, Eckstein et al. (2017) explain the eye's functioning in high detail.

As explained by Eckstein et al. (2017), the eye's pupils constrict in response to light and dilate in response to darkness. But, light and darkness are not the only factors causing changes in pupil size. Pupils also dilate during autonomic arousal. The reason for this is that pupil dilation is modulated by the noradrenergic locus coeruleus. The locus coeruleus is a small nucleus in the brainstem that plays important roles in the regulation of physiological arousal and cognitive functioning. It is a cluster of neurons that release norepinephrine, also called noradrenaline, which influences central and peripheral nervous system activity. Activity of the locus coeruleus and noradrenaline lead to pupil dilation because the locus coeruleus has direct inhibitory projections to the parasympathetic Edinger-Westphal nucleus, which is the origin of the pupil's constricting fibers. Locus coeruleus activity indirectly dilates the pupil by inhibiting the Edinger-Westphal nucleus and the pupil's constricting muscle. Noradrenaline also influences cortical processing globally, being a neuromodulator of brain activity, and has an important influence on cognitive processes. This influence on cognition is intrinsically linked with physiological arousal by the locus coeruleus and noradrenaline system. Being a neuromodulator of brain activity, noradrenaline modulates allocation of cognitive resources, and therefore, aroused and cognitive brain states such as anxiety correlate with pupil size through the noradrenergic locus coeruleus.

No pupil size biofeedback research with anxiety reduction has been conducted yet. This study will be the first study to do so. Other studies using pupil size as a biofeedback source mainly focused on metacognitive awareness (de Rooij, Schraffenberger & Bontje 2018) and cognitive information channels (Ehlers, Strauch, Georgi & Huckauf, 2016). These studies report correlations between pupil size and cognition, which could result in pupil size biofeedback showing correlations with anxiety reduction as well. Because respiratory rate biofeedback did not have an effect on anxiety (Morarend et al., 2011), but since pupil size shows correlation with cognition and anxiety, it would be interesting to research the effects of biofeedback from pupil size on the effectiveness of respiratory anxiety reduction.

3. Research question

The field of research covering self-regulation methods with biofeedback is broad but toning it down to anxiety reduction through breathing as a self-regulation method and pupil-size as the biofeedback source leaves an interesting new field of research to study. Passive mindfulness methods have been proven to be effective on self-regulation of emotions and anxiety (Brown & Ryan, 2003; Goldin and Gross, 2010; Manzoni, Pagnini, Castelnuovo & Molinari, 2008; Valenza et al., 2014), and biofeedback assistance has been proven to be more effective on anxiety reduction than passive relaxation methods (Aritzeta et al., 2017; Henriques, Keffer, Abrahamson & Horst, 2011; Sherlin, Gevritz, Wyckoff & Muench, 2009), incentivizing researchers to look into researching mindfulness methods with biofeedback to support and improve anxiety reduction with novel and under-researched methods. Since pupil size is not commonly used for biofeedback research (Eckstein, Guerra-Carrillo, Singley & Bunge, 2017) but correlates with anxiety (White & Depue, 1999), it would be interesting to study the effects of this novel biofeedback method on anxiety reduction through breathing exercises to find new insights to add to the current theory about biofeedback and mindfulness methods and to find new methods to effectively self-regulate one's anxiety. To study these effects, the following research question will be answered:

'*What is the effect of biofeedback from audible pupil size on the effectiveness of respiratory anxiety reduction?*'

4. Method

To answer the research question, an experiment was conducted with a 1+1 between subject design. Depending on the condition the participants were randomly assigned to, they were asked to reduce their anxiety levels with a breathing exercise while being assisted with biofeedback from audible pupil size or without a biofeedback signal. The experiment is a semi-replication of that of Wells, Outhred, Heathers, Quintana and Kemp (2012).

4.1 Participants

In total, forty-two participants (24 males and 18 females, aged 18-29 M = 21.33, SD = 2.91) participated in this study. The participants were recruited through convenience sampling via the Tilburg University's participant recruitment system, where participants could sign up for a time slot of their choosing and participate in exchange for study course credit. The participants were requested to not eat or drink two hours before the experiments, to abstain from caffeine products such as coffee on the experiment day to control for the physiological impact of these variables, to wear contact lenses instead of glasses if they had low vision and to not wear any eye make-up at the time of the experiment. Ethical approval was granted by the Research Ethics and Data Management Committee of the Tilburg School of Humanities and Digital Sciences. All participants signed informed consent. The consent forms can be found in Appendix A.

4.2 Materials and measurements

4.2.1 Audible pupil size

The physiological data that was used for this experiment was the participants' pupil size, which was measured through a Pupil Labs eye-tracker. For this study, a real-time mapping of pupil size to an audible sound was used. The pupil size data was streamed to the Cycling '74 MAX 7 system via the OSC protocol, where it was mapped to volume and frequency changes of a continuously playing sine wave. The experimental setup can be seen in Figure 1. Before the experiment, the minimum and maximum pupil size of the user was recorded to normalize the signal. The last known value was kept in situations of signal loss, for example due to blinking. The user also decided the volume (dB) of which the signal was mapped to, based on personal preference. The resulting sounds are referred to as *audible pupil size* and were part of the biofeedback condition.

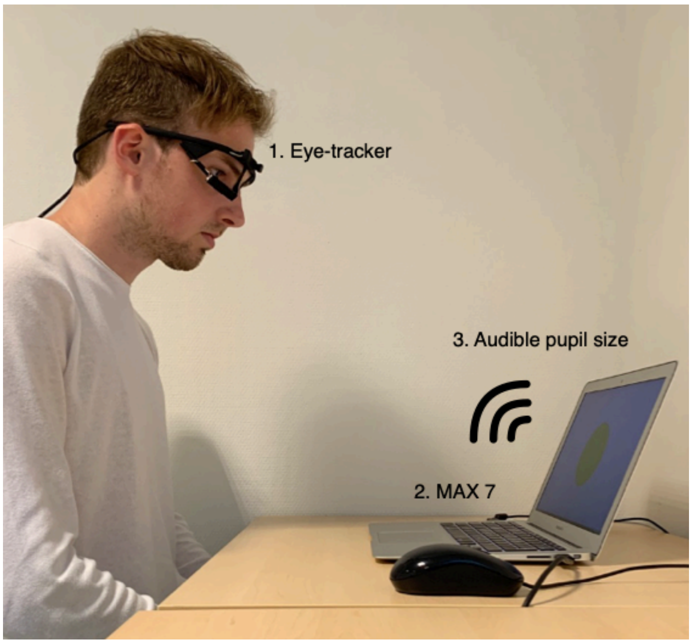

Figure 1. Experimental setup of the study. (1) The Pupil Labs eye-tracker registered the user's pupil size. (2) Through MAX 7, the pupil size data was mapped to volume and frequency changes of a sine wave in real-time, (3) which the user could hear.

