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Contemplations into Respiration

Effects of breathing and meditative movement on body and mind

Chapter 1

General introduction

Cognitive psychology studies fundamental functions and processes of the human mind; functions such as: perception, attention, memory, emotion, decision making, and language. It traditionally proposes mechanisms for these *cognitive functions* and captures their dynamics into *mental models*. These models are inspired, informed, and validated in empirical studies using (computerized) tasks. In these experiments, conditions are contrasted where these cognitive functions are assumed to be differentially affected. The conditions then should differ in how accurate or fast participants perform the tasks, or steps therein. Notably, measures of *reaction time* are taken to elucidate cognitive processes. Mental chronometry (Donders, 1868) is a research paradigm that harkens back to the finding that nerve pulses travelling through the body take time (Helmholtz, 1850). Recently, cognitive psychology has crossed over with neuroscience into the field of *cognitive neuroscience*, which attempts to couple cognitive models to brain anatomy and function.

Contemporary cognitive psychology is highly influenced by the metaphor of the human mind as an information processing machine: a computer (Fodor, 1968, 1975; Putnam, 1967, 1975). In this view cognition is *computation*. Also, in classic cognitive science — and classic artificial intelligence — there is believed to be a *central executive system* (Baddeley & Hitch, 1974), that makes decisions and directs the body top-down. In terms of the computational view this could be seen as both the processor and RAM-memory — working memory — of the information processing system. The executive system hierarchically controls how the information is processed. For a full review of the classic cognitivist view, see Dawson (2022). However, from the 1990s onwards a competing view to the computational conception has arisen: embodied cognition (Lakoff & Johnson, 1999; Varela et al., 1991).

Embodied cognition

In contrast to the classic computational approach, contemporary embodied cognition (Anderson, 2003) rejects that cognition solely happens in the brain, in isolation of the rest of the body and the outside world. In other words, humans are not simply run by a computer contained in their skulls. Next to the brain, the periphery and viscera of the body proper play an active, causal and critical part in cognition. In the embodied view there is no single central executive that directs all action top-down. The state of the body and its actions also influence cognition bottom-up. See Shapiro (2021) for a complete introduction to the embodied field. Three pioneers of the embodied mind need introduction for a better understanding of this work.

If we try to go back to the earliest form of any psychological theory then we usually end up at William James (1842-1910). For the embodied mind perspective, this is no different. In the *Principles of psychology* James (1890) pertains that the experience of an emotion is merely the mind's sensation of a bodily state. This view might now be described as belonging to the extremist stance of embodied cognition. Herein the causal relationship between body and brain is unidirectional, bottom-up — from the body towards the brain. Modern proponents of the embodied mind generally view this relation as being bidirectional; both bottom-up and top-down.

Clark (1997) — in his seminal book *Being there* — describes how the brain, the body and the environment are equal parties in producing effective action. The mind is not contained but extended: it also inhabits the body (and the environment). Cognition is not passive computation, but it is active and evolves in dynamic interaction with the environment. Cognition is rooted in the physical experience of reality (i.e. the body and the world) and operates in real time. Indeed, in many contexts *decentralized solutions*, solutions that rely on a close-coupled dynamic interaction between body and environment produce better adapted behavior and optimized performance than does centralized control; behavior as directed by a central executive or central nervous system. Very loosely summarized: doing trumps thinking.

Damasio (1999) makes the argument on neurological grounds — in his canonical work *the feeling of what happens* — that the body is central in constructing conscious awareness and the psychological self. The fundamental tenet of the claims in this work is the *somatic marker* hypothesis (Damasio, et al., 1996), which states that there is no cognition without emotion. All (higher) processing in the brain is stamped by emotion, which gives thoughts

their value and valence. Furthermore, goal-directed behavior without these emotional stamps is impossible. An individual would simply have no way of choosing a course of action; appropriate, preferred or otherwise. Similar to James (1890) this emotion starts in the body: the somatic part of the marker. Though the brain is able to create bodily states — through neural and hormonal pathways — it is also constantly itself being informed and influenced by these states, by the internal milieu (Bernard, 1865). For example, biomarkers on cardiopulmonary functioning, such as blood pressure and CO₂ blood levels, are constantly being monitored in the brainstem, sent upstream and change central functioning. In rats the vagus nerve not only monitors norepinephrine and epinephrine levels, but enhances memory function through projections to the forebrain (Williams & McGaugh, 1992, 1993). This bidirectional relationship is what Damasio calls the *body loop*. The brain influences the body, which returns feedback, changing brain states and leading to downstream adjustments, and so the cycle continues. Succinctly summarized: body states change brain states.

Let me give a concrete example of such bottom-up influences: the facial feedback hypothesis (Strack et al., 1988). According to this hypothesis facial muscles responsible for certain emotional expressions, can actually produce or inhibit these emotions themselves by being mechanically stimulated, or inversely by being impeded to contract. In the original study this was done by participants holding a pencil in their mouths, which makes muscle groups contraction and relaxation for certain emotional expressions impossible. The facial feedback hypothesis also maintains that manipulating expression influences cognitive processing higher up, such as in social recognition. Havas and colleagues (2010) showed in their infamous Botox study that paralyzing the specific muscles to an emotion like happiness, also hampers the recognition of this emotion in others. Thus, here we have an example of a peripheral cause not only directly influencing emotion, but also cognition. However, a slight disclaimer is in order on the facial feedback hypothesis. In a replication study by Wagenmakers et al. (2016) of the original study of Strack et al. (1988) the original results could not be replicated. Then again, a recent meta-analysis (Coles et al., 2019) has concluded that the facial feedback effect does exist, albeit small and heterogeneously produced.

It may be clear by now, that the current work is situated in the embodied corner of the debate. However, there is another trend in cognitive neuroscience that is of paramount importance to this work. Cognitive science has also branched out into an applied field. There cognitive enhancement is a main goal: leveraging the plasticity of the brain to improve cognitive functioning.

Cognitive enhancement

First, we need to make a critical distinction between the aforementioned *central executive system* and *executive functioning*, also known as *cognitive control* (Hammond & Summers, 1972). The first is a central component of a cognitive model that directs behavior like a little puppet master in the head, the latter are several distinct processes, that among others help steer behavior away from ingrained patterns and trodden tracks, inhibits socially inappropriate behavior, is necessary for doing tasks consisting of multiple serial and parallel steps (like following a recipe), manages working memory, and readapts strategies to new behavioral goals. In contrast to the central executive these executive functions are only cogs in a vastly complex machine, not their centerpiece (Miller, 2000). In other words: the first one's existence is in serious doubt, the second is clearly established. The existence of these control processes are easily made apparent when they fail. For example in attentional capture: paying attention to salient stimuli that are irrelevant in the current context; and in perseverance: continuing with prepotent behavior that has become inadapative or even dysfunctional (Monsell, 1996). Everyone knows the experience of being distracted by a prompt on your phone. Only to find yourself back in the present moment, having run the social media rabbit hole, that message led to and are left wondering what that very important thing was, you were in the middle of doing. This is a classic failure of cognitive control. Summarizing: executive functioning is something different from the central executive. This is relevant here because many cognitive enhancement attempts are oriented towards these functions, as are ours.

Cognitive enhancement (Juengst, 1998) — in the broad sense — has as its aim to improve any form of mental functioning by any means possible, such as improving mental health, language ability, social cognition in group dynamics. This can be achieved by means of computer training, physical exercise, diet, or even drugs. These all fall under the umbrella term cognitive enhancement. The means to this cognitive end can be categorized into three domains: pharmacological, physical, and behavioral (Dresler et al., 2018). See Marois and Lafond (2022) for a review of these different enhancement applications. In this work we will be concerned with the enhancement of fundamental cognitive functions, such as attentional and cognitive control, by the least invasive domain of intervention: the behavioral (Green et al., 2019).

Behavioral cognitive enhancement can take many forms. Some could be described as physically inactive, such as: gaming, training, language learning; some as active: physical exercise and mind-body exercises. As our focus on embodied cognition suggests, this work uses a family of applications that aims to intervene on both mind and body, though the way of change lies from the body towards the mind and back again (Kerr et al., 2013). These are the traditions of contemplation.

Contemplative practices

Contemplative activities come in many forms, the shape of which is usually passed down from (Buddhist) religious practices. Just the contemporary popular styles of meditation are myriad. To name a few: Vipassana, Transcendental, Zen, Compassion, and Loving-Kindness meditation. Then there are the meditative traditions that also include some form of physical exercise, like moving slowly and continuously or smoothly from stationary posture to posture. These mind-body exercises include practices such as: tai chi chuan, qi gong, walking meditation, and – with its diverse styles and by far the most widely practiced of all: yoga. For a systematic categorization of meditation practices see Lutz et al. (2017) and Matko and Sedlmeier (2019).

Varied as these practices may be, they do have a common thread: the meditative or contemplative aspect, commonly called *mindfulness*. The famous Vietnamese Zen Buddhist monk Thich Nhat Hahn, who passed away earlier in this year of writing 2022, describes mindfulness as: to pay attention to something, to remember being in the here and now, but also: to not judge in the present moment (Hahn, 1997). This attention in the present moment is key. It offers what to be mindfully attentive of. This attention can vary in its scope. This is where it diverges from the cognitive psychological construct of attention. In the work of Lutz and colleagues (2008) a division is made between focused attention meditation and open monitoring meditation, as a broad stroke dichotomy wherein most practices can be categorized. In focused attention meditation, attention is like the spotlight in psychological theory. It illuminates a single sensation, that can jump – like a saccade – from object to object in the visual field. For example, a practitioner might focus on a single part of their body, redirecting their attention back to it, gently, whenever distracted. In contrast, practicing open monitoring meditation means letting in awareness of any sensation, of any sensory modality (e.g., hearing, smell, pain), or thought or emotion, that comes to mind. Practitioners attempt

only to pay attention to these sensations fleetingly, to not dwell, before moving on to the next draw. It is believed that, through extended practice and experience, awareness can be spread and distributed across all sensory modalities and feelings in a singular moment. It turns out that training attention in these divergent ways matters: it affects attentional functioning longitudinally in the trained direction. In another study by Lutz and colleagues (2009) practitioners increased the stability of their (auditive) attention both behaviorally and neurally (EEG), after following a three-month training in focused attention meditation. However, a recent study showed that short bouts of meditation in either focused or open meditation had no effect on attentional function and this seemed to be explained by individual differences in mindfulness traits (Tanaka et al., 2021).

Whatever the scope of attention might be in any given exercise, one family of sensations in focus is always present: the bodily. In the body scan one goes through the whole body while sitting or lying down. Qi gong emphasizes proprioception, while moving slowly and with attention. During sitting meditation (like Samadhi) one looks for the feeling tone – the dominant mood or affective state – and is thus more preoccupied with interoception — emotional body feedback; as both positive or negative affect has biological markers that can be picked up by body awareness exercises. Whatever the focus of the particular practice, mindfulness starts in the body (Kerr et al., 2013). Within the practices that train awareness of the body, a special role is reserved for the sensations of respiration. Furthermore, respiratory patterns are also actively modulated in practice.

Breathing exercises

Contemplative activities frequently take breathing as the focus of awareness in their instructions. It is thus reasonable to assume that this will affect respiratory patterns and biomechanics. Furthermore, many contemplative traditions actively prescribe breathing exercises. Actively controlled breathing seems to have different neural routes than do autonomous respiratory drives, as in rats between respiration in fight-or-flight mode and when vocalizing (Subramanian et al., 2001). Breathing control in humans has been theorized to be the necessary evolution for human speech to be possible and indeed the respiratory innervation of the thorax in humans is comparatively increased with other primates (Lieberman, 1991).

The instructed respiratory patterns in contemplative breathing exercises vary in respiration rate, durations of inhalation versus exhalation, breath-holding, and locus of breathing (the location of intentionally directed muscle groups). Though these instructions vary, the vast majority of exercises does show a clear common thread: towards slower breathing, shifting to an abdominal locus and sometimes with extended expiration. Research into the effect of a lower respiration rates on autonomous nervous system activity has indicated an optimal rate of 6 breaths-per-minute (or lower) for increased relaxation (van Diest et al., 2014). This is reflected in a lower mean heart rate, blood pressure (but with more fluctuations and cardiac synchrony) and variability in heart rate (Russo et al., 2017). This suggests that specific respiratory patterns do not only reduce stress, but also increase systemic flexibility.

Systemic flexibility

Heart rate variability (HRV) is a metric that quantifies the moment-to-moment fluctuations in heart rate. The time interval between heart beats is never the same. The more this interval varies across a given period, the higher the HRV score, whereas less variation means a lower HRV score. Thus, two individuals might have the exact same heart rate – the average number of heart beats per minute – but may differ widely in its variability.

HRV is a useful indicator of individual cardiovascular health and overall physical condition (Singh et al., 2018). The higher resting state HRV, the better the cardiopulmonary condition is. Furthermore, HRV is a valid indicator of stress levels, both acute and chronic (Kim et al., 2017). This is because variations in heart rate are differentially produced by the branches of the autonomous nervous system: parasympathetic activity increases HRV, while sympathetic activity decreases HRV (Singh et al., 2018). The sympathetic branch of the autonomous nervous system is (partly) responsible for the “fight-or-flight” response. It thus increases the body’s readiness for action; heightening arousal and stress responses. The parasympathetic branch does the opposite. It prepares the system for “rest-and-digest”, dampening arousal and stress. Thus, physically, high HRV means low stress, low HRV means high stress. Chronic stress is characterized by a tonic decrease in resting state HRV, through prolonged activity of the sympathetic nervous system (Choi et al., 2017). In overview, HRV is a good indicator of the flexibility and functional adaptability of the organic system to energy demands. Next, it also seems to be an indicator of cognitive flexibility.

Thayer and colleagues (2000, 2007) were the first to make a clear link between HRV and cognition. In their neurovisceral integration model they showed that high HRV is associated with increased cognitive control and flexibility. The model couples the function of the autonomous nervous system with that of central nervous system, through projections of the vagal nerve into prefrontal cortex. The neurovisceral integration model was recently adapted to new data and expanded on (Smith et al., 2017). This link between HRV and central nervous system functioning has since then been corroborated through many studies and across many domains; in some of these studies it is also reestablished that breathing at the optimal rate increases HRV, which then has an effect higher up the neural chain (Schwerdtfeger et al., 2020). For a recent review of HRV in psychological science see Pham et al. (2021). Concluding, HRV is a suitable indicator of the flexibility of both body and mind.

Arousal, stress, and relaxation

In many scientific texts the terms arousal and stress are used interchangeably and seem to stand for the same psychological construct. Regarding this demarcation, the current manuscript is not much different. Likewise, relaxation is usually defined as the absence of stress or arousal. In reality, there are many parts to the stress system, both in terms of temporal dynamics and mechanics. It is not even a single system. The autonomic nervous systems' sympathetic branch (Langley, 1903) is a separate system from the hypothalamic-pituitary-adrenal (HPA) axis (Sheng et al., 2021; Smith & Vale, 2022). Effects on these systems might also diverge. For example, traumatized individuals show heightened sympathetic nervous system responses to acute stress, while the HPA axis response is dampened, as compared to healthy controls (Schuermans et al., 2021). Going even further, the effects on these separate stress systems can be divergent: patients suffering from post-traumatic stress disorder show tonically low salivary cortisol levels (a HPA axis hormone) in the majority of studies, while sympathetic stress levels are high. So it seems that physiologically speaking at the same time these stress levels can be both low and high (Wahbeh & Oken, 2013a-b). Though note here that HPA activity is assumed to still be high, through higher sensitivity of corticosteroid-receptors. Another example of different roles of these stress systems: in panic disorder the sympathetic nervous system was long held responsible in the etiology of this psychopathology. Instead adrenaline/epinephrine seems to

be the culprit of consensus currently; the autonomous nervous system no longer plays an explanatory role (Wilkinson et al., 1998).

The timing and duration of stress responses is of major consequence both in the processes triggered and in the effects sorted. For example, stress can both mobilize and immobilize the immune system depending on time scale (Sapolsky et al., 2000). The construct arousal also has very different mechanisms, that can work in concert or isolation, and do so in different timeframes. Even when the same chemical substance is involved, arousal can be reduced to different processes, depending on the site of action. Norepinephrine can act as a hormone or as a neurotransmitter. Both produce a form of arousal, but they diverge in timing and simultaneousness. In the relatively slow fight-or-flight response – effected by the hormone – the neurotransmitter is also acting on central nervous system sites. During the fight-or-flight response pupils dilate, a biomarker of central norepinephrine activity (Aston-Jones & Cohen, 2005; Gabay et al., 2011). But the involvement of the neurotransmitter here must be clearly disentangled from its neuromodulatory role in phasic arousal (Landman & Steenbergen, 2020), which acts on alertness moment-by-moment, or its function in the reticular activating system in waking up (Aston-Jones & Cohen, 2005; Berridge & Waterhouse, 2003, Kandel et al., 2000). These phenomena are not in concert with the endocrinal responses. Even within the fight-or-flight response, hormonal activity is associated more with the adrenocortical than the sympathetic norepinephrine response (Goldstein, 2010).

Relaxation is usually operationalized as the absence of stress or as a state of low arousal. Indeed, it is fair to say that if the HPA axis is not active or sympathetic activity is low, people are not stressed. However, concluding that this means that they are also relaxed is an inferential leap. For one, though the sympathetic and parasympathetic branch of the autonomic nervous system are mutually inhibitory and opposing in action, they can be active simultaneously. For example: when individuals recover from a stressor, both branches increase in activity (Weissman & Mendes, 2021). This realization leaves parasympathetic activity as the prime manner of assessing (active) relaxation and the development of valid measures of parasympathetic tone of heightened import.

As much as there are many possible definitions, interpretations and mechanizations of arousal, stress and relaxation, there are as many operationalizations. The measures intended to map these constructs are among others: pupillometry to measure central norepinephrine levels, blood sampling to measure stress hormones like cortisol, ECG for measuring vagal tone heart rate variability, ICG to measure sympathetic pre-ejection period, EEG to measure

the frequency bands of overall brain activity, electrodermal activity to measure manual sweat secretion, and questionnaires to measure self-reported stress levels. After this expose, it is apparent and safe to say that most of these methodologies tap into different aspects and processes of the terms arousal, stress and relaxation. What is relevant here are the golden standards: the metrics where a scientific consensus exists that they map a certain aspect of autonomic activity. Currently, the dominant measure of sympathetic (cardiac) activity is the pre-ejection period, obtained by using a combined ECG/ICG set-up. The dominant parasympathetic activity measure is vagal tone HRV by ECG. These are the HRV metrics that are claimed to represent vagal nerve activity. The most widely used of these is the root mean square of successive differences (RMSSD).

Again, this book is not the exception to the culture of muddling and conflating terms of arousal, stress and relaxation. Frequently, they are lacking clear neurophysiological or mechanistic definition in this book. However, when encountering these terms in the following text, the reader can safely assume that the authors are referring to autonomic activity, unless otherwise stated. Thus, when referring to arousal or stress this means high activity of the sympathetic nervous system, while relaxation refers to high activity of the parasympathetic nervous system, notably vagal tone. In my view, this will likely resolve most confusion, though probably not in all cases.

Bayesian statistics

In the empirical chapters of this dissertation all collected data is analyzed by Bayesian statistical counterparts of classical statistical tests. The reasons are obvious: Bayesian statistics are robust; have no issue with effects actually being a false positive or false negative result, if the study sample is large enough; no multiple comparison issue; and most importantly: confident statements about the actual strength of evidence can be made from these statistics (Wagenmakers et al., 2018). In short: it does not suffer from many of the ailments of classical p-testing and should be the statistics of choice in any probabilistic science. Certainly those sciences, such as psychology, that suffer a replication crisis and/or publication bias, where many canonical results can't currently be replicated or many null-results — or even adverse results — are not being published; should take advantage of the Bayesian approach to statistical evidence.

For the convenience of the reader, I provide the following very short primer. Any Bayesian analysis – in our chapters these are mostly Bayesian repeated measures ANOVAs – produces a Bayesian odd for each model under comparison: the Bayesian factor (BF₁₀). The factor gives the likelihood of the model under investigation being true, relative to the null-model. Note though, that it can also be compared to the best-fitting model in the complete analysis. In other words: this factor gives a quantification of the strength of evidence for any specific model, this in contrast to the all-or-nothing p-value. In the empirical chapters (**Chapter 3** and **Chapter 4**) we follow Jeffreys’ (1961, as adapted by Wetzels et al., 2011) qualification of Bayesian factor values. See table 1 for these qualifications of evidence load.

Table 1. Bayesian factors interpretation overview. BF₁₀ is the likelihood that H₁ is true over H₀. H₁ is model 1; H₀ is null-model. Table taken and adapted from Wetzels et al. (2011).

Bayes factor BF ₁₀	Interpretation
> 100	Extreme evidence for H ₁
30 - 100	Very strong evidence for H ₁
10 - 30	Strong evidence for H ₁
3 - 10	Moderate evidence for H ₁
1 - 3	Anecdotal evidence for H ₁
1	No evidence
1/3 - 1	Anecdotal evidence for H ₀
1/10 - 1/3	Moderate evidence for H ₀
1/30 - 1/10	Strong evidence for H ₀
1/100 - 1/30	Very strong evidence for H ₀
< 1/100	Extreme evidence for H ₀

Overview

Though the order of this work follows date of publication (or writing) it is not strictly speaking chronological. The experiment reported in **Chapter 3** was published two years after the theoretical work in **Chapter 2**. However, the experiment of **Chapter 3** was set up and conducted some years before the main ideas of **Chapter 2** were formulated. Thus, these ideas

were not set out to be tested in this experiment by design. The first experiment reported in **Chapter 4** was run during the time the manuscript of **Chapter 2** was being written. So the following chapters are anything but a smoothly flowing narrative.

Chapter 2 introduces a neurophysiological model that connects respiratory patterns with cognitive control, through mediation of the autonomous nervous system (Gerritsen & Band, 2018). The respiratory vagal nerve stimulation model (rVNS). One of the aims of the paper was to provide a framework from which to interpret and explain the many diverse findings of scientific publications on contemplative practices. For this aim, it also includes a selected review of that literature.

Chapter 3 presents a randomized controlled trial in a normally aging population (Gerritsen et al., 2021). The intervention consisted of a two-month online Tai Chi Chuan course and the experimental group was contrasted with an active control group that watched documentaries on similar topics of equal duration and frequency as the course. Participants were pre-post-tested on three executive functions: switching, updating and inhibition (Miyake et al., 2000); and (psycho)motorically scored on functional balance and motor speed.

Chapter 4 consists of two experiments that test some predictions of the rVNS model, that are expanded on in **Chapter 2**. Foremost, it was tested whether the locus of breathing – abdominal versus thoracic – has an effect on stress or relaxation (vagal tone) and on inhibitory control. In the first experiment the inhibition process under study is response inhibition, while in the second experiment it is cognitive inhibition (interference scores). Furthermore, the second experiment also tries to indirectly manipulate respiration rate and thus introduces another prediction of the rVNS model. As interventions, audio guided breathing exercises were used.

Finally, **Chapter 5** provides a summary of the previous three chapters. This is followed by a discussion which aims to give an in-depth analysis of the results and its implications. Lastly, the current state of contemplative science, but also of cognitive science in general, is critically discussed. **Chapter 5** has a Dutch copy right after the English version.

Chapter 2

Breath of Life: The Respiratory Vagal Stimulation Model of Contemplative Activity

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Abstract

Contemplative practices, such as meditation and yoga, are increasingly popular among the general public and as topics of research. Beneficial effects associated with these practices have been found on physical health, mental health, and cognitive performance. However, studies and theories that clarify the underlying mechanisms are lacking or scarce. This theoretical review aims to address and compensate this scarcity. We will show that various contemplative activities have in common that breathing is regulated or attentively guided. This respiratory discipline in turn could parsimoniously explain the physical and mental benefits of contemplative activities through changes in autonomic balance. We propose a neurophysiological model that explains how these specific respiration styles could operate, by phasically and tonically stimulating the vagal nerve: respiratory vagal nerve stimulation. The vagal nerve, as a proponent of the parasympathetic nervous system, is the prime candidate in explaining the effects of contemplative practices on health, mental health, and cognition. We will discuss implications and limitations of our model.

1. Introduction

The past fifty years have shown an increasing interest in eastern contemplative traditions in Europe and North-America. These traditions include meditation styles and mindfulness, as well as mind-body exercises like tai chi chuan (TCC) and yoga. What most of these practices have in common is not only their origin in eastern philosophy and religion, but also the goal to enhance individual physical and mental health. Scientific research has followed the popularity of contemplative activities (ContActs). *Figure 1* shows that the cumulative number of relevant publications since 1945 follows a quadratic pattern, with total number of publications within a decade increasing from 2412 between 1997-2006 to 12395 between 2007-2016 (Web of Science, February 2018). The number of clinical trials on meditation, mindfulness, yoga, TCC or qi gong each year alone increased from a little under 20 in the year 2000 to about 250 in 2014, citations in the same timeframe going up from 20 in 2000 to 7112 in 2014 (Web of Science, February 2018).

The current paper reviews scientific insights in potential health benefits. In particular, we investigate the role of particular breathing techniques (low respiration rate, long exhalations) integral to contemplative activities and show that these techniques are prime candidates in explaining the benefits of ContActs for health and mental health. Furthermore, we provide mechanisms and a neurophysiological model that can explain how respiratory patterns produce these effects; through vagal nerve stimulation.

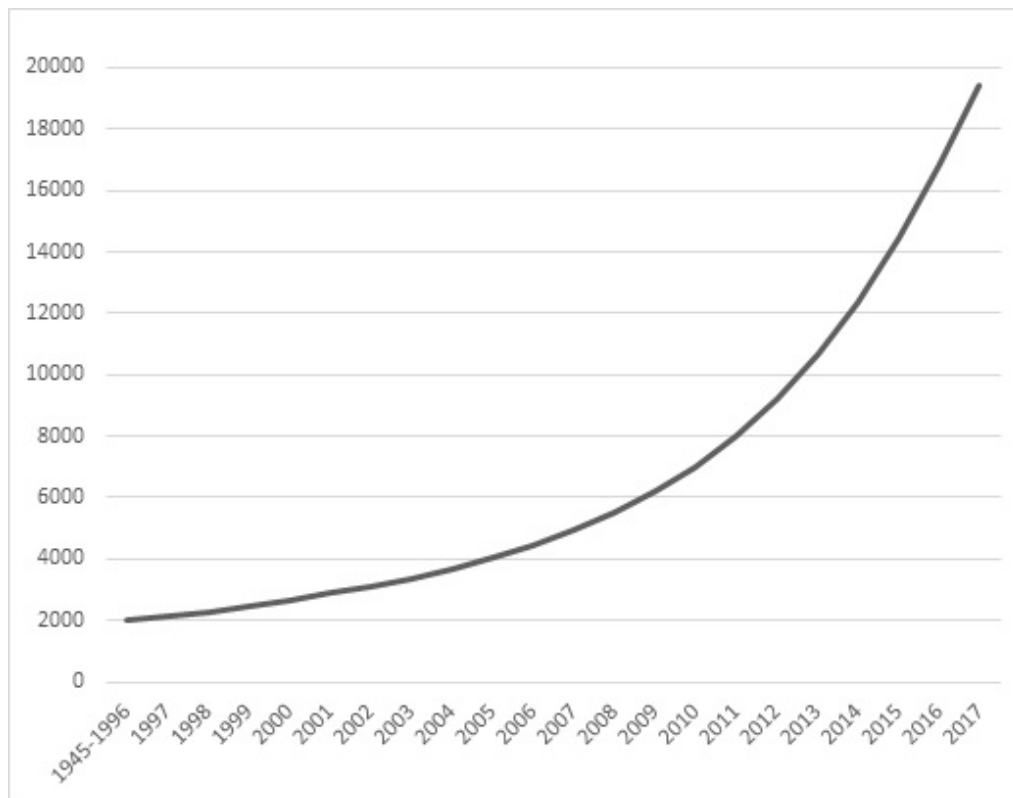


Figure 1. Cumulative number of scientific publications on ContAct, from the 1945-1996 bracket to 2017 per individual year. Obtained from Web of Science in January 2018 using the search terms: “mindfulness” OR “meditation” OR “yoga” OR “tai chi”.

Studies on contemplative practices have reported a plethora of positive effects on health, mental health, and cognition (for reviews, see Büssing et al., 2012; Forbes et al., 2013; Grossman et al., 2004; Lee & Ernst, 2012; Ospina et al., 2007; Shapiro et al., 2003; Wahbeh et al., 2008). However, not much literature has been devoted to revealing the mechanism underlying reported benefits. The current paper is intended to fill this gap. Hereby, we hope to give extra incentive for research on the mechanisms of ContAct action and inform traditional practices, thereby giving opportunity to innovate styles with new exercises and targeted interventions.

2. Contemplative traditions

For the purpose of this paper we define contemplative activities as activities that involve conscious and attentive exercise aimed at changing one’s mental state, contemplation in meaning comparable to ‘praying’ and ‘meditating’. We have deliberately not chosen the concept mindfulness because it is associated with particular practices, instructions and states, as we will discuss later. Despite the similarities between ContActs reflected in the definition,

a few differences among ContActs are worth explaining here, because they are also used to position interventions in research.

The most common, the most referenced, the most studied and largest subgroup of ContActs is *meditation*. Most meditation traditions come from east and south Asia and are originally Buddhist or Hindu in nature. Zen Buddhist meditation (originating in China from a marriage of Buddhism and Taoism), loving-kindness meditation (Tibet), vipassana (India) and transcendental meditation (India) are popular styles. Yet there are also European and Middle-Eastern forms such as acem from Norway, Christian monastic traditions (Egan, 1991; Studzinski, 2009) and Sufi Islamic meditation: muraqaba and whirling (Cakmak et al., 2011, 2017; Nizamie et al., 2013).

In an attempt to classify meditation traditions according to their differentiated instructions, a distinction has been proposed between two types: *focused attention* meditation (FA) and *open monitoring* meditation (OM, Lutz et al., 2008; Lippelt et al., 2014). FA instructions emphasize attention to a particular focus, almost always the breath, along with means how to handle distractions and refocus attention. Zen meditation is commonly seen as an archetypical form of FA. OM stresses the spreading of attention on multiple endogenic and exogenic stimuli, having fleeting awareness of multiple sensory modalities, emotions and thought. Vipassana is popularly regarded as an exemplar of this style. There is no strict separation between the two types in zen and vipassana, and most traditions blend one style into the other. Overall, OM is frequently seen as a more advanced level exercise than FA, and is thus practiced more by experts as compared to novices.

In a further categorization attempt by Lutz and colleagues (Dahl et al., 2015, for another three dimensional classification, see Lutz et al., 2015) more meditation styles are classified based on their most emphasized and practiced techniques. The resulting framework has three meditation families: the attentional, the constructive and the deconstructive. Both FA and OM styles belong to the attentional family. The constructive meditations are aimed at improving the well-being of oneself and others, exemplified by compassion and loving-kindness mediation. The deconstructive practices focus on breaking habits of perception, affect, thought and behavior: most *mindfulness meditation* falls in this category. Note however, that the term mindfulness has been used in a different meaning as well. It refers not only to a category of meditative practice; it can also refer to a mental state, or even the ultimate enlightenment goal of these practices. As a mental state, mindfulness refers to a state of *meta-awareness*, in which the practitioner observes emerging feelings and thoughts without

judgment (*non-judgmentality*). The state of mindfulness can actually be the target of both FA and OM exercises.

Another subgroup within ContAct, here referred to as *mind-body exercises*, is more multi-modal. It involves both meditation and physical exercise; such as stances, positions, complex movements and muscle relaxation techniques. Common traditions are the many styles of Indian yoga and Chinese styles like TCC and qi gong. Origins of these styles differ, but most also have religious or mystical roots like meditation traditions, even when developed as a martial art (e.g. Taoism in TCC).

3. The positive effects of ContAct

Research has found a vast array of beneficial effects in the three domains of physical health, mental health and cognitive performance.

3.1. Physical health

3.1.1. Cardiopulmonary effects

Multiple reviews on different ContActs report a decrease in *cardiometabolic risk factors* and an increase in *cardiopulmonary health and fitness*, according to a meta-analysis this is most consistently reflected in lowered heart rate, blood pressure and blood lipid profile across practices (Ospina et al., 2007). Reviews on separate practices confirm this for meditation styles such as mindfulness meditation and transcendental meditation (Grossman et al., 2004; Walton et al., 2004), as well as the mind-body exercises yoga (Büssing et al., 2012; Posadzki et al., 2014) and TCC (Jahnke et al., 2010; Lan et al., 2013), where one meta-analysis indicates increased aerobic capacity as well (Taylor-Piliae & Froehlicher, 2004). However, Cochrane reviews on transcendental meditation, TCC and qi gong state that even though there is suggestion to their positive effect, definitive conclusions on their efficacy cannot be drawn because of a lack of high quality long-term trials (Hartley et al., 2014a, 2014b, 2015).

3.1.2. Anti-Inflammation

ContAct reviews also report *immunological improvements*; most studies find functional *anti-inflammatory effects*, where meta-analyses indicate that the most commonly

reported decreases of pro-inflammatory markers are in C-reactive protein and pro-inflammatory cytokines, such as tumor necrosis factor- α (Bower & Iriwin, 2016; Morgan et al., 2014). Again, this enhancement can be seen with different ContActs: loving-kindness meditation (Hofmann et al., 2011), yoga (Black et al., 2013) and TCC (Jahnke et al., 2010; Lan et al., 2013).

3.1.3. Physical function

Like other physical exercises, mind-body exercises improve *general physical function*, most notably bone density, balance, strength and flexibility (Büssing et al., 2012; Jahnke et al., 2010). Mindfulness-based stress reduction, yoga and TCC seem to ameliorate (*chronic pain*) conditions, as indicated by pain scales in conditions such as: migraine, fibromyalgia and osteoarthritis (Büssing et al., 2012; Grossman et al., 2004; Wahbeh et al., 2008). As mindfulness-based stress reduction also includes yoga-like exercises, these results can be reserved to mind-body exercises and might best be interpreted as coming from the physical exercise part of these programs, as exercise-induced analgesia is well-established (Koltyn et al., 2014) and is even comparable to medication in chronic pain conditions according to a review of multiple Cochrane reviews (Geneen et al., 2017).

3.2. Mental health

3.2.1. Stress relief

Reviewed ContActs *decrease stress* and *negative affect*, and in parallel *increase well-being* and *self-efficacy*, as indicated by stress and (trait) anxiety rating scales and quality of life questionnaires (Eberth & Sedlmeier, 2012; Grossman et al., 2004; Jahnke et al., 2010; Keng et al., 2011; Kirkwood et al., 2005; Wang et al., 2010). Furthermore, a recent meta-analysis that collapsed studies using different meditation interventions, such as FA and OM, showed that they reduce multiple physiological stress markers across styles: heart rate, blood pressure, cortisol levels and inflammatory bodies (Pascoe et al., 2017).

3.2.2. Stress-related psychopathology

ContActs, and notably mindfulness-based cognitive therapy, *reduce symptoms in affective psychopathology* (Chiesa & Serretti, 2011; Kuyken et al., 2015). Most reviews and

meta-analyses report decrease in symptoms of depression, anxiety disorders and post-traumatic stress disorder, as measured by structured clinical interview and common clinical scales, such as the Beck Depression Inventory (Balasubramaniam et al., 2012; Chi et al., 2013; Cramer et al., 2013; Kim et al., 2013; Klainin-Yobas et al., 2012; Wang et al., 2013).

3.3. Cognitive performance

3.3.1. Cognitive control

Some studies show that ContActs enhance *executive functioning* and *working memory* or act as a buffer against age-related decline of executive functions and working memory: this applies to mindfulness meditation (Gard et al., 2013; Zeidan et al., 2010), yoga (Gothe & McAuley, 2015; Luu & Hall, 2016), and TCC (Wayne et al., 2014; Wu et al., 2013; Zheng et al., 2015). Most of the reported evidence for ContActs boosting executive functioning comes from cognitive inhibition tasks, such as the Stroop and flanker tasks, whereas the support for working memory improvement comes from span and n-back tasks. Furthermore, cognitive control states can be acutely affected through very short ContAct interventions (Colzato et al., 2012, 2015a, b; Gothe et al., 2013), although these effects are typically smaller and less robust than following prolonged ContAct practice.

3.3.2. Attentional control

ContActs also seem to have specific effects on *attentional control* and have been reported for FA and OM (Colzato et al., 2015b; Shapiro et al., 2003; Van Vugt & Slagter, 2014), mindfulness meditation (Chiesa & Serretti, 2010; Eberth & Sedlmeier, 2012), and yoga (Gothe & McAuley, 2015), with most studies reporting enhancement effects on attentional network task components and the attentional blink. These effects are differentiable according to specific practices and can be in opposite directions (Hommel & Colzato, 2017; Slagter et al., 2011). For example, practices high in FA are associated with better sustained attention, and those emphasizing OM support flexibility in allocation of attentional resources (Colzato et al., 2015b; Lutz et al., 2009; Van Vugt & Slagter, 2013). Jha and colleagues (2007) used the attentional network test (Fan et al., 2005) to compare FA with OM in terms of attentional subcomponents as distinguished by Posner and Petersen (1990). Jha et al. showed that FA has its effects on the alerting component (detecting a stimulus) and OM on the orienting component (allocating attentional resources). Surprisingly, they found no differences on

performance monitoring. Andreu et al. (2017), however, did find acutely enhanced performance monitoring by meditation. In people diagnosed with attention deficit hyperactivity disorder, meditation and mind-body exercises actually enhanced attentional functioning (Herbert & Esparham, 2017; Mitchell et al., 2015; Rubia, 2009), although an earlier Cochrane review was unable to draw conclusions due to a lack of clinical trials (Krisanaprakornkit et al., 2010).

