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A Short 15-Minute Pranayama Exercise: The Effect on Attentional Bias to Threatening Cues in Emotional Dot-Probe Task

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Abstract

Means to alleviate stress and stress-related illnesses have been on the rise. One of the core features of these techniques is maintaining focus and control over own breath when the mind starts wandering. This technique is known as Pranayama and studies examining the effect of Pranayama have successfully shown its benefits regarding stress, anxiety, and cognitive functioning. One of commonly used tools to assess stress and anxiety is attentional bias, which relates to faster recognition of threatening cues in one's surrounding. Present study examined the effect of a short 15-minute pranayama exercise on attentional bias towards threatening stimuli on the emotional dot-probe task. It was expected that this short pranayama intervention would decrease state anxiety levels and improve attentional processing, by showing slower reaction times for threatening cues and faster reaction times for pressing a start-button on the emotional dot-probe task. The experimental group (n = 20) and the control group (n = 21) consisted of students from Leiden University and were asked to perform the emotional dot-probe task twice, intervened by either 15 minutes of pranayama or 15 minutes of neutral state without relaxing. Results showed no effect of pranayama on attentional bias or state anxiety levels. Future research should focus on different pranayama rhythms and should assess levels of attentional control on forehand.

Key words: pranayama, attentional bias, emotional dot-probe

A Short 15-Minute Pranayama Exercise:
The Effect on Attentional Bias and Disengagement from Threatening Cues

In the modern fast-paste western world, the prevalence of stress and stress related illness is growing (Brown, Gerbarg, & Muench, 2013; Sengupta, 2012). In order to alleviate stress and anxiety levels, techniques such as mindfulness, yoga, and meditation have been on the rise. One of the core features of these techniques is related to the ability to bring one's attention back to breathing every time the mind starts wandering (Brown & Gerbarg, 2009). This implies that techniques such as these require attentional and emotional regulation strategies, which in turn require extensive training. There are plenty of breathing techniques that can be used for controlling respiration such as: breathing alternately through both nostrils, breathing through the abdomen, phased breathing, and other forms and combinations (Brown & Gerbarg, 2009). However, research has been conducted and found a common component within these diversities of practices: Yoga breathing, Sanskrit translation is *Pranayama,* maintaining control over ones breathing by repetitive paced inhalation and exhalation during a specific time period (Brown & Gerbarg, 2009; Sharma et al., 2014). Although research on pranayama is developing, there has been growing evidence for its benefits on psychological and physiological health and well-being (Brown & Gerbarg, 2009; Sengupta, 2012).

For instance, numerous studies have shown that yoga and Pranayama have an immediate decreasing effect on stress and anxiety levels and could, therefore, be seen as an effective tool for stress management (Brown et al., 2013; Sengupta, 2012; Telles, Sharma, & Balkrishna, 2014). In addition, Pranayama exercises showed positive effects on complex cognitive functioning (Gothe, Pontifex, Hillman, & McAuley, 2013; Sharma et al. 2014). Due to the beneficial effects of Pranayama breathing on stress and cognition, there has been growing inclinical and scientific interest in this practice and it's underlying neuro-physiological mechanisms (Yadav & Mutha, 2016).

Pranayama, Neuro-physiology and Stress

Homma and Masaoka (2008) suggested that respiration is important for maintaining a balanced state within the body and that there is a bi-directional relationship between breathing and emotion: conscious paced breathing changes emotional states and emotional states change the pace and depth of breathing. Interestingly, our respiration is governed by two systems: the first system, Homma and Masaoka (2008) explained, was as an emotional breathing system and translates to a change in respiration when emotional arousal levels alter. Importantly, this system is also susceptible to (internally and externally) triggered emotions and therefore we can observe immediate changes in our respiration pattern when we are stressed (Homma & Masaoka, 2008). Additionally, increased blood pressure and heart rate have been found to correlate with increased emotional states like anxiety and arousal levels, needing more oxygen and thereby altering breathing patterns (Homma & Masaoka, 2008). Some brain areas that have been found to relate with this system lay within the amygdala, that registers an increase in anxiety level or respiratory activity and consequently activates respiratory mechanisms or emotional responses respectively (Homma & Masaoka, 2008). This implies that the emotional state affects the functioning of respiration and in situations where this relation is bi-directional, we could affect our emotions by simply changing our respiration. The second respiratory system explained, is a behavioral breathing mechanism and is governed by hormonal chemoreceptors that automatically stimulate the muscles in the body making continuous breathing possible; this system is always activated in normal circumstances and fulfils the oxygen demand from the body. This idea of an emotional breathing system has been supported by study by Miyata, Okanoya, and Kawai (2015) who conduceted a study where participants with advanced mindfulness and yoga experience scored significantly higher on an inventory assessing well-being, positive affect, as compared to non-practitioners. In addition, a once a week 90-minute deep breathing practice for 10 weeks total induced better self-reported stress and positive affect levels within participants, compared to participants whom were told to sit down for 90 minutes without practicing any breathing technique (Perciavalle et al., 2017).