4.2.2 Understanding audible pupil size

In the biofeedback condition, pupil size was converted to an audible sound representing the physiological data. When setting up the eye-tracker, participants in this condition were given an explanation about the audible pupil size corresponding with their relaxation level. A higher-pitched and higher-volume sound represented an anxious state of mind, while a lower-pitched and lower-volume sound represented a relaxed state of mind. In the no biofeedback condition, also known as the control condition, pupil size was not converted to an audible sound, so no additional explanation was given.

The explanation placed emphasis on the correlations of pupil size with attentional focus and mental effort. Participants could also ask questions to the researcher to gain a further understanding. By doing this, people were aided in experiencing correlations between sounds and cognition, which was assumed to emerge the anxiety reduction.

4.2.3 Mathematics task

To be able to measure anxiety reduction in this experiment, participants' anxiety levels needed to be increased first. To induce anxiety in the participants, a timed mathematics task was used. Participants were instructed to solve five open ended math problems containing addition, subtraction, multiplication and division operations to the best of their ability. Participants were given fifteen seconds to solve a problem, after which the questionnaire would automatically continue to the next page. Between each math problem, a nine second break was implemented to prevent cognitive overload. The math problems used in this experiment were created for this study and were challenging enough so that participants could not finish them all in time. To make participants less at ease during the math task, the researcher would be seated behind them.

4.2.4 Breathing task

Participants completed a breathing task by performing a five-minute breathing exercise to reduce their anxiety levels. Participants had to inhale for four seconds, hold their breath for two seconds and exhale for four seconds. They performed this breathing exercise with a ten second interval for each breathing cycle to change their heart rate and arousal level, resulting in changes in the audible pupil size. Participants were visually aided by having the rhythm of the prescriptive breathing cycle displayed on a screen, which was showing a circle increasing in size when the participants were supposed to be inhaling, remaining the same size when the participants were supposed to hold their breath and decreasing in size when the participants were supposed to be exhaling. The original breathing exercise was edited to the lowest brightness settings possible while still visibly showing the circle presenting the breathing rhythm to limit the effects of color on pupil size. The original breathing exercise that was used to practice and the edited breathing exercise with lower brightness that was used for the recorded breathing exercise can be found in Figure 2.

Figure 2. Frames of the original breathing exercise and the edited breathing exercise.

4.2.5 Assessment of anxiety and anxiety reduction awareness

At three times during the experiment, anxiety states were assessed with the S-scale of the State-Trait Anxiety Inventory (Spielberger, 2010), a scale consisting of 20 items coded on a 4-point Likert scale, in which 1 indicated ‘not at all’ and 4 indicated ‘very much so’. The complete STAI consists of two sub-scales each consisting of 20 items measuring state anxiety (STAI-S) and trait anxiety (STAI-T). State anxiety is anxiety about an event and trait anxiety is anxiety as a personal characteristic. Since this experiment manipulates anxiety with a math task and a breathing task, state anxiety is manipulated. Therefore, the S-scale of the STAI is used since that sub-scale measures the anxiety that was manipulated in this experiment. The combined items on the scale add up to a score between 20 and 80, where 20 indicates a person is very comfortable and relaxed and 80 indicates a person is very anxious.

To assess whether audible pupil size resulted in anxiety reduction awareness, self-report methods were used. After the breathing task, participants in the biofeedback condition rated the statements “I was able to use the sound to become more relaxed” and “I heard the sound changing during this task” on a 5-point Likert scale, in which 1 indicated ‘strongly disagree’ and 5 indicated ‘strongly agree’. These items were used to control if the participants felt like they could control the sounds and to ensure that the sounds were actually perceived.

4.3 Procedure

Participants were randomly assigned to a condition before arriving at the experiment. Upon arrival, the participants were seated behind a laptop in an insoluminant environment, which was created by turning on the ceiling lights, closing the blinds in front of the windows and by placing the laptop in front of a white wall in the corner of the room, to prevent negative effects of ceiling light visibility and dark background colors. The participants were introduced to the study based on the condition and signed informed consent. The participants then answered socio-demographic questions about their age and gender, and questions about baseline anxiety using the STAI-S. In the biofeedback condition, the researcher helped the participants to understand the correlations between the sounds they would be hearing, pupil size and its effect on anxiety reduction. While setting up the eye-tracker, these participants were told that lower pitch sounds represented a relaxed state of mind and a higher pitch sound represented an anxious state of mind. In the control group, no audible pupil size was used. In the experiment introduction, these participants were informed that the study is about interest in the role of the eye during anxiety reduction through a breathing exercise, and that their pupil size would be tracked during the experiment. Using a nine-point calibration protocol, the eye-tracker was calibrated. The pupil size to sound mapping was calibrated by letting the participant stare at the white background of the Qualtrics survey while the researcher changed the screen’s brightness to the lowest and highest levels while reading the participant’s minimum and maximum pupil sizes to normalize the signal. When the eye-tracker was set up, the laptop’s volume was turned on and the participants in the biofeedback condition adjusted the overall sound volume to a comfortable and clearly audible level based on their preference. The researcher confirmed whether the participants perceived changes to the sound while changing the brightness of the screen. After that, the sound was turned off again, the screen's brightness was left on a neutral level and the participants were instructed of the next steps of the experiment. First, they would perform a timed math task with multiplication problems ranging from easy to hard difficulty. This was done while the researcher was seated behind the participant to increase the participants' anxiety levels, after which the STAI-S was used to assess the manipulation. Second, the participants would complete a five-minute breathing exercise. An example question of the math task is displayed, and the breathing exercise is practiced for a few cycles. The researcher then turns on the audible pupil size for the participants in the biofeedback condition and the pupil size recorder for participants in both conditions and the participants are instructed to start the math task. After completing the breathing exercise, the participants completed a questionnaire to assess the effectiveness of anxiety reduction using the STAI-S and participants in the biofeedback condition completed a questionnaire to assess the awareness of the role of the sounds. After that, the researcher stopped the recording and removed the eye-tracking glasses. The participants were debriefed by the researcher in a conversation and thanked for their participation. The study took approximately 25 minutes. The complete questionnaire can be found in Appendix B.