3.3.3. Global cognition & creativity

Attention and control are not the only psychological outcome variables of ContAct research. Shapiro et al. (2003) observed enhanced creativity through transcendental meditation, while the meta-analysis of Ospina et al. (2007) indicated increased verbal creativity as the most reliable cognitive outcome of diverse ContActs. However, these acute effects can be in different and even opposing directions, as shown by a study on convergent and divergent thinking that used short FA and OM interventions (Colzato et al., 2012). There is also evidence that *global cognitive functioning*, as measured by the mini-mental state examination or activities of daily living questionnaire is positively influenced by ContAct in aging populations with mild cognitive impairment or dementia. Studied ContActs include mindfulness meditation (Eberth & Sedlmeier, 2012) and yoga and TCC (Wu et al., 2013), as evidenced by a Cochrane review (Forbes et al., 2013).

4. Effective factors in ContActs

As a first step towards explaining the highly overlapping effects of diverse ContActs on physical, mental and cognitive health, a straightforward approach is to analyze what they have in common. Three of the common factors we distinguish were also proposed by Hölzel et al. (2011a) for mindfulness mediation, but we formulate them in terms of activities rather than goals and propose three additional factors: *attention training*, *affect training*, *metacognitive adjustment*, *body awareness training*, *physical exercise* and the central addition: *breathing techniques*. The first three can be seen as forms of mental training and the last three as more embodied cognitive exercises. We will cover the first five factors and other proposed models before we introduce the breathing exercises .

4.1. Attention training

The focus of attention in ContAct practices can involve many sensory or cognitive modalities; any of the external senses, the body, the breath, thoughts or feelings. Although explicit attentional training might be absent from some mind-body exercises, many ContActs are aimed at sustaining attention, handling distraction and refocusing or shifting and spreading of attention. These attentional techniques can explain the frequently reported effects on attention and perhaps some in the cognitive control domain. However, as stated earlier, these effects can be differentiated and in opposite directions according to specific instructions, showing increased sustained attention and decreased attentional flexibility by one manipulation (FA), and showing opposed functional differences by another (OM). Attention training in ContAct might thus be better described as resulting in a shift towards either more or less (attentional) control than as a unidirectional change. Both directions of change can be adaptive to the practitioner's intention because such metacontrol shifts result in either more persistence or more flexibility in thought (Hommel, 2015; Hommel & Wiers, 2017). A limitation to the effective strength of attention training is that transfer of effects to other contexts and untrained skills is known to be rather limited (Green & Bavelier, 2012; Keshavan et al., 2014; Seitz & Watanabe, 2005; Simons et al. 2016).

4.2. Affect training

Exercises we define as affect training are aimed at removing or transforming negative emotions or moods. These start with becoming aware and paying attention to negative feelings or thoughts. This is comparable to *exposure* therapy (Hölzel et al., 2011a). Subsequent instructions serve to modify the mental state. By *decentering* the meditator attempts to distance the self from the (negative) thought or feeling, trying to observe it as just a fleeting and subjective sensation, instead of a feeling that is taken personal, in effect trying to detach the observer from the observed (Bernstein et al., 2015). Associated with decentering is the attempt to treat thoughts and feelings as not necessarily representing an objective reality. This is known as *dereification*. Finally, the detachment that results from decentering and dereification helps the meditator to avoid judgment about invasive and recurring thoughts, feelings or external events, such as surrounding noise. This *non-judgmentality* is also explicitly instructed. Taking these three mental exercises together, it is easy to maintain that they can help to *reappraise* negative feelings (Hölzel et al., 2011a). Therefore, affect training, as comprising both exposure and reappraisal, could explain findings in the area of mental

health, and possibly by extension, through (chronic) stress reduction: immune function and cardiovascular health.

4.3. Metacognitive adjustment

Both the decentering and dereification techniques belong to the domain of meta-awareness and metacognition: being aware of awareness, thinking about thinking (Flavell, 1976). By metacognitive adjustment practitioners try to change the way they process information. Many thoughts and perceptions follow a default processing route, resulting in a default interpretation and categorization of what is perceived. ContActs that involve *thought monitoring* try to identify and deconstruct fixed thought pattern, thereby deviating from this default processing route. This effect may transfer to daily life in the form of a tolerance for ambiguity and an increase in mental flexibility. As such, some forms of ContAct can be seen as executive function training, possibly transferring to situations where overruling pre-potent responses, ignoring irrelevant information, switching between tasks and rules, or keeping working memory up to date is relevant. Therefore, metacognitive strategies can explain beneficial effects of ContAct in the cognitive control domain. However, even apparently very similar cognitive training or gaming paradigms show very little transfer of training attentional control or working memory (Green & Bavalier, 2012; Melby-Lervag & Hulme, 2012).

4.4. Body awareness training

Exercises instructing for attention to different parts of the body, mostly to the skin and muscles, but also the viscera, make up the *body awareness training* factor. These could be mistaken as being part of the attention training factor. However, body perception is also uniquely central in affective processing and the sense of self (Araujo, et al., 2013; Damasio, 2003; Ochsner et al., 2004). Some researchers even theorize that body awareness is central in the cultivation of empathy (Grossman, 2014). This makes us treat body awareness as a factor on its own. Kerr and colleagues (2008; 2011; 2013; 2016) have shown that interoception can be enhanced in practitioners of mindfulness meditation and TCC: tactile acuity goes up, and activity in related somatosensory and visceral cortical areas (S1, insula, anterior cingulate cortex (ACC)) shows a pattern of increased attention to specific body parts on instruction and filtering of irrelevant somatosensory information. They state that metacognition and cognitive enhancement starts in the body: somatosensory exercises are in their view early versions of

the techniques involved in attention, meta-awareness and metacognition. This being the case, body awareness could be involved in producing effects on emotional and cognitive levels.

4.5. Physical exercise

One could argue that *physical exercise* is the most likely candidate for broad enhancement. Many studies provide evidence that physical exercise of different kinds (aerobic, endurance, strength) is a strong cognitive control enhancer, resulting in better cognitive and response inhibition, and lower dual-task costs, though reviews on working memory performance are mixed (Berryman et al., 2014; Colcombe & Kramer, 2003; Roig et al., 2013; Smith et al., 2011; Voelcker-Rehage & Niemann, 2013; Voss et al., 2013; Wong et al., 2015). Exercise is also generally accepted as a cardiovascular health booster (Francescomarino et al., 2009; Heran et al., 2011; Korsager Larsen & Matchkov, 2016). However, the evidence for the supposed therapeutic effect of physical exercise on depression, anxiety and other stress-related conditions has been sparse, as indicated by a Cochrane review (Mead et al., 2009), despite high expectations and invested resources (Salmon, 2001). Most importantly, only a small minority of the reported ContActs provide any aerobic or endurance exercise quality: the mind-body exercises, and perhaps to a smaller degree the mindfulness-based clinical programs (i.e. mindfulness-based stress reduction). This seems to rule it out as the prime candidate.

4.6. Theories and models of ContAct efficacy

There is a large gap between the amount of research done on ContAct and the number and amount of detail of models proposed to explain the benefits (Schmalzl et al., 2015). (Neuro)cognitive models that have been put forward so far attribute the benefits of meditation to top-down factors such as *attention* and *metacognition*. For example, Vago and Silbersweig (2012) emphasized the role of the self in the effectiveness of mindfulness meditation, whereas Sperduti, Martinelli and Piolino (2012) highlighted the role of executive functions in all branches of meditation.

Models that describe the benefits of mind-body exercises (Clark et al., 2015; Gard et al., 2014; Wayne & Kaptchuk, 2008) incorporate *movement*, *mindfulness* and *attention*. One refers to TCC as “meditative movement”, clearly naming the two aspects of physical and mental training (Larkey et al., 2009). However, despite labelling movement as a functional

component, none of these models handle physical exercise as a full factor on its own. In the component ‘movement’, exercise is reduced to motor coordination and skill learning, or the training of physical strength. Even though TCC is classified as mildly aerobic (Chang et al., 2010), the benefits of its aerobic aspect are neglected. This is peculiar in light of the extensive support for the contribution of aerobic exercise to physical health, mental health and cognitive performance.

4.7. Breathing techniques

Two of the mind-body models also incorporate a factor that has been conspicuously absent from other models: *breathing techniques* (Gard et al., 2014; Wayne & Kaptchuk, 2008). In both of these accounts, one on yoga and the other on TCC, the breathing type described as effective is slow, deep and diaphragmatic.

4.7.1. Effects of respiration in theory and research

The *breathing techniques* used in ContAct include, but are not restricted to, slowing down respiration cycles, shifting to longer exhalations compared to inhalations, shifting the main locus of respiration from the thorax to the abdomen (diaphragmatic breathing), or paying attention to ‘natural’ breathing. Especially slow and deep breathing with emphasis on long exhalation is dominant across traditions, including zen and vipassana - though there are a few practices stimulating faster respiration patterns (i.e. the yoga technique “breath of fire”). In the physically active mind-body exercises respiration can be synchronized with movement techniques; moving with the breath. For example, in some TCC styles moving towards the body is performed on inhale and moving outward on exhale. Note that in yoga, qi gong and TCC moving is performed slowly, and thus so is the breathing cycle.

Although the word breathing is frequently mentioned in the scientific literature on ContAct, this is almost exclusively done in a purely descriptive and not an explanatory fashion. Indeed, research on breathing as a ContAct mechanism is sparse, though there are concrete physiological grounds to look at breathing as an effective factor (Brown & Gerbarg, 2005). As far as we know there are only a few papers looking at respiration in the context of ContActs directly, varying wildly in aims and measures. Danucalov and colleagues (2008) found increases in metabolism and oxygen uptake during yogic breathing exercises (*pranayamas*) in experts, as compared to rest and meditation conditions in a within-subjects

design. The breathing exercises used included holding the breath and extending exhalation. A similar slow breathing pranayama was used by Pramanik and colleagues (2009) showing reduced blood pressure and heart rate at post-measure. Brown and Gerbarg (2009) reviewed their own studies on the psychophysiological effects of various breathing techniques used in Sudarshan Kriya Yoga and reported a general tendency among the breathing exercises towards relaxation: activating the parasympathetic nervous system (PNS) and deactivating the sympathetic nervous system (SNS). Cysarz and Büssing (2005) observed increased cardiorespiratory synchronization with a slow breathing zen meditation intervention in naïve subjects. Though there is clearly a lack of studies on breathing in ContAct practice, fundamental neurophysiological studies on respiration mechanics and styles do abound.

In several studies respiration types have been manipulated in an attempt to influence autonomic nervous system functioning. A study of the effect of diaphragmatic relative to normal breathing on metabolism among male cyclists, before and after a meal found reductions in heart rate and glycemia, and increases in insulin (Martarelli et al., 2011). Bernardi and colleagues (2001) induced hypoxia in participants and found that slow breathing exercises not only increased blood oxygenation, but also down-regulated SNS activity. Similar results are reported by Critchley and colleagues' (2015) study on hypoxia. Many other studies show that slow and diaphragmatic breathing increases PNS activity, as measured by blood pressure, heart rate or heart rate variability (HRV) (Hirsch & Bishop 1981; Lee et al., 2003; Lehrer & Gevirtz, 2014; Mortola et al., 2015; Pal et al., 2004; Perciavalle et al., 2017; Tavares et al., 2017; Van Diest et al., 2014; for some conflicting results see Conrad et al., 2007 and Montgomery, 1994). In sum, experimental slowing of respiration seems to shift the balance between SNS and PNS activity towards the latter. Next, in light of these findings, we will look at the part of the nervous system responsible for such a shift: the vagus nerve and measures of its tone (i.e. HRV).

5. The vagus nerve and heart rate variability

5.1. Vagus nerve

The autonomic nervous system (Langley, 1903) is a dual-system divided in the SNS and PNS with mutual inhibitory connections, though the dual innervation can also work complementary in organs such as the heart (Jänig, 1983; McCorry, 2007). The SNS is responsible for the fight/flight mode of organisms. It raises heart rate, blood pressure and

indirectly respiration rate. It dampens currently irrelevant homeostatic processes, but stimulates immediate availability of energy. The PNS acts as an opposing force. It is the rest/digest system of the organism. It lowers heart rate, respiration rate and increases digestion. The vagus nerve is the main effector and effector of the PNS.

The VN is a cranial nerve complex with widespread afferents and efferents on glands and visceral organs (Berthoud & Neuhuber, 2000), consists of approximately 20% efferent and 80% afferent fibers (Agostoni et al., 1957) and has many independently operating functions (Chang et al., 2015). Overall it is well-suited for relaying relaxation from the central nervous system to the body and checking the arousal and homeostatic state of the viscera. VN activity is modulated by respiration. It is suppressed during inhalation and facilitated during exhalation and slow respiration cycles (Chang et al., 2015). Efferent and afferent VN functions overlap with the functional effects associated with ContAct practice. Therefore, the breathing exercise component of ContAct is a prime candidate mechanism behind the beneficial effects found on mental and physical health.

5.1.1. Cardiopulmonary control

Efferent VN fibers innervate the heart and the lungs. The pulmonary efferents regulate airway size and thus volume, they lower respiration rate and indirectly endocrine secretion (Yuan & Silberstein, 2016a). Exhalation is under direct control of VN (Chang et al., 2015), whereas VN activity is attenuated during inhalation (Eckberg & Eckberg, 1982; Canning, 2006). The vagal cardiac outputs to the sinoatrial node causes slowing of heart rate, whereas SNS innervation is responsible for heart rate increase. The SNS cardiac effector is under tonic inhibition of VN, indicating indirect control on heart rate increase (Olshansky et al., 2008).

5.1.2. Anti-Inflammation

There is evidence that VN also influences physical health by suppressing inflammation. An anti-inflammatory reflex, known as the cholinergic anti-inflammatory pathway has been put forward from findings in animal studies on rats (Pavlov & Tracey, 2015; Tracey 2002, 2007). This response is thought to inhibit a cascade of inflammatory activity and is triggered by vagal afferents monitoring immune status. However, an alternative sympathetic anti-inflammatory reflex has been proposed, explaining conflicting results in rat studies: the splanchnic anti-inflammatory pathway, where VN plays an afferent role at most

(Bratton et al., 2012; Martelli et al., 2014a, b, 2016). A complete discussion of these competing pathways lies outside the scope of this review. Suffice it to say that the VN seems to be involved in anti-inflammation in humans: studies using VN stimulation paradigms report anti-inflammatory effects as well (Browning et al., 2017; Johnson & Wilson, 2018). Furthermore, after vagotomy inflammatory activity goes up. This resembles the earlier mentioned tonic inhibition on heart rate (Borovikova et al., 2000). This merits the proposition that VN mediates effects of ContAct on immunological health, specifically anti-inflammatory, and potentially those on auto-immune diseases.

5.1.3. System State Monitoring

VN afferents reach the medulla from the heart, airways, liver and gastrointestinal track. It monitors cardiorespiratory, endocrinal and immune parameters (Berthoud & Neuhuber, 2000). Mechanoreceptors in the airways signal on airway size and thus on respiration cycle and style (Canning, 2006; Udem & Carr, 2001). VN afferents on the adrenal glands relay information on the release of stress hormones, such as epinephrine and the glucocorticoids (Coupland et al., 1989; Kessler et al., 2012; Nijima, 1992). The afferent branch of the VN constantly send up homeostatic parameters to the central nervous system (CNS), monitoring the state of the visceral system. This branch has been characterized as the “great wandering protector” (Andrews & Lawes, 1992).

Clearly, these functions all move the system towards the rest/digest mode of operation and away from fight-or-flight. Not only does VN control heart rate and slow deep breathing, slow respiration rates with extended exhalation could also activate the PNS by VN afferent function in the airways. This is a form of respiratory biofeedback. Slow breathing techniques with long exhalation will signal a state of relaxation by VN, resulting in more VN activity and further relaxation. Though VN involvement can explain effects on health and mental health, the link with cognition is less clear. One of the links between respiration and cognition is HRV.

5.2. HRV

5.2.1. Vagal Tone

HRV, the fluctuations in beat-to-beat intervals of the heart, has been related to VN and some measures are believed to reflect “vagal tone”. As only the VN cardiac output and not the

sympathetic innervation would be able to produce rapid changes in heart rate. HRV is used as an indicator for individual physical conditioning, general health, reactivity to and recovery from high stress levels. Higher HRV is related to lower stress levels, better health and disease outcomes (Thayer et al., 2012). A frequently used HRV metric to assess vagal tone is the respiratory sinus arrhythmia, by some maintained to be the best reflection of vagal tone (Porges, 2001, 2007). This refers to the acceleration of heart rate during inhalation and deceleration during exhalation.

HRV can either be obtained in the time or the frequency domain (Task Force, 1996; for a recent review of HRV methods see: Laborde et al., 2017). High frequency HRV (HF), also referred to as respiratory sinus arrhythmia, is seen as a measure of vagal tone, whereas low frequency HRV (LF) is thought to represent sympathetic activity. The ratio between the two (LF/HF) represents autonomic balance, where a smaller number indicates vagal dominance. However, studies have shown that vagal activity is also reflected in LF and furthermore that LF does not reflect the SNS (Martelli et al., 2014c), making the ratio unusable as an indicator of autonomic balance. Currently, most studies confirm that specific measures in the time domain (e.g. root mean square of successive differences, peak-valley method) best reflect vagal tone (Penttillä et al., 2001), though some studies state that HRV, notably respiratory sinus arrhythmia, is not a reliable indicator of vagal tone at all (Grossman & Taylor, 2007). Individual HRV varies widely through time and during various activities, such as physical exercise (Hottenrott et al., 2006). Three types of measurements in time can be defined: resting or baseline HRV, reactivity HRV and recovery HRV (Laborde et al., 2017). Resting HRV is obtained with the participant sitting down, not performing any specific activity and can be seen as an individual's baseline level. Reactivity HRV is obtained during an activity or intervention, such as a cognitive task or a breathing exercise. This short-term HRV tends to drop during a cognitive challenge (Wood et al., 2002). Recovery HRV refers to the return to baseline afterwards. In this paper, when we mention HRV we refer to resting state HRV measures that best reflect vagal tone (HF and aforementioned time-domain measures), unless stated otherwise. Changes in these measures thus reflect changes in tonic vagal tone. An exception consists of most of the reported studies on respiration patterns: in this case the HRV concerns reactive HRV and in some cases recovery, and thus phasic changes in vagal tone.

As stated earlier, HRV is regarded as an indicator of physical, but also cognitive health. Indeed, there is a concrete link between HRV and cognition, first sketched in the neurovisceral integration model of Thayer and colleagues (Thayer & Lane, 2000).

5.2.2 HRV and cognition

The neurovisceral integration model (Thayer & Lane, 2000; Thayer, 2007; Thayer et al., 2009) posits bi-directional cortical influences on autonomic functioning and integrates CNS and autonomic functioning. It builds on the work of Benarroch (1993, 1997) on the Central Autonomic Network (CAN), a network of brain areas for goal-directed behavior involved in modulating the viscera. These areas are mostly limbic and include the insula, ACC, amygdala and hippocampus. The neurovisceral integration model extends this to prefrontal structures (orbitofrontal, medial and lateral PFC). These regions are able to influence HRV and initiate endocrine responses through the VN. But the integration of CNS and autonomic nervous system also works bottom-up: projections from VN afferent medular termini reach limbic and cortical regions, affecting cognitive control. This framework provides a basis for a connection between executive functions on the one hand, and body relaxation on the other. Indeed, studies by Thayer and others show evidence of a positive association between HRV and PFC activity and subsequent improvements in executive functions, notably cognitive inhibition. PFC seems to exert tonic inhibition on heart rate (and the amygdala), and greater activity of the PFC is associated with higher HRV (Lane et al., 2001, 2009). Hansen et al. (2003, 2004) provided further evidence of this relation in individual differences studies: higher HRV is associated with better executive functions and working memory performance. In the view of Thayer and colleagues, HRV can be seen as a peripheral index of the adaptability of the nervous system and thus the organism. HRV increases with goal-directed behavior and emotion regulation, and reduced HRV is indicative of cognitive stress. Clearly, the CAN and these experimental findings give grounds for explaining executive function enhancement following ContActs as originating from VN, through upward projections producing functional and structural changes in the executive network.

In a recent update of the neurovisceral integration model (Smith et al., 2017), which adopts a hierarchical network architecture, the relative weight of top-down and bottom-up influences can be adjusted. This leaves room for learning or the training of autonomic responses. For example, a non-adaptive dysfunctional stress response can be modulated or go

extinct by the reappraisal of threat (top-down) or exposure to the stressor (bottom-up). This means that stress levels could be downregulated by lower level state feedback that is associated with unthreatening situations. In our account these are the pulmonary parameters: low respiration rate and long exhalation.

5.3. Vagal nerve stimulation

The many functions of VN have led researchers and clinicians to develop electrical or behavioral intervention techniques for VN stimulation (VNS). These techniques are promising for clinical application and for improving cognitive performance. At the same time, the pattern of results observed following VNS mirror those obtained by ContAct, making VN involvement likely, and thus breathing exercises a promising candidate for stimulation.

5.3.1. Electrical VNS

Electrical VNS (Groves & Brown, 2005; Henry, 2002; Yuan & Silberstein, 2016b) was originally used to treat epilepsy. However, because it also increased the mood of stimulated patients it found its way as an approved therapy for depression (Johnson & Wilson, 2018), especially treatment resistant depression (Müller et al., 2018). It is also used to treat cardiovascular disease (Das, 2011; Johnson & Wilson, 2018) and as mentioned earlier, VNS has also shown acute anti-inflammatory effects (Browning et al., 2017; Johnson & Wilson, 2018), possibly through the anti-inflammatory pathway (Borovikova et al., 2000).

VNS is also applied to respiratory conditions. A study on guinea pigs has shown that strength of stimulation makes a difference: high voltage produces the predicted VN effects bronchoconstriction, reduced heart rate and blood pressure, while low voltage only produces the pulmonary effects (Hoffmann et al., 2012). Studies in humans, in contrast, show that VNS can actually produce airway relaxation in asthma patients during acute episodes, as indicated by an increase in forced expiratory volume (Miner et al., 2012; Steyn et al., 2013). In other words: stimulating afferent branches of VN during exacerbations (shortness of breath) produces longer exhalations and therefore slowing of respiration rate.

VNS has been shown to affect cognitive functioning, for example memory consolidation and recognition (Clark et al., 1999; Ghacibeh et al., 2006; Vonck et al., 2014). Effects found on mood and memory can be interpreted through the vagal projections into the central autonomous network. It is also supposed that by vagal projections to the locus

ceruleus, norepinephrine levels are influenced in midbrain and forebrain structures. This proposition is paradoxical as norepinephrine increase is more associated with sympathetic than with parasympathetic activity, and indeed evidence for norepinephrine release by VNS is mixed (McIntyre et al., 2012; Guglietti et al., 2013). We propose that VNS actually increases PNS activity and that norepinephrine projections play a minor role, as shown by recent neuroimaging studies (Frangos et al., 2015). Clearly, VNS not only shows effects on well-documented afferent and efferent functions of VN, but also fits with the neurovisceral and CAN account of cortical VN projections.

5.3.2. Transcutaneous VNS

Transcutaneous vagal nerve stimulation (tVNS) is a new non-invasive tool that is used to electrically excite the auricular or cervical branches (afferent) through electrodes placed on the ear or neck. Though this line of research is in its infancy, preliminary results also show an association between tVNS and VN-related afferent functions and projections. Shiozawa and colleagues (2014) concluded from a review of neuropsychiatric studies that tVNS can reduce symptoms of depression. Furthermore, a recent study has shown that (cervical) tVNS indeed shifts autonomic balance from the SNS to the PNS in tinnitus patients, as indicated by the increase of multiple vagal tone HRV measures (Ylikoski et al., 2017). Neuroimaging studies have also shown that cortical and sub-cortical regions identified in CAN are activated during both cervical and auricular tVNS (Dietrich et al., 2008; Frangos et al., 2015).

Few studies have been conducted using tVNS to influence cognitive behavioral performance. However, two studies have shown phasic changes in associative memory (Jacobs et al., 2015) and in response selection (Steenbergen et al., 2015) following tVNS. Interestingly, tVNS also causes effects that would be expected if VN efferent function would be modulated, by increases in vagal tone. Multiple studies and reviews show an increase of PNS activity, and some also show a decrease of SNS activity (Clancy et al., 2014; Murray et al., 2016; Popov et al., 2013; Zhou et al., 2016). Furthermore, tVNS is also associated with anti-inflammatory effects (Wang et al., 2014; 2015). These results overlap strongly with those obtained in ContAct studies.

5.3.3. Behavioral VNS

There are also behavioral forms of VNS (*vagal maneuvers*), which are supposed to stimulate VN bilaterally. The Valsalva technique; pinching the nose closed and then trying to exhale through the nose, is best known for returning normal pressure to the inner-ear cavities when changing altitudes (Arnold, 1999). It is initiated by flexing the abdominal muscles and extending exhalation (in clinical or laboratory setting by blowing into a balloon), showing a strong similarity with breathing techniques in ContAct. Even further, extending, slowing and holding respiration are all considered vagal maneuvers on their own, stimulating the VN. All of these vagal maneuvers have been shown to slow heart rate (bradycardia). We propose that the breathing exercises of ContAct might be seen as a form of behavioral VNS.

Overviewing the functions and applications of VN, one can see its potential as a mediator between respiratory patterns employed in ContActs and the reported effects on health, mental health and cognition. This will be further outlined in our model.

6. The respiratory vagal stimulation model of contemplative activity

The model, as depicted in *Figure 2a-d*, has a number of assumptions, inductions and predictions. These can be roughly divided into: a) ContAct breathing, b) respiratory stimulation and c) long-term effects. This will be followed by a definition of terms and measures.

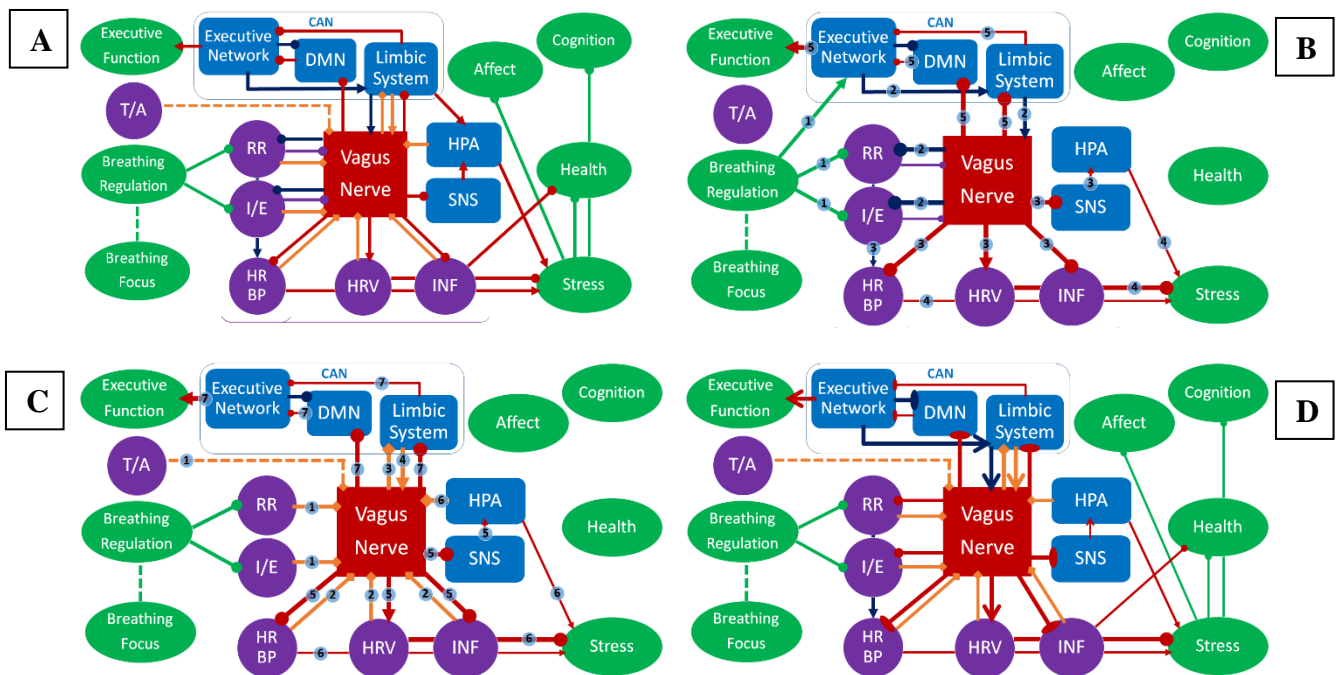


Figure 2. Figure 2a represents an overview of the rVNS model of ContAct. See the text body for more details. There are two pathways through which respiration style stimulates VN: direct and indirect (biofeedback through afferent projections), shown in *Figure 2b* and *2c* respectively. *Figure 2d* the tonic changes in the networks and the long-term effects. Color coding: red = VN, blue = other anatomy, purple = physiology, green = function, dark blue = direct route, orange = indirect route. Arrow-ends represent role: triangle = activating or increasing, circle = deactivating or decreasing, bladed = structural increase, ellipsis = structural decrease, diamond = afferent. Numbers on lines represent the temporal sequence during stimulation and the thickness of lines the phasic relative synaptic weight of the connection as a result. The dashed line represents the hypothetical afferent pathway of the thoracic/abdominal ratio to VN. RR = respiration rate, I/E = inhalation/exhalation ratio, T/A = thoracic/abdominal respiration ratio, HR = heart rate, HRV = heart rate variability, INF = inflammation state, SNS = sympathetic nervous system, HPA = hypothalamic pituitary adrenal axis, CAN = central autonomous network, DMN = default mode network.

6.1. ContAct breathing

ContActs are multi-modal interventions that can incorporate many different techniques and instructions. However, one of the most prominent and common ContAct techniques is respiratory regulation, in other words: breathing exercises. These breathing exercises have in common the instructions to focus on and slow down respiration, and/or extend exhalation. In *Figure 2* this is represented by the node “breathing regulation” inhibiting the nodes for respiration rate and inhalation/exhalation ratio, in other words: these exercises lower respiration rate and ratio. However, even exercises where the breath is just an attentional focus will lead to non-volitional adjustments of respiration. Practitioners just being aware of their breath enter slower and deeper respiration cycles. This can be caused by individuals’ previous experience with slow and deep breathing techniques, whose respiratory patterns will be automatically superimposed on current respiration. Another way is through the commonly slow pace of guided meditation instructions themselves: practitioners will sync their respiration to this rhythm. As focusing supposedly also leads to respiratory adjustments, similar to the breathing exercises, this is fit in the model overview (*Figure 2a*) by the node “breathing focus” showing a dashed line towards the node “breathing regulation”. Frequently adopting these respiration patterns (slowed and with longer exhalations) can explain a significant part of the efficacy found within ContAct practice. Though the ContActs are diverse, they have shown a similar pattern of beneficial effects on health, mental health and cognition: mostly in stress-related conditions and performance. This pattern can be explained by these controlled breathing exercises.

6.2. Respiratory stimulation

The main mediator of controlled breathing exercises on the described health, mental health and cognitive effects is VN. We posit that specific respiration patterns serve as

respiratory VNS (rVNS). The styles of respiration providing rVNS are controlled breathing techniques that slow and deepen respiration and extend expiration (Garcia III et al., 2013), and possibly those that put emphasis on relatively stronger diaphragmatic breathing. Note that rVNS is bilateral stimulation by nature, as opposed to unilateral electrical stimulation of VNS and tVNS. In *Figure 2* this is represented by the nodes respiration ratio and inhalation/exhalation ratio inhibiting VN. As such, rVNS is one of the main mechanisms of ContAct efficacy. rVNS can have two routes: direct and indirect. *Figure 2b* represents the direct stimulation and *2c* the indirect pathway of rVNS, which is temporally ordered by connection numbering.

6.2.1. Direct route

As can be seen in the dark blue path in *Figure 2b*, adopting a low respiration rate and small inhalation/exhalation ratio can directly stimulate VN, top-down from the executive network, by its own efferents (connections 2). This phasic increase in vagal activity increases reactive HRV, lowers heart rate and blood pressure (also by cardiorespiratory coupling), inhibits the SNS and indirectly the hypothalamic pituitary adrenal axis, and potentially activates an anti-inflammatory pathway (connections 3), resulting in a decrease of acute stress levels (connections 4). Critically, we also posit that VN activation statically increases cognitive control through CAN projections (connections 5).

6.2.1. Indirect route

Indirectly, afferent VN pathways constantly signal respiratory patterns upwards, in this case characterizing a state of relaxation and low-threat. As a result, efferent VN activation further increases vagal tone and produces associated physiological consequences (e.g. lowering heart rate, blood pressure, increasing HRV); a loop of relaxation ensues. In *Figure 2c* this route is represented by orange diamond arrows (afferent) that signal on respiratory patterns, possibly including the ratio of thoracic and abdominal expansion (connections 1), and the cardiac patterns influenced by the direct route (connections 2). This relaxed body state goes up from VN to the limbic system (connections 3), that in turn re-activates VN (connections 4), increasing its excitatory weight on cardiac and inflammatory patterns of physiological acute stress (connections 5 & 6) and statically enhances cognitive control (connections 7). The indirect route can be seen as a form of biofeedback and is responsible for

long-term changes in vagal tone. In this the respiratory patterns play a key role: a recent study using electroneurogram to map respiratory pattern signaling of the left VN, showed a near perfect overlap between this mapping and actual respiratory cycles (Sevcencu et al., 2018). Note that the left afferent VN is the locus of (t)VNS.

Diaphragmatic breathing might provide rVNS independent of respiration rate and inhalation/exhalation ratio, represented by the dashed inhibitory path of the thoracic-abdominal ratio to VN in *Figure 2c*. When oxygen demand is high during exercise or stress, SNS becomes active, and thoracic respiration goes up, and abdominal muscles are actively inhibited (Secher & Amann, 2011). When oxygen demand is low, in times of rest and digest, the vagal dominant state, the ratio shifts more towards abdominal respiration. The abdominal-thoracic respiration ratio of the rest-and-digest mode of the PNS should thus be similar to the ratio during ContAct practice. rVNS produces a wide range of effects in health, mental health and cognitive flexibility of the practicing individual, in the short as well as the long-term.

6.3. Long-term effects of respiratory stimulation

6.3.1. Stress reduction and anti-inflammation

Though rVNS produces a phasic change in PNS activity during and right after practice, in the long term it also results in a tonic shift in autonomic balance, shown in *Figure 2d*. As PNS activity goes up, SNS activity goes down. This shift is known as vagal dominance. In vagal dominance chronic stress and stress-related conditions are attenuated. Relaxation or rest and digest behavior increases. VN is responsible for the physiological effects of the red arrows in *Figure 2d*: heart rate, blood pressure and inflammatory response go down, whereas HRV goes up, which in turn also affects (chronic) stress. This works directly through tonal activity of the PNS, but also indirectly through inhibition of the SNS by VN. Specifically, reduction of the (chronic) stress response has positive effects on cardiovascular health and on stress-related psychopathology, shown by the stress node inhibiting the health and negative affect nodes, but also the general cognition node. Furthermore, vagal dominance also leads to better immune functioning and attenuation of inflammatory conditions. As can be seen in *Figure 2d*: VN inhibits the inflammation node, which inhibits the health node. These structural and tonic physiological changes in the networks are represented by bladed (activation) and elliptic arrows (inhibition) in *Figure 2d*.

6.3.2. Cognitive performance

rVNS increases vagal dominance in both resting state and in active states demanding behavioral and cognitive flexibility. The CAN (Benarroch, 1993) is a CNS network that receives its projections from VN and overlaps with the executive functioning network. The executive functioning network is not only dependent on autonomic balance for proper functioning, but can also be functionally and structurally changed by CAN activity. Enhancement of executive functions in ContAct practice results from rVNS of CAN, by structurally changing and activating the hubs of the executive functioning network and increasing their connectivity. In *Figure 2d* this is represented by the bladed red arrow path going up from VN to the limbic system to the executive network and then to the executive function node. Likewise, we hypothesize that default mode network (DMN, Raichle et al., 2001) hubs and internal functional connectivity are decreased, while DMN connectivity with the executive network is increased. The role of the DMN can be visualized as being inhibited by vagal projections and having a two-way inhibitory pathway with the executive network, as represented in *Figure 2*. These pathways are activated both phasically (*Figure 2b-c*) and tonically (*Figure 2d*).

6.4. Terms and Measures

1. Different forms of volitional control of respiration are defined as *controlled breathing techniques*. For this definition to be valid it should be possible for humans to put respiration under volitional control, overriding central pattern generated drive, and this has indeed proven possible for the diaphragm (Kolář et al., 2009).
2. *Vagal tone* is a construct relating to intra-individual tonal levels of PNS activity. Vagal tone can indirectly be indexed using HRV, notably respiratory sinus arrhythmia.
3. *Vagal dominance* refers to a relatively higher activity of PNS over SNS. Vagal dominance (PNS hyperactivity and SNS hypoactivity) should be observable in physiological measures of PNS (i.e.. HRV) and SNS (i.e. pre-ejection period, skin conductance, cortisol) activity. Vagal dominance can be increased both in acute and chronic time settings (Porges, 2001, 2007). However, in this work it is defined as a macro-state of autonomic balance, spanning minutes and hours, not a micro-state, changing millisecond to millisecond, for example: in heart node activation.