Apart from the decrease in subjective ratings of stress and anxiety, pranayama exercise was shown to display physiological alterations relating to stress reduction. For example, on a hormonal level, intensive prolonged three-month period of pranayama exercises decreased salivary cortisol levels in distressed women (Michalsen et al., 2005; Perciavalle et al., 2017). Studies by Telles, et al., (2013); Telles, Sharma, & Balkrishna, (2014) and Telles, Verma, Sharma, Gupta, & Balkrishna, (2017) demonstrated that both systolic and diastolic blood pressure significantly reduced after 10, 15 and 25 minutes of alternate-nostril pranayama. Moreover, reduced blood pressure has been found to correlate with lower physiological arousal, which in turn has been found to correlate with reduction of the activation of the Hypothalamic-Pituitary-Adrenal-axis (HPA-axis), the mechanism that activates flight-fight behavior during stressors in the environment (Sengupta, 2012). Telles et al. (2014) showed that pranayama has a direct effect on the autonomic nervous system thereby increasing heart rate variability. Increased heart rate variability is an indicator that the body anticipates better and faster on stressors via the sympathetic nervous system (increase in heart rate) but also is able to put a break to the stress response effectively and returns to a neutral state via the parasympathetic nervous system (heart rate decrease) (Sengupta, 2012; Telles et al., 2014). Therefore, it is suggested that the amplified HRV decreases blood pressure and causes the body to respond more flexibly towards stress indicators. Studies show that participants that employed pranayama techniques for 10-25 minutes increased their HRV and decreased their blood pressure and thus improved their autonomous functions. Moreover, on neurophysiological level, Cheng et al. (2018) found that pranayama related to an increase in frontal theta power compared with a control group. Frontal theta power is inversely related to anxiety, therefore an increase in frontal theta activity relates to a decrease of anxiety. In Figure 1, there is a schematic overview of the physical effects of pranayama and the interplay between perceived and physiological stress. Based on this evidence, it can be theorized that pranayama has a calming effect on emotional levels, relates to decreases in blood pressure, increases HRV and reduces HPA-axis stimulation, hence the stress mechanism is down-regulated and perceived stress and anxiety levels might drop after controlled deep breathing (Homma & Masaoka, 2008; Sengupta, 2012).

Effect Pranayama on Cognition

In addition to benefits in terms of anxiety and stress, pranayama exercise or experience with pranayama have been shown to increase cognitive functioning in various studies. Gothe et al., (2013) found that participants who performed yogic pranayama exercise for 20 minutes have demonstrated better performance on Flanker task and N-back task than participants that followed an aerobic exercise for 20 minutes without the pranayama component. The results imply heightened cognitive control and working memory for the pranayama group. Gothe et al. (2013) explained these results as caused by increased mood and relaxation after pranayama yoga exercise, hence better self-control, concentration and attention. In another study which compared performance of 40 participants on a motor-retention task, researchers found that a single 30-minute alternate nostril breathing exercise resulted in better retention of the newly learned motor skill than 30 minutes of resting thereby showing memory enhancing effects of pranayama (Yadav & Mutha, 2016). Furthermore, breathing exercise compared to breathe awareness activated P300 EEG-patterns related to sustained attention (Telles, Singh, & Puthige, 2013).

Recent work from Melnychuk, et al., (2018) linked these cognitive improvements of pranayama to the bi-directional coupling mechanism of the Locus Coeruleus (LC) whereby the LC was considered as the biological mechanism behind both attention regulation and respiration. The researchers hypothesized that due to the functional and physical overlaps of attentional and respiration mechanisms within the LC both mechanisms influence and stimulate each other. Therefore, pranayama exercises could alter the dynamics of the LC, resulting in a change in attentional span or respiratory pace (Melnychuk et al., 2018). EEG-studies showed that pranayama activates frontal attentional systems (Melnychuk et al., 2018) and frontal and medial theta wave patterns (Cheng et al., 2018) which both related to an increased strengthening of attentional networks. In addition, participants with extensive experience with pranayama showed more functional connections between brain areas that have been found to be part of the Default Mode Network. Therefore pranayama techniques stimulate brain regions that are involved during attentional processes and that are active during here and now awareness. (Taylor et.al, 2012).

Research on effects of pranayama have found positive results; however, duration of pranayama practice on changes in cognitive capacities has not received much attention. Cheng et al. (2017) compared the effectiveness between various lengths (5 minutes, 7 minutes, and 9 minutes) of pranayama on conflict monitoring in a Go/NoGo-task whereby participants were tested on conflicting stimuli and behavior inhibition (Cheng et al., 2017). Participants where visually supported and were shown a video of a flower increasing and decreasing its petals pattern and had to inhale and exhale on that same frequency of 6 breaths rhythm per minute respectively. Results showed that for the follow-up condition reaction times on cognitive control improved, especially for the 5-minute pranayama group (Cheng et al., 2017). In addition, the researchers found (i) frontal theta waves improved for both the 5-minute and 9-minute groups, indicating improved focused attention and reduced anxiety levels, (ii) increased central-located theta waves for the 7-minute and 9-minute groups, relating to an increased activation of the parasympathetic nervous system which has been found to be correlated with increased heart rate variability, (iii) at least 9-minute pranayama was needed to get neurophysiological results found in similar studies (Cheng et al., 2018). However, due to the relative small sample, the effects were relatively small. In addition, nine minutes was the longest exercise, therefore it is not clear whether longer exercises, show the same or even better results on cognition. The researchers concluded that there may have been a stronger effect of deep breathing if the duration was longer.

Although, literature shows promising effects of pranayama on cognition, physiology and stress related functions. To our knowledge there has not been yet research done examining the effect of short pranayama exercise on attentional biases towards negative or threatening stimuli.

Attentional Bias and Negative or Threatening Stimuli

Literature concerning attentional bias towards threat is a timely topic as it steers up lively scientific debate. Meta-analyses by Bar-Haim, Lamy, Pergamin, Bakkermans-Kranenburg, and van IJzendoorn (2007) has shown that anxious individuals show greater attentional bias towards negative and threatening stimuli as compared to non-anxious individuals (Bar-Haim et al, 2007). In consequence, Bar-Haim et al. (2007) stated that attentional bias toward threats in healthy individuals does not occur as it does for anxiety populations; contradicting earlier ideas that attentional bias toward threatening stimuli, like angry faces, occurs automatically to everyone (Van Rooijen, Ploeger, & Kret, 2017).

Ellenbogen et al. (2003) described in their paper the interplay between stress and attention and stated: "Selective attention is sensitive to affective-motivational states and that therefore biases in attention may occur in response to stress." Reasoning further, coping with stressors may be influenced by the strength of attentional mechanisms in selecting, maintaining, and disengaging from stressors. The researchers stated that participants disengaged their attention from negatively exposed words relatively fast, which was found not to happen for positive or neutral words. Ellenbogen et al. (2003) interpreted this as an adaptive coping mechanism toward negative stimuli.