4.4 Statistical analysis

Statistical tests were conducted using IBM SPSS Statistics 24. Data files that had 30% or more corrupted values within the used range of the data were removed from the dataset. To prepare the self-reported and pupil size data for the analysis, some variables had to be computed. Three variables were computed from the dataset to define effectiveness of respiratory anxiety reduction. The first variable defining effectiveness was the amount of anxiety reduced, which was computed for self-reported data by subtracting the post breathing exercise score from the post math task score. For pupil size data, the first variable was computed by subtracting the lowest pupil size value, which represents a low anxiety level, from the pupil size value at the start of the breathing exercise. The second variable defining effectiveness was the lowest pupil size value and thus anxiety level reached, which was computed for self-reported data by using the post breathing exercise score and for the pupil size data by using the minimum pupil size value. The third variable defining effectiveness was the time it took to reach the lowest anxiety level, which was computed for the pupil size data by using the second in which the lowest pupil size value was noted.

The first and second variables defining effectiveness of respiratory anxiety reduction were tested with the self-reported anxiety scores with Independent samples T-tests. Since the participants did not report their anxiety levels during the breathing exercise, the third variable was not measurable with the self-reported data. All three variables were tested with the recorded pupil size values representing anxiety levels with Independent samples T-tests and a Repeated Measures analysis.

5. Results

Pupil size data of fourteen participants (seven participants from the biofeedback condition and seven participants from the control condition) was removed from the dataset because the recordings of these pupil sizes were inconsistent and inaccurate. The eye-tracker recorded five pupil size values per second, but in the case of signal loss, the previous value was kept. Some pupil size recordings contained the same value for more than 30% of the used parts of the data. Because these recordings were too corrupted to be used for the analysis, they were removed from the dataset.

Because of this, the self-reports of the participants in the biofeedback condition that had inconsistent and inaccurate pupil size measurements were removed from the dataset as well, as the continuously playing sine wave that in these cases inaccurately represented their state of mind may have influenced their self-reports. Since the participants in the control condition did not hear this continuously playing sine wave, the self-reports of the participants in the control condition that had inconsistent and inaccurate pupil size measurements did not have to be removed.

5.1 Control variables

To control whether participants felt like they could use the biofeedback sound to become more relaxed, participants in the biofeedback condition rated a statement on a 5-point Likert scale, in which 1 indicated ‘strongly disagree’ and 5 indicated ‘strongly agree’. The participants showed to be indecisive (M = 3.00, SD = 1.18). Some participants felt they were able to use the sound to become more relaxed, whereas others felt like they could not use the sound.

To control whether participants actually heard changes in the frequency and volume of the biofeedback sound, participants in the biofeedback condition rated a statement of a 5-point Likert scale, in which 1 indicated ‘strongly disagree’ and 5 indicated ‘strongly agree’. The score showed that participants were able to hear changes in the sound (*M* = 4.43), *SD* = .85). Almost all participants indicated they could clearly hear the sound changing during the experiment.

5.2 Self-reported anxiety

5.2.1 Differences in self-reported anxiety

To test whether there were differences in self-reported anxiety between the biofeedback condition and the control condition during multiple stages of the experiment, Independent samples T-tests were used with condition as independent variable and the STAI-S scores at different stages of the experiment as dependent variables. The means and standard deviations of the STAI-S scores per condition are presented in Table 1.

Table 1.
Means and standard deviations of the STAI-S scores per condition.

Stage of experiment	Condition	Mean	St. Dev.
Upon entrance	Biofeedback (N = 14)	36.71	5.84
	Control (N = 21)	36.67	10.82
	Total (N = 35)	36.69	9.05
After math task	Biofeedback (N = 14)	42.79	9.02
	Control (N = 21)	44.62	10.43
	Total (N = 35)	43.89	9.79
After breathing exercise	Biofeedback (N = 14)	33.36	8.07
	Control (N = 21)	33.62	7.16
	Total (N = 35)	33.51	7.42

Since upon entrance no biofeedback signal was used yet, there is not supposed to be an actual difference between the conditions at this stage of the experiment. As expected, no differences are found between the STAI-S scores of the two conditions upon entrance of the experiment.

Independent samples T-tests were performed to test whether there were any differences between the conditions after completing the math task and after completing the breathing exercise. After the math task, on average, participants that heard a biofeedback signal (*M* = 42.79, *SD* = 9.02) reported lower STAI-S scores than participants that did not hear a biofeedback signal (*M* = 44.62, *SD* = 10.43). However, this difference in STAI-S scores between the conditions was not significant, M_{dif} = -1.83, $t(33) = -.537$, $p = .595$.

5.2.2 *Effectiveness of the breathing exercise*

A new variable was computed that defined the amount of anxiety reduced by subtracting the self-reported anxiety score after the breathing task of the self-reported anxiety score after the math task. On average, participants that heard a biofeedback signal (*M* = 9.43, *SD* = 7.40) reported a smaller amount of anxiety reduced than participants that did not hear a biofeedback signal (*M* = 11.00, *SD* = 9.80). However, this difference in amount of anxiety reduced between the conditions was not significant, M_{dif} = -1.57, *t*(33) = -.510, *p* = .614.

The lowest self-reported anxiety scores were reported after the breathing exercise. On average, participants that heard a biofeedback signal (*M* = 33.36, *SD* = 8.07) reported lower STAI-S scores than participants that did not hear a biofeedback signal (*M* = 33.62, *SD* = 7.16). However, this difference in STAI-S scores between the conditions was not significant, M_{dif} = -.262, *t*(33) = -.101, *p* = .920.

5.3 *Anxiety measured through pupil size*

5.3.1 *Effectiveness of the breathing task*

To test whether using biofeedback from audible pupil size during a breathing exercise increased the effectiveness of the anxiety reduction, the pupil size data was computed into three variables showing what the amount of lowered pupil size is when subtracting the lowest reached pupil size value from the value at the start of the exercise, what the lowest reached pupil size value is and after how many seconds in the breathing exercise the lowest reached pupil size was recorded. The mean value of pupil size lowered, the mean lowest pupil size value and the mean time to reach the lowest pupil size value can be found in Table 2.