4. *HRV* is also a suitable inverse measure of acute and chronic (psychological) stress (Porges, 1992, 1995). *HRV* can be used as an indirect indicator of intra-individual and possibly inter-individual differences in executive functions (Thayer et al., 2009).
5. As *rVNS* is a form of *VNS*, results obtained from studies using other modes of *VNS* should resemble those from *ContAct* studies in similar conditions, although not necessarily with perfect overlap.
6. The *stress release* refers to stress responses over larger scales of time; not the acute adaptive arousal employed in challenging situations, but the perseverative and chronic kind; in other words: the default stress response (Brosschot, 2017).

6.6. rVNS: evidence and possible mechanisms

What evidence is there for respiration as a mode of *VNS*? In our model there are two ways respiration can stimulate *VN*: directly and indirectly. In the direct route, slow breathing and extended exhalation are caused by vagal activity. This follows from the previously mentioned role of *VN* in respiratory affective and effective processing (slowing and exhalation). Controlled breathing in this form thus uses the vagal nerve as effector and increases its activity volitionally, if only momentarily. The indirect route involves stimulation through biofeedback and follows from physiological feedback theory: by adopting physiological body patterns associated with relaxation and low threat situations (i.e. slow breathing) vagal afferents project this state to the *CNS*, which interprets this as a reflection of the current contextual threat level, and proceeds by further adopting a rest-and-digest state top-down, again through *VN*. The indirect route is responsible for more long-term tonic changes of vagal tone. By either route, breathing styles with low respiration rate and low inhalation/exhalation ratio should show increases in vagal tone, though in slightly different timeframes (Keyl, 2001).

6.6.1. Evidence

Most experimental studies show higher *HRV* following breathing instructions, consistent with the involvement of *rVNS*. In particular, there is ample evidence that slow and deep breathing increase *HRV* indices of vagal tone (Critchley et al., 2015; Hirsch & Bishop 1981; Larsen et al., 2010; Lehrer & Gevirtz, 2014; Mortola et al., 2015; Pal et al., 2004 ; Tavares et al., 2017) and lowers stress markers such as: heart rate, blood pressure and salivary

cortisol (Lee et al., 2003; Perciavalle et al., 2017; Pramanik et al., 2009). Van Diest et al. (2014) looked specifically at the effects of different inhalation/exhalation ratio at either slow or normal respiration rate on different HRV measures (peak-valley, HF): higher HRV (both measures) was reported in the slow respiration condition, but only for extended exhalation, inhalation/exhalation ratio: 0.24, and not for extended inhalation, inhalation/exhalation ratio: 2.33. Though normal ratios were not included, this study most clearly shows the stimulating effects of the specific respiration styles on VN. For another example of extended exhalation, albeit with a completely different aim and context: a study on native American flute playing showed significant increases in HRV during playing, contrary to what one would expect during exerting activity (Miller & Goss, 2014). It needs no mention that playing any piping instrument involves extreme extended exhalation.

As far as we know, few studies report a decrease in HRV after controlled breathing, and these are primarily found outside the domain of ContActs. Sasaki and Maruyama (2014) gave instructions to participants to ‘control breathing’, without emphasizing a particular style (rate or ratio), and compared that to spontaneous breathing. This resulted in lower HRV, which may be the result of an increase in mental effort, stress, and thus SNS activity. Indeed, earlier reports also show a decrease of HRV when breathing is only “controlled” (Bernardi et al., 2000) as compared to directed in a specific direction. Note here the apparent contradiction with our own view that paying attention to the breath would result in lower respiration rate and possibly smaller inhalation/exhalation ratios: instructing to “control” versus “focus on” seems to have opposing results on autonomic balance.

As we are reviewing breathing techniques that are practiced in ContActs, studies that look into autonomic functioning through ContActs employing these kinds of techniques should report increased vagal tone. Indeed, HRV increases in almost all forms of ContAct, consistent with the rVNS hypothesis. Different forms of meditation (e.g. body scan, FA, OM acem, zen) and mind-body exercises such as yoga, all show increases in vagal tone HRV in healthy participants (Ditto et al., 2006; Markil et al., 2012; Melville et al., 2012; Nesvold et al., 2012; Phongsuphap et al., 2008; Tang et al., 2009; Telles et al., 2013; Wu & Lo, 2008). One exception is a study that involved the earlier mentioned ‘breath of fire’ (Peng et al., 2004) that showed a decrease in HF, LF and LF/HF ratio. This is not surprising and consistent with our biofeedback perspective, as breath of fire is strictly speaking controlled hyperventilation and would thus rather result in SNS activation and PNS inhibition. Though ContActs by great majority do not employ this particular rare technique, this nonetheless

stresses the importance of mapping actual practiced techniques in every ContAct to their outcomes. From these abundant, though correlational, findings on respiration and vagal tone we conclude that a form of rVNS plays a role in ContAct efficacy. However, less clear is what the exact physiological mechanisms of stimulation might be.

6.6.2. Possible mechanisms

The first possible physiological mechanism for these respiratory patterns to stimulate VN (as biofeedback) is by way of the baroreceptor reflex (Lehrer et al., 2003; Vaschillo et al., 2002). This reflex is responsible for regulating blood pressure and is triggered by stretch-activated mechanoreceptors (baroreceptors) in blood vessels, which leads to activation of the vagal branch of the heart node, that reduces heart rate and subsequently blood pressure. The threshold for triggering this reflex (cardiovagal baroreflex sensitivity) can be lowered by a respiration rate around 0.1 Hz or about 6 breaths per minute. Interestingly, this is exactly the same respiration rate that is reported in respiration studies as having the highest increase of HRV. Lowering the sensitivity results in more frequent reflexes, lower heart rate, and increased vagal tone (Song & Lehrer, 2003; Lehrer & Gevirtz, 2014; Lin et al., 2012; Wang et al., 2012, though see Tzeng et al., 2009 for an exception). This mechanism is a faster indirect route between respiration rate and heart rate, as mediated by VN, than the biofeedback route through VN afferent subcortical projections signaling broad relaxation.

The second possible feedback mechanism is even more direct and comes from the lungs themselves: the pulmonary mechanoreceptors. These VN afferents directly relay tidal volume upstream and are responsible for initiating particular physiological responses, notably the Hering-Breuer reflex (Breuer, 1868; Hering, 1868). The reflex is triggered by significant lung volume increase (e.g. during inhalation) and inhibits the central inflation drive, resulting in extended exhalation and slower respiration. In this way, when a practitioner starts a breathing exercise with a deep breath (a long inhalation), this immediately triggers the reflex, resulting both in activation of VN as well as the initiation of respiration styles that further relay relaxation. Furthermore, the dominant and supported view is that the mechanoreceptors, together with central pattern generated drive, are also responsible for respiratory sinus arrhythmia (Eckberg, 2003; Mortola et al., 2015; Taha et al., 1995).

The slowest of the indirect routes: biofeedback, where low respiration rate and small inhalation/exhalation ratio signal a resting state to the CNS is consistent with the James-Lange physiological feedback hypothesis of emotion and similar accounts (Critchley & Garfinkel,

2015; Levenson, 1994). The theory, independently proposed by William James and Carl Lange, maintains that the identification and experience of an emotion follows from peripheral physiological responses (e.g. arousal), instead of the other way around. The kind of emotion experienced depends on the interpretation of the physiological state and the appraisal of the context in which it is triggered. So, the physiological stress response precedes the subjective emotional experience of fear or sadness. Following this argument, bottom-up changes to dysfunctional emotional states can be produced by changing the physiological state of the body; in other words: relaxing the body relaxes the mind.

In sum, there is evidence that particular breathing exercises (with low respiration rate, small inhalation/exhalation ratio) are capable of stimulating the vagal nerve (rVNS), though the exact mechanisms of stimulation are proposed, not proven (i.e. baroreflex). The next question is how prolonged increase in vagal tone results in the beneficial effects found on health and mental health. Vagal dominance is contingent on consistent physiological relaxation. It therefore produces (chronic) stress release, and thereby prevents or ameliorates stress-related pathology and etiology.

6.7. Relaxation versus stress: health and mental health outcomes

Although SNS and PNS can be simultaneously active in a particular domain, they mostly operate as opposing forces (Berntson & Cacioppo, 1999; Freeman, 2006). SNS activity goes together with PNS inactivity and vice versa. Therefore, PNS hyperactivity (as indicated by HRV) also reflects SNS hypoactivity: vagal dominance. Plainly stated: relaxation means absence of stress. If ContActs work through relaxation by respiratory stimulation of the PNS, then stress should go down. This explains the observation that syndromes relieved after ContAct practice are often those associated with stress and SNS dominance.

The role of (chronic) stressors on the development of cardiovascular disease, through the cardiovascular response (Obrist, 1981) of the SNS causing atherosclerosis and hypertension, is well documented (Allen & Patterson, 1995; Rozanski et al., 1999; Thayer et al., 2010). That stress influences immune function is also well-known. Initially and acutely, stress suppresses immune function, but chronically it exacerbates immune response (Sapolsky et al., 2000; Haroon et al., 2012; Ménard et al., 2017). Additionally, stress seems to worsen auto-immune disease (Elenkov & Chrousos, 2002). Furthermore, there are indications that the two systems are related in their morbidity by SNS-PNS imbalance: recovery of both cardiovascular and immunological markers is impaired after stressors, when baseline vagal

tone is low (Weber et al., 2010). Also noteworthy is the existence of an inverse relationship between HRV and both inflammation and the risk of cardiovascular disease (Haensel et al., 2008). Bringing these findings together, HRV seems suitable as a multi-index of health: of physiological stress (Porges, 1995), as a measure of cardiovascular risk (Thayer et al., 2010) and of immunomodulation (Thayer & Sternberg, 2010).

In the mental health domain, mood disorders such as depression are widely recognized as being stress-related. They are often accompanied or triggered by acute or chronic life event stressors in the prodromal phase (Duman & Monteggia, 2006; Gold & Chrousos, 2002; Orosz et al., 2017). Depression has also shown a relation with the other stress-related diseases: there is a link of depression with occurrence of cardiovascular disease (Hare et al., 2014) and with the likelihood of having an overreacting immune system (Miller et al., 2009; Dantzer et al., 2008; Felger & Lotrich, 2013). All in all, these systems and their pathologies seem to be interrelated, wherein the common denominator is autonomic balance.

A healthy autonomic balance is vagally dominated and comes about by stress relief produced by PNS activation and SNS deactivation. If this is the way the aforementioned pathologies are positively affected, then there should be a clear negative relationship between vagal tone and the risk factors and symptoms of these conditions. Indeed, HRV shows a negative correlation with cardiovascular disease in children and adults (Oliveira et al., 2017; Tully et al., 2013) and even directly predicts hypertension (Schroeder et al., 2003). It has an inverse relationship with inflammation (Kemp & Quintana, 2013; Lampert et al., 2008), inflammation in depression (Carney et al., 2007), depressive symptoms in children and adults (Koenig et al., 2016; Sgoifo et al., 2015), perseverative cognition (Ottaviani et al., 2016), bipolar disorder symptomology (Faurholt-Jepsen et al., 2017), general anxiety and disorders (Chalmers et al., 2014; Cohen & Benjamin, 2006; Tully et al., 2013) and has recently even shown a negative correlation with schizophrenia (Clamor et al., 2016). Though schizophrenia is not considered a stress-related disorder, the role of HRV in schizophrenia is intriguing considering the interplay of dysfunctional emotional regulation and executive functions in its symptomology.

Stress has a negative association with executive or PFC function. Chronic stress has a degenerative effect on PFC structure and functioning (Arnsten, 2009, 2015; McEwen & Morrison, 2013) and seems to adversely affect its plasticity (McEwen et al., 2012). A study by Zhang and colleagues (2014), that builds on the correlational work of Nagai and others (2004, see Critchley & Garfinkel, 2015, for a review) shows a causal involvement of ventromedial

PFC in physiological arousal: when ventromedial PFC activity goes up, electrodermal activity (skin conductance level) goes down. In other words: prefrontal structures suppress stress. A conclusive review by the international behavioral neuroscience meeting (Radley et al., 2015) stated the negative effects of stress on the plasticity of the limbic network (amygdala, hippocampus and PFC) and its pivotal role in the etiology of aforementioned (mental) health conditions.

In sum, (chronic) stress is a significant negative mediator in all of the domains that benefit from ContAct. It is here proposed that these beneficial effects occur by (chronic) stress relief as a result of vagal dominance by rVNS. In other words: breathing exercises produce stress relief (Lee et al., 2003; Perciavalle et al., 2017; Pramanik et al., 2009). We have also already indicated that chronic and high levels of stress are negatively related to executive functions and PFC functioning. Next, we will show that there is also a positive relation between vagal tone, CAN areas and the executive functioning network, as predicted by the neurovisceral integration model, and between changes in CAN and ContAct practice as predicted by the rVNS model of ContAct.

6.8. CAN: regulated emotion & enhanced cognition

The link from VN to PFC (Ter Horst & Postema, 1997; Wager et al., 2009a, 2009b) is a critical element of the CAN in mediating rVNS effects of ContAct onto executive functions. Likewise, projections into limbic parts of the CAN allow ContAct to enhance positive affect via rVNS. If these projections are actually used, vagal tone should have a positive correlation i) with executive functions or PFC activation and ii) with emotional control or medial PFC activation. See Thayer and Lane (2000) for the CAN network as adapted in the original neurovisceral integration model.

6.8.1 HRV, cognitive control & PFC

There indeed is an association between HRV and executive functions, as first shown by Thayer and colleagues (2009), especially in emotional control: HRV predicts inhibition of attention to emotional stimuli (Park et al., 2012, 2013), it shows a positive relation to attentional control and negative relation to risk aversion in anxiety (Ramírez et al., 2015), predicts attentional lapses (Williams et al., 2016), and it is involved in cognitive inhibition, proactive cognitive control (Capuana et al., 2012, 2014) and emotional inhibition of

conditioned fear (Wendt et al., 2015). A recent meta-analysis also supports a relationship between HRV and cognitive control: executive functioning, pooled across the subdivisions of Miyake et al. (2000), showed a positive average association with HRV level, though the authors note a strong publication bias (Zahn et al., 2016). The effects can especially be observed in cognitively demanding settings. A brain imaging study shows that functional connectivity of the amygdala and medial PFC are associated with higher HRV in both younger and older people (Sakaki et al., 2016).

The link between HRV and the PFC seems to be very direct: they share a common genetic background (Thayer et al., 2009) and HRV and executive functions show a similar ontogenetic developmental trajectory; going up until early adulthood and going down again with advancing age (Umetani et al., 1998; Zelazo et al., 2004). This is expected if VN and PFC form a single system: CAN. Another clue to the involvement of VN in executive functions comes from the work of Van der Molen (2000) into the development of inhibitory control. During successful cognitive inhibition of action representations, heart rate deceleration can be observed, after which heart rate goes up again (Schel et al., 2013). As we have seen, heart rate slowing is under direct control of VN, indicating vagal dominance during employment of cognitive control.

6.8.2. Changes in CAN regions through ContAct

As we hypothesize that in ContAct rVNS is responsible for the emotional and cognitive enhancement by changes in CAN, studies looking at functional and structural changes in the brain in practitioners should show these changes along this whole network - in the limbic system and executive functioning network; in the levels of the updated neurovisceral integration model (see Smith et al., 2017). Studies on ContAct practice have shown this for the limbic part of CAN. For example, a decrease in volume and activity has been observed in the amygdala (Hölzel et al., 2010; Tang et al., 2015) and in the hippocampus (Luders et al., 2009; Luders et al., 2012a) in practitioners of different styles of meditation (both FA and OM). Insular cortex and posterior cingulate also increase in activity and volume in the same populations (Hölzel et al., 2011a, 2011b; Kirk et al., 2011; Lazar et al., 2005; Luders et al., 2012b; Tang et al., 2015). The practice of yoga shows the same pattern (Desai et al., 2015; Froeliger et al., 2012), while a TCC study shows the most significant morphological changes in the insula and dorsolateral PFC (Wei et al., 2013).

The ACC is a limbic structure, but is also part of executive functioning network and active in cognitive control, and is central in CAN. Notably its dorsal part has been implicated in autonomic control, as it modulates cardiovascular stress responses (Critchley et al., 2003). Following the previous argument, ACC should also be implicated in imaging studies of ContAct efficacy, and indeed, functional and structural enhancement in ACC has been reported in meditation styles and in mind-body exercises (Cahn & Polich, 2006; Hölzel et al., 2011a; Tang et al., 2009; 2010; 2013; Wei et al., 2013; Xue et al., 2011). The frontal end stations of CAN also show predicted structural changes: PFC gray matter density is increased by diverse meditation styles and mind-body exercises (Desai et al., 2015; Froeliger et al., 2012; Hölzel et al., 2011b; Lazar et al., 2005; Luders et al., 2009; 2012c; 2015; Tang et al., 2013; Wei et al., 2013; Yin et al., 2014). In sum, there is a large overlap between the brain regions changed by ContAct – amygdala, hippocampus, insula, ACC and multiple areas of the PFC – and those identified in CAN. Note, however, that these areas have been implicated in behavioral studies with very diverse tasks and contexts – not only in ContAct.

6.8.3. Default mode and executive network plasticity by VNS

In general, brain connectivity seems to increase by ContAct practice across multiple projections, commissures and associative networks, as shown by several diffusion tensor imaging studies (Luders et al., 2011; 2012b). Meditation practice (and trait mindfulness) is associated with greater connectivity between the executive, DMN and salience networks specifically (Brewer et al., 2011; Doll et al., 2015, Hasenkamp & Barsalou, 2012). Notably the DMN is implicated in neuroimaging studies among ContAct practitioners. DMN is active when external stimulation and work demand is low. Its main hubs are medial PFC, posterior cingulate cortex and parahippocampal region (Raichle et al., 2001); the last of which is believed to operate as the hub between DMN and limbic areas (Ward et al., 2013). The role of DMN in cognitive control can be seen as opposing that of the executive network; lapses of attentional control (i.e. mind wandering) are contingent on DMN activity over executive network activity (Gratton et al., 2018), in this way DMN can be viewed as a “task-negative” network (Fox et al., 2005). Studies on changes in connectivity by ContAct experience show deactivation of DMN hubs (i.e. posterior cingulate and medial PFC) and decreased functional connectivity between these hubs. At the same time the functional connectivity between the DMN and executive networks goes up (Brewer et al., 2011; Hasenkamp & Barsalou, 2012).

This mirrors what is consistently found in imaging studies that apply VNS to individuals with (treatment-resistant) depression.

Depression is associated with a disrupted DMN, particularly: hyperactivity and hyper-connectivity among DMN hubs, as well as hyper-connectivity between DMN and limbic system, and hypo-connectivity between DMN and executive network (Drevets et al., 2008; Gong & He, 2014; Kaiser et al., 2015). Clinical trials employing chronic VNS in patients with depression show a normalization of this etiology, obtaining results very similar to ContAct practice. One study on patients with depression not responding to regular treatment showed increased metabolism in the DMN hub ventromedial PFC (Pardo et al., 2008). While a similar study reported decreased activity (regional cerebral blood flow) in another DMN hub (posterior cingulate) and in the limbic system (insula), concurrently increasing activity in dorsolateral PFC of the executive network (Kosel et al., 2011). Another imaging study (on epilepsy), reports a decrease in regional cerebral blood flow in the DMN hub parahippocampus, as well as in the hippocampus and the thalamus by chronic VNS (Van Laere et al., 2002).

The few tVNS studies so far show a similar pattern as those obtained with VNS. One study of major depression reports that tVNS decreases the resting-state functional connectivity between main DMN hubs and parahippocampus - the DMN hub that connects to the limbic system - and anterior insula (Fang et al., 2016). Contrarily, it increases the resting-state functional connectivity of DMN with the precuneus and orbitofrontal cortex (executive network). In addition, all these connectomic changes were associated with reductions in depression severity. A fMRI study in a normal population shows that tVNS can acutely reduce activity in DMN hubs: parahippocampal and posterior cingulate (Kraus et al., 2013). There are also indications that tVNS produces changes in the executive network itself. Badran and colleagues (2018) are the first to show increases in metabolic activity between the main hub axis of dorsolateral PFC and ACC by tVNS. Another tVNS study produces changes between the executive network and limbic system, by decreasing functional connectivity between rostral ACC and medial hypothalamus in depression, all associated with clinical improvement (Tu et al., 2018). Somewhat paradoxically, a study on patients diagnosed with major depressive disorder showed a decrease in symptoms due to tVNS, but combined with an increase in resting-state functional connectivity between the amygdala and dorsolateral PFC, so between the limbic and executive systems (Liu et al., 2016).

Concluding: it has clearly been shown that activity in afferent branches of VN can affect areas and networks in the central nervous system, both acutely (e.g. by tVNS) and chronically (e.g. by chronic VNS). This notably affects the DMN, which is a critical CAN level in the latest version of the neurovisceral integration model (see Smith et al., 2017 for details). Central changes as a result of ContAct practice within DMN and between DMN and executive network are practically identical to those observed by (t)VNS studies. This makes vagal involvement and thus the mechanism of rVNS highly likely in producing these neurobiological effects and the concomitant improvements in cognition and affect. Concretely stated: DMN deactivation and increased DMN-executive network connectivity is caused by rVNS and will lead to improvements in cognitive control (e.g. cognitive inhibition) and performance monitoring.

7. Discussion

We have shown that ContAct practices, though diverse, have a number of components in common that can explain their efficacy in individual physical health, mental health, and cognition. Furthermore, one of these components: breathing techniques, is a prime candidate to explain the complete pattern of results, notably in the stress-related domain. We have further provided a neurophysiological model in which slow respiration and extended exhalation stimulate VN via two possible routes: rVNS. This results in PNS over SNS dominance, structural and functional changes in higher cortical areas through autonomic projections, and is thus responsible for aforementioned effects.

In these claims, one of the main arguments for the rVNS model of ContAct concerns the dovetailing between specific functions of VN with the pattern of effectivity shown by diverse ContActs; providing beneficial effects on cardiopulmonary fitness, immune function, psychological health, stress, anxiety and executive functions. The neurophysiological link between the two can be found in vagal tone: the existent relationship between the aforementioned functions and conditions with HRV and that of HRV with the VN. Evidently HRV is then an index of adaptability in a broad sense.

We realize that there might be more common factors involved in ContAct interventions than we have covered and categorized here. There also might be unique components to particular traditions, as well as emergent properties of specific combinations of components; almost all ContAct interventions are multi-modal. For example, many of the

covered traditions are not practiced in isolation, but in group sessions. Social, and even physical, contact could be a factor in relieving stress and in alleviating depression. Though we do not discount the other factors covered and those possibly left out, we believe that respiration style and vagal functioning fit the evidence best, and following Occam's razor, it stands out as the most parsimonious of explanations. However, also rVNS might benefit from a specific combination. For instance, combining rVNS with affect training: exposure and reappraisal procedures could be strengthened by the concurrent body relaxation brought on by rVNS, the biofeedback would weaken the stress response and negative emotion brought up by an aversive or traumatic memory.

Following the observation on the multi-modality of ContAct, we maintain that many null-findings and conflicting results in the literature could be ascribed to the presence or absence of particular effective components. For example, a yoga class only focusing on stretching and shifting positions might not have any executive functions benefits other than those stemming from some form of relaxation, but does show changes associated with mild exercise. Studies performing systematic analyses that compare functional ContAct elements, based on concrete predictions, are therefore sorely needed. Reported findings that show controlled breathing increasing SNS activity further underline the importance of making clear what kind of techniques are employed. This includes reporting on the exact instructions given and controlling for compliance to these instructions. Our predictions are only valid as far as interventions result in slow, deep (diaphragmatic) breathing - not in other breathing styles, such as fast and deep breathing during physical exercise. Thus, the described beneficial effects on health and cognition are predicted to occur more in ContActs with breathing exercises stressing relatively short inhale (SNS controlled) and long exhale (PNS controlled), than in ContActs that do not emphasize this distinction.

Some ContAct practitioners might proclaim that their particular tradition (notably FA) does not involve any breathing exercises. That the exercises only instruct to pay attention to the breath, and not to modulate respiration in any way; that instructions to change anything in breathing patterns are absent. They might also state that the breath is only one of many foci. For example: it could be a visual focus, such as a flickering candle flame or verbal, as in a mantra. But the fact is, that across these diverse FA traditions it usually is not another kind of focus, it usually is the breath, and we maintain that this is not arbitrary. As previously stated, we maintain that it is unlikely that focusing on the breath does not affect respiratory patterns. In our view, directed conscious awareness to breathing will slow respiration in expert and

layman alike, through direct and indirect experience with different breathing exercises and the implicit or explicit idea, an ideomotor representation if you will, what it should be like to meditate: meditating involves relaxed breathing. Furthermore, the rhythm of auditory instructions is in a slower pace than normal breathing, thereby slowing respiration as well. Of course, these are assumptions that should be tested in further experiments. But, if attention does slow respiration, this makes all these traditions fall under the explanatory umbrella of the rVNS model of ContAct.

So far the picture painted from rVNS has been optimistic. However, there might be circumstances and doses where no beneficial effects can be expected. For example: in chronically stressed individuals vagal activation might have such a high threshold that rVNS will have no noticeable effect; they might prove resistant to the intervention. rVNS might even have adverse effects, such as overstimulation. In a condition known as vasovagal syncope, vagal efferents reduce heart rate to such a degree that blood pressure drops to dangerous levels, resulting in fainting and symptoms of chronic fatigue. As indicated by VNS studies showing bronchoconstriction, stimulation might be dangerous for pulmonary pathology, such as chronic asthma. However, studies varying VNS voltage suggest that strength of stimulation could make the difference between beneficial or detrimental (Hoffmann et al., 2012). Respiratory VNS might be as beneficial and healthy as the individual baselines (e.g. autonomic balance) allow. By extension, these dangers could be present for ContAct practices as well. But as far as we know, there are no studies on adverse effects produced by ContActs. This does not deny their existence as the absence could be a result of publication bias.

On a similar note: higher vagal tone HRV is not always better. Much like arousal levels optimal levels of HRV for physical and mental functioning might follow an inverted U-shape, as in the Yerkes-Dodson law (Yerkes & Dodson, 1908). Individuals with a resting HRV at the right side of their personal curve might actually present adverse effects if HRV levels are further increased, autonomic balance shifting too far away from the sympathetic. In contrast, individuals with HRV levels falling at the left end of that curve might benefit the most from increasing HRV (by rVNS), being “parasympathetically compromised”. Also, there might be differences between populations in the shape of this curve and there might be different curves for different VN functions. For example, as first reported by Wang and colleagues (2005), but see Hill et al. (2015) for a review, African Americans’ resting HRV is on average higher than that of Caucasian Americans, while the prevalence of cardiovascular

disease is higher in African than in Caucasian Americans. This is counterintuitive if HRV is seen as a pure measure of vagal tone. At the same time, the relationship with anxiety and depression does follow the predicted direction: African Americans suffer less from these conditions than Caucasian Americans (Breslau et al., 2006). Further research has to address the question whether there is an inverse relationship between HRV and cardiovascular disease in this population, whether relatively higher HRV for this population is not optimal HRV regarding cardiopulmonary function, giving room for further enhancement, or perhaps whether this is a HRV methodological artefact.

In light of the necessity to report the specific instructions and exercises in every intervention study, mention has to be made of mindfulness meditation. When we and many other authors report on mindfulness meditation, this usually refers to two clinical programs: mindfulness-based stress reduction and mindfulness-based cognitive therapy. Both of these are multi-modal interventions, in which not only mindfulness meditation and other meditation techniques play a role, but also physical exercises (some form of yoga), mental strategies, and in mindfulness-based cognitive therapy: cognitive-behavioral therapy. Most studies that report on the effects of mindfulness or mindfulness meditation, that use these programs as interventions, should therefore strictly speaking not be taken as ‘pure’ mindfulness meditation; subsequently, caution is advised in interpreting result.

We want to note that HRV (respiratory sinus arrhythmia) as a valid measure of vagal tone, also maintained by Thayer and colleagues, is not without its critics (Grossman & Taylor, 2007). Grossman and colleagues have shown in their experiments that under different respiration conditions, respiratory sinus arrhythmia does not reflect changes in vagal tone accurately (Grossman & Kollai, 1993; Kollai & Mizsei, 1990; Taylor et al., 2001). They maintain that HRV measures should always be controlled for by respiration. Since these studies, there has been limited follow-up of these criticisms, as can be seen in a recent review of HRV methods (Laborde et al., 2017). We believe this issue should be addressed resolutely. More neuroscientific experiments directly assessing the relation between the different HRV measures and vagal tone are needed. As far as the implications for the rVNS model of ContAct: it includes assumptions made by the polyvagal and the neurovisceral integration accounts; and it loads evidence for the existence of rVNS on those assumptions. However, we maintain that HRV does not need to be a ‘pure’ measure of vagal tone for it to be useful as a measurement – a relational representation is enough. But even if respiration confounds on vagal tone are insurmountable, making HRV unusable as its measure, this will not affect the

core assumptions of our model and the predictions it makes. In the future, other measures of SNS/PNS activity and balance should be developed. In any case, the rVNS model of ContAct still provides testable and falsifiable predictions.

Though we are not able to definitively prove a causal link between breathing, VNS and improvements in body and mind, we believe we have provided ample evidence suggesting the existence of this link, largely by providing overlapping patterns of specific phenomena. Empirical studies need to put our hypotheses to the test. Furthermore, other concrete neurobiological mechanisms for the systems described in this work need to be proposed and charted by experimental studies, as prescribed for the field by Thayer and colleagues (2011). Studies on different respiration styles, using psychophysiological measures for PNS and SNS activity and tasks assessing executive functions acutely and longitudinally could provide concrete tests of our hypotheses. Imaging studies mapping structural and functional changes in the CAN following rVNS are critical for our hypotheses on cognitive functioning, especially: changes in DMN and executive network mirroring VNS results are expected. Other indirect tests include experiments comparing ContAct interventions with (t)VNS manipulations. As an established phenomenon, studies of rVNS dose-response relationships, with personal baseline levels, could follow. What is the “vagus code” of rVNS (Kwan et al., 2016)? What are observed differences caused by specific modes of stimulation? For instance, electrical VNS and behavioral rVNS differ also in laterality: unilateral versus bilateral stimulation, which might produce different effects (and strength of stimulation).

Lastly, as for the reasons why breathing techniques have gone pretty much unnoticed as a mechanism of ContAct efficacy, while being so prevalent and well-known, we propose that it perhaps has to do with this prevalence: it is such an unremarkable fact, so plainly observable and a starting point of practice. This could be coupled with a tendency to focus on ‘higher’ levels of consciousness among practitioners, and on higher level processes and structures in cognitive neuroscientific research. Relaxation might also be assumed and uninteresting, breathing exercises are automatically factored in, something for novices, hardly remembered by the expert. We hope that through this work future research on ContActs will recognize and study breathing techniques as an effective component, and that neuroscience will focus on rVNS respiratory patterns as potential cognitive and (mental) health enhancers.

Chapter 3

No Panacea?

Tai Chi enhances motoric but not executive functioning in a normal aging population

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Abstract

Many societies struggle with the immaterial and material costs of an increasingly large aging population. Therefore it is not surprising that lifestyle interventions that ameliorate individual decline in motor and cognitive functioning are actively sought. Tai Chi Chuan (TCC) is such a promising intervention, with a mix of different forms of physical exercise and meditative components. Though previous studies have shown benefits both in motoric and cognitive domains, how these effects are functionally related has hardly been studied. To try to untangle this relationship a randomized controlled trial was conducted in an aging population (55+), including two measures of motor functioning – on motor speed and functional balance – and three cognitive control measures – on shifting, updating and inhibition. The TCC condition consisted of an online 10 week 20 lessons video program of increasing level and control condition of educational videos of similar length and frequency. Counter to expectation no differences were found between TCC and control pre- to post-test on any of the cognitive measures. After weighing the evidence and the limitations of the TCC program we conclude that TCC indeed does not enhance cognitive control (in this timeframe). Furthermore, we suspect publication bias in the scientific field of this and similar traditions.

1. Introduction

Aging is affecting societies worldwide. The average life expectancy at birth has increased by 6.2 years from 1990 to 2013 (Murray et al, 2015): in the following decades it is expected that the world population of people aged 65 and over will have more than doubled (He, Goodkind & Kowal, 2016). One of the phenomena related to aging is individual functional decline, both in a physical and a cognitive sense, which has negative consequences both for the individual and society as a whole. Pathological aging conditions such as Alzheimer's disease are a large individual and societal burden. But even normal age-related cognitive decline and loss of mobility have far reaching consequences, for one on quality of life, which has received increasing attention (Hoang et al., 2020). In the current study, it is tested whether older adults performing a series of 20 Tai Chi Chuan exercise sessions improve their control of motor and cognition function relative to a control condition. Moreover, it is tested whether effects on motor and cognitive functions are functionally related.

1.1 Aging, cognition & motor function

For some cognitive functions, decline already starts around the age of thirty (Salthouse, 2009), such as cognitive control or executive functions (EF). EF, also known as the frontal functions by their association with the prefrontal cortex, is a broad construct that covers various cognitive functions to monitor and regulate thought, emotion and (automatic) behavior, including setting goals and the planning of their achievement. EF is not a unitary construct and is generally divided into three functional components: shifting, updating and inhibition (Miyake et al., 2000; Miyake & Friedman, 2012). Shifting refers to the switching between mental sets or task rules, updating to the monitoring and refreshing of working

memory representations according to contextual demands, and inhibition to inhibitory control of ambiguous and irrelevant information or pre-potent responses (cognitive inhibition) and prepared reactions (response inhibition).

Though EF is not an indivisible function, many accounts trying to explain age-related cognitive decline are common factor accounts. Salthouse (1996, 2000) has proposed that the decline of EF is caused by a general decline in processing speed. This account predicts that age-related cognitive decline affects all three EF components, though not necessarily to an equal extent. More recent common factor accounts provide neuroscientific frameworks. The vascular hypothesis (Spiro & Brady, 2011) holds decreases in blood flow across the brain responsible for the decline. Other accounts emphasize decreasing shared cognitive resources and compensatory activation by other brain areas (Cabeza, 2001; Davis et al., 2008; Li, Lindenberger & Sikström, 2001). In sum, various accounts explain age-related decline by general resource limitations (processing speed, perfusion and blood flow, recruitment of other brain areas) that affect not only cognitive, but also motor functions. As a result, shared resource accounts predict interdependence between cognitive and motoric performance. Reduced mobility is a common issue in aging populations (Tang & Woollacott, 1996) and with it comes an associated risk of severe falls: about one third of people aged 65 and older fall at some point, and in a third of the cases this results in mortality (Ambrose, Paul & Hausdorff, 2013). An important risk factor for loss of mobility and falling is dysfunctional balance and gait (Deandrea et al., 2010).

An important finding is that motoric and the cognitive decline indeed have a bi-directional relationship (Montero-Odasso et al., 2014, 2017). EF and attention play a critical role in the production of gait (Amboni, Barone & Hausdorff, 2013; Holtzer et al., 2006; Yogev-Seligmann, Hausdorff & Giladi, 2008) and vice versa: atypical gait seems to be a predictor of dementia or mild cognitive impairment (Hausdorff & Buchman, 2013).

Individuals at risk for dementia or suffering from mild cognitive impairment have a distinct motor signature (Montero-Odasso et al., 2014) and this differs for dementia subtypes (McArdle et al., 2017). This trade-off fits with shared resource accounts. In a review by Seidler et al. (2010) it is consistently shown that older people recruit EF more during motor tasks (e.g. walking tasks) than do young people. This is also reflected in larger recruitment of prefrontal cortex and basal ganglia, which can be interpreted as a form of compensatory activation. The functional decline might further be exacerbated as these are the brain structures most notably negatively affected by age-related neural decline (Heuninckx et al., 2005). The entanglement of EF and motor functioning in aging leads to an interesting conjecture: enhancing cognition might have the added benefit of improving mobility and thus reduce falls. But perhaps cognition itself might also be improved indirectly by training functional balance (Montero-Odasso et al., 2014). Any physical exercise intervention aiming at increased vitality would therefore be well advised to include functional balance.

1.2 Enhancement: buffering age-related decline

As it turns out, prospects for aging are indeed not all bleak: many lifestyle interventions provide a buffer against decline or even improve physical and cognitive functioning (see for a review Ballesteros et al., 2015). Tai chi chuan (TCC), a traditional Chinese contemplative practice, is such a promising practice (Larkey et al., 2009). It combines exercises in balance, slow, complex movement sequences, with breathing, concentration and relaxation techniques. TCC has low physical demands and is a light to moderate form of aerobic exercise (Taylor-Piliae & Froelicher, 2004). As TCC is usually accompanied by and highly similar to qi gong exercises, we will refer to them both under the common denominator of TCC (Larkey et al., 2009). As TCC has both a meditative aspect and a physical exercise component, it can be counted among the mind-body exercises. Both

physical exercise and meditation have shown to enhance EF and ameliorate age-related cognitive decline.