Similar as the results from Ellenbogen et al. (2003) Boal, Christensen, and Goodhew (2017) found that high anxious individuals disengaged from threatening stimuli compared to low-anxious participants whom where biased toward threatening stimuli. The researchers concluded that anxious participants avoided threatening stimuli after initially engaging with it and that this process occurs automatically and cannot be affected via top-down mechanisms, therefore showing attentional bias to be a bottom-up attentional process. Moreover, Elenbogen et al., 2003 tried to find attentional bias by inducing stress levels within participants. However, stress induced behavior did not seem to increase cortisol levels therefore not clear whether physiological stress was reached indeed and whether stress levels alter attentional bias.

These studies show that the level of anxiety influences whether there is a bias towards threatening stimuli or disengagement from it, with higher anxiety levels showing faster disengagement from threatening stimuli. However, the latter could be interpreted differently as a bias towards negative stimuli, since negative stimuli resulted in faster recognition and subsequently detachment. Interestingly, although their conclusion was clear about non-anxious individuals and attentional bias, Bar-Haim et al (2007) mentioned that non-anxious participants do show attentional bias when certain methodological factors were met, but these studies showed very low effect sizes. Studies using subliminal exposure towards threatening cues did show attentional bias within healthy participants when they were exposed to cues with high levels of threat (Bar-Haim et al., 2007). However, the meta-analysis had not studied the differences in threat levels between studies, therefore it stays unclear how cues propagate higher threat levels. Nonetheless, attentional bias is partly mediated by individual state anxiety and might occur within non-anxious participants under the right circumstances. In addition, attentional bias in low-anxious participants results in attentional focus towards threatening stimuli, while for high-anxious groups this bias results in shifting away from it.

Recent research from Basanovic, Notebaert, Grafton, Hirsch, and Clarke (2017) explained that it is two components of attentional control that affect the attentional bias towards emotional stimuli: attentional inhibition and control of selective attention. Put differently, it is the individual strength of cognitive control that affects the amount of attentional bias, whereby better control of inhibition and attentional selection prescribe better protection against it. Basanovic et al. (2017) concluded that the strength of attentional control predicted the degree of attentional bias. In other words, individuals with better attentional control showed less attentional biases; as they are able to direct their attentional selection more effectively as compared to individuals that show less attentional control and show a greater bias toward threatening stimuli.

Since pranayama has shown to be effective to alleviate stress and anxiety levels and alters attentional mechanisms, it was expected that attentional bias towards threatening stimuli in the environment would decline after practicing pranayama. Relatively few studies examined the effect of deep breathing on attentional bias towards threatening stimuli. There are varied findings in literature that explain attentional bias in high anxious or stress induced individuals. However, there seems to be a consensus about low-anxious and associated groups, whereby a bias towards threatening stimuli is found (Bar-Haim et al., 2007; Boal et al., 2017).

The effect of pranayama on attentional bias towards threatening cues in the environment is momentarily understudied. This study tried to contribute the scientific literature by gaining insight about attention alterations through short, paced respiration exercises. Better understanding of the effect of relatively short pranayama practice on attentional bias towards threat could benefit the general population by developing easily and quickly to learn exercises to alleviate stress levels and decrease bias toward threat-like events.

The aim of the current study was to investigate whether pranayama reduces attentional bias towards threatening stimuli or increases disengagement from threatening stimuli, measured by the emotional dot-probe task, possibly by inducing heightened cognitive control and reducing state anxiety levels. It was expected that reaction times for dots appearing after threatening cues were faster than reaction times for dots after neutral cues, thereby showing bias toward the threat, but that this effect will be reduced after 15 minutes of pranayama. Second, it was expected that pranayama led to better disengagement from threatening stimuli by showing faster reaction times for the start-dot that appeared after a threatening probe. Third, whether pranayama results in mood shifting towards a more relaxed level of the Affect Grid. At last, if 15 minutes of Pranayama resulted in less mind wandering activity. These questions led to the following hypothesis:

- H1: 15 minutes of Pranayama led to a decrease of the threat-index compared to control group while controlling for state anxiety levels.
- H2: 15 minutes of Pranayama increases disengagement from threatening cues on the dot-probe task, controlled for state anxiety levels, compared to the control group
- H3: 15 minutes of Pranayama improves mood valence levels on the Affect Grid compared to control group.
- H4: The pranayama group showed lower scores on the ARSQ, assessing mind wandering during resting state activity, than the control group.

Method

Participants

42 Students from Leiden University were recruited via the university's online SONA-system and for their contribution they could earn money or credits depending on their preferences. However, one student was unable to partake in the second dot-probe task due to technical malfunction and was therefore excluded from the data. Participants were allowed to partake in the study when they met the following criteria: (1) age within the range of 17-35 years old, (2) no previous experience with meditation, mind-body exercises and/or breathing exercises, and (3) no history of neurological and cardiovascular disorders (4) no heavy smoker. Participants were randomly assigned to the control or pranayama condition via randomized one-to-one placement. Leiden University Ethics Committee reviewed gave

approval to conduct the study at the university's Faculty of Social Sciences. All participation signed written informed consent.

Procedure

Participants sat down behind a computer screen in a seat with a comfortable pillow supporting their back and the lights dimmed. Participants were then given a briefing and informed consent form and instructed to call the experimenter when the program asked them to do. Participants were randomly assigned to either the control group or the pranayama group. Both of the conditions followed the same procedure: first, participants completed the affect-grid and STAI-6, followed by the first attempt of the dot-probe task. Prior to the dot-probe, participants were instructed by the experimenter to click the black dots as fast as possible, also the black start-dot in the middle of the screen, and performed three trials before starting the actual task.

Right after the dot-probe task participants received either the instructions for the pranayama condition or the control condition. In both conditions participants listened to relaxing music softly playing at the background. For the control group participants were instructed by the experimenter that they were about to stare at the screen and listen to music for 15 minutes. It was not allowed to close the eyes during the experiment and it was instructed that participants may not fall asleep. Participants were told that they could see the time they had left. When instructions were given the participants were told to put on the headphone and start the experiment. The pranayama condition received a different protocol and were told that they were about to perform a breathing exercise. The experimenter explained that the participant was about to see an altering flower and bar that followed a specific rhythm and that the participant had to follow this rhythm by inhaling when petals appeared on the flower and the bar filled up, followed by exhaling if the animation moved the other way. Thereby it was communicated that participants should inhale and exhale fully and through their abdomen. The experimenter would thereby demonstrate how to breathe by showing how to place the hands on the belly and chest and ask the participant if she could demonstrate this breathing method. Then, permitted by the experimenter, participants would start their task for 15 minutes.