Table 2.

Means and standard deviations of the variables defining effectiveness per condition.

Computed variable	Condition	Mean	St. Dev.
Amount of pupil size value lowered	Biofeedback (N = 14)	.42	.16
	Control (N = 14)	.53	.19
	Total (N = 28)	.48	.18
Lowest reached pupil size value	Biofeedback (N = 14)	.18	.13
	Control (N = 14)	.15	.11
	Total (N = 28)	.17	.12
Time to reach lowest pupil size value (in seconds)	Biofeedback (N = 14)	205.21	80.50
	Control (N = 14)	150.64	90.05
	Total (N = 28)	177.93	88.30

Independent samples T-tests were performed to test whether there were any differences between the conditions in the effectiveness of the breathing exercise. On average, participants that heard a biofeedback signal (M = .42, SD = .16) had a smaller amount of pupil size value lowered than participants that did not hear a biofeedback signal (M = .53, SD = .19). However, this difference between the conditions was not significant, M_{dif} = -.11, $t(26)$ = -1.72, p = .098.

On average, participants that heard a biofeedback signal (M = .18, SD = .13) had a higher lowest pupil size value than participants that did not hear a biofeedback signal (M = .15, SD = .11). However, this difference between the conditions was not significant, M_{dif} = -.03, $t(26)$ = .65, p = .523.

On average, participants that heard a biofeedback signal (M = 205.21, SD = 80.50) needed more time to reach the lowest value than participants that did not hear a biofeedback signal (M = 150.64, SD = 90.05). However, this difference between the conditions was not significant, M_{dif} = 54.57, $t(26)$ = 1.69, p = .103.

5.3.2 Differences at certain stages during the breathing exercise

To test whether there were differences in anxiety reduced, measured through pupil size, between the biofeedback condition and the control condition during the breathing exercise, a Repeated Measures analysis was performed with condition as a between subject factor and time as a within subject factor. To make 300 seconds of time work for a Repeated Measures analysis, the breathing exercise was split up in averages of 15 seconds. A detailed overview of the average pupil size per condition during the breathing exercise can be found in Figure 3 and the abstracted overview of the average pupil size per condition during the breathing exercise split up in 15 second averages can be found in Figure 4.

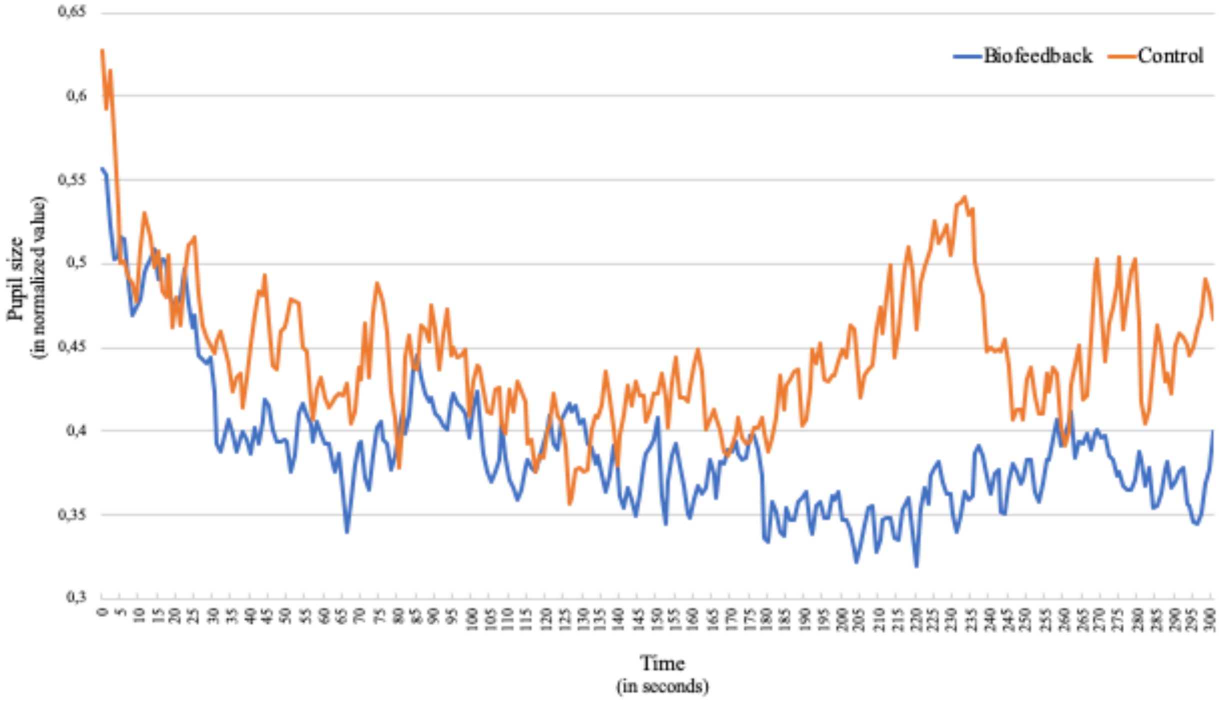

Figure 3. Average pupil size per condition during the breathing exercise.