Many studies provide evidence that physical exercise of different kinds – aerobic, endurance/strength and motor coordination training – is a strong cognitive control enhancer (Berryman et al., 2014; Colcombe & Kramer, 2003; Roig et al., 2013; Smith et al., 2011; Tse, Wong & Lee, 2015; Voelcker-Rehage, Godde & Staudinger, 2010; Voss, Vivar, Kramer & van Praag, 2013). In a cross-sectional study by Voelcker-Rehage, Godde and Staudinger (2010) physical fitness was associated with EF and not surprisingly, so was motor fitness: in speed, balance, coordination and flexibility. This study further showed that only motor fitness was related to perceptual speed. Lam and colleagues (2018) show in their systematic review that physical exercise improves strength, balance and functional mobility in people suffering from mild cognitive impairment and dementia. The effects of aging on brain and cognition can be reduced or slowed down by aerobic exercise (Colcombe & Kramer, 2003; Kramer, Erickson, & Colcombe, 2006). A recent review shows that a mix of endurance training and aerobic exercise might provide the most benefit (Northey et al., 2018). Also just coordination training has shown similar effects on cognition in aging, across the three components, as has aerobic exercise (Voelcker-Rehage, Godde & Staudinger, 2011; Voelcker-Rehage & Niemann, 2013; Tsai et al., 2017). It might also be the case that different forms of exercise have specific effects on specific components, for example aerobic fitness relating to inhibitory control (Boucard et al., 2012). In a comparison between coordination and endurance training regimens Tsai and colleagues (2017) showed that both increased performance on a task switching task (shifting) and the n-back (updating), but shifting benefited more from coordination exercise and updating more from endurance exercise. TCC combines all three types of exercise: aerobic, endurance and coordination. Consistent enhancement of EF across components fits with common factor accounts of aging such as the vascular hypothesis (Spiro

& Brady, 2011). Indeed, exercise intervention studies have shown that exercise leads to increases in growth factor responsible for angiogenesis, neurogenesis and synaptic growth (Cotman, Berchtold & Christie, 2007; Vonderwalde & Kovacs-Litman, 2018).

Meditation, another feature of TCC, has been shown to act as a buffer against EF and working memory decline (Gard, Hölzel & Lazar, 2013; Zeidan, Johnson, Diamond, David & Goolkasian, 2010). Furthermore, meditation might even slow the frontal cortex atrophy in aging (Lazar et al., 2005) and increase grey matter density in other brain areas, notably the hippocampus, after meditation interventions (Chiesa & Serretti, 2010; Hölzel et al., 2011). A recent review on mindfulness meditation indicated that all three EF components have been reported to be enhanced by different studies, however most consistently the inhibition factor (Gallant et al., 2016). According to a recent model - the respiratory vagal nerve stimulation model (rVNS) - a way these practices are able to produce these effects is through system relaxation and stress relief (Gerritsen & Band, 2018). This could certainly apply to aging populations as the prefrontal cortex is less resilient to stress in aging individuals (Arnsten, 2015). Gerritsen and Band further state in their rVNS model that a single component in these different practices could account for most of these effects: breathing regulation. TCC, especially in qi gong, prescribes highly similar breathing exercises both in motion and in meditative stance, as discussed by these authors.

In conclusion: TCC practices, especially those including multiple forms of physical exercise (e.g. aerobic and endurance training), together with meditation and breathing techniques, will be expected to lead to enhanced cognition and to combat age-related decline. The types of exercise combined in TCC also seem ideally suited for enhancement; as it is a combination of open coordination exercises, strength and endurance training with a moderate aerobic aspect. Furthermore, there are indications that multi-modal interventions are better suited to protect or enhance EF than any single intervention, showing additive effects

(Burgener, Yang, Gilbert, & Marsh-Yant, 2009). Indeed, prior studies on TCC seem to confirm its potential as a cognitive enhancer. However, a Cochrane review of studies in aging populations with dementia (Forbes et al., 2015), could not find evidence for enhancement of cognitive functioning by various exercise programs including TCC, though activities of daily living did show improvement, and also noted that experimental designs overall were of poor quality. In normal (aging) populations TCC enhances EF and working memory or acts as a buffer against EF and working memory decline (Chang et al., 2014; Laird et al., 2018; Wu et al., 2013; Zheng et al., 2015) and this is reflected in their neural substrates (Hawkes et al., 2014). TCC even seems to have a larger effect on cognition than just physical exercise, as expected from its multi-modal design (Wayne et al., 2014). It is less clear whether EF components are equally affected, as a controlled study including all three factors is absent.

Perhaps less surprising: functional mobility is also enhanced by TCC practice. Several studies and reviews demonstrate the value of TCC for physical health (Jahnke et al., 2010; Lee & Ernst, 2012) and in increasing functional mobility (Rogers, Larkey & Keller, 2014; Sun et al., 2015; Voukelatos et al., 2007; Yeh et al., 2006). According to a meta-review by the Cochrane Collaboration, TCC reduces the risk of falling (Gillespie et al., 2012). Specifically, TCC has been shown to lead to greater muscle strength (Chen et al., 2012; Li, Xu, & Hong, 2009; Lu, Hui-Chan, & Tsang, 2013) and dynamic balance (Wong et al., 2001, 2011). Long term practitioners have better postural control than matched controls (Lu et al., 2013). This is also the case for clinical population such as Parkinson patients (Song et al., 2017). There are also indications that TCC practitioners have a generally higher motor speed (Tsang et al., 2013). These results might not be surprising given the physical exercise component in TCC and the specific exercises aimed at balance and strengthening the lower body. However, it is still unclear whether EF and mobility enhancement by TCC are functionally related, as we

would expect from shared resource accounts and findings of their bi-directional influence. Perhaps gait improvement comes by way of enhanced cognitive functioning.

1.3 Current study

A systematic comparison between EF components and motor functioning was performed within a randomized controlled design in an aging population. The aim was to 1) replicate findings on motor and cognitive enhancement by TCC 2) study whether potential positive effects on physical and cognitive functioning are related 3) fit these patterns with common factor or shared resource accounts of aging. This leads us to the following questions: does TCC practice enhance any of the EF subtypes: shifting, updating and inhibition, in aging populations? Does shifting, updating or (response) inhibition, as measured by the tasks-switching task (TST, switch costs), 2-back (sensitivity) or stop-signal task (SST, stop-signal reaction time), respectively, improve more from pre-test to post-test in a TCC than in a control condition? Does TCC enhance functional mobility, as measured by the Timed Up and Go test (TUG), or motor speed, as measured by the finger-tapping task (FTT)? If so, is EF a modulator of this effect, or vice versa?

Our expectation is that functional balance will be improved for the TCC group, as seen in a greater improvement in TUG-time. We also expect general motor speed will be enhanced, as seen in a greater increase in finger taps. This would fit with the common factor account of declined processing speed by Salthouse (1996), as the decline in processing speed should be reflected in general motor speed (Salthouse, 2000), which has found support in neuroscientific studies (see Eckert, 2011 for a review). From both common factor and shared resource accounts (Li & Lindenberger, 2000) we expect all three EF components to be enhanced. We expect both TUG and FTT scores to be improved significantly more in TCC than control.

Furthermore, we expect EF enhancement to be a moderator of improvement in TUG scores: individuals showing EF enhancement will improve more in motoric functions, as predicted from a compensatory perspective. In contrast we expect EF to be modulated by FTT scores, as motor speed indirectly measures processing speed, and thus the Salthouse common factor. Note that as long as the motoric effects are present these predictions also fit with a brain growth perspective of exercise efficacy. In the absence of any motoric effects - and thus perhaps physical challenge - our predictions for EF remain the same from the rVNS model, by way of stress relief through respiratory control. However, we do not control directly for any of these (additive) effects.

We will use a randomized controlled trial design to try and answer these questions. The active TCC intervention constitutes a 10 week 20 session online video program. The control condition is an online program of watching videos on health and contemplative practices of the same length, duration and frequency as the active condition. After each session participants fill in self-reports on compliance, difficulty, both physical and mental, and specific understanding of the specific practice. Pre- and post-measures are taken in the lab.

2. Materials and methods

2.1. Participants

Participants were recruited by flyers posted at locations frequented by the target population, such as community centers and libraries; through advertisements in local and regional media; and by e-mail to local organizations focused on elderly interests. Participants were required to be 55 and over, have normal or corrected-to-normal vision and no history of severe psychiatric or neurological disorders. The screening was done by e-mail, phone or face-to-face meeting. In total, 55 applicants were recruited and randomly assigned to either the intervention group (TCC) or the active control group (health education). Five participants dropped-out due to personal reasons, two because of an unrelated injury and one due to an unreported disability (in visual acuity). Three participants dropped out while reporting the intervention as cause: two in the TCC group, one of which was unable to follow the instructions and the other got agitated by the tone of instruction; the drop-out in the control condition reported a disbelief in its efficacy. The program was completed by 20 participants in the TCC group and 24 participants in the control group. Preliminary analysis led to the exclusion of one participant from the control group because of a low score (22 out of 30) on the MMSE, leaving 23 participants in the control group. See table 1 for descriptive statistics on both groups. This study has been approved by the ethics committee of the Leiden University institute of psychology. All participants gave informed consent prior to participation and were debriefed afterwards. Before enrollment they also signed a medical declaration confirming their general, neurological and cardiopulmonary health; and intact functional mobility. Participants were intrinsically motivated to participate and received no compensation for their participation. Similar expectations on health and cognitive benefits were induced in both groups by similar phrasing.

2.2. Procedure

2.2.1 Testing

After screening, participants were enrolled in the randomized controlled trial. Pre- and post-tests were performed in psychology labs. The interventions were followed at home and online. At pre-test participants were informed of the procedure and asked to sign the informed consent form and the medical declaration. Next, participants had to fill out a questionnaire inquiring about demographics, and current and previous experience with contemplative practice and frequency of practice (e.g. meditation, yoga). Furthermore, the International Physical Activity Questionnaire (IPAQ, Craig et al., 2003), the Mini-Mental State Examination (MMSE, Folstein, Folstein, & McHugh, 1975) and the State-Trait Anxiety Inventory (STAI, Laux et al., 1981) were presented. The MMSE was taken verbally and the rest of the questionnaires on the computer (Qualtrics). Next, a One Legged Standing Test with open eyes (OLST, Ekdahl, Jarnlo, & Andersson, 1989) and a Time-to-get-Up-and-Go test (TUG, Mathias, Bayak & Isaacs, 1986) were conducted to test functional balance. Lastly, the FTT and three cognitive tasks and were performed on the computer: the task-switching task, N-back, and stop-signal task. All cognitive tasks were presented on a computer screen, which was at 70 cm distance from the participant, in a quiet lab-space without distractions. At post-test appointment STAI-T, OLST, TUG and the cognitive tasks were performed again. However, by error of omission of one of the experimenters only half the sample retook the STAI-T and the OLST at both measurement points, resulting in insufficient statistical power for repeated measures comparison. After the post-test participants were debriefed: they were informed to which group they were assigned, and the complete goal of the study and expected results were explained. The pre-test lasted approximately 2.5 hours and the post-test approximately 1.75 hours.

2.2.2 Intervention

Both TCC and control group followed a home-based online video program over the course of 10 weeks, that consisted of 20 sessions of about 45 minutes each – so 2 sessions or 90 minutes per week. The TCC intervention was designed and guided on screen by a licensed instructor of the Dutch Academy for Taijiquan and Qigong. Videos were recorded at a martial arts training center in Oegstgeest, the Netherlands. The lessons consisted of beginner level TCC principles and techniques in adapted Yang style (Zheng Man Qing form) and Dao Yin Qi Gong techniques (Ma Wang Dui form), which acted as a recurring warm-up. Every session built on previous lessons and scaled in difficulty. At the end of the course practitioners were expected to know and be able to move independently through half of the Zheng Man Qing short form, which has 37 movements in total. The Qi Gong warm-up consists of exercises combining endurance training with stretching, muscle relaxation and breathing techniques - inhaling and exhaling synced with movement - all these aspects recurred in the TCC instructions as well. The edited videos were made available as Youtube videos with restricted access. The control group watched health educational videos on public online broadcast for the same amount of time in total per week (~1:30 hours). These were obtained from the open access Dutch public broadcast network on www.npo.nl. Per session there could be 1-2 different programs ranging from 30-60 minutes per session. The subjects of educational video's matched themes and aims of the TCC interventions, for example: interviews with health professionals on lifestyle and healthy aging or a documentary on the mental benefits of meditation. There was no particular order of programming and thus in scaling of sessions, but this was held constant for each participant in the control group.

Once per week, participants individually received an email reminding them of their participation and asked to either follow the next two lessons (TCC) or watch two educational

programs (control). They were instructed to follow these lessons a few days apart and were urged to watch and participate with their full attention, to the best of their abilities, without forcing anything. Participants in the TCC condition were expected to stand in front of their computer, in a quiet room and follow the instructions, imitating the movements of the trainer.

2.2.3 Session Questionnaires

At the end of each session participants were required to fill out online questionnaires. These questionnaires inquired about self-assessed performance and the main aim was to check for compliance and to assess the difficulty curve. Two questionnaires were designed: for TCC and for control. The questionnaires were identical after each session. For the TCC group this included questions about difficulty, attention and alertness, breathing, mindfulness/meditation, physical strain, balance and TCC principles. The questionnaire for the control group was designed to test general understanding and investment to a similar degree, albeit not oriented towards the specific television episode. These queried on the interest in the topic, its difficulty, attention and alertness, physical arousal and whether they learned something or were going to apply anything from the video into their own lives. Adherence to the video session was assumed when the questionnaire was filled in.

2.3. Measurements

2.3.1. Questionnaires

2.3.1.1. Demographics. An online Qualtrics questionnaire was designed to assess the demographics: sex, age and level of education. Education level was remapped to a scale from 1-6.

2.3.1.2 MMSE. The MMSE (Folstein, Folstein, & McHugh, 1975) is a questionnaire designed to measure clinical cognitive functioning. The MMSE was taken on paper; the continuous value (max. 30) was obtained and compared to the cut-off point to indicate mild cognitive impairment (<27, O’Caoimh, Timmons & Molloy, 2016) or dementia (<23, Kochhann et al., 2010). It was also used to compare pre-intervention differences in cognitive functioning between groups.

2.3.1.3 STAI-T. STAI-T (Spielberger et al., 1983) was administered to map baseline levels in trait anxiety and used to check differences between the intervention groups at pre-test. The STAI-T consists of 20 statements each of which can be likened to how participants generally feel on a 4-point Likert scale. These add up to a to a single trait anxiety score ranging from 4 to 80.

2.3.1.4 IPAQ. Current physical activity was assessed by the IPAQ questionnaire (Craig et al., 2003). The IPAQ short form contains questions about walking, moderate and vigorous activities, as well as questions addressing time spent sitting down. It allows for calculating a continuous score of metabolic equivalents of task minutes per week (MET-min), whereby participants can be divided into three main levels of physical activity: low, moderate, and high. To obtain MET-min, answers are weighed according to the intensity and duration of the activity: low intensity = 3.3 x minutes x days per week; average intensity = 4.0 x minutes x days per week; and vigorous intensity = 8.0 x minutes x days per week. The METs per week are added up and can be compared to cut-off points for three different categories of activity: low, average and high.

2.3.1.5 Contemplative activities. Current contemplative activity was assessed by an online questionnaire at the end of the demographic questionnaire (Qualtrics). Two categories were distinguished in the questionnaire: *meditation & mindfulness* and *mind-body exercises* (yoga, qi gong, TCC). Participants answered whether they currently practiced and if so they reported frequency of practice: *every day, 2-6 times a week, once a week, 1-2 times a month, 5-10 times a year, 1-4 times a year*. Since all provided answers were either: *never, 1-2 times a*

month, once a week or 2-6 times a week, the values 0-3 were attached to these answers in corresponding order. The sum of the two scores represents the current level of contemplative activity and ranges from 0-6. If participant reported that they were not currently practicing in either category, a question inquired about their previous practice using the same frequency scale as above. As none of the participants reported previous practice these were left out of analysis.

2.3.1.6 Session self-report. After each session a self-report questionnaire was presented in both groups. This online questionnaire, administered through Qualtrics, had a number of statements, 26 for TCC and 18 for control, about the previous session, with responses on a 5-point Likert-scale (“not at all” – “very much so”). These self-report scores provide insight into the degree of difficulty participants experienced while following the TCC or the control group program. The TCC group self-report questionnaire consisted of 26 questions that address topics that pertain to the previous session, like comprehension of instructions, relaxation, attention, breathing, physical capability, and pain. The control group self-report questionnaire consisted of 14 questions addressing topics like comprehension of the video, attention while watching, breathing and interest in the subject of the session. The two questionnaires contained questions specific to the condition and questions that were constant in both questionnaires, such as on relaxation and attention. Though the self-reports were mainly created to check and nudge compliance, these could also be used to extract scores on factors such as effort, attention and motivation; and to follow progression through the sessions. After unexpected null-results in the cognitive domain on all predicted EF components this was done in the TCC group as a manipulation check on the level of challenge and physical exertion. Five scores ranging from 1 to 5 were obtained. For challenge: ease and effort; for physical exertion: aerobic quality, heart rate and muscle ache. See Appendix A for questions and scoring.

2.3.2. Motor function tasks

2.3.2.1 One-legged standing test.

OLST was performed by participants in order to assess standing balance (Ekdahl, Jarnlo, & Andersson, 1989). However, erroneously only half the sample performed the OLST pre-test and post-test, where the other half did neither. Therefore, the OLST was left out of all analyses.

2.3.2.2 Timed up and go task.

The TUG (Mathias, Bayak & Isaacs, 1986) was used to assess gait speed and functional balance. A chair is placed facing a wall at a distance of three meters. The participant is seated in the chair and asked to walk to the wall without touching it, walk back to the chair and return to a seated position. The experimenter counts down from 3, at which point the participant should start. Time is measured by stopwatch. Two practice rounds and three test rounds were performed. In between rounds there is no instruction towards increased speed. The final score on the TUG is the mean score of the three test rounds.

2.3.2.3 Finger tapping task.

FTT (Reitan & Wolfson, 1985) was implemented in Inquisit and used to assess motor speed. Participants were asked to tap the spacebar on the keyboard with either their right or left index finger as fast as they could and as many times as they could within trials of 10s. The rest of the hand should remain immobile. Each participant received between 5 and 10 trials for the participant's dominant hand and 5 to 10 trials for the non-dominant hand. After the first five trials, if the scores of these rounds were not within 5 taps of each other, another trial was added until there were 5 trials with scores within this range. There was an upper limit of 10 trials in total. The mean of these 5 trials was the final score. In between trials there were

breaks of 10s or 60s after every 3 trials. During a practice trial the experimenter monitored correct procedure.

2.3.3. Cognitive measures

2.3.3.1. Task-switching task.

The shifting component (Miyake et al., 2000) was assessed by a task-switching task and implemented with Inquisit software. It measures an individual's ability to efficiently switch from one set of task rules to another, as quantified in the switch cost (Rogers & Monsell, 1995). Participants had to respond to a dyad comprised of a letter, number or symbol combination within a 2x2 white grid made of evenly divided squares, against a black background. The dyads were presented clockwise in one of the 4 squares. The participant was required to respond to only one stimulus of the dyad. The two stimuli of the dyad could either be a digit, letter or a symbol, but never two symbols (e.g. A7, #b, 2!). The task consisted of 3 different conditions: the letter, number and mixed condition; and set into four blocks of 120 trials each - 2 pure blocks (letter and digit) and 2 switch blocks. The order of block types was counter-balanced across subjects. In the pure blocks no switch had to be made between task rules: participants had to always respond to only one of the two stimuli, either the letter or the digit. In the digit condition, they were either required to respond by pressing "Z" with the left index finger on the keyboard if the presented digit was smaller than 5, and to respond by pressing "M" with the right index finger when the digit was greater than 5. In the letter condition, participants had to respond by pressing "Z" when the presented stimulus was a lowercase letter, and to respond with "M" when the stimulus was a capital letter, using either the left or right index finger respectively. This was not counterbalanced for the purpose of online distribution. In the two switch blocks the participants had to switch between these rules

(and thus the target stimulus), when the dyads alternated down or up. They were required to respond to the digit when stimuli were presented in the top two squares, and to the letter when presented in the bottom two squares. Trials could be either congruent (both the target and distractor stimulus signaling the same response), incongruent (both stimuli signaling different responses) or neutral (the distractor being a symbol that cues no response). Switch costs in accuracy and reaction time can be either global (between pure and switch blocks) or local (between repeat and switch trials within the switch blocks). Practice rounds were included for all blocks. Participants were asked to respond as accurately and fast as possible at the start of the task, after practice rounds and in between each block.

2.3.3.2. N-back.

To assess working memory performance and the updating component of cognitive control (Miyake et al., 2000) an n-back paradigm (Gevins & Cutillo, 1993) was employed (in Inquisit). In a single trial, a series of stimuli was presented on a computer screen and the participant was asked to press the spacebar on the keyboard when the stimulus shown on the screen at a given time was the same stimulus as the one 2 stimuli back ($n=2$). Each trial started with a 250ms delay, after which the stimulus was presented for 500ms with a fixed response window of 2500ms starting at stimulus onset, thereafter the trial finished with another 250ms delay before the next stimulus (i.e. inter-stimulus interval = 3000ms). There were 8 blocks of 40 trials, so a total of 320 test trials. Half of the blocks, 4 blocks of 40 trials each, consisted of a letter task, where the identity of the stimulus was to be remembered (A, E, G, M, U, X, Y,Z). The other half of the blocks concerned a location task, where the location of a blue square (6.8 cm x 6.8 cm) was to be remembered. Order of the tasks was randomized for each participant, but always stringed together (e.g. 4 position blocks followed by 4 letter blocks). In the letter task letters (4.2 cm height by 3.1 cm max. width on screen) were presented in sequence in the middle of the screen in a grey-lined square (7.5 cm x 7.5

cm). In the location task squares were shown in a 3x3 grey-lined matrix (each field 7.5 cm x 7.5 cm) where the middle field was used for a fixation cross only. All stimuli were presented in white against a black background. Blocks were stringed together according to task, the order of which was counterbalanced. For both forms of stimuli there were several practice rounds, where $n=1$ and $n=2$, with and without feedback on accuracy. Participants were asked to respond accurately, but also as fast as possible, and were given these instructions between blocks and after practice rounds. Feedback on accuracy and reaction time was provided between the test blocks, but not during. There was an opportunity for a short break in between test blocks. As an indicator of updating the sensitivity index of accuracy, d' was obtained by subtracting the false alarm rate from the hit rate. Though the task was originally designed to function as a dual n-back with higher levels of n and both tasks concurrently, this single 2-back was chosen because an early pilot showed that higher levels were too taxing and this 2-back was challenging enough for this age bracket.

2.3.3.3. Stop-signal task.

Response inhibition, which according to the horse-race model is the inhibitory force in a race between a stop and a go process (Band, van der Molen, & Logan, 2003; Logan & Cowan, 1984), was taken as a representation of the inhibition component of cognitive control. For this end, a stop-signal paradigm (Lappin & Eriksen, 1966) was implemented in E-prime to assess stop-signal reaction time (SSRT), a quantitative value of response inhibition performance. SSRT here represents an estimate of the time needed to suppress a go response (Verbruggen & Logan, 2008). Participants performed a choice RT task in 3 blocks of 35 trials each, where participants responded to a go stimulus, either an “X” or an “O”, presented in black over a white background on the computer screen, by pressing the corresponding button, either “C” or “N”, on the keyboard (counterbalanced across subjects). In 25% of the trials the target stimulus was followed by a stop signal, an auditory tone presented through headphones,

which indicated that participants had to withhold their response. The time between the go stimulus (“X” or “O”) and the stop signal is defined as the stop signal delay (SSD). A staircase tracking procedure was used, which altered SSD dynamically after each trial according to whether the participant was able to inhibit the response (Verbruggen & Logan, 2008). As preliminary analyses showed that a majority of the participants had a commission rate of under 35% and thus likely waited with their responses (reaction time was relatively high on go trials as well) the nth method of obtaining SSRT was used (Ridderinkhof, Band & Logan, 1999). Also the recommendations of Verbruggen and colleagues (2019) were used: SSRT was only calculated if commission error rates were between .25 and .75. There was one practice round, which could be repeated as many times as the participant needed to in order to understand the task. In actual testing this was never repeated more than three times.

2.4. Statistical analysis

Statistical analyses were done in JASP 0.10.2.0 for all (Bayesian) statistics (Wagenmakers et al., 2018).

Bayesian Statistics. All statistical analysis were performed with their Bayesian counterparts. The main reason being that with Bayesian statistics inferences can be made on the actual evidence load (its strength) and it does not have the weaknesses or issues associated with classical p-testing, such as multiple comparisons (Gelman & Tuerlinx, 2000) and insufficient power (as long as the Bayesian factor is low or high enough, there is enough power). Two types of comparisons were made: every possible single model (excluding null) versus the null-model and a comparison of every possible model (excluding null) with a particular effect to every possible model without that effect taken together. Bayesian odds BF_{10} (the relative likelihood of the H_1 being true over H_0) or BF_{incl} (the relative likelihood of

all the inclusive models being true over all the exclusive models) are reported respectively. The last type of comparison was only added when there were more than two factors, which results in an exponential increase in comparisons, and always targets the expected interaction effects of *time*group*. We follow Jeffreys (1961) Bayesian factor cut-off points for strength of evidence, notably: a factor of 3 or above or 1/3 or below for moderate evidence for or against H_1 . When the strength of evidence falls in other categories this will be noted. R scale priors are set to 0.5 (equal prior likelihood of both hypotheses being true)

T-tests. Bayesian independent sample t-tests were used to test for group differences in age, education level, MMSE, STAI-T, IPAQ MET and contemplative practice.

Repeated-measures ANOVA. Bayesian general linear model repeated-measures ANOVA was conducted for all tasks to compare pre-intervention performance to post-intervention performance between the TCC group and the control group. Intervention *group* (TCC/ control) was taken as a between-subjects factor and *time* (pre-test/post-test) as a within-subjects factor in every analysis. In comparisons with more than two factors BF_{10} is only reported for the top model and the strongest with *time*group*. The BF_{incl} of the *time*group* interactions is then also reported (the Bayesian factor comparing all models including that effect with all models without that specific interaction effect).

3. Results

3.1. Questionnaires

3.1.1 Demographics & descriptives

Table 1 shows the demographic means of the sample. Bayesian independent samples t-tests were performed to test for average group differences between TCC and control for: *age*

[BF₁₀ = 0.32, error % = 0.02]; *education level* [BF₁₀ = 0.33, error % = 0.02]; *MMSE score* [BF₁₀ = 0.41, error % = 0.02]; *STAI-T score* [BF₁₀ = 0.33, error % = 0.02]; *IPAQ MET-min* [BF₁₀ = 0.39, error % = 0.02]; and *contemplative activity level* [BF₁₀ = 0.30, error % = 0.02]. There was no evidence for difference between any of the means. However, strength of evidence varied: whereas *age*, *education level*, *STAI-T score* and *contemplative activity level* all indicated moderate evidence against a difference in means, *MMSE score* and *IPAQ MET-min* showed only anecdotal evidence against a difference in means.

Table 1. Descriptive means \pm SD of both intervention groups (TCC/Control). Bayesian factor (BF₁₀) is shown for independent t-tests. M=Male; F=Female; MMSE=Mini-Mental State Examination; STAI-T=State-Trait Anxiety Test – Trait; IPAQ=International Physical Activity Questionnaire; MET-min=Metabolic Equivalents of Task minutes per week; ContAct=Contemplative Activities.

Measures	Group		
	TCC	Control	BF ₁₀
Sample Size	20	23	
Sex (M/F)	11/9	12/11	
Age	63.95 \pm 7.25	63.17 \pm 7.73	0.32
Education level	4.55 \pm 1.47	4.74 \pm 1.05	0.33
MMSE	29.45 \pm 0.89	29.17 \pm 1.15	0.41
STAI-T	35.90 \pm 7.09	36.96 \pm 8.08	0.32
IPAQ (MET-min)	3581.1 \pm 3038.1	4481.2 \pm 4197.7	0.39
ContAct level	0.65 \pm 1.39	0.65 \pm 1.15	0.30

3.1.1 Session self-report

In terms of the level of *Challenge* in the TCC group, the reported *Ease* of exercises and instructions over all sessions was M: 3.51 (SD: 0.25). Reported overall put-in *Effort* was M: 3.79 (SD: 0.11). The *Physical Exertion* level was assessed by *Aerobic* aspect M: 1.64 (SD: 0.15), higher *Heart Rate* M: 1.64 (SD: 0.15) and expectation of *Muscle Ache* M: 1.14 (SD: 0.08). As far as *Adherence* is concerned: all questionnaires were filled out for each participant and each session.

3.2 Motor function

3.2.1 TUG

Figure 1 shows the TUG scores pre- to post-test for TCC and control conditions. Bayesian repeated-measures ANOVA [$time(2)*group(2)$] resulted in a $BF_{10}(time) = 264.8$, error % = 1.3; $BF_{10}(group) = 0.6$, error % = 0.6; $BF_{10}(time+group) = 153.3$, error % = 1.7; and $BF_{10}(time+group+time*group) = 713.5$, error % = 1.5. In other words: the complete model including the $time*group$ interaction effect is the strongest model and 714 times more likely to be true, than null model. Any factor above 100 is in the highest category: extreme evidence for H_1 (Jeffreys, 1961). Concluding: both groups decreased their TUG scores over time, where TCC scores decreased more than that of control, from pre-test to post-test [TCC: $5.64 \pm 0.91s$ to $4.98 \pm 0.78s$ vs. *control*: $5.64 \pm 0.97s$ to $5.45 \pm 1.16s$].

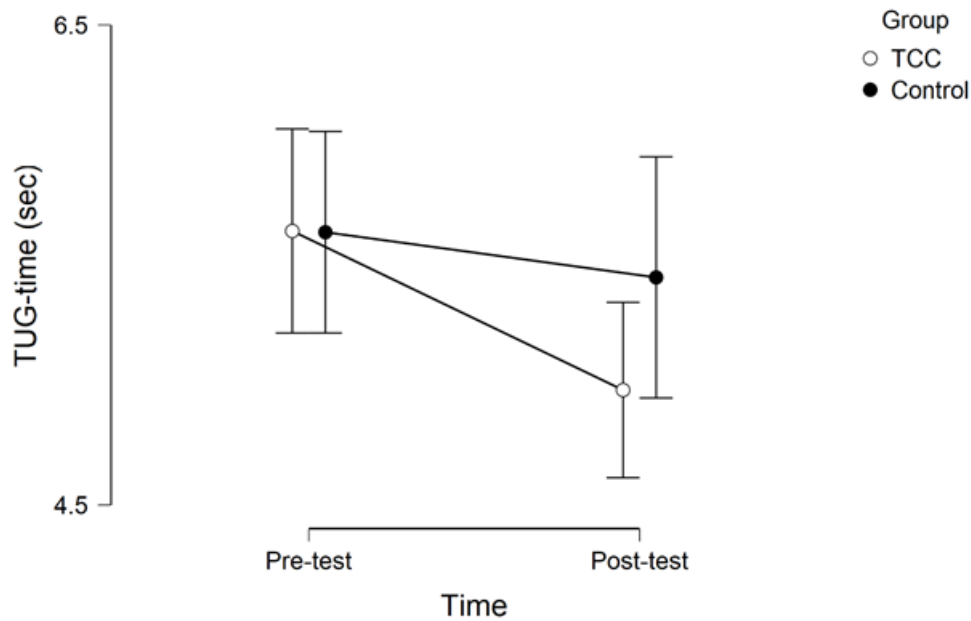


Figure 1. TUG scores for TCC and control conditions between pre-test and post-test (in seconds).

3.2.2 FTT

Figure 2 shows the FTT count averaged across hands pre- to post-test for TCC and control conditions. Bayesian repeated-measures ANOVA [$time(2)*group(2)$ resulted in a $BF_{10}(time) = 1.7$, error % = 1.2; $BF_{10}(group) = 0.7$, error % = 8.9; $BF_{10}(time+group) = 1.1$, error % = 2.7; and $BF_{10}(time+group+time*group) = 3.1$, error % = 3.7. The complete model including interaction $time*group$ is the strongest model, but is barely in the moderate evidence category. Tentatively concluding: FTT count increased more than that of control, pre-test to post-test, from a higher baseline level [*TCC*: 61.52 ± 8.95 to 63.78 ± 9.27 vs. *control*: 59.83 ± 9.47 to 59.88 ± 8.94].

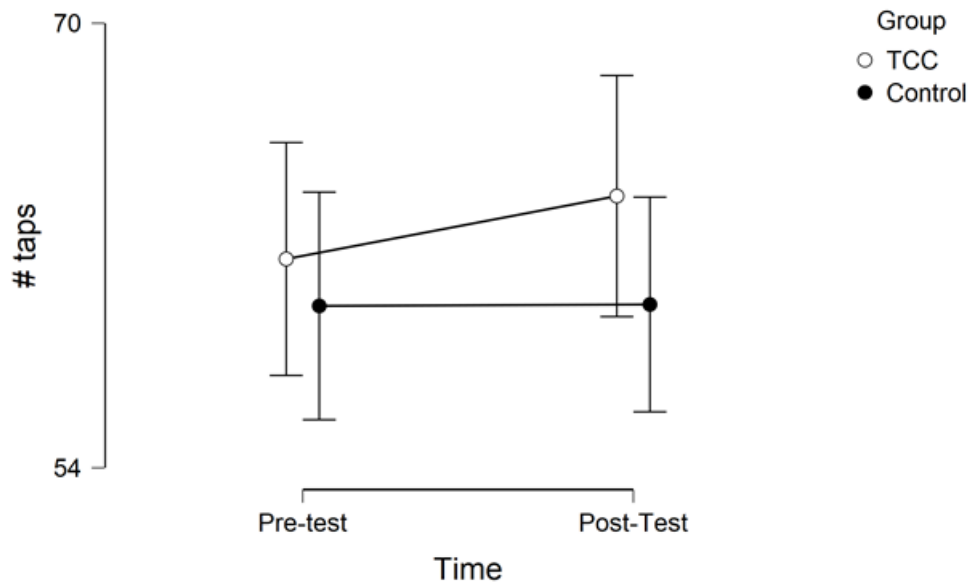


Figure 2. FTT number of taps averaged over both hands for TCC and control conditions between pre-test and post-test.

3.3. Cognitive measures

3.3.1. Task-switching task

3.3.1.1. Global switching costs.

Accuracy. Bayesian repeated-measures ANOVA [$time(2)*block(2)*group(2)$] on accuracy means revealed the top model to be $time+block$ [$BF_{10} = 16.5$, error % = 3.7]: there is strong evidence for both these main effects over null. In comparison, the strongest model with the expected interaction was $time+block+group+time*group$ [$BF_{10} = 1.3$, error % = 2.1], this together with a $BF_{incl}(time*group)$ of 0.14, indicates that there is moderate evidence against any model with the interaction, as opposed to without the interaction effect, this is very strong for the three-way [$BF_{incl}(time*block*group) = 0.02$]. Concluding: accuracy overall increased from pre-test to post-test [$93.9\% \pm 9.3$ to $96.2\% \pm 6.3$], accuracy was higher in the repeat block than in the switch block [$96.2\% \pm 6.9$ vs. $93.9\% \pm 8.8$] and there were no interaction effects of $time*group$.

Reaction time. Bayesian repeated-measures ANOVA [$time(2)*block(2)*group(2)$] on reaction time means revealed a similar result: the top model was again $time+block$ [$BF_{10} = 1.15*e^{21}$, error % = 5.0]: there is extreme evidence for both these over null. The strongest model (8th) with interaction was again $time+block+group+time*group$ [$BF_{10} = 1.07*e^{20}$, error % = 4.6] and $BF_{incl}(time*group) = 0.14$ indicates that that there is moderate evidence against models with this interaction over those without, this is very strong for the three-way [$BF_{incl}(time*block*group) = 0.03$]. Concluding: reaction time overall decreased from pre-test to post-test [942ms \pm 258 to 893ms \pm 221] , responses were much faster in the repeat block than in the switch block [787ms \pm 165 vs. 1049ms \pm 234] and there are no $time*group$ effects.

3.3.1.1. Local switching costs.

Accuracy. A Bayesian repeated-measures ANOVA [$time(2)*trial(2)*congruency(3)*group(2)$] on accuracy showed the top model to be $time+trial+congruency+trial*congruency$ [$BF_{10} = 2.97*e^{27}$, error % = 5.2]: extreme evidence for this combination of effects over null. The strongest model (6th) with interaction was $time+trial+congruency+group+trial*congruency+time*group$ [$BF_{10} = 4.95*e^{26}$, error % = 4.6]. The $BF_{incl}(time*group) = 0.13$ indicates that that there is moderate evidence against models with this interaction and this is extreme for both three-ways and the four-way [$BF_{incl} < 0.01$]. Concluding: overall accuracy increased from pre-test to post-test [92.9% \pm 10.3 to 95.1% \pm 8.6], accuracy was higher in the repeat trials than in the switch trials [95.5% \pm 8.6 vs. 92.4% \pm 10.1], accuracy was lower for incongruent trials [*neutral*: 95.3% \pm 8.9; *congruent*: 95.0% \pm 8.6; *incongruent*: 91.7% \pm 10.5] and there was an expected $trial*congruency$ interaction (see table 2 for these values). All effects involving $time*group$ were absent.