Subsequently, participants started the same dot-probe task without the training trial and filled in the affect grid and STAI-6 for a second time. Thereafter they filled the Amsterdam Resting-State Questionnaire. The whole experiment took approximately 40 minutes to complete.

Apparatus

Software used for the experiment was E-prime Professional 2.0. All tasks and questionnaires were designed in E-prime. A Dell laptop was used to run the program. In total five questionnaires, one attentional task, and one intervention were programmed into the E-prime experiment since the data was gathered for additional future studies as well. However, current study used the following questionnaires: STAI-6, Affect Grid, and the Amsterdam Resting-State Questionnaire.

Spielberger State-Trait Anxiety Inventory with 6 items. State anxiety levels within participants was measured with the Spielberger State-Trait Anxiety Inventory with six items (STAI-6), which is the shortened version from the complete Spielberger State-Trait Anxiety Inventory (Marteau & Bekker, 1992) whereby the full version measures both state anxiety; anxiety feelings in the present, and trait anxiety levels; anxious feelings in general. The STAI-6 was developed to quickly assess self-reported presence and severity of state anxiety. This scale quickly measures how participants feel right now and is therefore thought to differentiate between state anxiety before and after pranayama. The scale has three anxiety-absent ("I feel calm", "I am relaxed", and "I feel content") and three anxiety-present questions ("I am tense", "I feel upset", and "I am worried") with a 4-point Likert scale, therefore the anxiety-absent questions need to be rescored. Total scores were multiplied by 3.33 to get the final index score: the highest possible score was 79.92 and implies a high state anxiety, in contrast the lowest score is 19.98, implying no current state anxiety feelings. The complete STAI can be used in clinical settings and for chronic medical conditions (Julian, 2011). However, the current study merely aims to find change in state anxiety and is not concerned with its clinical predictive value. The STAI-6 is sufficiently perceptive in detecting differences in state anxiety, gives similar scores compared to the full-item scale, has a high reliability Cronbachs' alpha of.82 and shows good construct validity compared to the full scale (Marteau & Bekker, 1992). Participants' STAI-6 scores before the intervention were used as indicator for state anxiety levels.

Affect Grid. In order to assess mood levels and whether mood shifts to relaxation the Affect Grid was used to describe participants' current mood. The Affect Grid is a single-item matrix with two dimensions: pleasure-displeasure and arousal-sleepiness (Russell, Weiss & Mendelsohn, 1989). Participants were asked: "Please rate your mood as it is right now" and had to click on a place in the matrix that corresponds with their mood along these two dimensions. The Affect Grid gives two scores in the range 1-9 for both Pleasure and Arousal whereby scores indicate the position on the grid. Moreover, scores can be divided in four subcategories: (1) low arousal and low pleasantness, like depression, (2) low arousal and high pleasantness indicating relaxation, (3) high arousal and unpleasant feelings as indicator for stress, and (4) high arousal and high feelings of pleasure, meaning excitement. Although the grid seems multi-interpretable and lacks a consistent framework for terminology (Feldman Barrett & Russell, 1999), the two dimensions pleasure and arousal are reasonable for indicating mood as it is in the moment. In addition, the Affect Grid has evidence for convergent validity with other mood questionnaires, shows good discriminant validity, and scores showed that the scale is reliable for quickly assessing mood although slightly less reliable than multiple-item scales (Russell, Weiss & Mendelsohn, 1989). However, the Affect Grid showed its evaluating value in previous studies and was "capable of assessing the continuous flux of affective response" (Russell, Weiss & Mendelsohn p.499).

Amsterdam Resting-State Questionnaire. Diaz, Van der Sluis, Moens, Benjamins, Migliorati, Stoffers, and Boomsma (2013) developed the Amsterdam Resting-State Questionnaire (ARSQ) as quick 27-item self-reported assessment method for resting-state cognition. The whole assessment takes approximately four minutes to complete. ARSQ measures resting-state cognition in seven different subscales: Discontinuity of Mind, Theory of Mind, Self, Planning, Sleepiness, Comfort, and Somatic Awareness and was developed for clinical and non-clinical purposes (Diaz et al., 2013). Current study used ARSQ to assess the amount of mind wandering during the pranayama exercise and the control condition. Although the latter group did not partake in a high-cognitive task, their data was used to find out to which extend attention drifts away within 15 minutes of pranayama compared to 15 minutes of being absent from any attentional task. Re-test data showed great reliability for testing in different settings like EEG and fMRI (Diaz et al., 2013). High scores indicate the strength of resting-state cognition and scores on the distinctive subscales offer insights in the content of it.

Emotional Dot-Probe task. The dot-probe task was designed to capture attentional bias toward threatening laden stimuli and is widely used for doing so (Van Rooijen et al, 2017). The images for dot-probe task used in current study were retrieved from E. R. Kimonis, University of New South Wales, Australia. The task consisted of one block of 40 picture pairs that represented either a neutral or an emotion evoking setting. Cues were shown for 500 ms and were immediately followed up by a dot that could appear behind either the neutral or threatening cue. Participant's goal was to click with the mouse button as fast as possible on the dot. Responses that took longer than 2000 ms resulted in an automatic continuation of the next trial. Images corresponding with the neutral or threatening cue were chosen by the researchers and contained a social setting containing human characters, or non-social setting whereby images represented tools, animals, or other non-human related stimuli. Before the experimental block in the first attempt of the dot-probe task, participants completed a short trial block containing three pairs of stimuli in order to have experienced the procedure of the task. The whole task took about three minutes and was done twice in the experiment. The reaction time-index on the dot-probe after a neutral cue minus the reaction time on dot after the threatening cue was used to assess the amount of attentional bias towards threatening stimuli. Negative index-scores showed slower response for threatening cues. The reaction time on the start-dot that appeared in-between trials was used to measure quality of disengagement for threat-like cues, whereby negative index-scores showed slower response for the start-dot when it appeared after a threatening cue.