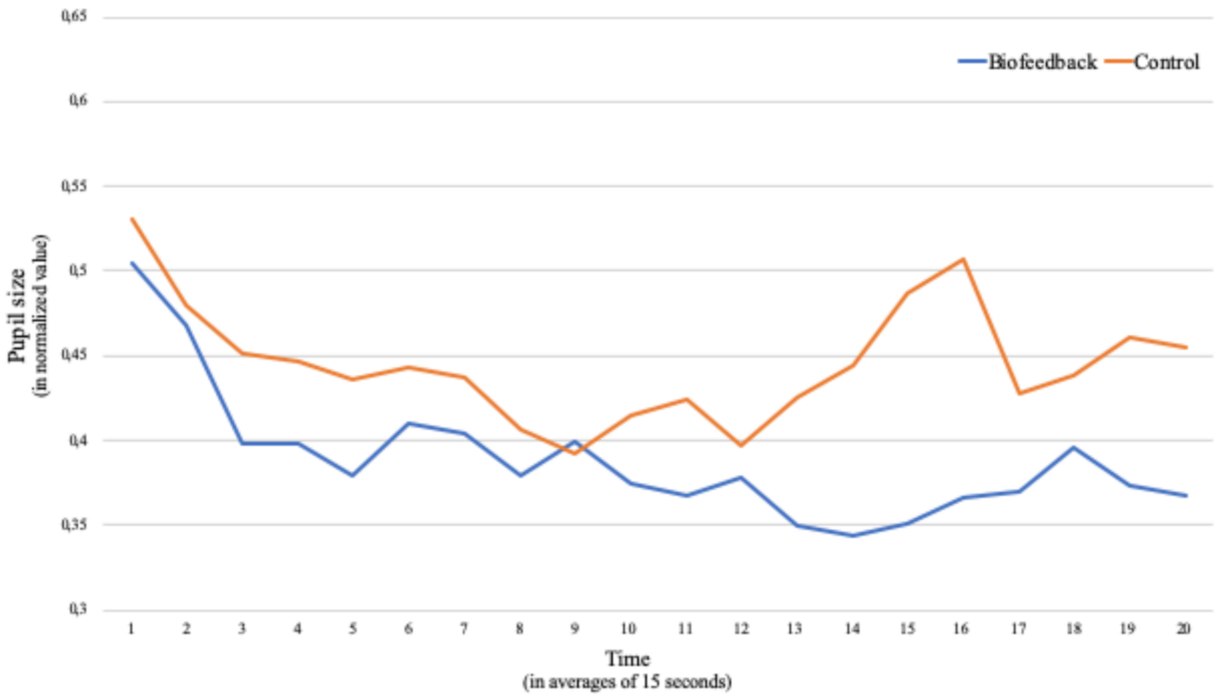

Figure 4. Average pupil size per condition during the breathing exercise split up in 15 second averages.

Mauchly's test showed that the assumption of sphericity was violated ($\chi^2(189) = 409.05$, $p < .001$). Because of this, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon = .28$). A main effect of time was found. The pupil size changed over time, F (19, 494) = 3,21, $p = .007$, $\eta^2 = .110$. No main effect of condition was found. There was no significant difference of the average pupil size over the entire breathing task between conditions, F (1, 26) = 1.00, $p = .327$. No interaction effect time * condition was found. There were no significant differences of the average pupil size at any of the 15 second sections of the breathing task, F (19, 494) = 1,46, $p = .203$.

6. Discussion

6.1 Conclusion and interpretation results

To answer the research question '*What is the effect of biofeedback from audible pupil size on the effectiveness of respiratory anxiety reduction?*', an experiment with eye-tracking glasses, a biofeedback signal and a breathing exercise was conducted. Both self-reported anxiety and anxiety measured through pupil size were used to assess the effectiveness of respiratory anxiety reduction. Effectiveness was defined as the amount of anxiety lowered, the lowest anxiety level reached and the time it took to reach the lowest anxiety level from the start of the breathing exercise.

The self-reported anxiety scores showed that the anxiety reduction was effective, but no significant differences between the conditions were found. Participants that heard a biofeedback signal during the experiment did not report more anxiety reduced and did not report lower anxiety scores than the participants that did not hear a biofeedback signal during the experiment. This result does not align with prior biofeedback studies on self-reported anxiety reduction. Henriques, Keffer, Arbrahamson and Horst (2011) and Artizeta et al. (2017) studied self-reported anxiety reduction with biofeedback assistance and found a significant decrease in anxiety scores over time. A possible reason for this difference between the current study and the prior studies is that the prior studies used biofeedback and relaxation training for multiple weeks, whereas the current study lets the participants perform without training.

The anxiety levels measured through pupil size showed that the anxiety reduction exercise was effective. The recorded pupil size value changed over time during the breathing exercise. However, no significant differences between the conditions were found. Participants that heard a biofeedback signal during the experiment did not reduce their pupil size and thus anxiety more, did not reach lower pupil size values and thus anxiety levels and did not reach this lowest pupil size value and thus anxiety level faster than participants that did not hear a biofeedback signal during the experiment. No prior research has been conducted on anxiety reduction measured through pupil size, so no comparison between the current study and prior studies can be done. Even though de Rooij, Schraffenberger and Bontje (2018) and Ehlers, Strach Georgi and Huckauf (2016) reported correlations between pupil size biofeedback and cognition, this does not mean there are also correlations between pupil size biofeedback and anxiety reduction.

These results show that there is no effect of biofeedback from audible pupil size on the effectiveness of respiratory anxiety reduction. Using a biofeedback signal does not significantly increase nor decrease the amount of anxiety reduced, does not help nor hinder you reach a lower anxiety level and does not speed up nor slow down the anxiety reduction process. These results do not align with the prior studies on biofeedback and anxiety reduction. Most studies using biofeedback in anxiety reduction found positive effects of biofeedback on anxiety reduction (Henriques, Keffer, Arbrahamson & Horst, 2011; Artizeta et al., 2017). A possible explanation is that the method used in the current study differs too much from biofeedback methods used in prior studies that found effects of biofeedback on anxiety reduction. As pointed out in the introduction and theoretical framework, there is little research done with pupil size as a biofeedback method, especially in an anxiety reduction through breathing exercise context. There might be differences in functionality between for example heart rate biofeedback methods and pupil size biofeedback methods, or differences in the respiratory domain compared to other biofeedback and mindfulness methods. However, Morarend et al. (2011) specifically studied respiratory rate biofeedback on anxiety reduction and they did not find any effects of the biofeedback signal either. Even though respiratory rate biofeedback and respiratory anxiety reduction methods are not the same, the results of both studies are comparable. This could imply that the respiratory domain is not compatible with biofeedback technology and anxiety reduction.

6.2 Limitations and future research

There are a few factors that could have threatened the validity and reliability of the results. Firstly, the eye-tracker was very vulnerable to changes in viewing angle and thus to changes in sitting position of the participant. The eye-tracker was calibrated at the start of the experiment for the sitting position and viewing angle at that time, but as the experiment moved on, some participants took different sitting positions which resulted in different viewing angles. When the eye-tracker was calibrated for participants sitting straight up, they would slightly look down to the screen. But, when participants suddenly leaned back on their chair, the viewing angle changed and the eye-tracker would sometimes not be able to accurately track the pupil size of the participant. Participants most commonly changed their sitting posture during the breathing exercise. The unstable pupil size measurements resulted in some participants being excluded from the dataset.