Reaction time. Bayesian repeated-measures ANOVA [$time(2)*trial(2)*congruency(3)*group(2)$] showed highly similar effects, the top model

being *time+trial+congruency+trial*congruency* [$BF_{10} = 5.96 * e^{98}$, error % = 3.9]: extreme evidence for this combination of effects over null. The strongest model (10th) with interaction was again *time+trial+congruency+group+trial*congruency+time*group* [$BF_{10} = 3.77 * e^{97}$, error % = 9.0]. The $BF_{incl}(time*group) = 0.06$ indicates that that there is strong evidence against models with this interaction and this is extreme for both three-ways and four-way interactions [$BF_{incl} < 0.01$]. Concluding: overall reaction time was higher in the pre-test, than in the post-test [$1090ms \pm 311$ to $1014ms \pm 255$], reaction time was faster in the repeat trials than in the switch trials [$920ms \pm 217$ to $1185ms \pm 286$], there was a *congruency* effect [*neutral*: $987ms \pm 258$; *congruent*: $1094ms \pm 300$; *incongruent*: $1103ms \pm 309$] and there was an interaction effect of *trial*congruency* (also see table 2 for these values). Again there were no effects involving *time*group*.

Table 2. Means and standard deviations of local switch costs. Split for trial type and congruency. SD=standard deviation; Acc=accuracy; RT=reaction time.

		Neutral		Congruent		Incongruent	
		Mean	SD	Mean	SD	Mean	SD
Acc	Repeat	96.1%	8.4%	96.5%	7,8%	94.0%	9.4%
	Switch	94.5%	9.4%	93.4%	9.1%	89.4%	11.2%
RT (ms)	Repeat	867	193	939	219	953	231
	Switch	1091	255	1228	282	1236	300

3.3.2. N-back

The repeated-measures ANOVA on the sensitivity score of the N-back (hit rate – false alarm rate) [$Time(2)*Task(2)*Group(2)$] resulted in a single main effect of *Time* [$F(1, 41) =$

6.36, $p < 0.05$, $\eta^2_p = 0.13$]. Sensitivity went up from pre-test to post-test [78.5% \pm 18.8 to 83.3% \pm 14.7]. None of the other main effects or interactions were significant.

A repeated-measures ANOVA of reaction time on the target was performed [Time(2)*Task(2)*Group(2)]. This also showed a single main effect of Time [$F(1, 41) = 15.38$, $p < 0.001$, $\eta^2_p = 0.27$]: reaction time went down from pre-test to post-test [727ms \pm 177 to 657 \pm 196]. None of the other main effects or interactions were significant.

3.3.3. Stop-signal task

After calculating the chance of commission error p(Com) per participant and per test 13 participants were excluded from SSRT analysis based on values below .25 or above .75 for either test, as recommended by Verbrugge et al. (2019). This led to 6 exclusions from the TCC group, leaving 14, and 7 exclusions from the control group, leaving 16. A repeated-measures ANOVA was conducted on SSRT(nth) of the remaining sample [Time(2)*Group(2)]. Only a main effect of Time [$F(1, 28) = 7.46$, $p = 0.01$, $\eta^2_p = 0.21$] was found, where SSRT went down from pre-test to post-test [321ms \pm 69 to 283ms \pm 64]. The interaction effect of Time*Group was non-significant. All relevant values for the SSRT sample can be seen in table 3.

Table 3. Means and standard deviations of stop-signal task variables. Split for TCC/control and the two time points. TCC=Tai Chi Chuan; SST1=pre-measure stop-signal task; SST2=post-measure stop-signal task; SD=standard deviation; Go=go trials; Com=commission error; p(Com)= commission error rate; Acc=accuracy; RT=reaction time; SSD=stop-signal delay; SSRT=stop-signal reaction time.

			Go Acc	Go RT	p(Com)	Com RT	SSD	SSRT
TCC	SST1	Mean	95,1%	628	41,1%	522	261	308
		SD	5,7%	111	8,4%	68	78	50
	SST2	Mean	92,9%	612	41,7%	524	277	285
		SD	18,3%	94	10,9%	85	88	64
Control	SST1	Mean	95,8%	645	41,8%	549	256	331

	SD	6,0%	100	12,2%	75	103	82
SST2	Mean	96,9%	636	38,4%	553	288	280
	SD	4,2%	123	9,1%	97	89	67

4. Discussion

This randomized controlled trial was conducted to investigate the relationship between motoric and cognitive effects of the multi-modal mind-body exercise TCC. As such we expected to replicate findings on enhancement of EF in aging populations and specifically sought to elucidate which of the components of EF – shifting, updating or inhibition – is targeted by this exercise and how and in which direction this relates to any motoric enhancements. The cognitive results did not meet any of our expectations. None of the three components showed a larger improvement for TCC than control from pre-test to post-test; not statistically, but also not numerically in trend. This was the case for global and local switching costs in both accuracy and reaction time in the TST (shifting), sensitivity in the n-back and SSRT in the SST. Another aim of this study was to replicate beneficial effects of TCC on motor function and look into the possible interaction of cognitive and motoric factors. Here the results did meet posed expectations. Both functional balance, as measured by TUG score, and motor speed, as measured by FTT count, were significantly enhanced more in TCC than in control. This last result could be interpreted as a form of cognitive enhancement as motor speed is related to processing speed, which makes the null-results on cognitive control the more tantalizing.

One explanation for our null findings on the three different types of EF could be that our online intervention did not provide a sufficient quality of TCC, because the instructions were not followed correctly or compliance with practice was low – as one of the drop-outs indicated annoyance as a reason. However, we find this to be unlikely as the self-reports were filled in after every planned session for all but one of the participants (who missed one session). It could also be that the exercises were not challenging and exerting enough. In the scores of the session self-report questionnaires a low difficulty and low physical exertion was reported, while the effort put-in was mid to high. But even low physical exertion would be

expected to lead to cognitive enhancement and most telling: motor function was enhanced, both in functional mobility and in motor speed. The most likely explanation of this enhancement is from diligently following the TCC exercises.

So what are possible reasons for this absence of cognitive effects? It might be that the intervention was too short for positive effects on cognitive control to surface (as opposed to motoric improvements), in other words: dosage by duration. Most TCC studies use longer intervention periods of 3 to 6 months, whereas this program took 2 months. This explanation cannot be ruled out; however, effects on EF have been reported with similar (e.g. meditation), but much shorter interventions and most clinical programs such as mindfulness-based stress reduction, show significant effects after just 8 weeks.

Another explanation is an absence of certain factors in this online TCC implementation which might be present in TCC intervention of studies reporting cognitive enhancement, such as: 1) spirituality, 2) social contact and 3) personalized training. There are indications that a spiritual dimension is a key component of contemplative practices. In a study comparing a secular and a spiritual style of meditations during a two-week intervention it was shown that the spiritual group had a greater decrease in anxiety and increase in positive mood, than the secular control group (Wachholtz & Pargament, 2005). A similar assessment of the contribution of spirituality to cognitive effects is not known to us. The second absent factor is social contact. In a regular TCC practice session the practitioner has bidirectional interaction with a teacher and is part of a group that performs exercises in unison. There might also be contact outside of the classroom, at least before and after. Social contact can provide a buffer against cognitive decline (Kuiper et al., 2015). This factor might even include physical contact, for example: teachers might put their hand on the lower back to check posture or practitioners might engage in the one-on-one exercise known as “sticky hands”. The third factor of note is the lack of personalized teaching. There is no direct monitoring of the level

and progress of practitioners and therefore the lesson material is not adapted to the individual case. Both personalized scaling of difficulty and the shaping of behavior (by reward) is largely absent, although the lessons do scale in difficulty and encouragement is offered. We know from computerized cognitive training studies that personalized training works better than general computer games (Peretz et al., 2011). However, previous studies showing effects of TCC also do not incorporate personalized scaling and shaping. In conclusion: though we acknowledge spirituality, social contact and personalization to be absent factors in this intervention, we regard this to be insufficient cause for the null-results found in this study, as two factors that are sufficient cause for cognitive enhancement are clearly present: physical exercise and meditation. Indeed, as outlined in the introduction, either of these two factors in isolation should be sufficient for cognitive enhancement to occur.

Might there be a ceiling effect in this sample? The sample might be in the top tier of cognitive health from the start. The sample does seem to be physically fit: the participants report a high level of physical activity, as can be seen in the demographics. Then again, there was a main effect of time and it could be seen that both groups improved in the three cognitive components. Then there could only be a ceiling effect if this end state was the highest achievable level of this sample, overshadowing any additive effect of TCC. Looking at the absolute numbers and comparing these to other studies with aging populations, together with the absence of any numerical direction of effect; we find this to be unlikely as well.

Therefore, we tentatively conclude that TCC does not have notable effects on EF. This could imply that a physical exercise factor (e.g. aerobic challenge) *and* the meditation factor are not sufficiently present in TCC. Another implication might be that the scientific literature on these domains exaggerates the cognitive effects of these types of interventions and there might be a publication bias, such as has been indicated in studies of mindfulness-based programs (Coronado-Montoya et al., 2016). We lean towards this last explanation and thus

regard the publication of studies reporting null-results, such as this one, of paramount importance. We have three suggestions for the field. Firstly, submit and publish all null-effects produced by experiments of sufficient quality of design. Secondly, actively seek to replicate previous findings in these domains. Thirdly, to isolate common factors in TCC - and other contemplative practices - and test their individual and mixed efficacy on both cognition and motor functioning. The question whether TCC can help to obtain a buffer against cognitive decline can only be answered by accumulating sufficient representative results.

Appendix A

Original language was Dutch. Questions and scale translated into English. Only relevant questions reported.

All items had a 5-point Likert scale: 1: Completely not; 2: Somewhat; 3: Neutral; 4: Moderately; Very much so.

Multiple questions per aspect were averaged per session per participant (for ease and effort).

All session aspect scores were averaged across the 20 sessions to obtain grand mean aspect scores.

Introductory text:

“Below you will find a number of questions about your experiences in the previous session. Read every question thoroughly and report to what extent this applied to you. There are no right or wrong answers. Don’t think too much and respond with your first associations.”

Challenge – Ease

“Was it easy to follow the instructions?”

“How hard or difficult was this session?”

Challenge – Effort

“Did you have the feeling that you were doing Tai Chi?”

“Do you have the idea that you participated well?”

Physical exertion – Aerobic

“Did you have a higher breathing rate?”

Physical exertion – Heart rate

“Did you have a higher heart rate?”

Physical exertion – Muscle ache

“Do you think you will have muscle aches tomorrow?”

Chapter 4

Breath of life: the acute effects of respiratory locus and rate on autonomic activity and inhibitory control

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1. Introduction

Respiration has a central and critical function in complex organisms. It serves as a pillar of homeostasis, in other words: being able to live. By its essence of transporting oxygen to organs and muscles, is absolutely necessary for any function to function properly, dynamically and optimally. Respiratory processes are also highly complex themselves and even today the neural mechanisms and even substrates remain unresolved (Ashhad et al., 2022; Feldman & Del Negro, 2006). Despite the central position of respiration in homeostasis and the popularity of bottom-up explanations of complex behavior (e.g. embodied cognition, ideomotor theory), studies of its role in emotion and cognition have been lacking. A field of study that lends itself well to look for potential respiratory effects is that of contemplative practices. Contemplative practices, such as mindfulness meditation, employ a variety of breathing techniques that serve as a manipulation of respiratory parameters and have shown to impose a wide range of beneficial effects on mental health (Schlechta Portella et al., 2021; Smart et al., 2022) and cognition, notably on attentional control and executive functions (Casedas et al., 2020; Verhaeghen, 2021). Even short-term mindfulness training seems to benefit executive functions, notably inhibition and updating, though the reported results are mixed (Zhou et al., 2020). However, the potential mediating or moderating effects of respiratory control on these dependents has been understudied. To fill this gap and the lack of focus on respiration in general, Gerritsen and Band (2018) provided a framework: the respiratory vagal nerve stimulation (rVNS) model of contemplative activities. According to this model respiratory patterns stimulate the autonomic nervous system, notably the vagal nerve complex, to a relaxed and flexible state. Through this state cognition becomes more adaptive to current demands, and can enhance it on the long-term. This study was set up to test some of its predictions.

1.1. rVNS model

The main claim of the rVNS model is that the slow breathing exercises inherent in many contemplative tradition can directly lead to many of the reported benefits, by phasically and tonically stimulating the parasympathetic branch of the autonomous nervous system. Hereby the system shifts away from an arousal dominated stance towards a more relaxed one. This ameliorates stress-related pathology and enhances cognition in overly demanding contexts. Where normally the system would be taxed (stressed) beyond optimal settings, it can now

remain inside these bounds. In our model, there are three respiratory parameters that influence parasympathetic tone, or rather autonomic state, in order of evidentiality: 1) rate: slowing breathing down increases relaxation, with an optimal rate of 6 bpm on average (Laborde et al., 2017; You et al., 2021); 2) ratio: extending exhalation relative to inhalation relaxes (Cregg et al., 2017;; van Diest et al., 2014), for example: due to respiratory sinus arrhythmia (Hirsch & Bishop, 1981); 3) locus: breathing by moving the abdomen relative to the thorax moves the autonomic state from fight/flight to rest/digest (Amann, 2012), as thoracic respiratory musculature is both inspiratory and expiratory, but abdominal musculature is only expiratory (Aliverti, 2016). Though note that automatic changes of rate and ratio might be confounding herein.

The rVNS model proposes the vagal nerve complex as the neural mediator between respiratory patterns and emotional/cognitive effects and adopts heart rate variability (HRV) as a valid indicator of vagal tone. The association between HRV and cognition was first proposed by Porges (1995, 2001) and further elaborated upon in the neurovisceral integration model by Thayer and Lane (2000). In the neurovisceral integration model, cardiac flexibility, reflected in HRV, stimulates mental flexibility (notably cognitive inhibition), by projections of the vagal nerve towards (ventromedial) prefrontal cortex. This connection is also the hypothesized way slow breathing exercises lead to cognitive enhancement of executive functions in the rVNS model. However, note that vagal mediation is not necessary in our model – contrary to what is suggested in its name. In our view, specific respiratory patterns may lead to an optimal state on the arousal/relaxation spectrum that will benefit cognition in any specific context. This means that controlled hyperventilation could also boost executive functioning, if the context demands it; for example due to an individual having a high state of relaxation in an arousal demanding setting. For a detailed discussion of the rVNS model and vagal tone measurements we refer to the original work (Gerritsen & Band, 2018) and to a subsequent work by unaffiliated authors that posits a more detailed neurophysiological foundation (Noble & Hochman, 2019). Since the publication of the rVNS model, a number of studies have been conducted that indirectly or directly try to test some of our premises.

1.2. Studies linking respiration, HRV, and cognition

Zaccaro and colleagues (2018) performed a systematic review of actively controlled trials that employed slow breathing exercises and included HRV measures,

neurophysiological measurements, psychological/behavioral tests, or combinations of these. Following slow breathing interventions, vagal tone HRV increased, while relaxation went up and anxiety down. Zaccaro et al.'s main conclusion is that the evidence for a link between parasympathetic tone and favorable behavioral outcomes exists, but the evidence is scarce. They also note that there are conflicting findings and that the operationalizations of vagal tone HRV are highly divergent. For one, most studies seem to employ frequency domain measures of HRV, though the scientific consensus currently is that time domain measures are more valid as a vagal tone approximation, notably: the root mean square of successive differences (RMSSD). Lastly, the reviewed studies did not directly test for correlations between vagal tone HRV and behavioral/psychological outcomes.

Spangler and colleagues (2018) tested predictions of the neurovisceral integration model in ecologically valid low and high stress conditions in soldiers (virtual shooter simulator). They reported that higher HRV variability was associated with less successful response inhibition (false alarms), contrary to findings that show enhanced inhibition in high HRV conditions. However, they stated that this not contrary to their expectations, because in high risk environments (life threatening) the cost of a miss is relatively higher, making downgrading inhibition the adaptive setting, and thus flexible response. Note however that reaction times were not reported and therefore any potential speed-accuracy tradeoff was hidden. Next to this, if one follows the reasoning that a liberal signal detection threshold was adaptive in the high risk setting and adaptivity is reflected in HRV variability, we would not only expect more hits in this condition, but also a positive correlation between HRV variability and hit rate, which was not found. Finally, the vagal tone measure of choice was high-frequency HRV, which is as stated above, not considered a reliable vagal tone marker (Laborde et al., 2017). Nonetheless, in this study a relationship between HRV and (response) inhibition was (re)established.

Two other studies linking slow-paced breathing and vagal tone (RMSSD) need mention. The first tried to disentangle slow-paced breathing with and without HRV biofeedback (Laborde et al., 2022). They found no difference between these conditions on RMSSD (went up) and self-reported emotional valence (more negative), arousal (went down) or control (went up), from pre-test to post-test. Clearly, slow-paced breathing on its own is sufficient to produce effects on vagal tone. The second studied the dose-response relationship of slow-paced breathing exercises with four different durations (You et al., 2021). They found no differences on RMSSD by dose either during intervention, or the resting state post-

measurement. RMSSD did go up as compared to control during the intervention phase. Evidently, even short bouts of breathing exercises are sufficient to produce effects on vagal tone. Although it seems that these effects are short-lived, as the return to baseline is apparent across conditions.

Another study looked into the complete chain of prediction: respiration, vagal tone HRV and inhibition (Laborde et al., 2019). This study used physical exertion as a psychological stress inducer, had a slow breathing exercise either before or after exercise (6 breaths-per-minute), and included both an inhibition task (Stroop) and valid vagal tone marker (RMSSD). The results showed that Stroop interference scores (accuracy) were lower after slow breathing and RMSSD went up accordingly. Interestingly enough, the cognitive effects were not mediated by RMSSD, which casts doubt on the vagal tone complex mechanism. Again note that this does not rule out relaxation as potential candidate, by other mediators.

Bonomini and colleagues (2020) looked at two other executive functioning subtypes: shifting and updating (Miyake et al., 2000). In two experiments, using a 2-back and task switching task, they tested whether a slow breathing exercise (<6 breaths-per-minute) – contrasted with two other different breathing exercises – changed autonomic state and through this influenced executive functioning. The authors report an increase of low-frequency HRV and increased general success rate in both tasks after slow breathing. No effects are found on reaction time in both tasks or on switch costs in the task switching task. Clearly, this is not convincing evidence for increased executive functioning by slow breathing, nor is the low-frequency HRV easily interpreted as a marker of vagal tone, arguing against involvement of the vagal complex. Furthermore, it has to be noted that this study was underpowered and of weak/unclear design. For example: the duration of the breathing exercise was not reported.

To our knowledge there are no (recent) studies looking at either the ratio of inhalation/exhalation or locus, and their associations with cognition and/or autonomous state. However, there is a growing body of research on divergent effects on cognition of respiration phase (inhalation vs exhalation), for example: on memory processes (Heck et al., 2019) and perception and production of quantities (Belli et al., 2021). Studies that report on locus, by referring to abdominal breathing, only include instructions towards these incorporated in a slow breathing condition, and are not contrasted with a thoracic locus condition. In our first attempt at testing the predictions of the rVNS model, we therefore set out to investigate the influence of the locus factor on vagal tone and executive functioning of the inhibition subtype, while mapping the indirect influence of the two factors – rate and ratio – as well.

1.4. Current study

The study consisted of two experiments, both randomized controlled trials, of two different samples and design.

Experiment 1 was a within-subject pre-test post-test design with three conditions in three sessions: abdominal breathing (AB), thoracic breathing (TB) and focused breathing (FB). The acute effects of short breathing exercises, which manipulated locus of breathing, were studied on phasic parasympathetic tone (RMSSD) and phasic sympathetic tone (pre-ejection period, PEP), using ECG/ICG measures; and on response inhibition (stop-signal reaction time, SSRT) by stop-signal task (SST). Locus was manipulated through audio recordings of 3 different guided breathing exercises, including a tutorial. In AB the participants were instructed to expand and retract their abdomen during inspiration and expiration respectively, engaging the diaphragm more; during TB intervention the participants had to expand and retract the sides of the ribcage instead; and in FB they only had to pay attention to the locations of their breathing and were instructed to not change anything (semi-control condition). Respiration parameters, respiration rate (RR) and inhalation/exhalation ratio (I/E), were checked by pneumography. The research question here is: do short locus of breathing exercises (AB, TB, FB) lead to distinct autonomic states, as reflected in RMSSD and PEP changes, that in turn influence the efficiency of response inhibition, as measured by SSRT?

Our expectations were that autonomic state would be parasympathetically dominated in AB and sympathetically dominated in TB. This should be reflected in a lower RR, higher RMSSD and higher PEP (PEP score is inversely related to sympathetic tone) for AB intervention and higher or unchanged RR, lower RMSSD and lower PEP for TB intervention. Furthermore, we hypothesized that these autonomous states transfer to the post-test and lead to an increase in response inhibition, as indicated by a lower SSRT, in AB, relative to TB, from pre-test to post-test. In FB we expected these physiological and cognitive parameters to be (relatively) unaffected. However, our expectations of Experiment 1 were not met. Importantly, RR went down during all three interventions, while RMSSD and SSRT were seemingly unaffected. Interestingly, PEP went down during AB (from a much higher baseline), as compared to TB and FB, indicating an increase in sympathetic tone. Clear

conclusions could not be drawn from these results, especially by the decrease of RR across conditions. To provide more clarity, the second experiment was set up.

In Experiment 2 the semi-control condition FB was dropped, as an orthogonal design was deemed sufficient. The guided audio was rewritten and instructions for pacing of breathing were added. In AB intervention participants were instructed to breathe slower than normal, while in TB it was stressed to breathe slightly faster than normal. In both recordings the pacing of the instruction itself, matched the direction of the explicit instructions (i.e. slow pace of instruction to breathe slower and vice versa). Note that, with this design the unique contribution of locus can't be studied, as RR changes are a supposed and likely confound. The physiological measurement for sympathetic tone was changed to electrodermal activity (EDA) and skin-conductance level (SCL) used as its marker, by reason of feasibility and ease of experimentation. A third physiological resting state measurement time was added after intervention to determine carry-over of autonomic changes (recovery phase). SST was substituted for the Simon task, as there is debate on the reliability of SSRT (Wöstmann et al., 2013) and thus whether changes therein actually reflect enhancement effects. The Simon task was chosen as it is theorized to be the most pure measure of cognitive inhibition, by lack of stimulus-response overlap (Hommel, 2011). But perhaps most importantly, the Simon task does not suffer from excluded data as the SST does. Experimentation with SST frequently has a large proportion of the sample that does not comply with the instruction to not wait for the stop-signal. Another change to the cognitive testing was the inclusion of a pre-test for each session, instead of only one baseline on the first session. Finally, as a self-report measure of (psychological) relaxation/stress the affect grid was added. The research question of Experiment 2 is: do short locus of breathing (AB, TB) exercises lead to distinct autonomic states, as reflected in RMSSD and SCL changes, that in turn influence the efficiency of cognitive inhibition, as measured by the Simon task?

The expectations for Experiment 2 are highly similar to that of Experiment 1. We expect RR to go down in AB and up in TB, which in this context is a manipulation check. Furthermore, we expect RMSSD to go up and SCL to go down during AB, with the reverse observed direction of RMSSD and SCL during TB. Furthermore we expect that these levels are mostly maintained during recovery, thus do not immediately return to baseline. We expect the affect grid to show a more positive and relaxed affect state from pre- to post-test in AB condition as compared to TB pre- to post-test. Lastly, we hypothesize that this autonomic state persists into the cognitive post-test, resulting in a more or less flexible and efficient

performance. Concretely: we expect a larger decrease in Simon interference effect from pre- to post-test in AB, than in TB, in both reaction time and accuracy.

2. Experiment 1

2.1. Method

2.1.1. Participants

Participants were recruited at the Faculty of Social Sciences at the University of Leiden by flyer or by online registration through a research participation system. Participation was restricted by the following selection criteria: no experience in meditation, other contemplative practices and/or breathing exercises, no neurological or cardiopulmonary disease; and no medication that affects cardiac or respiratory systems. Power analysis (G*power, $1-\beta=.8$) based on the measure with the least iterations (SST, 4 measurements), suggested that 24 participants needed to be recruited. Note that, at this time planned statistical analysis was to be classical, instead of Bayesian. In the end, 29 participants were recruited: 23 females (M = 22 years, 18-34) and 6 males (M = 31 years, 21-58). There were no drop-outs. Participants were asked to abstain from strenuous exercise (same day), alcohol (24 hours), caffeine (12 hours) and nutrition (1 hour) before testing. Screening took place at the start of each session; no participant was excluded (on these grounds). These experiments have been approved by the ethics committee of the Leiden University institute of psychology (V1-1215 and V1-1337). Participants received either course credit or financial compensation for participation, the majority of which were (psychology) students.

2.1.2. Procedure

2.1.2.1. Design

Experimental sessions were held between 8:00 and 20:00 at a lab in the Faculty of Social Sciences in Leiden. Each participant completed three sessions, one session every week, each session corresponding to one of the three breathing interventions (AB; TB; FB). The order of sessions was counter-balanced. Testing time between sessions was held constant (max. deviation: 1 hour). At the start of the first session of the experiment participants were informed on the experiment and provided informed consent. Then a short survey was used to screen on the selection criteria.

2.1.2.2. Physiological measures

At the start of a session participants were seated in the testing booth and equipped with ECG/ICG electrode leads (Kendall foam electrodes) and two respiratory bands (transducer belts). The ECG/ICG set-up consisted of the standard 11 electrode configuration (3-way ECG and 8-way ICG lead). For ECG, one electrode was placed just below the right collar bone, 4 cm to the right of the sternum; one on the right and one on the left side of the abdomen just under the ribcage. For ICG, four electrodes were placed at both sides of the neck 3-4 cm apart from each other within the vertical plane and four electrodes were placed at both sides of the trunk. Two at each side, with one approximately at the 7-8th intercostal space and the other 4 cm above, at the 5-6th intercostal space. The respiratory bands were placed around the thorax (at the 4-5th intercostal space) and the diaphragm (slightly above the navel). After complete configuration, ECG/ICG and respiratory signals were monitored for noisy signals and equipment adjusted accordingly. ECG/ICG and respiration data were collected through Biopacs at 1000 HZ and recorded on computer (Acqknowledge 4.4.1).

2.1.2.3. Testing

The experimental session was scripted and conducted through the computer (E-prime 2.0), though verbal instructions and prompts were offered by the experimenter if needed. Sessions always started with the respiration, ECG and ICG baseline physiological measurements (resting state), where the participant was instructed to sit still for five minutes in an upright position (with hands resting on their legs) and a grey screen was shown on the monitor. Only during the first session was this followed by a baseline SST, as the design with pre-post measurements in every session was deemed too time invested. The respiratory intervention followed next and took ~10 minutes. During the intervention phase physiological measurements were taken, these are the active condition measurement (reactive). The experimental session finished with the post-intervention SST and a debriefing at the last session.

2.1.3. Interventions

The three different interventions (AB; TB; FB) were performed in as much comparable circumstances as possible. Participants were instructed to sit upright with their feet flat on the floor and about hip distance apart, knees bent. Breathing exercise instructions

were presented in audio through headphones. The audio scripts were written, spoken and recorded by the second author. The intro and outro were standardized across interventions. Each different protocol started with a tutorial (~2:30 min), followed by the experimental phase (~5:30 min), during which the intervention physiological measurements were taken. See Appendix A for a complete transcript. Visual prompts on the exercise were provided at 0:45, 1:15 and then every 1:00 min on screen.

2.1.3.1. Abdominal breathing (AB)

The aim of the AB protocol was to change the locus of expansion towards the diaphragm and away from the chest. Thereby, we expected an increase in RMSSD and decrease in PEP. We expected respiration rates to go down and I/E to remain the same. In the AB tutorial this was taught by instructing participants to put their hands on their abdomen, flanking the navel, and during exhalation pull their navel in (by flexing the abdominal muscles) and release muscle tension and feel the abdomen expand naturally during inhalation. During the experimental phase participants were instructed to place their hands on their knees, while continuing the abdominal style of breathing. Thus participants breathed without receiving feedback from the hands during the intervention.

2.1.3.2. Thoracic breathing (TB)

The aim of the TB protocol to keep the locus of expansion in the chest area or to move it there if the diaphragm was naturally more involved. Through this exercise we expected an increase in sympathetic activity (PEP) and decrease in in parasympathetic activity (RMSSD). We expected respiration rates to remain the same or go up and I/E to remain the same. In the TB tutorial participants were instructed to put their hands on the side of their chest, flanking the breastbone, and breathe into their ribs, under their hands; and feel their ribs expanding and contracting. Again: during the experimental phase they were instructed to retain this breathing style, but with their hands on their knees.

2.1.3.3. Focused breathing (FB)

The FB protocol was designed as a semi-control condition, with the aim to control for attentional effects on autonomous nervous system activity and cognition. Participants were instructed to focus their attention on their breathing and be aware of the expansion and contraction of abdomen and chest, without changing anything in their breathing. We expected RR to remain the same or go down, as we hypothesize that paying attention to breathing slows down breathing (see Gerritsen & Band, 2018) while the I/E is expected to remain stable. During the FB tutorial participants were instructed to place their left hand on the abdomen and the right on the side of the ribs and proceed to experience movement of their hands during breathing, while paying attention to their breath. During the experimental phase this protocol was repeated again, but without the hand placement, in other words to just experience their breathing.

2.1.4. Stop-signal task

To represent inhibitory control SSRT was taken to indicate response inhibition - the inhibitory process in a race between go and stop processes according to the horse-race model - as measured by SST (Band, van der Molen, & Logan, 2003; Logan & Cowan, 1984). As the SST we used the same task as in a previous experiment (Gerritsen et al., 2020), constructed in E-prime 2.0. The task consisted of a practice block (9 trials) and 3 experimental blocks of 36 trials each. One third of trials were *stop trials* and two thirds *go trials*. Target stimuli were either 'X' or 'O' and had to be responded to by left or right hand (counter-balanced) as fast as possible. On *stop trials* a tone was sounded signaling participants to refrain from responding. The stop signal delay between target and stop signal was dynamically altered by staircase tracking procedure (30ms steps), which should result in a proportion of responding of ~50% (Ridderinkhof et al., 1999; Verbruggen & Logan, 2008). Participants were explicitly instructed to not wait for the tone, and feedback on screen prompted to not wait and speed up if responses were slow, after every trial. SSRT was obtained by the nth method. We follow the recommendations of Verbruggen and colleagues (2019). Because in 10 of the 29 participants at least 1 of the 4 measurements the p(respond) was lower than .25 or higher than .75, we had to exclude them from further analysis, resulting in n=19.

2.1.5. Physiological analysis

All physiological channels of every session were loaded into PhysioDataToolbox 0.50 (Sjak-Shie, 2021) from Acqknowledge 4.4.1 files (Biopac Systems, Inc, USA) and converted to .physioData file format. Two epochs were made per session: baseline and condition, of 5 min. each, as the recommended minimum for a reliable HRV measurement by the HRV Task Force (Malik et al., 1996). All pre-analyses were done by the dedicated PhysioDataToolbox analyzers.

2.1.5.1. Respiration

Respiration channels were analyzed for respiratory patterns by respiration analyzer (moving-mean of intercepts), with the following parameters: low-pass filter: 4 Hz; moving-mean multiplier: 2; min-max inhalation & exhalation duration: 1-20s; outlier filter threshold-span: 3.5s-100s; slope gradient threshold: 10%; smoothing window width: 5s. In about a quarter of epochs analyzer was unable to detect respiration peaks. For these, settings were changed to an inhalation minimum of 0.5s and outlier threshold of 5s. All signals of both respiration channels were checked for artefacts by eye. Peaks were removed on the basis of three criteria: 1) multiple peaks in one waveform, 2) large drift (lopsided) and 3) peaks detected in a flat curve (false positives from adjusted settings); as these resulted in under- or overestimation of RR or duration of inhalation vs exhalation. Mean inhalations, exhalations, breath duration and amplitude were extracted for further analysis for each band. Breath duration was computed to breaths-per-minute (RR). I/E was computed by dividing mean inhalation duration by mean exhalation duration. Note that, the duration is of either is relative and not absolute. The flat interval of the respiration curve is (arbitrarily) counted towards exhalation, as the analyzer times the start of inhalation by the rising curve. Changes in ratio are therefore the only analysis of interest. Tho/Abd ratio was determined by dividing the mean amplitude of the thoracic band by the mean amplitude of the abdominal band. Note that, the absolute value of respiration amplitude is in itself not an informative measure and it can vary widely even within-subjects, e.g. by the way it has been attached in a specific session. Therefore, it is only used to look for relative changes in the locus within a single session. But it has to be noted that even then other sources of change cannot be ruled out, such as band movement.

2.1.5.2. ECG

Heart rate (HR) and IBIs were obtained from the PhysioDataToolbox ECG analyzer, that automatically detects R-peaks, with the following settings: high-lowpass filter: 1-50 Hz; min. R-peak: 0.35 mV; min. R-R: 0.3s; min-max IBI: 0.3-2s. In 3 epochs (of 174) the amplitude of the R-peak was frequently too low to be detected. Here the R-peak min. was set to 0.3mV. All ECG signals were checked by eye and artefacts removed manually. This was always done by removing IBIs from the signal and caused by: 1) missing R-peak or 2) wrong peak detection (noise or missing R). HR and RMSSD were extracted for further analysis.

2.1.5.3. ICG

PEP (Newlin & Levenson, 1979) was obtained by ICG ensemble analyzer. The Q-point was manually selected in the ECG ensemble on the last downward peak before R (Q-peak) and B-point in the dZ/dt ensemble by picking the lowest point before the upwards slope towards C-peak. PEP is widely seen as a valid indicator of sympathetic tone (Berntson et al., 1994; Cacioppo et al., 1994). See Sherwood and colleagues (1990) for further details on ICG measures and analyses.

2.1.6. Statistical analyses

All analyses were done in JASP 0.15.0.0 with Bayesian counterparts of classic statistical tests. The models were always compared to null and the best-fitting model was deemed to have ample evidence for or against it if $3 < BF < 0.33$. If the top model was within these bounds no definitive statement can be made between it and null (underpowered sample). Two Bayesian repeated measures ANOVA analyses were performed as manipulation and sanity check, on Tho/Abd ratio (Time) for each session and HR respectively ($\text{session} \times \text{time}$). Main tests of our hypothesis were Bayesian repeated measures ANOVA ($\text{session} \times \text{Time}$) on RR, I/E, RMSSD, PEP, SSRT, Go RT and Go Accuracy, with *session order* as a covariate. If there are three models or less in the analysis, all BF are reported, if there are more only the most dominant three are reported, when necessary followed by an analysis of relative effect. Analyses of respiration was performed on Tho channel as this had the strongest signal and least noise. Difference scores (D-scores) were obtained for each of the main psychophysiological dependents: RR, I/E, RMSSD and PEP, by subtracting baseline score from intervention score (Dt1t2). A Bayesian correlation matrix was constructed, testing for a

relationship for any possible combination of these D-scores. If the session*time model was found to be dominant for the cognitive measure in the ANOVA, this matrix would be done a second time with SSRT for each time point, but only for the participants included in SST analysis.

2.2 Results

2.2.1. Respiration

2.2.1.1. Thoracic/abdominal ratio

Figure 1 shows Tho/Abd for all 3 interventions, from baseline to condition. Bayesian repeated-measures ANOVA [*time(2)*] of AB session with *session order(6)* as a covariate indicated *time* as the best-fitting model with $BF_{10}(time) = 58.9$, error % = 1.9; followed by $BF_{10}(time + session\ order) = 30.6$, error % = 2.5; and $BF_{10}(session\ order) = 0.53$, error % = 1.4. So there is very strong evidence for Tho/Abd going down during the condition measurement, in other words: locus of respiration moved towards the abdomen in AB. The Bayesian repeated-measures ANOVA [*time(2)+ session order(6)*] of TB session indicated *session order* as the best-fitting model with $BF_{10}(session\ order) = 1.4$, error % = 52.1; followed by $BF_{10}(time) = 0.26$, error % = 1.2; and $BF_{10}(time+ session\ order) = 0.18$, error % = 2.4. So there is evidence against Tho/Abd changing from baseline to the condition measurement and it is unclear whether order had an effect. It seems that locus of respiration remained thoracic in TB. Lastly, a Bayesian repeated-measures ANOVA [*time(2)+ session order(6)*] of FB session indicated *null* as the best-fitting model, followed by *time* $BF_{10}(time) = 0.71$, error % = 1.4; $BF_{10}(session\ order) = 0.47$, error % = 0.6; and $BF_{10}(time + session\ order) = 0.34$, error % = 1.1. There seems to be no clear evidence either for or against changes in Tho/Abd from baseline to condition in FB.