Pranayama breathing exercise. Participants were instructed to perform the pranayama exercise while following the pace of an altering flower and a bar appearing on the screen in front of them. The animation was designed with E-prime professional software. At the center of the screen there was an orange flower with six petals appearing and disappearing; the time for each petal was set to 833 ms therefore, one full cycle took 10 second in total. When the first six petals appeared, participants had to inhale and subsequently had to exhale when the petals disappeared. A green bar at the right center of the screen increased and decreased in volume with the same rhythm as the flower. Participants had to follow this pace of breathing continuously, preferably without breaks. They were explicitly instructed to breathe in through their abdomen without moving up their chest; hereby they were allowed to place their hands on both their chest and abdomen as helpful guidance. In the bottom left corner there was a small descending timer; the rationale behind this was that participants could see how much time they had left and therefore stimulating to remain their effort in doing the pranayama exercise. The video was made by the researchers themselves but shows resemblance with the happy face from Cheng et al., (2017).

Design and analysis

Testing whether pranayama decreases attentional bias towards threatening stimuli controlled for state anxiety levels, a mixed design ANCOVA has been used. Hereby, the reaction time on the dot-probe task between both conditions was used as measurement for attentional bias towards threatening stimuli whereby the participants average reaction time for threatening cues was subtracted from the average reaction time on neutral cues. The calculated threat facilitation index displayed attentional bias towards threatening cues in milliseconds whereby negative values showed a slower response for threatening targets; demonstrating decrease of attentional bias towards threat. Disengagement facilitation index was used to measure disengagement reaction times for the start-dot appearing after the cues and showed whether participants were faster or slower after threatening compared with neutral cues. It was calculated by subtracting participants mean reaction time for the start-dot after threatening cues from average reaction time neutral cues.

To answer the first two questions, the state anxiety levels were analyzed to see whether change in anxiety occurs over time as well as between groups. This was done in order to determine whether state anxiety levels should be included as covariate in the model. Consequently, a mixed-design ANCOVA was used with between-subject factor Group, and within-subject factor Time for the dot-probe threat facilitation index (or disengagement facilitation index) before and after the intervention, and as covariate state anxiety before intervention. For the third hypotheses, a between subject design will be used. The last hypothesis will be tested with a between subject independent-samples t-test.

Results

Preliminary tests were conducted to test whether state anxiety scores differed between groups before and after the intervention. The STAI-6 showed high reliability and validity (Cronbach's Alpha = .82). A paired-samples t-test was conducted to compare the state anxiety scores before the intervention and after the intervention. There was a significant difference in the scores for pre-state anxiety (*M*=38.86, *SD*=10.32) and post-state anxiety (*M*=28.62, *SD*=8.94) conditions; t (40) = 8.192, p < .001. These results suggest that regardless of Group participants showed a decline in state anxiety after intervention. However, an independent-samples t-test was conducted to compare state anxiety scores between pranayama group and control group before intervention. There was a non-significant difference in the pre-state anxiety scores for pranayama (*M*=38.73, SD=8.53) and control (*M*=39, *SD*=12.14) groups; t (39) = -.083, p = .935. Another paired-samples t-test was conducted to compare the scores after intervention; t (39) = .660, p = 513. These results show that between both groups there is no difference in state-anxiety scores before and after intervention. Figure 2 shows a bar graph of state anxiety scores between groups and before and after intervention.

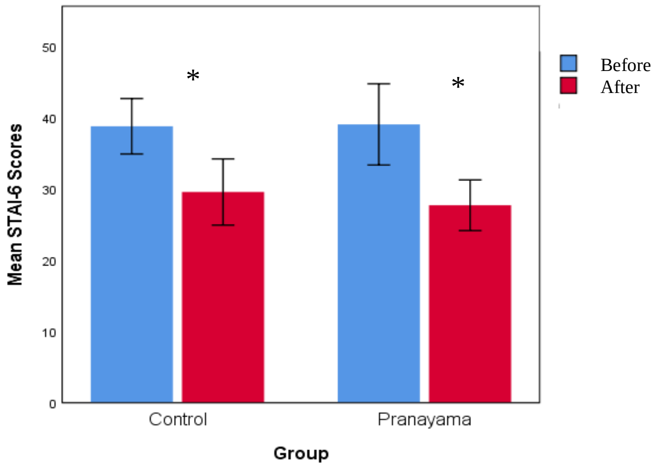

Figure 2: Mean STAI-6 scores per group before (blue) and after (red) intervention. Stars indicate significance on .001-level

A 2x2 mixed-design ANCOVA with Group (pranayama, control) as between-subject factor, Time (threat facilitation index before intervention, threat facilitation index after intervention) as within-subject factor, and state anxiety scores before intervention as covariate, was conducted to compare reaction times-index. Table 1. shows the mean reaction times in milliseconds (RT) for both the group and type of cue (threatening or neutral) before and after the intervention. Preliminary data-analysis showed one outlier; however, its reaction times did not exceed 2000 ms. and was not excluded from the analysis. Mauchly's test indicated that the assumption of sphericity had been violated (χ^2 (0) = 1, p < .001), therefore degrees of freedom were corrected using Huynh-Feldt estimates of sphericity (ε = 1). Test for parallelity showed non-significant relationship between state anxiety before intervention and Group. Levene's Test was significant (*p* = .666) indicating equal variances. Main effect Group on threat facilitation index was not significant, *F*(1, 38) = .136, *p* = .714, ηp^2 = .004. There was a significant main effect of stimuli type F(1,38) = 5.137, *p* = .029, ηp^2 = .116 , indicating overall attentional bias towards threat for both groups. Main effect of Time showed no effect for threat facilitation index, *F*(1, 39) = .022, p = .883, ηp^2 = .001. There was no significant interaction effect of Time x Group; *F*(1, 39) = .077, *p* = .782, ηp^2 = .002. These results suggest that there is no difference in reaction times on threatening and neutral cues between the two groups and between threat facilitation index before and after the intervention when controlled for state anxiety scores. The non-significant interaction effect illustrates that there is no difference in reaction time-index between groups over time. Participants in both groups showed attentional bias towards threatening stimuli.