Secondly, pupil size functioning is different for every participant. It is difficult to gauge the correct minimum and maximum pupil size for all participants. Changing the brightness of the screen worked for most of the participants, but some participants went above and below the defined range pretty quickly after starting the recording. This resulted in the exclusion of these participants from the dataset because the pupil size recording used a normalized signal. Values above and below the range are not measured, so the data could not be used. Future research could look into taking more time to correctly measure each participant's pupil size range.

Thirdly, not much was given to the participants to grow accustomed to the biofeedback signal and the breathing exercise. In prior biofeedback studies (Henriques, Keffer, Abrahamson & Horst, 2011; Aritzeta et al., 2017), participants followed weekly practice programs to get used to the biofeedback technology and in prior passive respiratory anxiety reduction studies (Valenza et. Al, 2014; Chen, Huang, Chien & Cheng, 2017), participants followed multiple long practice sessions to get used to the controlled breathing programs. In the current study, participants received a short explanation about the correlation between their pupil size and the sound they were hearing, and the changes is the sound were made evident by changing the brightness. However, in this practice part, participants did not experience changes in the sound due to their internal state but due to brightness changes, which made the changes in the sound during the math and breathing tasks their first real experience with the biofeedback signal. Not all participants indicated they were able to use the sound to become more relaxed, which is a requirement for biofeedback to be effective. The participants might have been able to use the sound better to become more relaxed if they had been more familiar with the signal. This could have threatened the construct validity of the study. Future research could look into providing more training and practice with biofeedback technology and breathing exercises.

Fourthly, the biofeedback signal in this study was a continuously playing sine wave. Prior studies used mainly visual biofeedback signals (Henriques, Keffer, Abrahamson & Horst, 2011; Aritzeta et al., 2017), but since the breathing exercise used in the current study is a visual cue already, a sine wave was used in the current study. However, listening to a high-pitched sine wave for ten minutes straight is not a pleasurable experience. The signal itself does certainly not stimulate relaxation and that might have carried over in the effects of the breathing exercise. Since pupil size can change very quickly, these changes can be heard in the audible pupil size as well. This could explain heavy fluctuations in data in short amounts of time but could also explain the sudden anxiety increase participants were experiencing during the breathing exercise. When participants were focusing on keeping the sound low-pitched, a slight increase in pitch of the signal made them panic slightly which resulted in the pitch going up even higher. This may have made it difficult for participants hearing the biofeedback signal to relax, which could have threatened the construct validity of the study. Future research could look into using a different biofeedback signal such as a different type of sound or even a visual indicator.

Lastly, there was a big difference in measurement between the biofeedback and control condition. The eye-tracker was set up to measure five values per second, and in the biofeedback condition the eye-tracker worked perfectly fine. However, in the control condition, the eye-tracker measured inconsistently, resulting in randomly having only zero to four values measured per second. This has threatened the reliability of the study, as inconsistent measurements make replication difficult. To account for this, averages per second were computed for the biofeedback condition with formula and averages per second for the control condition were computed manually. Seconds that were missing measured pupil size values had a value computed from the averages of the values of the adjacent seconds. This made the data usable so comparisons between conditions could be made, but the control condition averages were based on less stable measurements than the biofeedback condition measurements. This could have threatened the internal validity of the study.

Future research could also delve deeper into measuring anxiety through pupil size, since that has not been done before. Perhaps measuring anxiety through pupil size during anxiety reduction tasks in a different domain than the respiratory one could result in new insights to the current theory on biofeedback and anxiety reduction.

6.3 Implications

Despite these limitations, this study has an interesting contribution to the discussion in the biofeedback and anxiety reduction research. This is the first study that uses both subjectively self-reported anxiety and objectively anxiety measured through pupil size to test for effects of biofeedback from audible pupil size on the effectiveness of respiratory anxiety reduction. Previous biofeedback studies used the biofeedback as a signal to influence the self-reported anxiety, but since pupil size correlates with anxiety, in this study the anxiety level of participants is also measured through their pupil size.

The study has both scientific and practical implications. A scientific implication is the new insights that this study adds to the current theory, since both self-reported anxiety and anxiety measured through pupil size are used in this experiment. No differences between these subjective and objective measurements were found, which means that a breathing exercise accurately reduces anxiety both subjectively as well as objectively. A practical implication is that the results of this study imply that breathing exercises in their current format are sufficient to reduce one's anxiety. Both for self-reported anxiety and anxiety measured through pupil size did the breathing exercise help reduce the anxiety levels. Therefore, designers of breathing exercises do not need to incorporate pupil size biofeedback in their exercises.

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8. Appendices

8.1 Appendix A.

8.1.1 Biofeedback version

Consent form

Study: Stress reduction through mindfulness

Researcher: Jannick Vogel

Supervisor: Alwin de Rooij

By means of this form we will inform you about the purpose of this research and what you can expect if you decide to participate in this experiment.

Goal of the research: Anxiety is a brain state coming from stress and fear that is becoming more common in society. To reduce anxiety, people can use mindfulness methods such as mediation, relaxation or breathing exercises. While these methods have been proven to work, they do not measure anxiety during the process. Measuring and presenting anxiety levels during a mindfulness method can possibly support and improve these methods. A technology that can do this, is biofeedback. That is why we are interested in the effects of pupil size biofeedback on anxiety levels during a breathing exercise. The goal of the research is to better understand the effects and potential benefits of pupil size biofeedback on anxiety reduction methods.

Duration of research: This experiment will be conducted over a period of 30 minutes. During this time, each participant will fill in some questionnaires about demographics and anxiety levels. The participant will have the eye-tracker set up and receive a briefing by the researcher. After that, the participant will complete a math task and a breathing exercise. For participation, participants will receive 0.5 credit point. This will be granted after the experimental task is completed.

Privacy and confidentiality: All data collected will be treated with the utmost confidentiality and will only be used for research purposes only. After data collection, your ID number will be deleted from the set, and your identity will in no case be linked to the results. After the experiment, the data you will give us will be stored for 10 years so that they can be used for further research. You have the right to stop the research at any time if you no longer wish to participate without giving an explanation. The research was tested and approved by the ethics committee of TSHD.

Voluntary participation: You are not obliged to participate in this study. If you agree to participate, you can cancel your participation at any time without having any consequences and your withdrawal does not require provision or an explanation. You are not obliged to answer questions that you do not want to answer.