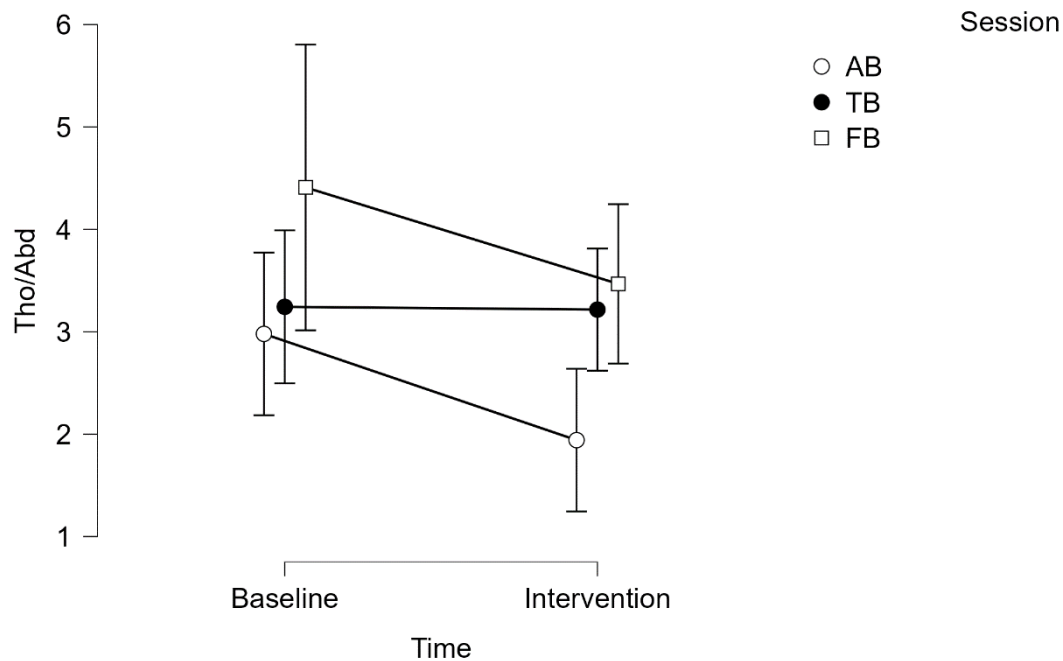


Figure 1: Ratio of thoracic amplitude / abdominal amplitude for all three conditions, between baseline and intervention measurement. AB = Abdominal breathing; TB = Thoracic breathing; FB = Focused breathing; Tho= Thoracic amp (mV); Abd = Abdominal amp (mV).

2.2.1.2. Respiration rate

Figure 2 shows RR development for all interventions. The Bayesian repeated-measures ANOVA [$session(3)*time(2)+session\ order(6)$] showed $session+time$ as the best-fitting model with $BF_{10}(session+time) = 2.5*e^{21}$, error % = 7.2, but is very closely followed by $time$: $BF_{10}(time) = 2.4*e^{21}$, error % = 1.7; and then $BF_{10}(time+session\ order) = 1.6*e^{21}$, error % = 3.0. Analysis of effects produces a $BF_{incl}(time)$ of infinity, a $BF_{incl}(session) = 0.85$ and $BF_{incl}(session\ order) = 0.50$. Clearly, RR goes down in all three conditions in the intervention phase, as there is extreme evidence for this main effect.

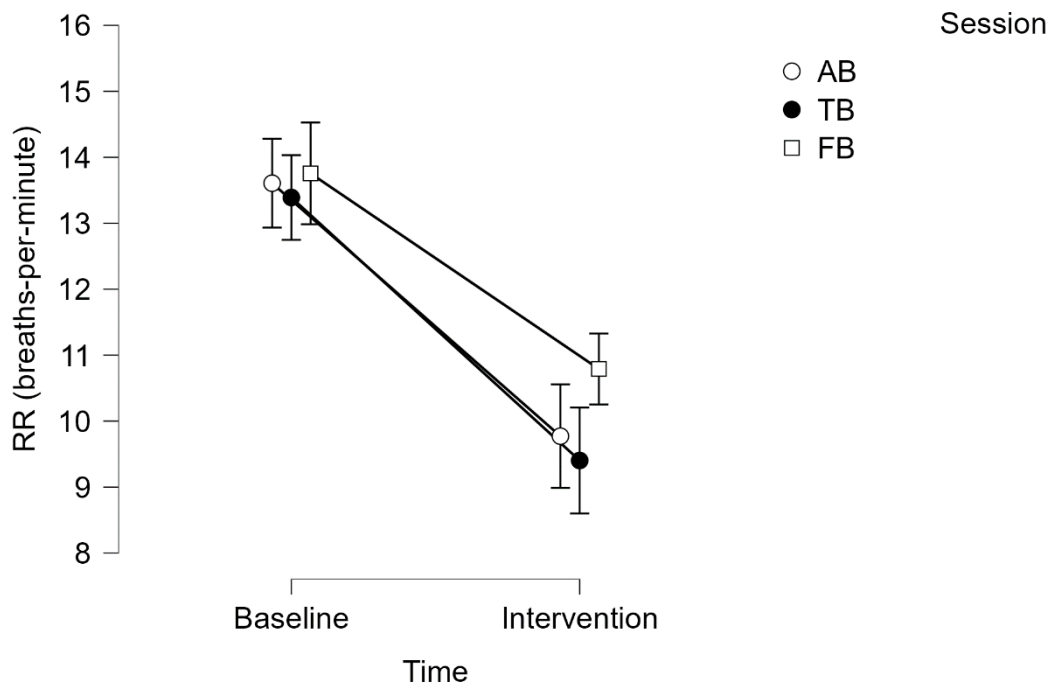


Figure 2: RR for all three conditions, between baseline and intervention measurement (in breaths-per-minute). RR = respiration rate.

2.2.1.3. Inhalation/exhalation ratio

Figure 3 shows the I/E ratio across conditions and measurement points. Repeated measures Bayesian analysis [$session(3)*time(2)+session\ order(6)$] again shows dominance of $BF(time) = 4.4*e^6$, error % = 0.8; followed by $BF(time+session\ order) = 1.8*e^6$, error % = 1.3; and $BF(session+time+session*time) = 1.3*e^6$, error % = 2.7. Analysis of effects shows a contribution of $BF_{incl}(time) = 4.4*e^6$, a $BF_{incl}(session) = 0.37$ and $BF_{incl}(session*time) = 0.93$. In all three conditions participants increased inhalation as compared to exhalation duration, from baseline to intervention.

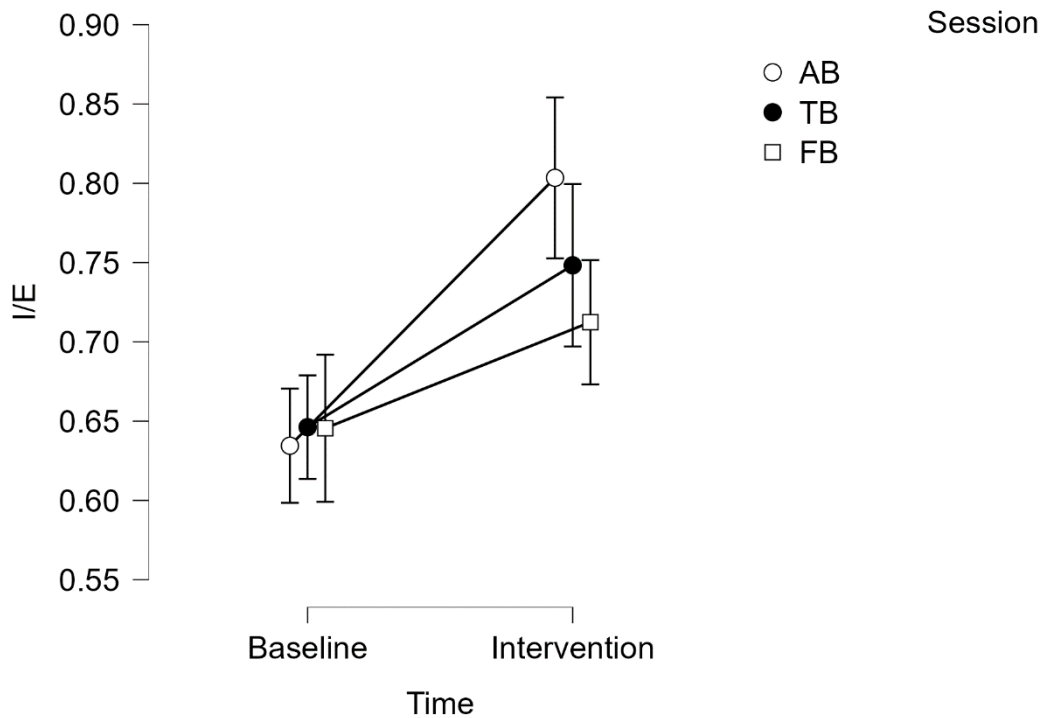


Figure 3: I/E ratio for all three conditions, between baseline and intervention measurement. I/E = inhalation duration (ms) / exhalation duration (ms).

2.2.2. ECG

2.2.2.1. Heart rate

As a sanity check HR was analyzed by Bayesian repeated measures ANOVA [$session(3)*time(2)+session\ order(6)$]: *null* was dominant, followed by $BF_{10}(session\ order) = 0.54$, error % = 2.3; $BF_{10}(time) = 0.23$, error % = 1.2; and $BF_{10}(time+session\ order) = 0.13$, error % = 2.9. HR did not change from baseline to condition measurements points. It is unclear whether session order had an effect on HR.

2.2.2.2. Heart rate variability

Figure 4 shows RMSSD per condition developing in time. A Bayesian repeated measures ANOVA [$session(3)*time(2)+session\ order(6)$] indicated that here also *null* was the strongest model. The next model was that of *time*: $BF_{10}(time) = 0.95$, error % = 2.9; then $BF_{10}(session\ order) = 0.60$, error % = 0.6; thirdly, $BF_{10}(time+session\ order) = 0.54$, error % = 1.2. Counter to expectation there was no evidence that HRV was affected by any intervention.

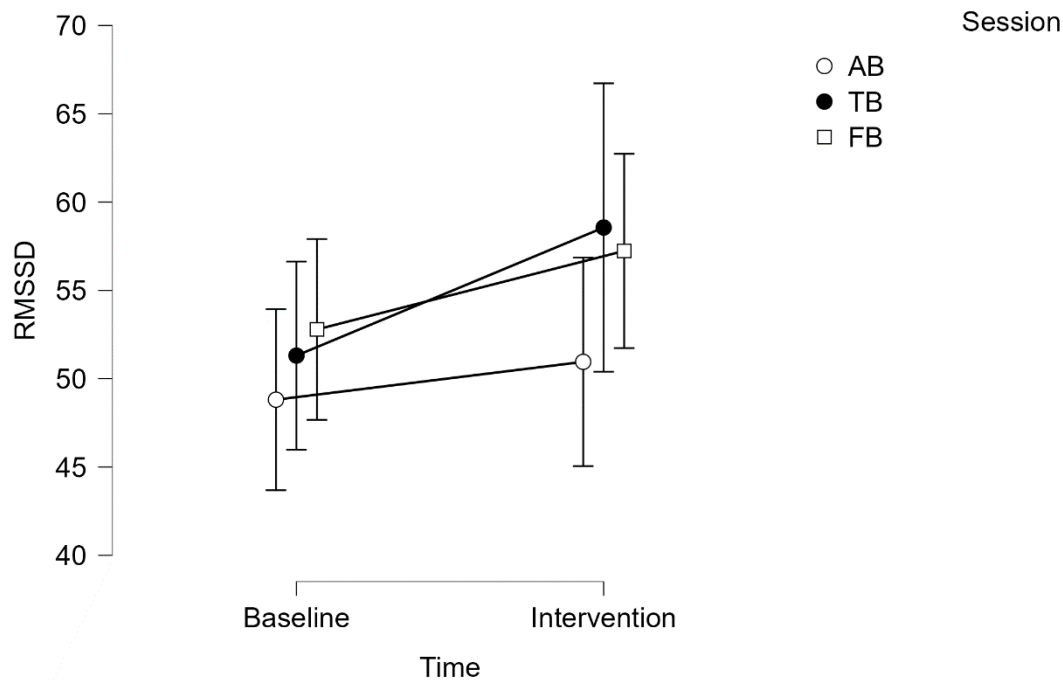


Figure 4: RMSSD for all three conditions, between baseline and intervention measurement. RMSSD = root mean square of successive differences.

2.2.3. ICG

2.2.3.1. PEP

Figure 5 shows changes in PEP from baseline to condition, for each condition (in ms). The Bayesian repeated measures ANOVA [$session(3)*time(2)+session\ order(6)$] did find evidence for effects. The fittest model being: $BF_{10}(session+time+session*time) = 1.2*e^6$, error % = 31.7; then $BF_{10}(session+time+session*time+session\ order) = 5.5*10^5$, error % = 3.3; thirdly, $BF_{10}(session+time) = 2.1*10^3$, error % = 1.6. Analysis of effects showed the contribution of $BF_{incl}(session) = 3.7*10^5$; $BF_{incl}(session*time) = 1.1*10^3$; $BF_{incl}(time) = 387.0$; and $BF_{incl}(session\ order) = 0.45$. Though error is very high it seems there is extreme evidence for the complete model. Figure 4 shows that AB starts out relatively low in sympathetic activity, but this goes markedly up during intervention (PEP going down). While sympathetic activity during TB and FB seems unaffected, if anything it goes up in TB (PEP going down). This pattern of results is the opposite of our predictions.

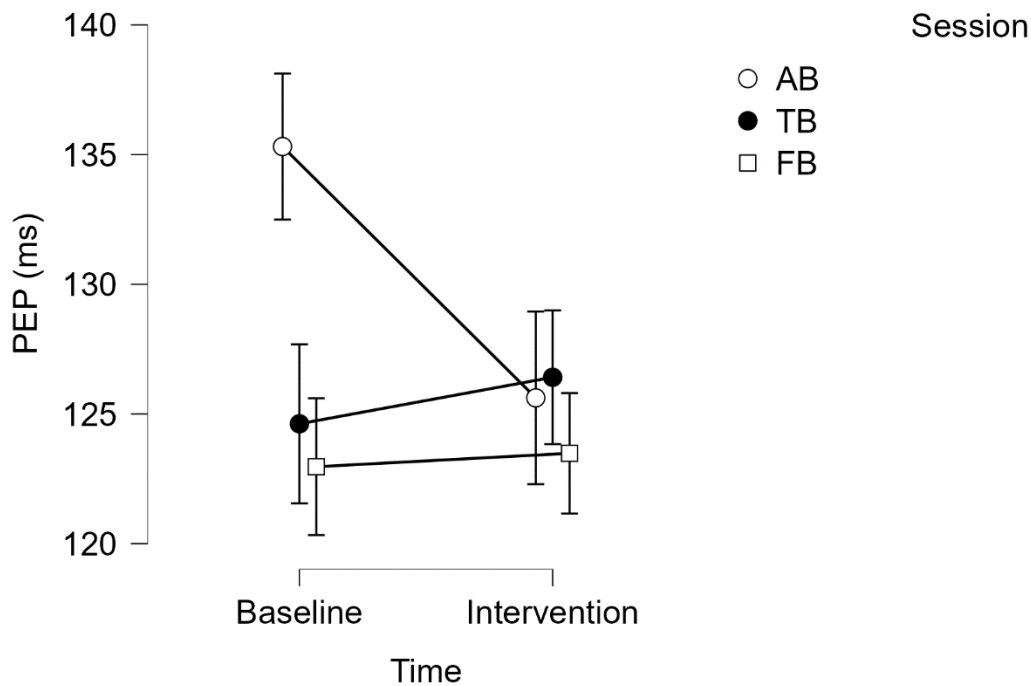


Figure 5: PEP for all three conditions, between baseline and intervention measurement. PEP = pre-ejection period.

2.2.4. Stop-signal task

Bayesian repeated-measures ANOVA [$time(4) + session\ order(6)$] of Go trial accuracy indicated *null* as fittest model. There is strong evidence that both *time* and *time+session order* have no effect on Go accuracy. The same analysis on Go RT, produces similar results: $BF_{10}(session\ order) = 1.05$, error % = 1.9; $BF_{10}(time+session\ order) = 0.32$, error % = 22.1; and $BF_{10}(time) = 0.22$, error % = 0.6. There is evidence that neither *time* nor the complete model explains Go RT results.

Figure 6 shows SSRT across the four conditions. Bayesian repeated-measures ANOVA [$time(4) + session\ order(6)$] indicated the complete model as fittest $BF_{10}(time+session\ order) = 1.9$, error % = 1.3; followed by $BF_{10}(time) = 1.9$, error % = 0.4; and lastly $BF_{10}(session\ order) = 0.93$, error % = 0.8. There is insufficient evidence that *time* had an effect on SSRT in either direction, which is not surprising as 10 participants were excluded because of waiting: $p(\text{respond}) < .25$, and thus the sample was underpowered. However, looking at Figure 6 a pattern emerges where SSRT goes down across conditions from baseline, but markedly after AB.

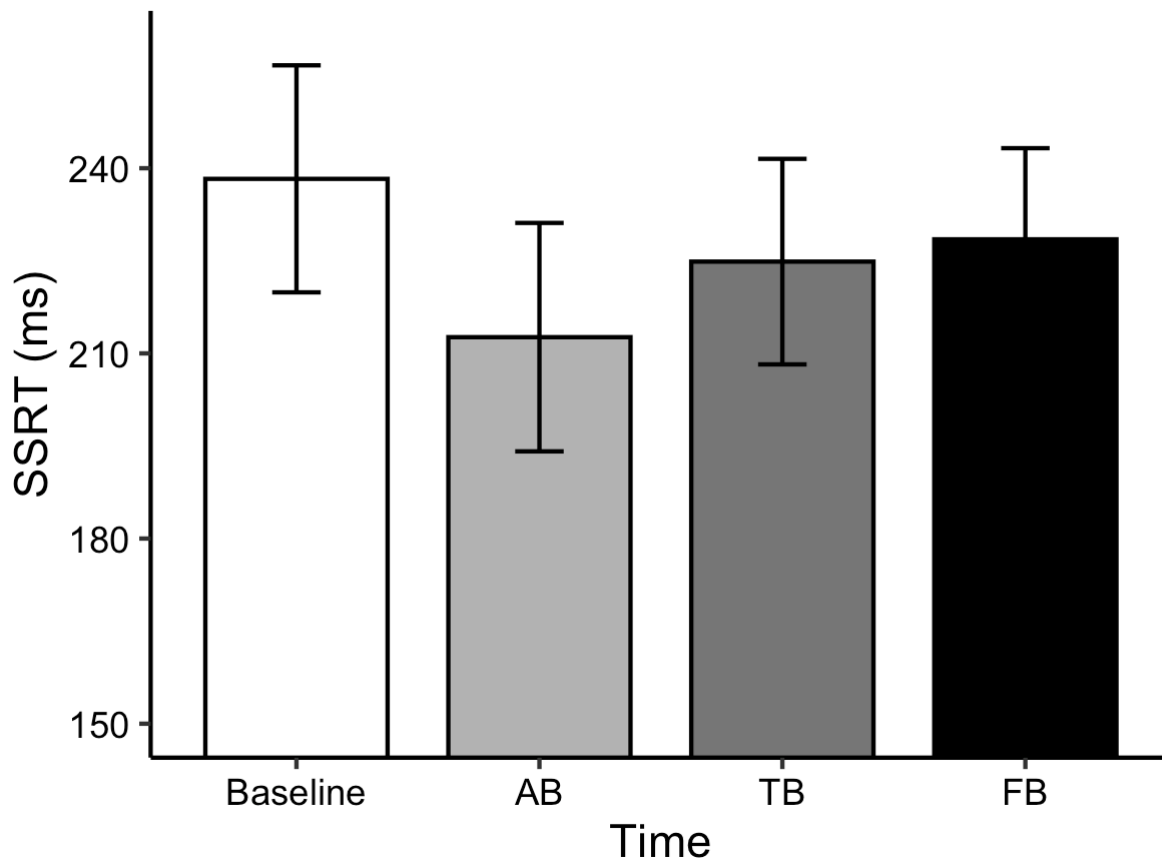


Figure 6: *SSRT across the four measurements: baseline (first session) and after AB, TB, FB. SSRT = stop-signal reaction time in ms.*

2.2.5. D-score correlations

See Appendix B Table 1 for the complete matrix. Only four of the possible correlations had a $BF_{10} > 10$. All of which were uninformative, for example: correlations of RR D-scores between sessions. An important note: there was no relation found between respiration and RMSSD D-scores, which was unexpected.

3. Experiment 2

3.1. Method

If not reported, the methodology is the same as in Experiment 1. Only deviations from the design and procedure are mentioned in this section.

3.1.1. Participants

In total 41 participants were recruited. The first 3 participants were dropped because the ECG was not set up correctly (parameter settings). Another 4 had to be dropped because the ECG signal was lost during the session (at a measurement point), making it unusable for analysis and interpretation. This left N=34 for analysis, including 3 males (M=25.3, 23-28) and 31 females (M=20.7, 18-28). Next to the prescriptions described above, participants were given 180 ml of water before each session and not allowed to drink anything during the session, with the aim to level effects of hydration on HRV (Heathers et al., 2018).

3.1.2. Procedure

3.1.2.1. Design

Participants completed only two sessions: AB and TB. Each session took ~75 minutes.

3.1.2.2. Physiological measures

Participants were equipped with 3 ECG and 2 EDA electrodes (on the left hand), after skin preparation. Two respiration bands were used in the same way as described above.

3.1.2.3. Testing

The experiment started with the baseline resting state physiological measurement of respiration, ECG and EDA (~5 min); followed by the pre-intervention affect grid and Simon task. Then the intervention commenced, during which condition physiological measurements (reactive) were taken. Another resting state respiration, ECG and EDA followed (recovery), after which the post-intervention affect grid and Simon task finished the testing phase.

3.1.3. Interventions

AB and TB scripts were rewritten and recorded by the first author, using another voice-actor as audio guide. The main change was that AB protocol now explicitly emphasized slower breathing than normal and TB protocol emphasized slightly speeded breathing. This

was also reflected in the pace and tone of the voice recording. See Appendix A for the complete transcript.

3.1.4. Behavioral measures

3.1.4.1. Affect grid

The affect grid (Russell et al., 1989) was taken immediately pre- and post-intervention to assess self-reported changes in arousal and pleasure (positive-negative affect). It consists of two dimensions on the axes of a 9x9 grid, where a participant places a marker on the grid to signal current state. The middle of the grid, center square, reflects a neutral stance, while moving down or left moves to unaroused or negative and moving up or right adjusts to aroused or positive, respectively.

3.1.4.2. Simon task

The Simon effect – interference scores of conflicting stimulus spatial dimensions on stimulus and response in accuracy and RT – was taken as indicator of cognitive inhibition. Simon task (Simon, 1969) consisted of a practice block of 40 trials and 3 testing blocks of 60 trials each. Trials started with a centered black fixation square presented for 1000ms (against the light grey background). Then either a blue or green circle target appeared to the left or right of fixation for 1500ms or until a response was made. Participants had to respond to the color of the stimulus, ignoring the location, with either Q or P keys (counter-balanced). Accuracy and reaction time data is compared for congruent and incongruent trials, where congruent trials are defined as corresponding location of stimulus and response (e.g. left on screen; Q response) and incongruent trials as juxtaposed positioning (e.g. left on screen; P response). Note that stimulus location is irrelevant to the task goals. The interference here (Simon effect) is an operationalization of cognitive inhibition. Each session had a pre-test and post-test Simon task. Pre-test followed baseline physiology and preceded affect grid pre-test; post-test followed post-test affect grid.

3.1.5. Physiological analyses

PhysioDataToolbox 0.6.1 was used instead. Three epochs of 5 min. were analyzed for each channel: baseline resting state, during the intervention (reactive) and resting state post-intervention (recovery).

3.1.5.1. Respiration

Inhalation min. and max. were set to 0.5 and outlier threshold of 5s for all epochs.

3.1.5.2. ECG

Identical to Experiment 1.

3.1.5.3. EDA

EDA channel was checked for quality of signal. No changes were made. Skin conductance level (SCL) was obtained per epoch.

3.1.6. Statistical analyses

Almost identical to Experiment 1, apart from SCL substituting for PEP and Simon effects replacing SSRT. Furthermore, a second D-score (Dt2t3) was calculated between measurement point 2 and 3, by subtracting the intervention values from the recovery values.

3.2. Results

3.2.1. Respiration

3.2.1.1. Thoracic/abdominal ratio

Figure 7 shows Tho/Abd for the 2 interventions, from baseline to recovery. Bayesian repeated-measures ANOVA [*time(3)*] of AB session with *session order(2)* as a covariate indicated *time* as the best-fitting model with $BF_{10}(time) = 12.5$, error % = 0.7; followed by $BF_{10}(time + session\ order) = 4.6$, error % = 2.3; and finally *session order*: $BF_{10}(session\ order) = 0.34$, error % = 1.8. There is strong evidence that the relative amplitude of Tho and

Abd changes across AB measurements. As seen in Figure 7, Abd locus increases during intervention and recovers during recovery to higher Tho locus.

The Bayesian repeated-measures ANOVA [$time(3)+session\ order(2)$] analysis of TB session had *null* as dominant, followed by *session order*: $BF_{10}(session\ order) = 0.72$, error % = 1.7; followed by $BF_{10}(time) = 0.46$, error % = 0.8; and finally $BF_{10}(time + session\ order) = 0.34$, error % = 1.5. For TB, there is no evidence for any effect of any of the factors.

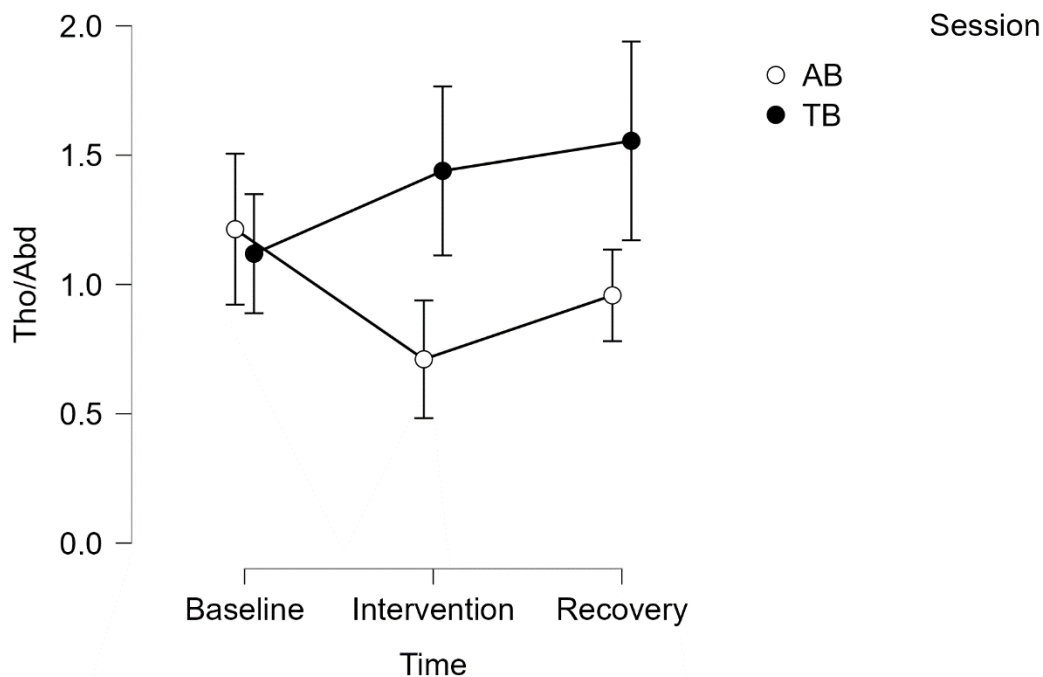


Figure 7: Ratio of thoracic amplitude (mV) / abdominal amplitude (mV) for session AB and TB, across the 3 measurement. Tho=Thoracic amp; Abd=Abdominal amp.

3.2.1.2. Respiration rate

Figure 8 shows RR development for both sessions. As a manipulation check a Bayesian repeated-measures ANOVA [$session(2)*time(2)+session\ order(2)$] analysis was performed, It showed the complete model as best-fitting: $BF_{10}(session+time+session\ order+session*time) = 5.1*e^{22}$, error % = 2.7; followed by $BF_{10}(session+time+session*time) = 3.5*e^{22}$, error % = 2.1; and thirdly by all main effects: $BF_{10}(time+session+session\ order) = 1.3*e^6$, error % = 2.3. In the analysis of effects all BF_{incl} are infinity, except for $BF_{incl}(session\ order) = 1.5$. Looking at Figure 8 and the extreme evidence for the complete model it can be concluded that RR goes down during the intervention phase in AB, in contrast to TB where it

goes up. In both sessions it returns towards baseline during the recovery phase. Clearly, participants followed the instructions and performed according to expectation in Experiment 2, as compared to Experiment 1. However, note that, in AB even during intervention the “golden” RR of 6 breaths-per-minute (van Diest et al., 2014) is not reached on average, by far.

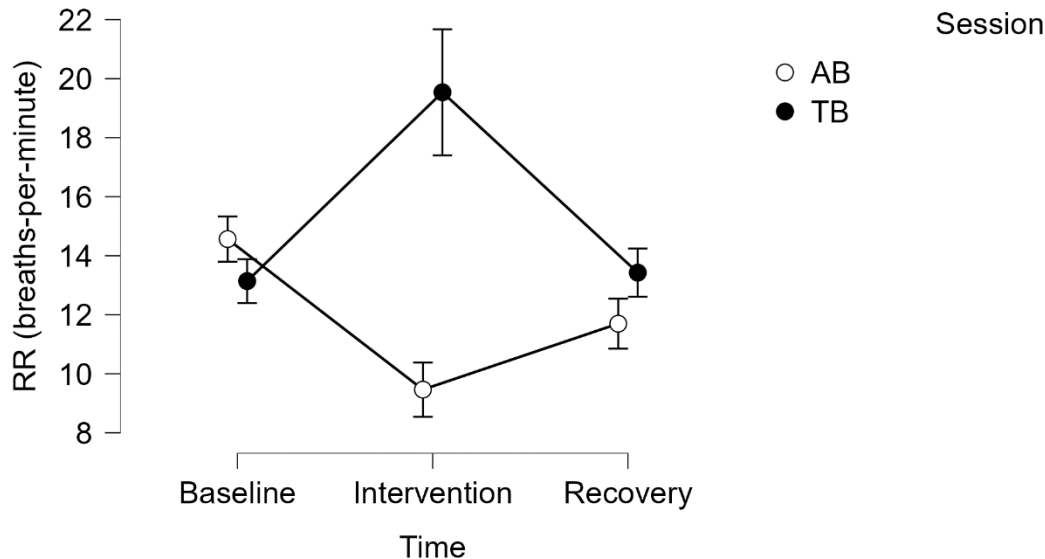


Figure 8: RR for both sessions, across measurement points (in breaths-per-minute). RR=respiration rate.

3.2.1.3. Inhalation/exhalation ratio

Figure 9 shows the I/E ratio, for the three measurement points baseline, condition and recovery for each session. A Bayesian repeated-measures ANOVA

[*session(2)*time(2)+session order(2)*] analysis indicated as best-fitting:

$BF_{10}(session+time+session*time) = 1.7*e^6$, error % = 1.8; followed by the complete model:

$BF_{10}(session+time+session\ order+session*time) = 4.9*10^5$, error % = 4.2; and then *time*:

$BF_{10}(time) = 4.7*10^6$, error % = 6.3. The analysis of effects gives the strongest contribution of

time: $BF_{incl}(time) = 1.1*e^6$; then *session*time*: $BF_{incl}(session*time) = 9.6$; thirdly *session*

$BF_{incl}(session) = 2.7$; and finally: $BF(session\ order): 0.29$. Evidently, ratio changes across

measurement where inhalation length goes up during both interventions, though more strongly for TB, and goes down again in recovery.

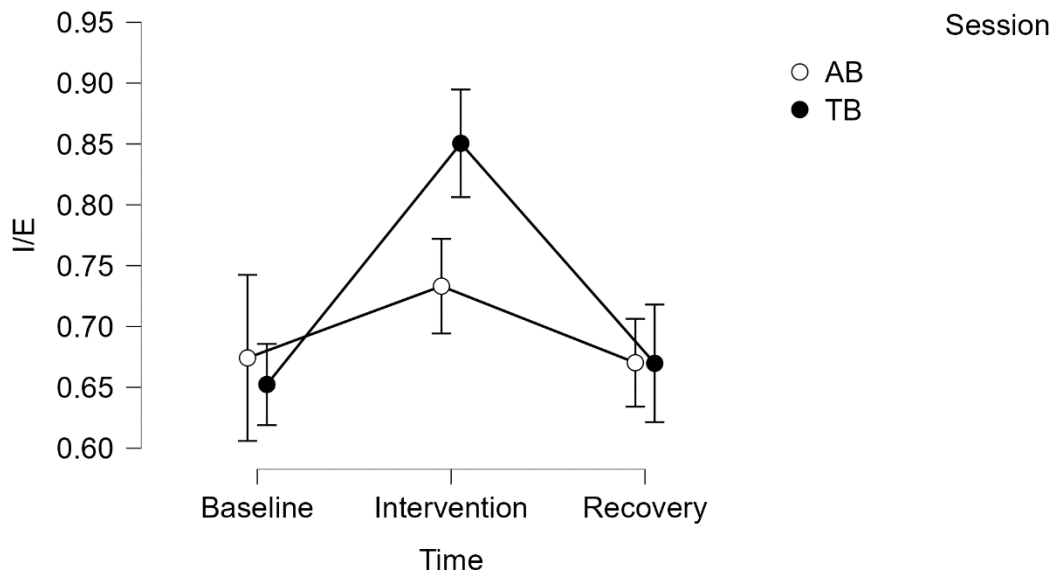


Figure 9: I/E ratio for AB and TB, from baseline to recovery measurement. I/E ratio = inhalation duration (ms) / exhalation duration (ms).

3.2.2. ECG

3.2.2.1. Heart rate

Heart rate was analyzed by Bayesian repeated measures ANOVA [$session(2)*time(2)+session\ order(2)$]. The model with main effects *session* and *time* was dominant: $BF_{10}(session+time) = 747$, error % = 1.6; followed by all main effect model: $BF_{10}(session+time+session\ order) = 379$, error % = 3.7; and thirdly: $BF_{10}(session+time+session*time) = 116$, error % = 2.3. Analysis of effects showed a relative higher contribution of *session*: $BF_{incl}(session) = 55.1$; than *time* $BF_{incl}(time) = 11.8$. Clearly, heart rate is higher during the TB than AB session and it goes up during both interventions, going down again during recovery. Apparently, both interventions are taxing. See Figure 10 for a visualization.

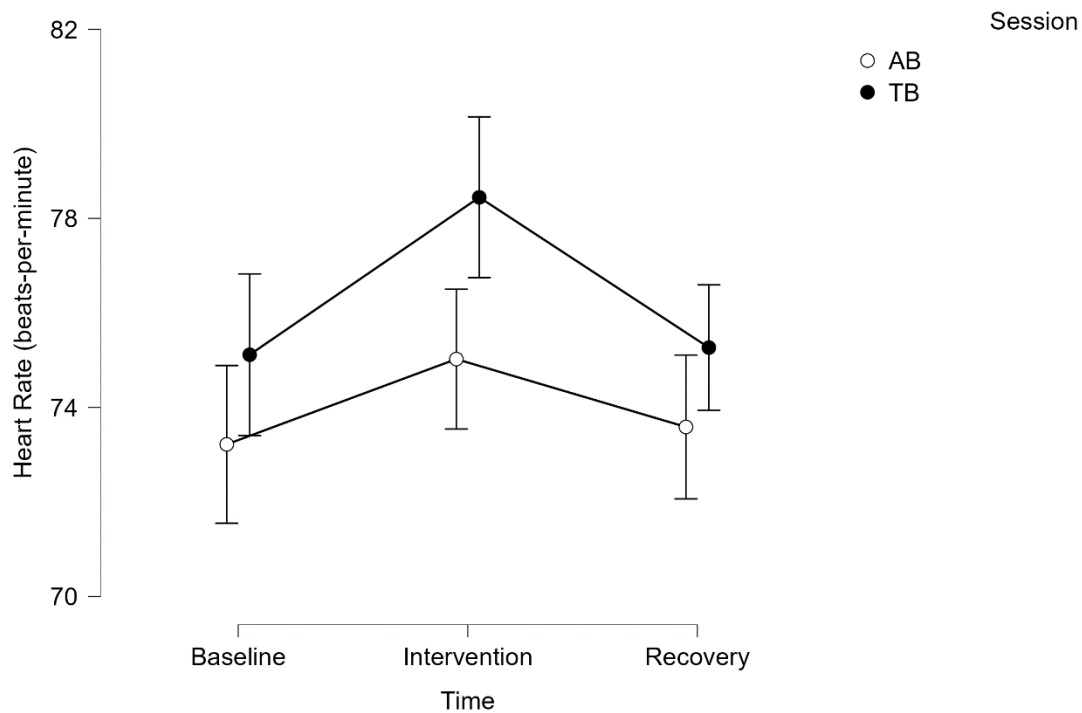


Figure 10: Heart rate (in beats-per-minute) for both session, between baseline and recovery measurement.

3.2.2.2. Heart rate variability

RMSSD was analyzed by Bayesian repeated measures ANOVA [$session(2)*time(2)+session\ order(2)$], see Figure 11. The model with only the main effect *session* was dominant: $BF_{10}(session) = 55.5$, error % = 7.1; followed by: $BF_{10}(session+session\ order) = 24.6$, error % = 2.6; and thirdly: $BF_{10}(session+time) = 13.2$, error % = 1.7. An analysis of effects only showed a significant contribution of *session*: $BF_{incl}(session) = 38.1$. Our expectations regarding HRV changes were not met. It seems that overall RMSSD was higher in the AB than TB session, even before intervention. In Figure 11 a dip can be seen in RMSSD during intervention, but this is not reflected in the analysis by an interaction effect.