Table 1

Mean Reaction Times on the dot-probe task for each group before and after the intervention, split between threatening congruent and neutral congruent

Group	Time	Cue	Mean RT in ms.
Pranayama	Before	Threatening	553 (67)
		Neutral	541 (69)
	After	Threatening	550 (67)
		Neutral	525 (80)
Control	Before	Threatening	563 (76)
		Neutral	554 (90)
	After	Threatening	566 (87)
		Neutral	547 (110)

A 2x2 mixed-design ANCOVA was used to estimate the effect of between-factor Group (pranayama, control), within-factors Time (disengagement facilitation index before intervention, disengagement facilitation index after intervention), and covariate state anxiety before intervention. In Table 2, there is an overview of all reaction times, on each cue-type and for each time (before and after intervention). Preliminary data analysis showed one extreme observation on disengagement facilitation index after intervention (RT-index = 195 ms.), compared to the second largest disengagement facilitation index after intervention (70 ms.), although its reaction times did not exceed the 2000 ms. its Cook's distance was much larger than the second largest value (.82 vs.; .19) and standardized residual was much more than 4 below zero. Therefore, this case was excluded from the analysis. Mauchly's test indicated that the assumption of sphericity had been violated (χ^2 (0) = 1, p < .001), therefore degrees of freedom were corrected using Huynh-Feldt estimates of sphericity (ε = 1). Levene's Test was significant (*p* = .471) indicating equal variances. Main effect Group on disengagement facilitation index was not significant, $F(1, 37) = 1.045$, $p = .313$, $\eta p^2 = .027$, main effect Time was significant for disengagement facilitation index, $F(1, 37) = 5.530$, p = .024, $\eta p^2 = .13$, with a lower index before intervention (*M*= -1.104, *SD*= 8.94) than for after the intervention (*M*= 5.7, *SD*= 4.5). There was no significant interaction effect of Time x Group; $F(1, 37) = .522$, $p = .475$, $\eta p^2 = .014$. These results show that there is no difference between groups on their reaction time for the start-dot after a neutral or threatening cue.

However, controlled for state anxiety, results show that after the intervention participants showed faster reaction times for the start-dot after threatening cues, compared with disengagement facilitation index before the intervention. This means that participants were better in disengaging from threatening cues on the dot-probe task after both 15-minute interventions. Time explained 2,4% of the variance on disengagement facilitation index, which is a low effect size. No interaction effect was found between Time and Group. In Figure 3 a bar graph with disengagement facilitation index between before and after attempt was shown.

A paired-samples t-test was conducted to compare the reaction times for neutral cues with reaction times on threatening cues on the dot-probe task before the intervention. There was no significant difference in reaction time for threatening cues (*M*=558, *SD*=71) and neutral cues (*M*=548, *SD*=80) before intervention; t (40) = 1.364, p = .18. These results suggest that there was no difference in reaction times between cues before both interventions, implying no attentional bias towards threatening cues before the intervention regardless of groups.

Table 2

Mean Reaction Times on the start-dot for each group before and after the intervention sorted by 'after threatening' cues and 'after neutral' cues

Group	Time	After cue-type	Mean RT in ms.
Pranayama	Before	Threatening	378 (85)
		Neutral	385 (89)
	After	Threatening	316* (69)
		Neutral	323* (74)
Control	Before	Threatening	371 (109)
		Neutral	363 (95)
	After	Threatening	290* (105)
		Neutral	303* (85)

Note: * = significant at .05-level

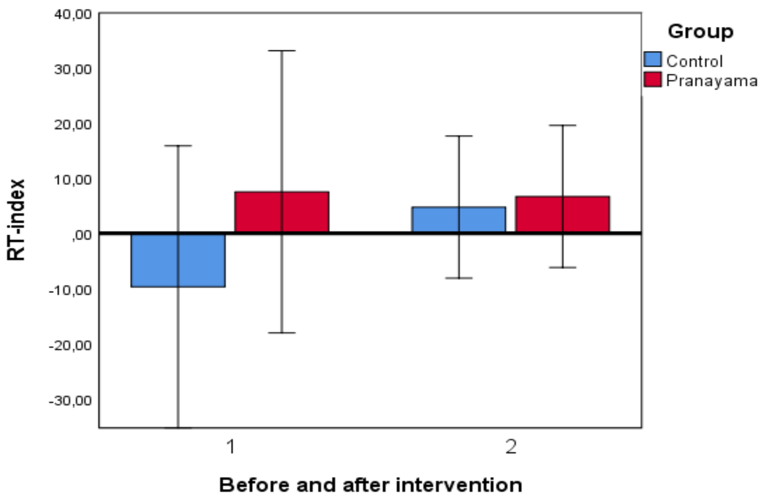

Figure 3: Bar graph showing RT-index scores for start-dot before (1) and after (2) intervention for both groups. Error bars represent standard deviations.

A paired-samples t-test was conducted to compare the reaction times for probes after neutral cues with reaction times on threatening cues on the dot-probe task after the intervention. There was a significant difference in reaction time for threatening cues (*M*=559, *SD*=78) and neutral cues (*M*=536, *SD*=96) conditions; t (41) = 2.734, p = .009. These results show that regardless of groups, there was a significant slower response for threatening cues than for cues that were neutral after both interventions. Therefore, there was no attentional bias towards threat, compared to neutral cues, found in general for the second dot-probe task.