Contact: If you still have questions after completing this research, you can contact Alwin de Rooij, the researcher who is responsible for this study. You have also the possibility to contact Jannick Vogel, the Master's student in charge of data collection.

Permission

I have had the opportunity to read this statement and the investigation has been explained to me. I had the opportunity to ask questions about the research and my questions were answered. I am willing to participate in the current research.

__________________________ ____________________

Signature participant Date

____

Name participant

__________________________ ____________________

Signature researcher Date

8.1.2 Control version

Consent form

Study: Stress reduction through mindfulness

Researcher: Jannick Vogel

Supervisor: Alwin de Rooij

By means of this form we will inform you about the purpose of this research and what you can expect if you decide to participate in this experiment.

Goal of the research: Anxiety is a brain state coming from stress and fear that is becoming more common in society. To reduce anxiety, people can use mindfulness methods such as mediation, relaxation or breathing exercises. While these methods have been proven to work, they do not measure anxiety during the process. Measuring anxiety levels during a mindfulness method can possibly help improving these methods. Since pupil size correlates with anxiety, your anxiety level will be measured through your pupil size. The goal of the research is to better understand the role of pupil size during anxiety reduction methods.

Duration of research: This experiment will be conducted over a period of 30 minutes. During this time, each participant will fill in some questionnaires about demographics and anxiety levels. The participant will have the eye-tracker set up and receive a briefing by the researcher. After that, the participant will complete a math task and a breathing exercise. For participation, participants will receive 0.5 credit point. This will be granted after the experimental task is completed.

Privacy and confidentiality: All data collected will be treated with the utmost confidentiality and will only be used for research purposes only. After data collection, your ID number will be deleted from the set, and your identity will in no case be linked to the results. After the experiment, the data you will give us will be stored for 10 years so that they can be used for further research. You have the right to stop the research at any time if you no longer wish to participate without giving an explanation. The research was tested and approved by the ethics committee of TSHD.

Voluntary participation: You are not obliged to participate in this study. If you agree to participate, you can cancel your participation at any time without having any consequences and your withdrawal does not require provision or an explanation. You are not obliged to answer questions that you do not want to answer.

Contact: If you still have questions after completing this research, you can contact Alwin de Rooij, the researcher who is responsible for this study. You have also the possibility to contact Jannick Vogel, the Master's student in charge of data collection.

Permission

I have had the opportunity to read this statement and the investigation has been explained to me. I had the opportunity to ask questions about the research and my questions were answered. I am willing to participate in the current research.

____________________________ _____________________

Signature participant Date

____

Name participant

____________________________ _____________________

Signature researcher Date

8.2 Appendix B.

Questionnaire experiment

(All text between parentheses in italics font was not visible to participants. The text is used here to provide additional information)

Q1. Participant condition (completed by researcher before participant enters room)

- o Biofeedback condition
- o Control condition

Q2.1. (If biofeedback condition is selected at Q1)

Thank you for coming to this experiment. You will first be informed of the nature of the experiment. Please read the following text:

Anxiety is brain state that is becoming more common in present-day society. It often comes from stress and fear, which are feelings of emotional tension. To reduce anxiety, people can use mindfulness methods such as mediation, relaxation or breathing exercises. But, while these methods have been proven to work, they do not measure anxiety during the process. When users learn about their actual anxiety levels during a mindfulness exercise, they might be able to make anxiety reduction more effective. A method that can be used to do this, is using biofeedback from pupil size.

The size of your pupil changes very often. The most important factor that changes the size of your pupils, is light. However, anxiety also correlates with pupil size. This is where biofeedback comes into play. Biofeedback is a real-time presentation of physiological data, in this case pupil size. By making your pupil size audible you can indirectly 'hear' your anxiety level, so you can use that information to help you regulate your anxiety.

In this experiment, we will measure and record your pupil size with eye-tracking glasses and convert the pupil size to a sound that changes based on your pupil size. We ask you to complete some scales during the experiment and perform certain tasks. More information about this will be provided later.

If you want to continue with the experiment, please read and sign the consent form.

Q2.2. (*If control condition is selected at Q1)*

Thank you for coming to this experiment. You will first be informed of the nature of the experiment. Please read the following text:

Anxiety is brain state that is becoming more common in present-day society. It often comes from stress and fear, which are feelings of emotional tension. To reduce anxiety, people can use mindfulness methods such as mediation, relaxation or breathing exercises.

The size of your pupil changes very often. The most important factor that changes the size of your pupils, is light. However, anxiety also correlates with pupil size. It is therefore interesting to study the eye's functioning during an anxiety reduction task such as a breathing exercise to learn more about how the pupil size can potentially aid the user with anxiety reduction.

In this experiment, we will measure and record your pupil size with eye-tracking glasses. We ask you to complete some scales during the experiment and perform certain tasks. More information about this will be provided later.

If you want to continue with the experiment, please read and sign the consent form.

Q3. Please enter the ID code that the researcher will hand you.

Q4. What is your gender?

- Male
- Female

Q5. What is your age?

Q6. (STAI-S 1)

A number of statements which people have used to describe themselves are given below. Read each statement and then indicate how you feel right now, that is, at this moment. There are no right or wrong answers. Do not spend too much time on any of the statements but give the answer which seems to describe your current feelings best. Some phrases have synonyms added between parentheses. If there are more phrases you don't know the meaning of, you can ask the researcher.

	Not at all	Somewhat	Moderately so	Very much so
I feel calm	O	O	O	O
I feel secure	O	O	O	O
I am tense	O	O	O	O
I am regretful	O	O	O	O
I feel at ease	O	O	O	O
I feel upset	O	O	O	O
I am presently worrying over possible misfortunes	O	O	O	O
I feel rested	O	O	O	O
I feel anxious	O	O	O	O
I feel comfortable	O	O	O	O
I feel self-confident	O	O	O	O
I feel nervous	O	O	O	O
I am jittery (nervous; unable to relax)	O	O	O	O
I feel "high strung" (anxious; tense)	O	O	O	O
I am relaxed	O	O	O	O
I feel content	O	O	O	O
I am worried	O	O	O	O
I feel "rattled" (nervous; worried)	O	O	O	O
I feel joyful	O	O	O	O
I feel pleasant	O	O	O	O

Q7. Please call the researcher. The eye-tracker will be set up and it's functioning will be explained.

Q8. Do you think you can wear these eye-tracking glasses for the next 10 minutes?