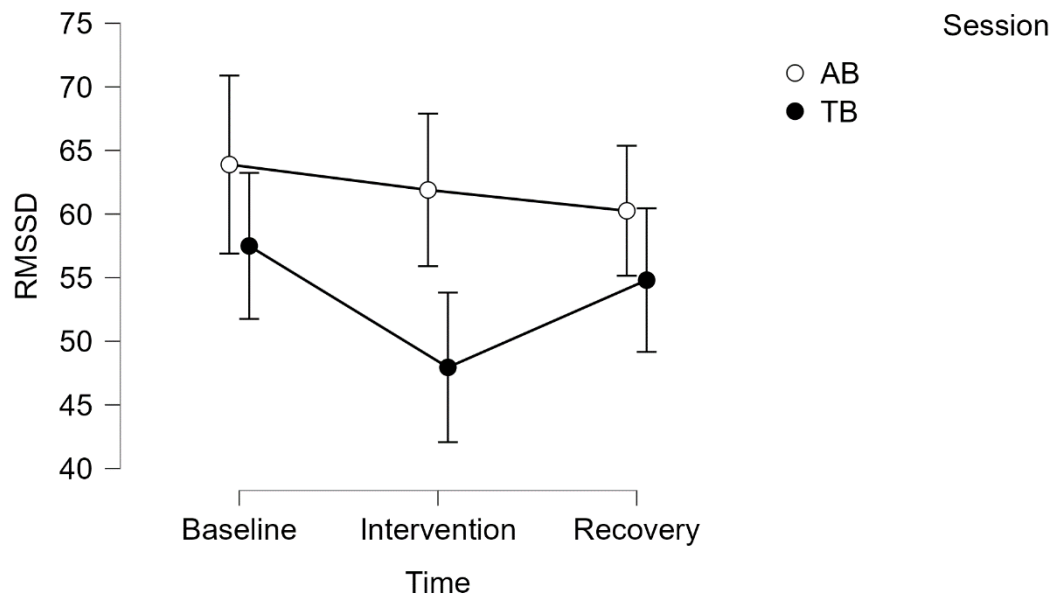


Figure 11: *RMSSD for the two sessions, between baseline and recovery measurement. RMSSD = root mean square of successive differences.*

3.2.3. EDA

3.2.3.1 SCL

See Figure 12 for a visualization of SCL mean development. A Bayesian repeated-measures ANOVA [$session(2)*time(2)+session\ order(2)$] analysis indicated as best-fitting the *null*, followed by: $BF_{10}(session\ order) = 0.77$, error % = 2.2; and thirdly: $BF_{10}(time) = 0.17$, error % = 1.0. Expectations on stress development were also not met. Although Figure 12 suggests an increase in SCL during the TB session and after intervention for AB session, there is actually clear evidence against all effects apart from session order and extreme evidence against this interaction: $BF_{incl}(session*time) = 0.008$.

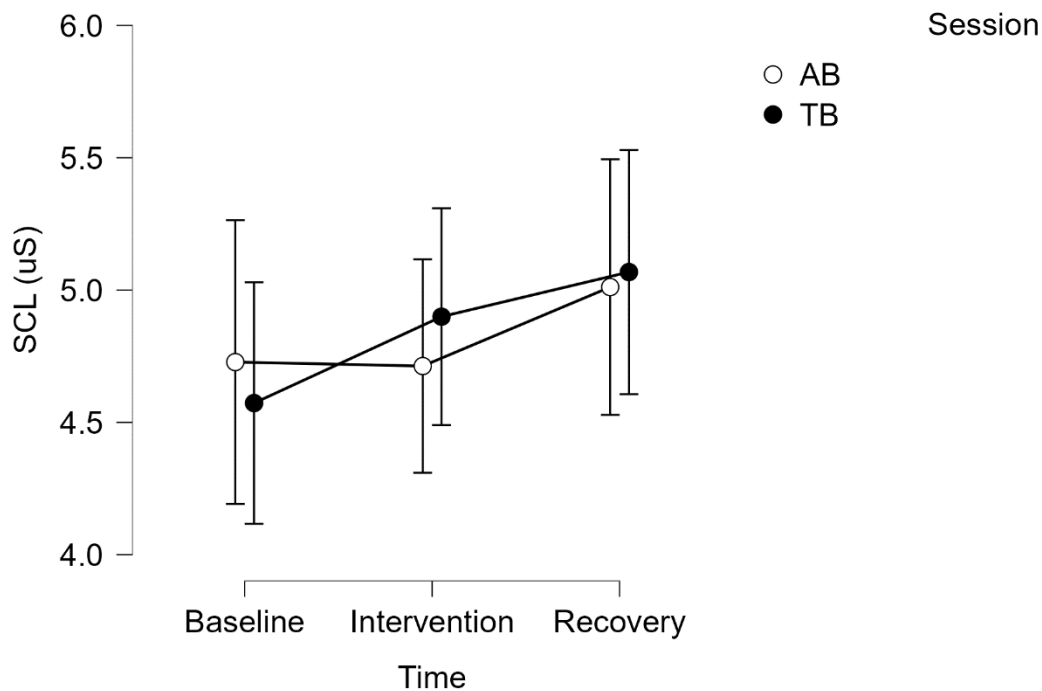


Figure 12: SCL for the two sessions, between baseline and recovery measurement. SCL = Skin conductance level in microSiemens.

3.2.4. Behavioral measures

3.2.4.1. Affect grid

Figure 13 shows plots for the self-reported arousal and pleasure components of the affect grid, during both sessions. Bayesian repeated-measures ANOVAs [$session(2)*time(2)+session\ order(2)$] were performed for both dimensions.

In arousal the analysis indicated $BF_{10}(session+time) = 8.3*10^3$, error % = 4.2 as best-fitting; followed by: $BF_{10}(session+time+session*time) = 3.8*10^3$, error % = 2.0; then thirdly: $BF_{10}(session+time+session\ order) = 2.5*10^3$, error % = 2.6. The major contribution according to analysis of effects here is *time*: $BF_{incl}(time) = 2.7*10^3$; with a moderate effect of *session*: $BF_{incl}(session) = 3.7$. Clearly, arousal goes down after the intervention and there might be an overall lower arousal level in AB, as compared to TB, that according to the second model might be because of the interaction effect: after AB arousal goes down more than after TB.

For pleasure the most dominant model was $BF_{10}(session+time+session*time) = 2.4$, error % = 3.1; followed by the complete model $BF_{10}(session+time+session\ order+session*time) = 0.9$, error % = 3.3. This does not merit any conclusion regarding these

effect, as the sample is underpowered. However, when looking at the analysis of effects and Figure 13 an interaction effect can be seen: $BF_{\text{incl}}(\text{session}*\text{time}) = 5.3$. There is slight indication that AB produces pleasure, while TB diminishes it.

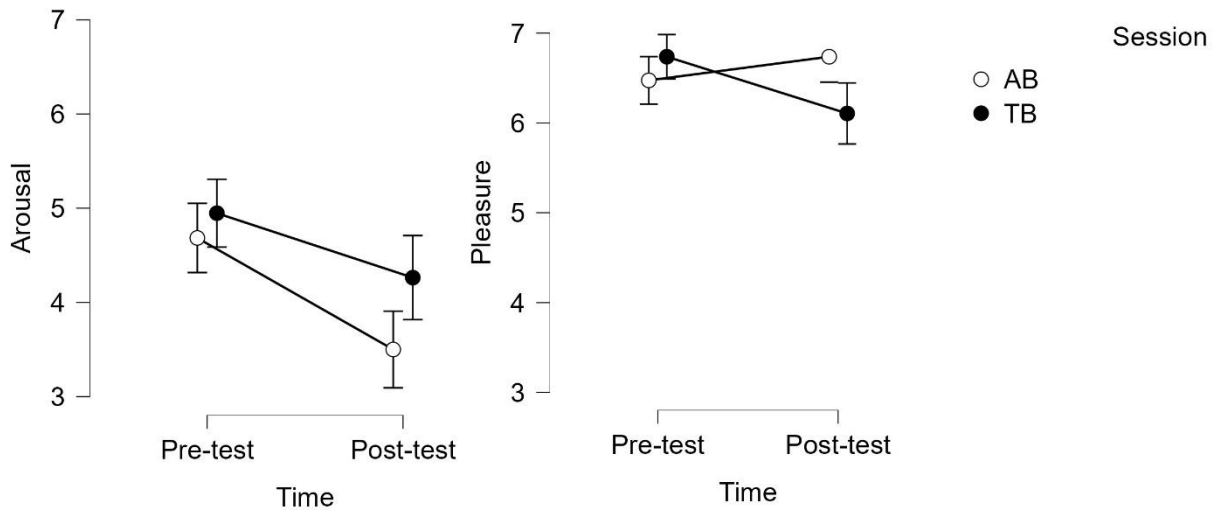


Figure 13: Affect grid dimensions for arousal (left) and pleasure (right) for both sessions, pre-test to post-test.

3.2.4.2. Simon task

3.2.4.2.1. Accuracy

A Bayesian repeated-measures ANOVA [$\text{session}(2)*\text{time}(2)*\text{congruency}(2)+\text{session order}(2)$] was performed on the accuracy data of the Simon task. The dominant model was: $BF_{10}(\text{session}+\text{time}+\text{congruency}+\text{session}*\text{time}) = 1.3*10^4$, error % = 3.0; then by: $BF_{10}(\text{time}+\text{congruency}) = 7.1*10^3$, error % = 2.0; and closely followed by: $BF_{10}(\text{congruency}) = 6.8*10^3$, error % = 1.0. Analysis of effect shows the dominant factor to be *congruency*: $BF_{\text{incl}}(\text{congruency}) = 4.1*10^3$. Figure 14 plots these accuracy means. Clearly, congruency had an effect where overall congruent trials were more accurate. The dominant model and Figure 14 also suggest an interaction effect, where after TB accuracy goes down, while after AB accuracy seems unaffected or even goes slightly up for congruent trials.

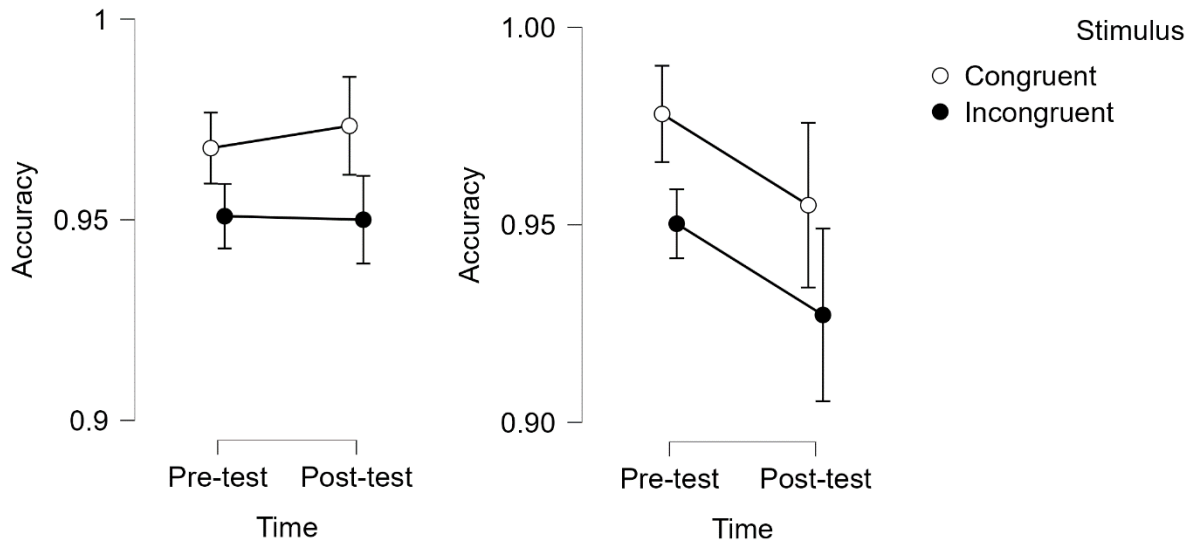


Figure 14: Simon task accuracy data. Left graph Abdominal session and right graph Thoracic session.

3.2.4.2.2. Reaction time

A Bayesian repeated-measures ANOVA [$session(2)*time(2)*congruency(2)+session\ order(2)$] was performed on the reaction time data. The dominant model was:

$BF_{10}(congruency) = 2.3*e^6$, error % = 0.9; followed by: $BF_{10}(congruency+session\ order) = 1.8*e^6$, error % = 2.7; and then by: $BF_{10}(session+congruency) = 4.7*10^5$, error % = 1.3. Also here, analysis of effect showed the single dominant factor to be *congruency*: $BF_{incl}(congruency) = 3.8*e^7$. Figure 15 visualizes this data. Evidently, only congruency had an effect on reaction times, where congruent trials were faster than incongruent trials across all conditions.

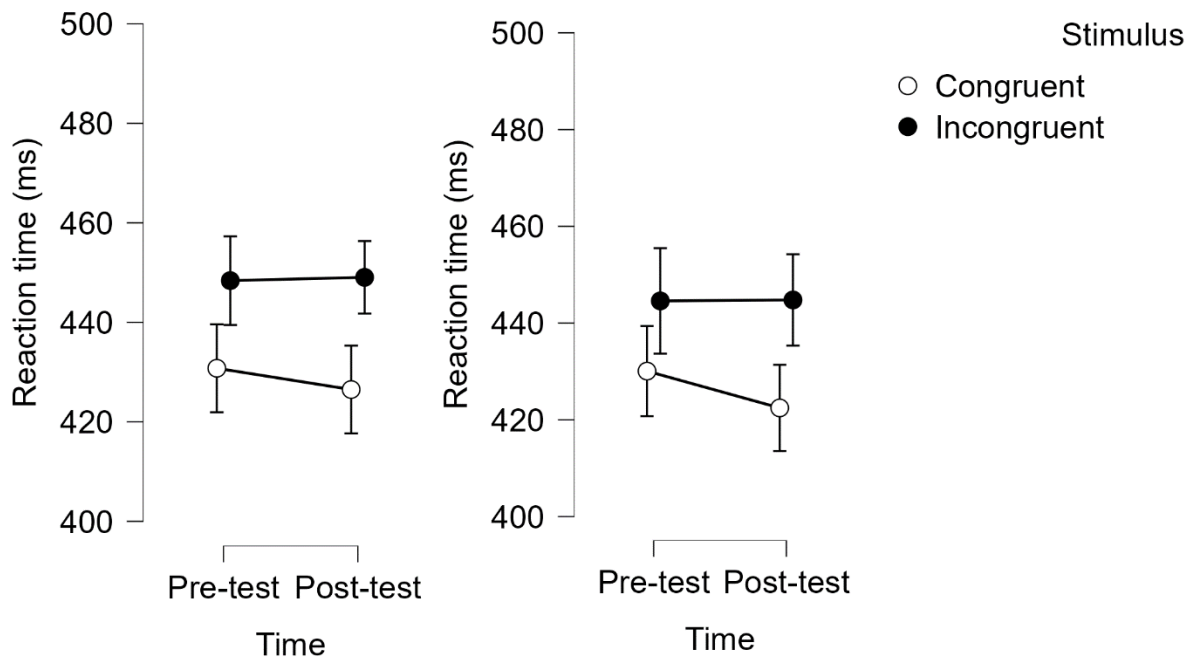


Figure 15: Simon task reaction time data. Left graph Abdominal session and right graph Thoracic session.

3.2.5. D-score correlations

See Appendix B Table 2 for the full matrix of both Dt1t2 and Dt2t3 scores. Seven of the possible correlations had a $BF_{10} > 10$, all of which were equally uninformative as in Experiment 1, for example: correlations of RMSSD D-scores within a session. Also here, not a single correlation was found between respiration and RMSSD D-scores, violating expectation.

4. Discussion

For this study we set out to test predictions of the rVNS model (Gerritsen & Band, 2018) onto cognition; more specifically the indirect influence of the locus of breathing. It was predicted i) that abdominal as compared to thoracic breathing would lead to relatively more activity of the parasympathetic and less of the sympathetic nervous system, as reflected in HRV and PEP/SCL respectively; ii) that increased parasympathetic activity would be associated with more efficient inhibitory control, as reflected in the speed of response inhibition and the ability to resist Simon interference. Overviewing the results of both experiments, we can safely conclude that these predictions have not been met.

In Experiment 1, instructions emphasizing thoracic or abdominal breathing were not accompanied by a relative and divergent change in RR. In all three conditions, including semi-control FB, RR went down: participants slowed down their breathing during any intervention. This violated the rationale behind the expectation that these manipulations could induce a parasympathetic dominated abdominal condition versus a sympathetically dominated thoracic condition. In our view, shifting the locus towards the abdomen would signal a more relaxed state, making RR go down and this would lead to a higher RMSSD and thus vagal tone.

Then, with slow-paced breathing present in each condition, our expectation would be that RMSSD would go up during all interventions. Surprisingly, there was no main effect of condition on RMSSD. So, not only was the expected interaction effect on RMSSD of *session*time* absent, vagal tone did not go up at all during any intervention, which would be expected from previous studies due to a slowing of breathing. Do note that, though RR went down, it did not reach the golden rate of 6 breaths-per-minute (van Diest et al., 2014): both AB and TB went down to 9-10, while FB went down to 11. Simply stated, breathing pace might have not gone down low enough to produce effects on vagal tone.

Similarly, sympathetic tone also showed a markedly different pattern than hypothesized. During AB sympathetic tone went up (PEP decreased) instead of down from baseline levels, as compared to TB and FB. Though do note that, this was from a higher baseline level and PEP seemed to normalize during AB (see Figure 5). Apparently, participants' stress levels were not comparable at the start of the three

sessions, though the design was within-subjects and sessions counter-balanced in order. Perhaps, the participants expected abdominal breathing to be relaxing and showed anticipatory relaxation effects. But this was followed by a manipulation that is somewhat stressful itself, at least for beginners, that drove down PEP. Another possibility, is that manual picking of Q-point and R-point was systematically different and thus temporarily skewed for one session over the others, making these results an artefact. Either way, it is safest to not connect definitive conclusions to the PEP findings in Experiment 1.

The predicted differential effects on cognition were also not observed in the SST. This might be expected from the respiration and vagal tone results. However, we might still expect differences in response inhibition or overall reaction time, as there are divergent effects on sympathetic tone. It has to be noted that a numerical trend in the predicted direction was observed for SSRT. The baseline measurement had the slowest response, followed by FB, TB; and lastly and most markedly faster: AB. In other words, there is a slight suggestion that participants were a bit faster at stopping after the AB intervention. A reason why the interaction model did not meet a sufficient evidence level (Bayesian factor above 3) might be that the sample was severely underpowered. Of the original sample, 10 of the 29 participants had to be excluded. Because they waited for the stop signal, regardless of repeated verbal instructions and visual prompts to not do so. Also, note that the results are difficult to interpret as there was only a baseline measurement at the first session.

In explaining the unpredicted pattern of results, there is no reason to question the compliance. The instructions aimed at manipulating the locus of breathing seem to have been followed, as Tho/Abd went down during AB, while it remained the same during TB. In Experiment 1, explicit instructions to change RR were absent. However, the pace and rhythm of the (sparse) audio instruction were similar for all three conditions: a slower pace than in a conversation and in a relaxed tone of voice. Unconscious imitation of these non-verbal cues may have resulted in RR going down across all breathing conditions in Experiment 1. To address abovementioned issues and to retest the predictions, Experiment 2 was set up.

The main change of design in Experiment 2 was in the interventions. The scripts for audio guidance were rewritten and added explicit instructions to contrast AB and TB in breathing pace. The FB session was dropped. As the cognitive task,

SST was replaced by the Simon task for multiple reasons. SSRT is not very sensitive to enhanced inhibition (Wöstmann et al., 2013). Furthermore, participants have to be excluded from the analysis if they deviate from the instructions aimed at approximately 50% stop success, as often happened in Experiment 1. Lastly, interference costs in Simon task are also viewed as a more valid measure of (cognitive) inhibition (Hommel, 2011). Another addition was the affect grid: a self-report measure of arousal and pleasure. As the final change, another physiological resting state measurement was introduced after the intervention (recovery), to test for wipe-out of possible RMSSD increases and thus transfer of a state of mental flexibility to the cognitive task.

However, Experiment 2 also did not show the predicted interaction effect on any of the main measures: RMSSD, SCL and Simon effect. Experiment 2 did pass the manipulation checks, so compliance was also not the issue here. As expected, Tho/Abd decreased during AB intervention, meaning participants shifted their breathing towards their abdomen. Furthermore, RR went up during TB and down during AB, then returning to baseline in recovery measurement. In other words, participants breathed at a slower pace during AB and at a faster pace during TB, than they did at baseline. However, similar to Experiment 1, it has to be noted that mean RR was not anywhere near the optimal rate of 6 breaths-per-minute (van Diest et al., 2014) during AB intervention, but rather around 10 breaths-per-minute. Perhaps for this reason, vagal tone did not show any effect of RR going down during AB.

There seemed to be no arousal differences between sessions in Experiment 2, at least as picked up by SCL. Then again, inhalation duration increased relative to exhalation duration during both interventions, most markedly for TB, and returned to baseline levels in recovery. The same pattern can be observed in heart rate. This is relevant, as respiration characterized by prolonged expiration triggers a relaxation response, while the reverse suppresses parasympathetic activity (Benson, 1975; Komori, 2018). Clearly, both interventions are cardiopulmonary taxing. This is not necessarily an indication of the scale of vagal involvement, but could hide enhancement effects that rely on relaxation. Interestingly, self-reported arousal on the affect grid goes down after both interventions. Taken together, it is hard to make definitive statements on the extent of sympathetic nervous system involvement, due to our manipulations. As a side note, one could debate on whether SCL is a valid

indicator of sympathetic tone, or is only suited to monitor acute psychological stress and anxiety (Fowles, 1981, 1986). Arousal and stress are many-faceted phenomena, with different mechanisms, timing and dynamics. For example, a recent study that used ambulatory tools to measure physiological stress levels found a negative relationship between SCL and self-reported stress (Tutunji et al., 2021). Here, high self-reported stress was associated with decreased SCL instead of the predicted reverse relationship.

The Simon task results did not provide stronger statistical support for our hypothesis than the SST, though the statistical power was higher. On the contrary, the numerically lower SSRT found in Experiment 1 after AB, was not reflected in Experiment 2 by a smaller Simon effect. What does stand out is the accuracy trend in the TB session (Figure 14). Accuracy on both congruent and incongruent trial types goes steeply down for TB, from pre- to post-test. We suggest that this is due to the high variability in this condition. Though the increased cardiopulmonary stress during TB intervention – as seen in I/E ratio and heart rate – might also play a role. Overviewing the lack of cognitive effects we conclude that there is no evidence for cognitive enhancement of inhibitory control. Furthermore, on the basis of these null-results, we can also not conclude that Simon effect is a preferable inhibition enhancement measure over SSRT.

To further investigate the lack of finding a relationship between a respiration condition and vagal tone and look at the associations of the main physiological independents and mediators of the rVNS model, the D-score correlation matrixes were made for both experiments. This led to the most striking of unexpected results: in neither experiment a correlation between RR and RMSSD was found. A number of directions offer an explanation for this pattern of results.

Firstly, the interventions were short (~10 min). This duration may have been too short to induce an rVNS effect, reflected in vagal tone measures during intervention. Let alone a carry-over effect to recovery phase and the proposed transfer to the cognitive post-test phase. This issue of dose might be exacerbated by the inexperience of participants. The active duration of vagal stimulation might even have been briefer by sub-optimal adoption of respiratory patterns. As an example of a study with a longer duration: Laborde and colleagues (2019) employed a slow deep breathing exercise of 15 min., with 2 min. breaks every 5 min. They did find an

increase in RMSSD and cognitive inhibition (Stroop interference). Though note that, the rVNS mechanism was absent in their analysis as well: RMSSD did not mediate the cognitive effects. On the other hand, the aforementioned study by You and colleagues (2021) showed no differences in RMSSD increase by the duration of breathing exercise, opposing this dose-response interpretation of null-findings. Their shortest intervention was just 5 min. and the longest 20 min. So if 5 min. of slow-paced breathing is enough to produce an increase in RMSSD, we would still expect an increase in vagal tone as RR goes down, which we did not find.

Secondly, another explanation for the absence of association between RR and RMSSD, might be that AB did not bring breathing pace down sufficiently. Even though there was a response to the manipulation in AB in Experiment 2 (RR went down), the pace did not come close to reaching the optimal rate of 6 breaths-per-minute. Only six participants showed an RR lower than 7, of which four have an RR under 6. The optimal rate might be a necessary condition for observing changes in RMSSD, on any timescale. Supporting this is that all the studies that were reported in the aforementioned systematic review that showed an increase in vagal tone, used a breathing exercise that went down to at least 6 breaths-per-minute (Zaccaro et al., 2018). This also applied to the trial with the different and ultra-short duration (You et al., 2021). Then again, if we assume this threshold exists: that the optimal rate has to be reached to observe a vagal response; then we would still expect some correlation between $Dt1t2$ scores of RR and those of RMSSD. At the very least, for the individuals that do lower their pace towards the golden rate, we would expect the trends of RR and RMSSD to covary. To observe this, we would need a larger sample.

Thirdly, we suggest that the association between RR and RMSSD might still be present in our study, but remains hidden. This could be due to a parallel increase in sympathetic activity, which down-regulates parasympathetic tone. In other words, the respiration interventions, and especially AB, produce stress. An indication for this stressfulness is the increase in heart rate and relative inhalation duration during both interventions in Experiment 2. Do note that, there was no clear increase in sympathetic tone as picked up by either PEP or SCL in Experiment 1 and 2, respectively. Still, our findings do suggest that consciously controlling ones respiratory patterns produces some cardiopulmonary stress for the inexperienced. We propose that when participants are asked to manipulate their locus of breathing – and thus intentionally change

muscle coordination to patterns that are underused in laymen, that have little experience in breathing exercises or meditation – this produces sympathetic activity. This might especially apply to abdominal localized breathing, as intentionally using diaphragmatic and abdominal musculature is an unusual way to breathe. That TB is the default setting of breathing can be seen in our data. In TB sessions Abd/Tho does not change from baseline to intervention. In other words: the locus did not shift further towards the chest. The normal way of breathing is already dominated by thoracic (and diaphragmatic) musculature. The finding of a numerically faster SSRT after AB, might also fit this interpretation. An increase in arousal is accompanied by faster responses and shorter stopping reaction times. Summarizing our inferences: consciously manipulating respiratory musculature and especially changing its locus taxes cognitive resources and might produce psychological and physiological stress.

Fourthly, to explain the null-results on inhibitory control, we suggest that acute state enhancement effects on cognition might not be feasible. This short time scale might be insufficient to produce acute but linearly static effects on cognitive control. This is regardless of whether these potential effects are mediated by autonomous nervous system activity. Of course, if a phasic vagal tone increase is necessary to produce cognitive effects we would not observe them in these studies. So from our findings we can't conclude the reason for this null-result. There are studies finding no effects on cognition. For example, a recent high powered study on all three executive functioning components – shifting, updating and inhibition - found no enhancement on any, by a brief mindfulness meditation intervention (Baranski, 2021). We are certainly not suggesting that cognitive functioning does not dynamically fluctuate. Respiratory phase effects on cognition are well-documented (Belli et al., 2021; Heck et al., 2019). Increase and decrease in performance that matches the sinus waveform of inspiration and expiration would likely not produce a bottom-line increase in cognition. Though testing whether relative increases of inhalation/exhalation phases could contribute to a linear increase in performance would be an interesting line of enquiry.

Fifthly, these null-results might be a symptom of the replication crisis. Failure to replicate (cognitive) effects might be because the conclusions of previous studies were based on type 1 errors. The failure to replicate previous findings might actually be increasing significantly. This will be due to more studies being conducted that aim to replicate and that more studies reporting null-findings are published. But also

simply by the statistics that are widely used. An increasing amount of researchers are currently using Bayesian statistics, instead of the classical p-testing. A new wave of null-findings might be therefore be caused by stronger more robust and evidence-based statistics (Fucci et al., 2022). Clearly, this study is also using the Bayesian counterparts of classical statistical tests. However, many of the recent studies we have reported without null-findings are still using classical statistics (e.g. Laborde et al., 2021; You et al., 2021). This might be a reason for conflicting findings.

Lastly, both our experiments' samples are underpowered. There might be simply too little variance to show relationships between respiration, vagal tone and cognition.

Through all these limitations, it is too soon to conclude that the predictions of the rVNS model under study here, have been falsified. However, we do conclude that this study provides no evidence at all that confirms rVNS predictions. Furthermore, we conclude that the selective study of the locus of breathing factor is not very promising. At least, when studied acutely with a short intervention.

Future studies should take into account the lessons learned from these two experiments. Firstly, if researchers want to study the influence of the locus component in breathing patterns or even just include a deep abdominal respiration style, we suggest including a biofeedback set-up. Either using a respiratory band (placed at the diaphragm) or an ECG for HRV feedback might make it easier to teach these complicated motor patterns to inexperienced participants. The tutorial should then also be lengthened, as biofeedback needs many training rounds. Indeed, a study using such a respiratory feedback set-up in a virtual reality environment has shown promise (Blum et al., 2020). Another recent case study on police officers in a zombie shooter also shows great potential (Michela et al., 2022). It studied the effects of slow-paced breathing biofeedback on decision making under stress. Participants being able to control their breathing patterns were superior in performance, due to decreased physiological stress.

Secondly, if the aim is to study phasic changes in autonomic functioning and their connection to cognition, we suggest to use longer interventions than 10 min. We suggest 15 min. and over, with breaks every 5 min, following Laborde et al. (2019). We believe a longer duration, or a higher dose, will produce changes in autonomic

tone with higher fidelity, longer carry-over to subsequent phases and thereby will increase the chances of transfer to cognitive functioning.

Thirdly, we deem audio guidance unsuited as a manipulation of respiratory patterns. Even when the pace of instruction matches the intended respiration rate, there will be non-compliance and too much variation. This makes aiming at a given RR, like the optimal rate of 6 breaths-per-minute, next to impossible. Stimuli that have the potential to more directly manipulate RR and optionally I/E are warranted. Simple two-dimensional visual cues will suffice. For example: a colored ball that expands and contracts, reflecting inhalation and exhalation respectively, can be used to visualize the respiratory goal parameters. When a biofeedback virtual reality design is used, stimuli can go far beyond that in visuals and dynamics. Added benefits of these stimuli is that even breath holding can be easily incorporated. Furthermore, potential confounds and noise, such as emotional reactivity to the audio guide's voice will be eliminated.

Fourthly, when testing for more enduring changes in the autonomic nervous system and cognitive enhancement, a longitudinal design with many intervention sessions is needed. We believe bringing about tonal changes has the most promise in producing the proposed beneficial effects of the rVNS model.

Fifthly, another option for progress in the respiratory cognition field is to eliminate the proposed mediator of rVNS entirely. We predict that without involvement of the vagal nerve complex, respiratory patterns should still produce phasic and tonic changes in autonomic functioning and thereby influence cognition. For this end we might simply substitute HRV with breath rate variability (Ashhad et al., 2022). The temporal dynamics of the respiratory system are like the cardiac parameters a valid indicator of the flexibility and responsiveness of the organism at large, including behavior and neural state (Glass, 2001). Soni and Muniyandi (2019) made an attempt to produce a breath rate variability measure and were able to differentiate meditators from non-meditators by it. It was even superior to HRV on the short time scale.

Finally, another promising line of enquiry might be mapping individual respiratory patterns in different natural and ambulatory contexts. Through this an individualized respiratory parameter, like breath rate variability, can be extracted. Then it would be possible to offer personalized breathing exercises. Interventions can

be adapted to a specific individual in a specific setting. For example: one person might systematically be on the low end of the arousal spectrum, which negatively affects their performance on a cognitively demanding task. This individual might actually benefit from an intervention that increases RR, instead of lowering it to the one-size-fits-all optimal rate. Which in this case might actually have adverse effects instead; lowering the arousal state further. Clearly, we are still at the start of the study on the potential benefits of breathing.

Appendix A

Intervention instructions experiment 1:

Color coding: **Visual (PC Monitor)** **Audio**

General introduction

We gaan zo beginnen met een ademhalingsoefening die zal bestaan uit 2 onderdelen: een tutorial en de ademhalingsoefening zelf. Probeer de gehele ademhalingsoefening zo natuurlijk mogelijk te blijven ademhalen terwijl je de instructies volgt.

Je mag nu eerst de koptelefoon opzetten. De instructies zullen zo voortgezet worden via een audio-bandje middels de koptelefoon. Volg de instructies zo zorgvuldig en nauwkeurig mogelijk.

Mocht er iets niet duidelijk zijn tijdens de tutorial laat dit dan aan het eind van de tutorial, dus voordat je begint aan de ademhalingsoefening weten aan de begeleider. Mocht je voor nu nog vragen hebben kun je deze aan de begeleider stellen.

Succes!

Introduction audio (1.5 min.)

Ga ontspannen, maar rechtop zitten, plaats beide voeten op de grond en je knieën op heup breedte of iets breder.

Probeer niet tegen de achterkant van de stoel te leunen.

Zit recht op, zo recht op mogelijk, en rust de palmen van je handen op je bovenbenen.

Breng nu je schouders langzaam omhoog richting je oren en laat ze daarna weer zakken.

Vergeet niet zo ontspannen en rechtop mogelijk te blijven zitten.

Plaats nu je linker hand zacht tegen je buik en je rechter hand tegen je borst.

Voel voor een moment hoe de buik en ribben naar binnen bewegen op een uitademing.

(8sec)

Voel nu ook voor een moment hoe de buik en ribben naar buiten bewegen op een inademing.

(8sec)

Plaats dan nu je handen terug op je bovenbenen.

Start of the tutorial round (2.5 min)

- TB:

Welnu, plaats beide handen tegen de zijkant van je ribben ter hoogte van je borstbeen.

Met de volgende inademing, adem in via de zijkant van je ribben, zodat je voelt dat je handen en ribben naar de zijkant bewegen.

Wanneer je uitademt bewegen de ribben naar binnen, je voelt dat je handen en ribben naar binnen bewegen..

Als je inademt, adem volledig in, dus zorg dat je met elke ademhaling volledig in en volledig uit ademt met je borstkas.

Breng dan nu je handen van je ribben naar je bovenbenen, zit ontspannen en probeer zonder de feedback van je handen via de zijkant van je ribben te blijven ademen voor de volgende dertig seconden.

(30sec)

Uitstekend, dit was het einde van de oefening, probeer ontspannen te blijven zitten met beide voeten op de grond.

Wanneer je er klaar voor bent mag je op de spatiebalk klikken om de ademhalingsoefening voort te zetten. Dit keer zal je op dezelfde manier ademen maar voor een langere periode. Probeer de ademhaling niet te forceren, probeer zo natuurlijk mogelijk te blijven ademen terwijl je de instructies opvolgt.

- AB:

Welnu, plaats beide handen op je buik met je middelvingers ongeveer ter hoogte van je navel.

Met de volgende inademing, adem in via je buik, zodat je voelt dat je handen en buik naar voren bewegen.

Wanneer je uitademt trek je je buik en navel in, zodat je voelt dat je handen en buik naar binnen bewegen.

Als je inademt, adem volledig in, dus zorg dat je met elke ademhaling volledig in en volledig uit ademt met de buik.

Breng dan nu je handen van je buik naar je bovenbenen, zit ontspannen en probeer zonder de feedback van je handen via je buik te blijven ademen voor de volgende dertig seconden.

Uitstekend, dit was het einde van de oefening, probeer ontspannen te blijven zitten met beide voeten op de grond.

Wanneer je er klaar voor bent mag je op de spatiebalk klikken om de ademhalingsoefening voort te zetten. Dit keer zal je op dezelfde manier ademen maar voor een langere periode. Probeer de ademhaling niet te forceren, probeer zo natuurlijk mogelijk te blijven ademen terwijl je de instructies opvolgt.

- FB:

Welnu, plaats je linker hand terug op je buik en je rechter hand op je borst.

Probeer bewust te worden van je inademing zonder er iets aan te willen veranderen, zodat je voelt dat de buik en/of ribben uitzetten.

Probeer daarnaast ook bewust te worden van de uitademing, zonder er iets aan te willen veranderen, zodat je voelt dat de buik en/of ribben naar binnen bewegen.

Zorg ervoor dat je elke in- en uitademing opmerkt, wanneer je merkt dat je afgeleid bent, probeer dan weer je gedachten naar je ademhaling te brengen.

Breng dan nu je handen van je buik en borst naar je bovenbenen, zit ontspannen en probeer zonder de feedback van je handen bewust te blijven van je ademhaling voor de volgende dertig seconden.

Uitstekend, dit was het einde van de oefening, probeer ontspannen te blijven zitten met beide voeten op de grond.

Wanneer je er klaar voor bent mag je op de spatiebalk klikken om de ademhalingsoefening voort te zetten. Dit keer zal je op dezelfde manier ademen maar voor een langere periode. Probeer de ademhaling niet te forceren, probeer zo natuurlijk mogelijk te blijven ademen terwijl je de instructies opvolgt.

Breathing exercise

- *TB*:

Welnu, ga opnieuw ontspannen zitten, met een rechte rug en je handen rustend op je bovenbenen.

Probeer nu zo goed mogelijk adem te halen via de zijkant van je ribben, in je eigen tempo voor de volgende 5 minuten.

30 (seconden)

Blijf ademen via de zijkant van je ribben.

30 (seconden)

Probeer recht op te blijven zitten terwijl je in- en uitademt via de zijkant van je borstkas.

(1 minute...)

Ga zo door, blijf zo goed mogelijk via de zijkant van je borstkas in- en uitademen, blijf te allen tijde ontspannen.

(1 minute...)

Vergeet niet volledig in- en uit- te blijven ademen met de zijkant van je ribben.

(1 minute...)

Nog 1 minuut te gaan, blijf ademen via de zijkant van je ribben.

(1 minute...)

Uitstekend, dit is het einde van de ademhalingsoefening, blijf rustig zitten met beide voeten op de grond.