The third hypothesis tested whether 15 minutes of pranayama resulted in less stress-like mood levels compared with the control group. Arousal was not normally distributed with significant Kolmogorov-Smirnov (p = .008). Therefore, a Wilcoxon Signed-ranks test was used and indicated that median arousal levels were higher before the intervention (*Mdn* = 5) than after the intervention (*Mdn* = 3). There was a significant reduction of median arousal over time. However, no significant difference between groups was found. Pleasure scores were not normally distributed (p = .002) therefore non-parametric Wilcoxon Signed Rank Test was used. Results showed a significant increase in median pleasure scores over time whereby the median pleasure score increased from 6 to 8. No significant effect for median difference between groups for pleasure was found. The results suggested that Affect Grid scores shifted from the center of the grid (5-6) towards the subcategory ‘relaxation’ (3-8). For the last hypothesis tested was the expectation that 15 minutes of pranayama resulted in lower scores on the ARSQ than the control group. Measurement of reliability showed a medium reliability score on the 27-item ARSQ (Cronbach's Alpha = .66), and was therefore considered as acceptable but of moderate predictive value. Nevertheless, PCA showed similar component scores for the seven dimensions as was established in previous research. An independent-samples t-test showed no significant effect between groups for ASRQ-scores: *t* (39) = .628, p = .534. Results suggest that there is no difference in mind wandering between 15-minutes of pranayama and control group.

Discussion

The aim of this study was to find whether employment of 15 minutes pranayama breathing exercise was effective in reducing attentional bias towards threatening cues in the dot-probe task, namely it was hypothesized that this short breathing technique would lead to an improvement of disengagement from threatening stimuli. Furthermore, we hypothesized to see reduction in self-reported state anxiety and stress levels. Positive results could then be used to develop an easy to implement and achievable breathing exercise to alleviate stress and reduce attentional biases towards negative cues in ones surrounding.

However, results showed that there was no significant difference between the experimental and control group on attentional bias towards threat-like cues before and after intervention, while controlling for state anxiety levels before intervention. In other words, the threat facilitation index did not differ among the two groups. Neither did we find a change in threat facilitation index after pranayama intervention. However, over both attempts on the dot probe it seemed that participants showed attentional bias towards the threatening stimuli. Furthermore, we investigated whether pranayama led to better disengagement from threatening stimuli measured by disengagement facilitation index. We found no effect for 15 minutes pranayama on disengagement from threatening stimuli, although current outcomes suggest that both groups showed faster reaction times returning to the start-dot after a threatening as compared to neutral cue. However, as is shown in Figure 3, this improvement in disengagement was primarily due to change within the control group and the decrease of variance in both groups. As for attentional bias measured by the difference between mean reaction time for threatening cues and neutral cues regardless of groups, we found no difference for the first dot-probe task, but there was a significant slower response after threatening cues after both interventions on the second dot-probe task. Although we cannot state that attentional bias decreased over time, results suggest that both interventions led to slower response for threatening cues. In addition, both groups did not seem to differentiate on:

state anxiety levels before and after the intervention, pleasure and arousal levels measured by the Affect Grid, and scores on the ARSQ measuring mind wandering. However, for both the experimental and the control group STAI-6 scores significantly reduced over time and median outcomes on the Affect Grid showed that mood shifted towards the relaxation subcategory.

One possible explanation for the absent effect of pranayama on dot-probe task is that this particular pranayama rhythm and length is not suitable for activating the brain regions responsible for attentional control (Cheng et al., 2017; Melnychuk et al., 2018). Present study imitated the experimental pranayama set-up from Cheng et al. (2017) and is therefore suitable for comparing results. Cheng et al. (2017) found promising effects for 5-, 7-, and 9-minute pranayama on the Go/NoGo-task and proposed that lengthening the pranayama duration might show stronger effects as it would show better resemblance with other mindfulness and yoga techniques. However, even though the length of the exercise used in our study was between the three and two times longer, no results were found. Contrastingly, previous studies that have used longer 20 to 30-minute experimental pranayama conditions were able to show effects of pranayama on cognitive functions (Gothe et al, 2013; Yadav & Mutha, 2016). These studies suggest that the lack of effect was not caused by the duration of the pranayama exercise used in present study but that the cause should be searched elsewhere. Moreover, Cheng et al. (2017) used six seconds in- and six seconds exhale pranayama breathing pattern which slightly deviates from the rhythm used in our study. To our knowledge the effect of the breathing rhythm has not been studied thoroughly, although Sharma and his colleagues (2014) found different and corresponding effects of fast versus slow pranayama breathing. However, discrepancies between fast and slow pranayama were clear (Sharma et al., 2014) though it is currently imprecise whether minor differences as used by Cheng et al (2017) and in current study are truly of predictive value.

In addition, the pranayama exercise used in our study did not have the expected effect on subjective anxiety and stress levels as it did not differ from subjective state anxiety and mood levels in the control group. Contrasting to previous studies (Telles, et al., 2013; Telles, Sharma, & Balkrishna, 2014; and Telles, Verma, Sharma, Gupta, & Balkrishna, 2017) who found physiological changes relating to stress reduction, our study did not find any effect of pranayama on subjective affect changes. Although it was not intended to compare physiological studies with the subjective questionnaires used in our study, data showed that both groups became less anxious and more relaxed. Therefore, this study might have illustrated that it is not pranayama but the use of relaxation music or the dimly lit room that stimulates relaxation within participants.

Another possible explanation for the lack of effect on attentional bias could be the different tasks used for studies on cognition and pranayama. Previous research on pranayama and cognitive enhancement (Cheng et al., 2017; Gothe et al., 2013; Yadav & Mutha, 2016) used either the Flanker task, N-Back task and the Go/NoGo task whom measure more specific cognitive functions or rely on late stages of attentional mechanisms compared to the dot-probe task which measures early stages of attentional performance (Van Rooijen et al, 2017). Vago and Nakamura (2011) examined the effect of an 8-week mindfulness-based meditation training on attentional bias toward pain related stimuli, using emotional dot-probe task with pain related and neutral words. Interestingly, Vago and Nakamura (2011) and Van Rooijen et al. (2017) found that it mattered whether cues were presented for 100 ms or 500ms, whereby the first corresponded with bias towards pain related words, while the latter related to better disengagement from it. This may explain the reason there was no effect for the 15-minute pranayama on attentional bias, since 500 ms already activates later attentional mechanisms and allows attention to shift between cues. Moreover, the cues used in our dot-probe task demonstrated both social and non-social settings and presumably have both their own effect on attentional stages (Van Rooijen et al., 2017). Van Rooijen et al. (2017) stated in their review that it is not clear whether human and non-human stimuli used for the dot-probe have the same effect on attentional bias since previous studies had find opposing results. However, further research was needed to estimate the effect of different categories of cues.