- Yes
- No

  (*If no is selected, go to the end of the questionnaire*)

Q9. (Task explanation)

Next you will be performing two tasks. The first task consists of solving mathematics problems and the second task is completing a breathing exercise. You will first see an example of the mathematics task and then you will practice a few cycles of the breathing task with the researcher.

An example of a mathematics problem can be found on the next page. The problems are open ended questions and will contain the following symbols:

+ (addition)
- (subtraction)
* (multiplication)
/ (division)

Q10. (*Math task example*) 42 + 26 =

Q11. (Breathing exercise practice)

The breathing exercise will be 5 minutes long. Please try to match your breathing to the rhythm provided by the circles below. The actual breathing exercise will use the less visible circle, because the clearly visible one influences your pupil size too much. However, you can practice getting the hang of it with the clearly visible one. Try a few breathing cycles with the researcher until you think you can perform the breathing exercise with the less visible circle as well.

(*GIF version of the original breathing exercise to practice*)

(*GIF version of the edited breathing exercise to practice)*

Q12. The researcher will now activate the eye-tracking glasses and start the recording of your pupil size.

Please focus on the screen for the remainder of the experiment to minimize the chance of other factors influencing your pupil size.

Q13. The mathematics task consists of 5 problems with ranging difficulties. Please try your best to answer them correctly. You have 15 seconds to answer each problem. After these 15 seconds, you will automatically continue to the next page. Between each problem there is a 9 second break. Good luck!

Q14. (This question lets you record and manage how long a participant spends on this page. This question will not be displayed to the participant. (auto-advance after 15 seconds, enable submit after 16 seconds))

Q15. 123 * 6 =

Q16. (This question lets you record and manage how long a participant spends on this page. This question will not be displayed to the participant. (auto-advance after 9 seconds, enable submit after 10 seconds))

Q17. Break

Q18. (This question lets you record and manage how long a participant spends on this page. This question will not be displayed to the participant. (auto-advance after 15 seconds, enable submit after 16 seconds))

Q19. 57 – 31 + 92 =

Q20. (This question lets you record and manage how long a participant spends on this page. This question will not be displayed to the participant. (auto-advance after 9 seconds, enable submit after 10 seconds))

Q21. Break

Q22. (This question lets you record and manage how long a participant spends on this page. This question will not be displayed to the participant. (auto-advance after 15 seconds, enable submit after 16 seconds))

Q23. 21 * 37 - 8 =

Q24. (This question lets you record and manage how long a participant spends on this page. This question will not be displayed to the participant. (auto-advance after 9 seconds, enable submit after 10 seconds))

Q25. Break

Q26. (This question lets you record and manage how long a participant spends on this page. This question will not be displayed to the participant. (auto-advance after 15 seconds, enable submit after 16 seconds))

Q27. 681 – 168 / 7 =

Q28. (This question lets you record and manage how long a participant spends on this page. This question will not be displayed to the participant. (auto-advance after 9 seconds, enable submit after 10 seconds))

Q29. Break

Q30. (This question lets you record and manage how long a participant spends on this page. This question will not be displayed to the participant. (auto-advance after 15 seconds, enable submit after 16 seconds))

Q31. 430 – 203 / 7 + 179 =

Q32. (This question lets you record and manage how long a participant spends on this page. This question will not be displayed to the participant. (auto-advance after 9 seconds, enable submit after 10 seconds))

Q33. This is the end of the mathematics task. You will automatically proceed to the next page.

Q34. (STAI-S 2)

A number of statements which people have used to describe themselves are given below. Read each statement and then indicate how you feel right now, that is, at this moment. There are no right or wrong answers. Do not spend too much time on any of the statements but give the answer which seems to describe your current feelings best.

	Not at all	Somewhat	Moderately so	Very much so
I feel calm	O	O	O	O
I feel secure	O	O	O	O
I am tense	O	O	O	O
I am regretful	O	O	O	O
I feel at ease	O	O	O	O
I feel upset	O	O	O	O
I am presently worrying over possible misfortunes	O	O	O	O
I feel rested	O	O	O	O
I feel anxious	O	O	O	O
I feel comfortable	O	O	O	O
I feel self-confident	O	O	O	O
I feel nervous	O	O	O	O
I am jittery	O	O	O	O

I feel "high strung"	O	O	O	O
I am relaxed	O	O	O	O
I feel content	O	O	O	O
I am worried	O	O	O	O
I feel "rattled"	O	O	O	O
I feel joyful	O	O	O	O
I feel pleasant	O	O	O	O

Q35. Next you are asked to perform the breathing exercise for 5 minutes.
You will automatically continue to the next page when 5 minutes have passed.

The breathing exercise starts on the next page.

Q36. (This question lets you record and manage how long a participant spends on this page. This question will not be displayed to the participant. (auto-advance after 300 seconds, enable submit after 301 seconds))
Q37. (GIF version of the edited breathing exercise)

Q38. (STAI-S 3)
A number of statements which people have used to describe themselves are given below. Read each statement and then indicate how you feel right now, that is, at this moment. There are no right or wrong answers. Do not spend too much time on any of the statements but give the answer which seems to describe your current feelings best.

	Not at all	Somewhat	Moderately so	Very much so
I feel calm	O	O	O	O
I feel secure	O	O	O	O
I am tense	O	O	O	O
I am regretful	O	O	O	O
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I feel upset	O	O	O	O
I am presently worrying over possible misfortunes	O	O	O	O
I feel rested	O	O	O	O
I feel anxious	O	O	O	O
I feel comfortable	O	O	O	O
I feel self-confident	O	O	O	O
I feel nervous	O	O	O	O
I am jittery	O	O	O	O
I feel "high strung"	O	O	O	O
I am relaxed	O	O	O	O
I feel content	O	O	O	O
I am worried	O	O	O	O
I feel "rattled"	O	O	O	O
I feel joyful	O	O	O	O
I feel pleasant	O	O	O	O

Q39. (If biofeedback condition is selected at Q1)

Please score the following statements

	Strongly disagree	Disagree	Undecided	Agree	Strongly agree
I was able to use the sound to become more relaxed	O	O	O	O	O
I heard the sound changing during the tasks	O	O	O	O	O

End of Survey

This is the end of the experiment. Thank you for your participation!

Please call the researcher. The recording will be stopped, and the eye-tracking glasses will be removed.

The researcher will debrief you and make sure you get your participation credit afterwards.