Wanneer je er klaar voor bent, klik op de spatiebalk om door te gaan naar de volgende opdracht.

- *AB*:

Welnu, ga opnieuw ontspannen zitten, met een rechte rug en je handen rustend op je bovenbenen.

Probeer nu zo goed mogelijk adem te halen via je buik, in je eigen tempo voor de volgende 5 minuten.

(30 seconds...)

Blijf ademen via je buik.

(30 seconds...)

Probeer recht op te blijven zitten terwijl je in- en uitademt via de buik.

(1 minute...)

Ga zo door, blijf zo goed mogelijk via de buik in- en uitademen, blijf te allen tijde ontspannen.

(1 minute...)

Vergeet niet volledig in- en uit- te blijven ademen via de buik.

(1 minute...)

Nog 1 minuut te gaan, blijf ademen via de buik.

(1 minute...)

Uitstekend, dit is het einde van de ademhalingsoefening, blijf rustig zitten met beide voeten op de grond.

[Wanneer je er klaar voor bent, klik op de spatiebalk om door te gaan naar de volgende opdracht.](#)

- *FB*:

Welnu, ga opnieuw ontspannen zitten, met een rechte rug en je handen rustend op je bovenbenen.

Probeer nu zo goed mogelijk je ademhaling te volgen, zorg er voor dat je bewust bent van elke in- en uitademing zonder er iets aan te willen veranderen, voor de volgende 5 minuten.

(30 seconds...)

Blijf met je gedachten bij je ademhaling.

(30 seconds...)

Probeer recht op te blijven zitten terwijl je je bewust bent van je ademhaling.

(1 minute...)

Welnu, als je gedachte afgedwaald waren, breng je aandacht opnieuw naar de ademhaling.

(1 minute...)

Vergeet niet bewust te zijn van je ademhaling, zonder hier iets aan te willen veranderen.

(1 minute...)

Nog 1 minuut te gaan, blijf met je gedachten bij de ademhaling.

(1 minute...)

Uitstekend, dit is het einde van de ademhalingsoefening, blijf rustig zitten met beide voeten op de grond.

[Wanneer je er klaar voor bent, klik op de spatiebalk om door te gaan naar de volgende opdracht.](#)

Appendix B

Table 2: Correlation matrix for main physiological variables of experiment 2. AB=abdominal breathing session; TB=thoracic breathing session; Dt12=difference score variable between: measurement time 2 (intervention) - measurement time 1 (baseline); Dt23=difference score variable between: measurement time 3 (recovery) - measurement time 2 (intervention); BMP=breaths-per-minute; I/E ratio = inhalation duration (ms) / exhalation duration (ms); RMSSD= root mean square of successive differences; SCL=skin conductance level.

Variable	AB Dt12 BMP	AB Dt12 I/E	AB Dt12 RMSSD	AB Dt12 SCL	AB Dt23 BMP	AB Dt23 I/E	AB Dt23 RMSSD	AB Dt23 SCL	TB Dt12 BMP	TB Dt12 I/E	TB Dt12 RMSSD	TB Dt12 SCL	TB Dt23 BMP	TB Dt23 I/E	TB Dt23 RMSSD	TB Dt23 SCL
AB Dt12 BMP	Pearson's r	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
AB Dt12 I/E	B _{F₁₀}	0.12	—	—	—	—	—	—	—	—	—	—	—	—	—	—
AB Dt12 RMSSD	Pearson's r	0.26	—	—	—	—	—	—	—	—	—	—	—	—	—	—
AB Dt12 SCL	Pearson's r	-0.09	0.01	—	—	—	—	—	—	—	—	—	—	—	—	—
AB Dt12 SCL	B _{F₁₀}	0.23	0.20	—	—	—	—	—	—	—	—	—	—	—	—	—
AB Dt12 SCL	Pearson's r	0.01	0.18	-0.18	—	—	—	—	—	—	—	—	—	—	—	—
AB Dt12 SCL	B _{F₁₀}	0.20	0.36	0.35	—	—	—	—	—	—	—	—	—	—	—	—
AB Dt12 SCL	Pearson's r	-0.49 *	0.00	0.09	0.04	—	—	—	—	—	—	—	—	—	—	—
AB Dt12 SCL	B _{F₁₀}	23.52	0.20	0.23	0.21	—	—	—	—	—	—	—	—	—	—	—
AB Dt12 SCL	Pearson's r	-0.09	-0.30	-0.03	-0.22	0.01	—	—	—	—	—	—	—	—	—	—
AB Dt12 SCL	B _{F₁₀}	0.23	0.96	0.21	0.46	0.20	—	—	—	—	—	—	—	—	—	—
AB Dt12 SCL	Pearson's r	0.28	-0.25	-0.60 ***	-0.03	-0.28	0.21	—	—	—	—	—	—	—	—	—
AB Dt12 SCL	B _{F₁₀}	0.81	0.61	455.16	0.21	0.82	0.44	—	—	—	—	—	—	—	—	—
AB Dt12 SCL	Pearson's r	0.10	0.28	-0.46 *	0.39	0.05	-0.23	0.15	—	—	—	—	—	—	—	—
AB Dt12 SCL	B _{F₁₀}	0.24	0.79	12.80	3.55	0.21	0.50	0.29	—	—	—	—	—	—	—	—
AB Dt12 SCL	Pearson's r	-0.01	-0.09	0.05	-0.13	0.10	0.20	-0.22	0.01	—	—	—	—	—	—	—
AB Dt12 SCL	B _{F₁₀}	0.20	0.23	0.21	0.27	0.24	0.40	0.49	0.20	—	—	—	—	—	—	—
AB Dt12 SCL	Pearson's r	0.17	0.05	-0.18	-0.03	-0.12	-0.03	-0.12	0.47 *	—	—	—	—	—	—	—
AB Dt12 SCL	B _{F₁₀}	0.33	0.21	0.36	0.21	0.26	0.21	0.34	15.27	—	—	—	—	—	—	—
AB Dt12 SCL	Pearson's r	-0.15	0.00	0.36	-0.24	0.08	-0.23	-0.20	-0.34	-0.16	—	—	—	—	—	—
AB Dt12 SCL	B _{F₁₀}	0.30	0.20	2.24	0.57	0.23	0.50	0.69	1.75	0.32	—	—	—	—	—	—
AB Dt12 SCL	Pearson's r	-0.07	0.01	-0.17	0.34	0.03	-0.07	0.18	0.18	-0.09	-0.29	—	—	—	—	—
AB Dt12 SCL	B _{F₁₀}	0.22	0.20	0.33	1.62	0.21	0.22	0.36	0.34	0.23	0.95	—	—	—	—	—
AB Dt12 SCL	Pearson's r	0.15	0.18	0.02	0.01	-0.14	0.19	-0.08	-0.93 ***	-0.53 **	0.28	-0.17	—	—	—	—
AB Dt12 SCL	B _{F₁₀}	0.30	0.37	0.20	0.20	0.28	0.37	0.56	1.24 * e14	51.18	0.78	0.32	—	—	—	—
AB Dt12 SCL	Pearson's r	0.01	0.41	0.11	0.03	-0.01	0.11	-0.09	-0.13	-0.43	-0.06	0.02	0.29	—	—	—
AB Dt12 SCL	B _{F₁₀}	0.20	4.97	0.25	0.21	0.20	0.25	0.23	0.27	6.83	0.22	0.20	0.93	—	—	—
AB Dt12 SCL	Pearson's r	0.14	-0.29	-0.41	0.30	0.00	-0.01	0.25	0.36	0.19	-0.57 ***	0.25	-0.43	-0.26	—	—
AB Dt12 SCL	B _{F₁₀}	0.28	0.91	4.99	0.96	0.20	0.20	0.63	5.02	7.13	148.19	0.58	7.13	0.69	—	—
AB Dt12 SCL	Pearson's r	-0.01	0.21	0.10	0.28	0.03	-0.17	-0.08	-0.36	-0.26	-0.05	-0.20	0.39	0.24	-0.03	—
AB Dt12 SCL	B _{F₁₀}	0.20	0.43	0.24	0.85	0.21	0.33	0.23	2.13	0.67	0.21	0.40	3.54	0.57	0.20	—

* B_{F₁₀} > 10, ** B_{F₁₀} > 30, *** B_{F₁₀} > 100

Chapter 5

General Discussion

Summary

In this book we venture into the world of contemplative practices, or meditative exercises and the effects these have on cognition and stress. The main focus lies on a subset of meditative techniques, namely breathing exercises, or how respiratory patterns change body and mind. The general introduction in **Chapter 1** covers the historical distinction between classic cognitive science and the embodied cognitive perspective, adopted here. It also introduces the contemplative practices and the concept of mindfulness. It also provides a short primer on Bayesian statistics, as used throughout this book. Because the data collection in **Chapter 3** predates the theoretical work in **Chapter 2**, breathing is not a central topic in that empirical study.

Chapter 2 contains a peer-reviewed theoretical paper (Gerritsen & Band, 2018). It starts with a selected review of studies on contemplative traditions. In the review it was shown that the majority of reported beneficial effects on physical health, mental health and cognitive/emotional functioning, were within the stress-related domain. We have proposed that these benefits could be explained through a decrease in individual (chronic) stress levels and that breathing exercises, by deliberately or passively lowering respiration rates, play a critical role in achieving these effects. Because of the prevalence of breathing exercises in contemplative traditions common findings across interventions could be attributed to this factor. We have further maintained that also exercises that just prescribe focused attention on breathing change respiratory patterns and slow its rate. The main route by which respiratory patterns affect stress-related conditions is by their effects on the autonomous nervous system. This nervous system is both responsible for arousal and relaxation, for fight-or-flight and rest-and-digest modes of operation, by its sympathetic and parasympathetic branch, respectively.

Furthermore, in **Chapter 2** we have proposed a neurophysiological model: the respiratory vagal nerve stimulation (rVNS) model of contemplative activity that tries to explain the aforementioned emotional and cognitive effects and capture several possible mechanisms of effect. The three main predictions of the rVNS model are: 1) that respiration rate; 2) the relative duration (ratio) of inhalation versus exhalation; and 3) the relative locus — thoracic versus abdominal — of respiratory motor activity influence both acute and chronic stress levels, and thus cognition and (mental) health. Finally, we suggested a number of mechanisms focusing on the role of the vagus nerve as possible biofeedback mechanisms in producing these effects, acting also as the mediator between respiration and cognition.

The primary proposition of **Chapter 2** is that by lowering respiration rates, extending exhalation and shifting the locus to the abdomen, relaxation increases and stress goes down. Subsequently, a more flexible psychological state is reached whereby complicated tasks characterized by high mental workload — tasks that tax *executive functions* — can be performed more efficiently. In other words: breathing exercises indirectly enhance cognitive control. In **Chapter 4**, two of the main predictions (on respiration rate and locus) are empirically tested.

Chapter 3 describes an empirical study on aging, published in a peer-reviewed journal (Gerritsen et al., 2020). Here, we conducted a randomized controlled trial that examines the effects of Tai Chi Chuan (TCC)— a mind-body exercise characterized by slow movement and body awareness— on motoric and executive functioning in an aging population sample. Though the TCC intervention did include some instructions aimed at breathing, none of the predictions of the rVNS model were directly tested, because the study was conducted well before the publication of the model described in **Chapter 2**. In total 55 participants between the ages of 53 and 85 were assigned either to a TCC group or a control group, of which 43 were in the final analysis. The TCC group participated in an online 10-week, 20-lesson video program of increasing difficulty level, while the control group watched educational videos of similar length and frequency. Two measures of motor functioning: motor speed, as measured by the finger tapping test, and functional balance, as measured by the timed up and go test; and three measures of executive functioning: *shifting*, as measured by the task switching test, *updating*, as measured by the 2-back task, and *inhibition*, as measured by the stop-signal reaction task, were used to evaluate the effects of TCC. We found that there were no differences in executive functioning on any of the measures between the TCC group and the control group pre-to-posttest. However, we found extreme evidence for TCC benefits on functional balance and moderate evidence for increased motor speed. Thus, we concluded that while TCC may be beneficial for improving motoric functioning in older adults, it may not have a significant impact on executive functioning.

Chapter 4 contains a first attempt at testing some predictions of the rVNS model (unpublished manuscript). Foremost, the prediction is that a phasic shift of respiratory locus from the thorax to the abdomen leads to acute relaxation, increases parasympathetic (vagal) tone, and enhances executive functioning. The chapter includes two experiments. In the first experiment, 29 people participated in three experimental sessions, where they were exposed to three different breathing interventions by audio guidance: abdominal breathing, thoracic

breathing and focused breathing (semi-control condition). Participants were tested on changes in respiratory parameters, parasympathetic and sympathetic tone, as well as response inhibition. Although participants complied with the interventions as shown by a change of the ratio between thoracic and abdominal circumference in accordance with the instructions (participants breathed relatively more with their stomach in the abdominal condition), expectations made by the rVNS model did not materialize. Parasympathetic and sympathetic tone, as measured by the root mean square of successive differences metric (RMSSD) of heart rate variability (HRV) and pre-ejection period, respectively, did not change pre- to post-test; and neither did executive functioning (response inhibition), as measured by the stop-signal task. However, because breathing slowed down during all three interventions, the effect of breathing locus instructions may have been mitigated. Also, response inhibition, as measured by the stop-signal task, might not be sensitive enough as an executive function to show acute enhancement — a short bout of respiratory modulation might not transfer to an increase in this functional component of cognitive control in laymen. Therefore, a second experiment was run.

In the second experiment of **Chapter 4** an attempt was made to address the two aforementioned issues present in the first experiment. Firstly, focused breathing was dropped and instructions for the other two were rewritten to prompt participants to slow their breathing in the abdominal condition — and slightly speed it in the thoracic condition. Secondly, the stop-signal task was dropped in favor of the Simon task. The form of (cognitive) inhibition mapped by the Simon task is assumed to be more sensitive to acute changes than response inhibition, as mapped by the stop-signal task. Thirdly, the pre-ejection period indicator of sympathetic activity was substituted for skin conductance level (SCL) for reason of feasibility of experimental set-up. Finally, a self-report on affect was added as an indication of subjective stress levels (affect grid). The data of thirty-four participants were analyzed. The results of the second experiment were highly similar to the first. Though participants adjusted their respiration in the instructed direction, none of the major predictions came to pass. Cognitive inhibition was not improved from pre-test to post-test in the abdominal condition and even general speed (reaction time) did not differ between breathing conditions in the Simon task. Furthermore, autonomous tone, whether sympathetic (RMSSD) or parasympathetic (SCL), was equally unaffected. Only the self-report matched expectation (affect grid): after abdominal breathing people indicated that they felt more relaxed. Though clearly both experiments were underpowered, and this might hide the effects, there also was

no numerical trend in the expected direction. Therefore, we concluded that there is no change in autonomous tone and cognitive functioning in this timeframe due to these specific breathing exercises.

Discussion

Null-results

This dissertation contains two experimental chapters, both of which showed null-results on the main variables under study. The TCC intervention of **Chapter 3** did not lead to improvement in any of the executive functions under measurement — shifting, updating and (response) inhibition —as compared to control. The breathing exercises of the two experiments described in **Chapter 4** also did not affect executive functioning, as indicated by response and cognitive inhibition measures (stop-signal task and Simon task). Nor did the breathing exercises lead to the predicted state changes in autonomic functioning. A number of possible explanations for these null results have been put forth in the discussions of the previous two empirical chapters. These share a common pattern.

First, the issue might have been *dosage*. The interventions might have been too short for predicted and previously reported effects to surface. This might notably be the case in the breathing studies. TCC, on the other hand, might have suffered from a lower quality and lack fundamental components such as meditative aspects. The breathing instructions in the respiration studies were auditory and did not directly manipulate respiration to a certain respiration rate, like slowing it to 6 breaths-per-minute or lower. Both breathing exercises and TCC participants might have shown failures to engage, or have other compliance problems to the instructions, for example: by difficulty level. Our breathing studies were run on young people without much *experience*, so for many participants this will have been their first dose of meditation or breathing exercise. This might have also compounded the issue of compliance. In all these cases, the dose of the effective components might have been too low to lead to a response in cognition (or autonomic functioning).

Second, there are *sample* issues. All three empirical studies were statistically underpowered: the number of participants in the samples were too low. There might also be sample characteristics that influenced the results. The aging TCC sample was highly (physically) active and might therefore have a ceiling effect on the gains that can be made

from TCC's physical exercise aspect. The participants in the breathing studies were relatively young and inexperienced with these techniques. This might have led to incompletion due to motivation and ability. Indeed, many were unable to lower respiration rates significantly.

The aforementioned causes might hide effects in our studies that do exist. However, it might also simply be the case that the interventions just do not lead to the predicted effects. Thus these null-results are a valid description of reality. Then we have to conclude that 1) the rVNS model is not a valid description of human neurophysiology and cognition; 2) the failure to replicate previous findings of TCC benefits on executive functioning suggests that these benefits do not exist. If we follow this latter conclusion, it remains mysterious why many studies on TCC do find positive results. Perhaps findings on the beneficial effects of contemplative practices on cognitive functioning are inflated or overstated in the literature.

Is there a *publication bias* in contemplative science? According to two recent meta-analyses on the cognitive effects of mindfulness meditation there is no evidence for publication bias within the study field on these contemplative practices (Casedas et al., 2020; Gill et al., 2020). However, the findings of the two reviews do diverge strongly otherwise. Casedas and colleagues (2020) concluded that mindfulness meditation has a small to medium effect on executive functioning as a whole, though the authors acknowledge that these are preliminary findings as only 13 studies met the selection criteria and could be included. In contrast, Gill and colleagues (2020) only found a small to medium effect on higher-order functions, but no effect on executive functioning itself; and reported that overall, studies had many methodological flaws. A highly relevant difference between both studies for our discussion here, is that Gill et al. (2020) solely focused on brief mindfulness interventions (in beginners). This corresponds to our own null-findings with short interventions in laymen. But even taken together these two reviews do not provide strong confidence that contemplative practices have beneficial effects on cognition, specifically on executive functions. A randomized controlled trial published after these reviews echoes this sentiment (Baranski, 2021). This experiment investigated the acute effects of mindfulness meditation on the three executive functioning components (shifting, updating, inhibition) and found no benefits on any of the three. Note that these are the same functional components as were under investigation in the TCC study of **Chapter 3**. The author makes the suggestion that practitioners and scientists should lower their expectations on cognitive enhancement by meditation. Another study, by Paap and colleagues (2020), reports that mindfulness meditation practice does not predict executive functioning, at least as measured by the

interference scores used in multiple cognitive inhibition tasks, like the Stroop, flanker and Simon task. In this correlational study, the authors report a Bayesian statistical analysis, where extreme evidence was found, against a (positive) relationship between meditation practice and inhibitory functioning. In conclusion, aforementioned studies cast significant doubt on whether contemplative practices can enhance executive functioning, especially on the short-term; and that any potential existent effects are likely small.

This brings us to an overarching issue. Null-results and failures to replicate, even within a well-established field, where there is no indication of publication bias, are not a problem *per se*. Instead, they can be highly informative. Following Chalmers (1976) in his adaptation of Poppers (1959) falsificationism: a *falsification* is most useful when it provides evidence against a *dominant* hypothesis or paradigm. While *confirmation* still has value when finding evidence in favor of a bold claim (hypothesis): if it goes against the common consensus of scientific knowledge. Of course, in our studies we have not provided evidence against the dominant hypotheses (e.g. that contemplative practices benefit cognitive functioning), as our Bayesian evidence load was insufficient, due to lack of power, to make a confident claim against a model. So instead we were only unable to confirm the dominant hypotheses. Thus, I assuredly do not claim that any of the results described in this work constitute a falsification proper. But complete rejection of the hypotheses and conclusions of previous studies into contemplative activities is not and never was the aim here; and also not in any way necessary, I believe, because there is another way to look at these results.

The problem might be that we — as cognitive scientists — opt for all or nothing answers: a statement is either false or true. We would like to have a clear cut answer to the (implicit) question: does this specific effect *exist*? While the question should be: does this specific effect *occur*? The difference between these two questions is context: the particular circumstances inherent in, and perhaps unique to, a particular research setting. This means that if a study is well-designed, conscientiously conducted and the inferential statistics allow, the results stand, no matter the apparent conflict with previous findings. Current scientific background knowledge does not invalidate these results. Do note here, that when encountering an anomalous result, a scientist should always start with critically assessing their own work, such as the design and methodology of the study; this is simply the most parsimonious and likely explanation for the anomaly. But if the data has been correctly and reliably obtained, then clearly there are conditions where this specific effect does *not* manifest itself. Previous findings must have been collected in a critically different setting, albeit

different in a minor matter. These critical differences in context merit discovery, analysis and further investigation. This is another reason why Bayesian statistics should be preferred over classical statistics. Classical statistics invites a dichotomous binary way of thinking: about an effect's existence or non-existence, by simply rejecting or accepting a hypothesis, while in Bayesian statistics there is a quantification of the strength of evidence, — against or for — a particular model. Just by laying findings against this grey scale, a binary mode of inference becomes less likely. Next, I will discuss a number of circumstances that are candidates in affecting the likelihood of the *occurrence* of respiratory effects.

Contextual factors: timeframes, demographics and individual differences

As stated in the previous chapters' discussions, effects on stress systems and cognition might only show up after long-term application of the intervention under study. Specifically on the effects of breathing patterns, as of yet, there have been no longitudinal studies. There are indications in our work that some of these effects do occur, but are short-lived. For example, during the two experiments described in **Chapter 4** we observed a brief increase in parasympathetic tone (or rather HRV) in a subset of participants during the intervention — when they were breathing slowly — but HRV returned to baseline soon afterwards. Certainly, these effects did not last into the next experimental phase. Thus, the benefits of relaxation may only develop through continued daily practice and might only then be reflected in a more parasympathetic dominant autonomic balance and perhaps an increased cognitive flexibility. In short, acute effects of respiratory modulation on autonomic and cognitive functioning might not *occur* (or even *exist*) because the physiological effects are too short term and do not transfer to tests of cognitive performance.

Certain demographic aspects might also function as moderator variables in the chain effects of respiratory patterns, through autonomic functioning to cognition, as predicted by the rVNS model. These demographic differences — between groups of people — might affect (psycho)physiological outcomes significantly, and thus what beneficial effects can be expected to *occur* from any intervention targeting these. Indeed, especially in the autonomous nervous system, differences between groups of people might be large, as it has recently been established that autonomic functioning differs between certain populations; and thus the (supposed) mediating role of stress systems might be impacted. Some of the following population effects might have also been a factor in our experimental work.

Firstly, there seem to be sex differences in autonomic functioning. It has become increasingly more apparent over the past decade, that the female body has not been taken as the default medical model and that physiology and symptomology may vary widely between sexes. As an illustrative example, let's look at symptoms of myocardial infarction. The "atypical" symptoms, such as: stomach ache and nausea, of myocardial infarction are far more common in women and have been historically underreported and overlooked in favor of the common symptoms in men: chest pain and upper arm ache, by both science and society. To this day, knowledge that these symptoms are signs of acute infarction have been shown to be lacking in the general population (Birnbach et al., 2020). Differences in cardiac functioning between sexes do not stop at cardiac arrest. There are also strong indications that cardiac autonomic functioning differs between sexes. A meta-analysis of studies that have gathered HRV measurements (Koenig & Thayer, 2016) reports that women have higher resting state heart rate and lower HRV, within the time-domain (e.g. RMSSD). However, women show lower power in the low frequency band of HRV, but higher power in the high frequency band than do men, which is also reflected in a lower low/high frequency ratio. This suggest that cardiac activity is parasympathetically dominated (by vagal means) in women and sympathetically dominated in men. The authors conclude that this is a likely reason for the cardiac health benefits and longevity women enjoy, as compared to men. My added conclusion is that this might also have implications for the choice of HRV metric in skewed samples of the sexes. A more recent meta-analysis has made a slightly conflicting discovery in settings of social stress (Hamidovic et al., 2020). Paradoxically, women show lower reactive HRV during a public speech task than men, as well as slightly lower HRV during anticipation and recovery. Apparently, when (social) stressors are introduced, female participants show more arousal than men, while their resting states are conversely more relaxed than men. Clearly, cardiac autonomic control differs between the sexes. Relevant here is that this implies that studies with a proportional high number of women might have difficulty finding enhancement effects due to an already healthy autonomous balance, unless a strong stressor is introduced. These studies might suffer from a ceiling effect, where HRV responsivity in females is limited by a high baseline. The two breathing experiments in **Chapter 4** have a far higher number of females than males: the sample of experiment 1 consisted of 23 females to 6 males and experiment 2 of 31 females to 3 males. It is thus not unreasonable to assume that this HRV ceiling effect might have been present in in our experimental work. Then this might have resulted in *non-occurrence* of cognitive effect as well.

Secondly, there are developmental differences in autonomic functioning. Lipsitz and Novak (2012) report a number of affected functions that occur in normal human aging. Overall, sympathetic nervous system activity goes up, while — paradoxically — sympathetic functioning actually decreases, due to desensitization of its receptors by spillover and lowered clearance of sympathetic neurotransmitters and hormones. In parallel, parasympathetic tone goes down. The decrease in parasympathetic functioning is also reflected in lower resting state HRV, especially in the high frequency domain; a vagal tone indicator. Sensitivity of the baroreflex — a blood pressure regulating mechanism — and the cardiac neuroreceptors decreases. Overall blood levels of norepinephrine (noradrenalin) increase, also because of diminished clearance of its hormonal form (Pflughaupt et al., 2006). Conversely, blood levels of epinephrine (adrenaline) decrease, in response to noxious stimulation (pain), as compared to younger people. In other words: while resting state levels of stress hormones are high, the adaptive responsiveness to actual stressors decreases instead (the function of these substances). A review by Hotta and Uchida (2010) therefore concludes, that autonomic reactivity decreases in normal aging and that this also results in a decreased cerebral blood flow control. So additionally, brain regions that increase in activity and demand a higher oxygen supply, are served less efficiently. From this short summary, it can be concluded that the autonomous nervous system becomes less functionally reactive overall with increasing age. This implies that expectations from interventions trying to target this system in aging populations will have to be lowered, accordingly. The TCC study in **Chapter 3** was performed in an aging population. If potential cognitive enhancement by TCC should go through an autonomic route, as suggested by the rVNS model, then this implies that especially the target population of the elderly will have a cap on its benefits by cause of a changing autonomic nervous system and would need a very large sample (power) to show these incremental effects. As stated earlier, the sample in our TCC study was small and thus autonomic non-responsivity might be a reason for the null-results. Thus, this does not rule out the *existence* of autonomic effects, but only shows that they do not *occur* in this specific sample.

Thirdly, there might be lifestyle factors, that limit the expected gain of a meditative or breathing interventions, for example: *physical conditioning*. Similar to aging, people with a low physical fitness show a decline in autonomous functioning (Fu & Levine, 2012). People who abstain from physical exercise show a disbalance in autonomic functioning characterized by vagal withdrawal and thus suffer from an overactive arousal system (Besnier et al., 2017). Furthermore, dysfunctional autonomic balance predicts, in turn, the ability and motivation to

partake in physical activity; in other words: cardiac vagal activity determines an individual's ability to do exercise (Gourine & Ackland, 2018). Concluding, physical inactivity leads to a downward spiral of autonomic dysfunction and thus most likely affects the range of benefits a respiratory modulation can be expected to produce. It is currently unknown whether progressive vagal withdrawal is reversible (by respiratory modulation). Looking back at our studies, the TCC study had a highly physically active sample, which implies two things. First, that the participants probably had a healthy stress system (and autonomic balance), that could be receptive to intervention. However, the question then remains whether there was room for improvement. Second, that the physical component of TCC is likely to have been not challenging enough for further benefits on cognition. If this is the case, then a lack of improvement of executive functions in our study might also be led back to the meditative component of TCC. If enhancement could not be reached through way of physical exercise, the expected cognitive enhancement might still be caused through meditative practice, as reported by previous studies. This leads me to conclude that: either, the meditative instructions of the intervention were of insufficient quality (or not complied to), or the meditative aspects present in TCC, such as body awareness and breathing exercises, do not enhance cognitive control factors (in isolation).

The null-results might also be a results of differences in *individual baselines*. As described in the discussion of **Chapter 4**, the effects of breathing interventions might very well depend on individual variations in respiratory patterns, stress states and the context they are applied in (whether ecologically valid or not). For instance, some individuals might have a high respiration rate at rest and might benefit from a manipulation that lowers their rate, decreasing stress and thereby performance on a cognitive task, as prescribed in the rVNS model. In contrast, another person might have a very relaxed state characterized by a relatively lower respiration rate and might be adversely affected by a further slowing of breathing. Instead this person might benefit from an activating manipulation, like slightly speeded breathing. A one-size-fits-all approach to modulating autonomic balance, as applied in our studies, might thus not work for everyone and this might hide the effects on those by whom it does. This would also explain null-results in our experiments. As these differences could overshadow differences between groups, or rather experimental conditions.

Fourth, human stress systems are highly individually divergent in general and in their *stress responsivity* specifically. People widely diverge in their perception of, response/adaptation to stress and susceptibility to adverse and pathological conditions,

resulting from stress exposure (Ebner & Singewald, 2017; Sapolsky, 1994). The parameters of individual stress systems are already set prenatally and predispose the amplitude of adult stress responses, as was shown by the longitudinal studies of the Dutch famine that occurred during the end of World War II (Carroll et al., 2012; de Rooij, 2013). Inversely, large variation in the responsivity of individual stress systems diminishes what can be expected from manipulations of the autonomic system at the group level, for example by adjusting respiratory patterns. How far an individual is baseline stressed or reactive to stress might impact the effects of a relaxation exercise. If many non-responders – individuals that are not (much) influenced by parasympathetic activation — are present in a sample, this also might hide the effects of the intervention, especially with a small sample. However, we do not have any indication that stress responsivity (and thus relaxation responsivity) differed or was skewed in our samples.

Lastly, the acute responsivity of individuals to respiratory interventions might very well be contingent on unknown mediating factors. This would explain why we do not find any acute effects on the stress system and cognition. However, the conclusion would then be that these acute effects do not *exist*. A prominent and necessary mediator of these effects might be *sleep*. The quantity and quality of sleep has a huge influence on all supposed affected variables of the rVNS model: cognition and (mental) health within the stress-related domain. Sleep not only plays a role in memory function, like sleep consolidation, and the development of executive functions, but a lack of it also increases the risk of developing many stress-related pathologies, such as: dementia, cardiovascular disease and immune system dysfunction. Actually, there is not much a healthy sleep pattern does not benefit (Walker, 2017). Specifically, sleep tones down the sympathetic nervous system and restores autonomic balance (Meerlo et al., 2008). Sleep is also a necessity for healthy executive functioning (Tucker et al., 2010). Thus without a good night of sleep, in quantity and quality, after an intervention aimed at these systems and before the effects are tested, there might be no benefits. Sleep consolidation is then necessary to observe the effects we are expecting to occur. Then, *acute* effects of respiration, do not *exist*. Next, I will make up the tally for the rVNS model from our current state of knowledge.

Respiration, rVNS and cognition

When we look back at the rVNS model in **Chapter 2**, we must conclude that we have not obtained any evidence for any of its predictions. For an overview and discussion of likely explanations, I refer to **Chapter 4** and the past sections. The identification of these factors leads to avenues of scientific enquiry that can either make, break or force adaptation of the rVNS model. At this point, it is too early to judge whether rVNS has any veracity and thus scientific merit. However, there are a few new studies by other authors on respiration and psychological effects that therefore deserve mention.

Grund and colleagues (2022) showed that when the onset of a tactile stimulus is synchronized to an individual's respiratory phase, its detection is increased. The rationale behind this occurrence is that due to the phenomenon of respiratory sinus arrhythmia — the speeding of heart rate during inhalation and the slowing of heart rate during exhalation — there is an optimal timepoint in the respiratory cycle for stimulus detection. This is when heart rate is at its local peak (near the end of the inhalation phase) and thus arousal is at its highest (sympathetic tone). This is indeed what they found. Note that simply the existence of the phenomenon of respiratory sinus arrhythmia indicates that respiratory modulations should have effects on arousal, stress and relaxation. Also, the uptake of oxygen — by respiratory rate and depth — is dynamically coupled with cardiac output: when breathing quickens, heart rate goes up, and vice versa (Rowell, 1993). A study by Klink and Pruessner (2023) convincingly showed that slow diaphragmatic breathing following a physiological stress induction (cold pressor test) leads to relaxation, as reflected in lowered heart rate and an increase in HRV. Clearly, respiratory patterns have an effect on autonomic functioning; on stress and relaxation. Only, this effect has not *occurred* in our experimental studies. However, the road from breathing exercises to effects on cognition remains less clear and especially the mediating role of the vagus nerve is currently causally unsubstantiated.

There is a substantial amount of literature on the link between vagal tone HRV and cognitive/emotional control or flexibility. This literature has been extensively described in **Chapter 2**, with some updates in **Chapter 4**. However, most of these studies are correlational or cross-sectional in design. This has ramifications for the conclusions and inferences that can be made from their results. As the famous maxim states: correlation does not imply causation. So, for example, Spangler et al., (2018) found that human beings with higher resting states of HRV show lower response inhibition rates in a threatening situation. Though this study had an experimental design it cannot be concluded from these results that higher HRV causes or

mediates these fluctuations in executive functioning, as they do not manipulate HRV levels and it thus remains a correlational finding.

In an intervention study that did have an experimental controlled design, De Smet and colleagues (2023) performed a transcutaneous auricular vagus nerve stimulation study, where they measured effects on HRV and on perseverative cognition (cognitive inflexibility). Surprisingly enough, though they did find a decrease in perseverative cognition due to the active stimulation as contrasted with sham stimulation, they did not find an overall increase in HRV during active versus sham conditions. However, participants who did respond to the stimulation with increases in HRV also showed the largest increase in cognitive flexibility. Concluding, the relationship between afferent stimulation of the vagus nerve (as is the biofeedback of slow breathing), HRV and cognition is not linear and is modulated by individual differences in the autonomous system.

When overviewing the literature domain on the link between HRV and cognition, it is remarkable that most to all of these studies come from the same lab or are at least co-authored by scholars who have introduced the neurovisceral integration model (Thayer & Lane, 2000). This observation is not meant to suggest that these studies are in any way unsound, unreliable or invalid. But we can conclude that hardly any other author, has either studied these effects or deemed their studies fit for publication. We certainly have not found any relationship between HRV and cognition in our empirical work.

Though the mediating role of HRV between autonomic manipulations and cognition is at this point unsure, this does not necessarily mean that the vagus nerve is not involved. Relaxation or activation due to respiratory adjustments can be mediated in other ways. The vagus nerve is a vast complex with many afferent and efferent routes, as well as projections upwards into the central nervous system. This is not all reflected in the measurement construct of HRV. In other words: the connection between respiratory patterns and specific adaptive cognitive parameters still seems to have promise, even within a rVNS framework. For instance, the route of transcutaneous vagus nerve stimulation might go through the locus coeruleus, suggested by studies that have shown synchronization between stimulation and neuronal firing in the locus coeruleus (Hulsey et al., 2017). This alternative route is especially promising as a rVNS pathway because respiratory activity also synchronizes with the locus coeruleus (Melnychuk et al., 2018).

Aforementioned limitations do not mean that taking HRV measurements is without any merit. It might be functional as an indicator of autonomic responsiveness as suggested by the results of De Smet and colleagues (2013). Another recent study (Manser et al., 2021) found HRV reactivity to be a predictive biomarker for responsiveness in normal cognitive decline to intervention load. Individuals with higher HRV reactivity show (larger) increases in cognitive functioning due to enhancement exercises, than do those with low reactivity.

Breathing exercises might also take other indirect ways of long-term benefits to cognition and (mental) health, for example: by increasing overall psychological well-being. Well-being is a strong predictor for many effects, such as delaying or preventing cognitive decline (Zhang et al., 2022) and all-cause mortality (Tamosiunas et al., 2019). All these aforementioned avenues deserve further study.

Conclusion

We have found no evidence for the claim that TCC enhances executive functioning, while there is strong evidence that it improves physical functioning, notably functional balance. Though the *non-occurrence* of the cognitive effects might be due to certain contextual factors, such as sample characteristics and compliance rates, we lean towards the conclusion that these cognitive effects do not *exist*.

Also, we were not able to find any evidence for the predictions of the rVNS model in our experiments. However, a number of studies that have been conducted by other labs have found confirmatory evidence for claims made by the rVNS model, specifically: that slow deep breathing increases HRV. This together with the presence of contextual factors that can lead to cognitive effects to not *occur*, that are mentioned above, leads us to conclude that the rVNS model still holds promise and the *existence* of these predicted effects can still be shown to *occur* in other circumstances. However, we do suggest that *acute* enhancement effects (by respiration) of cognition are unlikely to *exist*.

We started out this dissertation with a brief introduction to the history of cognitive psychology. The contrasting views on the mind between classical and embodied cognitive science were discussed in **Chapter 1**. Have we come any further in this reading? Not really. Our null-results do not give grounds to state something on this with any amount of confidence. The most I can say, is that we also did not find any cognitive effects without

changes in more peripheral systems. Because we did not find any effects at all. Though we have not covered much ground on this topic, I do still maintain that cognition cannot be completely identified by computation and is therefore unsuited to be studied as such. The human mind is embodied, embedded and extended into its umwelt. But, as always, further study is required. For now, my suggestion to you, is instead of hooking up to the grid and disappearing into cyberspace, is to move into your body, experience, go outside with another human being, listen, talk.. and breathe deeply.

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