To interpret the luck of findings from the self-reports, it is possible that because the ARSQ was developed to measure subjective resting state activity for fMRI and EEG studies (Diaz et al., 2013), the observed corresponding ARSQ-scores for both groups suggested no difference between subjective resting state activity after a neutral state without relaxing or a pranayama exercise. As Melnychuk et al. (2018) and Taylor et al. (2012) proposed, a different effect on attentional performance was found when one was in a passive resting state activity or a focused performance task. Present study expected that 15 minutes of pranayama would relate to an increase of attentional performance and therefore decrease of resting state activity, while 15 minutes of neutral state would result into a passive resting state, hence different scores between groups on the ARSQ were expected. Apparently, either the pranayama technique used in our study was unable to discriminate with 15 minutes of neutral state without relaxing on resting state, or the control condition did not increase the subjective resting state as predicted.

Although present study did not find the expected results, it did illustrate that increased relaxation and decreased subjective state anxiety levels related to: slower reaction times for threatening cues, and faster disengagement after threatening cues on the post-intervention dot-probe. The fact that data showed increase of attentional disengagement from threat-like cues, is interesting when compared with findings in the study of Ellenbogen et al. (2003). Ellenbogen et al. (2003) designed their study to find attentional bias for stress induced groups and discovered that within this group participants showed faster disengagement from negative words. Contrastingly, data from present study showed that decreased state anxiety levels and induced relaxation within the participants resulted in disengagement from threatening cues faster. These two findings seem to contradict each other whereby both stress provocation and stress/anxiety reduction cause participants to disengage faster. Nevertheless Ellenbogen et al. (2003) used a spatial cueing task with negative and positive words, while present study used the emotional dot-probe task, therefore no hard conclusions can be made. In addition, the absence of attentional bias on the first dot-probe task might be a result from stronger attentional control abilities (Basanovic et al., 2017). Participants in our study were non-anxious individuals whom potentially possessed strong attentional inhibition on forehand.

In terms of practical relevance for this field of research, present study illustrates that induced relaxation does not decrease attentional bias. Although there was a difference found between reaction times on threatening cues versus neutral cues on the second dot-probe task, this was not applicable as indicator for attentional bias, since attentional bias was measured by subtracting reaction time on threatening cues from reaction time on neutral cues. Nevertheless, is seems either that induced relaxation makes people less biased towards threats or more susceptible for neutral cues in one's environment.

One of the limitations of this study is the little number of participants in both groups. In order to get better insight into the effect of pranayama on attentional bias, further studies should have a larger sample size. Another limitation is the underestimation of the effect of music used within both conditions which might have contributed to the results. Potentially, it could have been the music used in both conditions that acted as confounder on reduction of state anxiety. Future research should take this effect into account and use music or sounds that do not interfere with performance on the experiment or use music as another experimental group to understand the impact of music on dot-probe tasks. Other factors that may have contributed as confounders are the dimly lit room and the fact that the room temperature was warm during the whole study. Combined, these two factors already may evoke relaxation and should therefore kept constant in new research. In addition, current study used a five seconds inhalation and five seconds exhalation breathing rhythm for a total of 15 minutes. However, it is not tested whether this rhythm is feasible for this duration neither it was tested if other rhythms are more comparable to yogic breathing techniques found in literature. Dot-probe used for this study was not piloted before the onset of the study and therefore reliability and validity were not estimated. The mixture of non-human and human stimuli could also have affected the non-significant result and therefore future studies should estimate the effect of these different types of cues. In current study data was controlled for state anxiety before intervention.

In sum, this study tried to find an effect of a 15-minute five seconds inhale and five seconds exhale pranayama exercise on attentional bias towards threatening cues. Data showed no effect of pranayama on attentional bias and disengagement from threatening cues as compare to control group. Presumably, music and relaxation inducing atmosphere in the lab acted as confounding factors within each group that led to decreased state anxiety levels for both groups. Moreover, the emotional dot-probe task used in this study might have used problematic combinations of stimuli, which both seem to have different effects on attentional mechanisms. Potentially, sufficient attentional control and low non-anxious state-anxiety levels within the participants present on forehand contribute to the absence of significant effect of this type of pranayama. Future studies should address the importance of complementary but sufficiently different control groups for research on pranayama and attentional control.

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Appendix

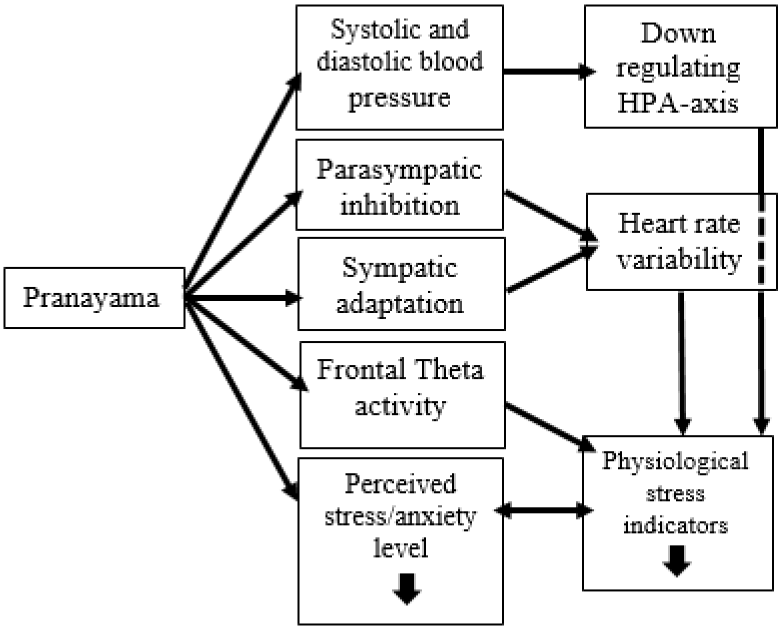

Figure 1. Schematic overview factors facilitated by pranayama and their effect on the reciprocity between perceived stress levels and physiological stress indicators.