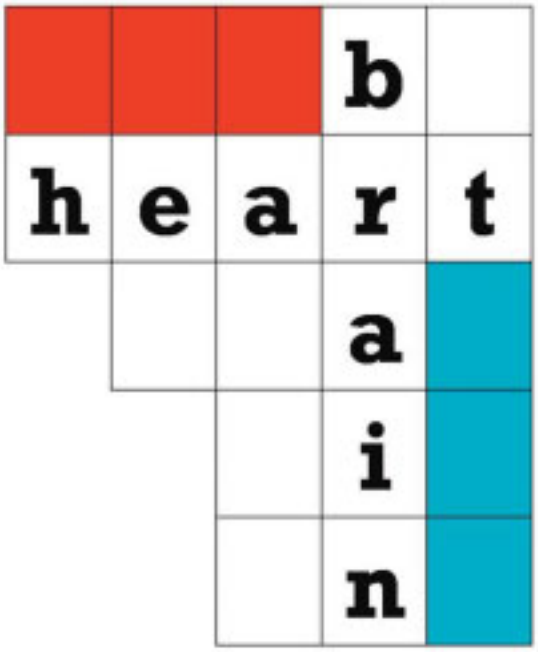
Roberto La Marca

Vagal functionality as indicator for biopsychological stress responsiveness and beneficial effects of auricular electrical stimulation on vagal activity



Cuvillier Verlag Göttingen
Internationaler wissenschaftlicher Fachverlag

Roberto La Marca

Vagal functionality as indicator for biopsychological stress responsiveness and beneficial effects of auricular electrical stimulation on vagal activity

Cuvillier Verlag Göttingen
Internationaler wissenschaftlicher Fachverlag

Bibliografische Information der Deutschen Nationalbibliothek
Die Deutsche Nationalbibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über http://dnb.ddb.de abrufbar.
1. Aufl. - Göttingen : Cuvillier, 2009
Zugl.: Zürich, Univ., Diss., 2009

978-3-86955-070-1

Die vorliegende Arbeit wurde von der Philosophischen Fakultät der Universität Zürich im Frühjahrssemester 2009 auf Antrag von Frau Prof. Dr. rer. nat. Ulrike Ehlert und Herrn Prof. Dr. rer. nat. Markus Heinrichs als Dissertation angenommen.

Nonnenstieg 8, 37075 Göttingen
Telefon: 0551-54724-0
Telefax: 0551-54724-21
www.cuvillier.de

1. Auflage, 2009
Gedruckt auf säurefreiem Papier

978-3-86955-070-1

CONTENTS

Acknowledgements I

Abstract III

Tables V

Figures VI

Abbreviations VIII

1. INTRODUCTION 1

2. THEORETICAL BACKGROUND 5

2.1 The vagus nerve: structural and functional properties 5

2.1.1 The autonomic nervous system 5

2.1.1.1 The central autonomic nervous system 6

2.1.1.2. The peripher autonomic nervous system 8

2.1.2 The vagus nerve 9

2.1.2.1 Vagal innervation of the heart 9

2.1.2.2 Vagal innervation of the ear 11

2.1.2.3 Indicators of vagal activity: markers not referring to the heart 12

2.1.2.4 Indicators of vagal activity: electrocardiographic markers 13

2.1.2.5 Determination of vagal functionality 20

2.1.2.6 The polyvagal theory 21

2.1.2.7 The neurovisceral integration model 25

2.2 Factors negatively associated with vagal activity 28

2.2.1 Risk factors 28

2.2.1.1 Age 29

2.2.1.2 Body mass index 30

2.2.1.3 Gender 30

2.2.1.4 Alcohol consumption 31

2.2.1.5 Smoking 31
2.2.1.6 Air pollution 32
2.2.1.7 Little or no exercise 33
2.2.1.8 Low socio-economic status 33
2.2.1.9 Circadian rhythm 34
2.2.1.10 Psychological factors 35
2.2.1.11 Stress 36
2.2.1.11.1 Definition 37
2.2.1.11.2 Stress response 39
2.2.1.11.2.1 Cortisol 39
2.2.1.11.2.2 Alpha-amylase 41
2.2.1.11.2.3 Heart rate 43
2.2.1.11.2.3 Heart rate variability 44
2.2.2 Morbidity and mortality 46
2.2.2.1 Depression 48
2.2.2.2 Anxiety disorders 48
2.2.2.3 Eating disorders 49
2.2.2.4 Schizophrenia 50
2.2.2.5 Cardiovascular events, disorders and mortality 51
2.3 Interventions increasing vagal activity 54
2.3.1 Pharmacological interventions 54
2.3.2 Invasive vagus nerve stimulation 55
2.3.2.1 General introduction 55
2.3.2.2 Neuroanatomical basis of VNS action 56
2.3.2.3 Treated disorders 57
2.3.2.4 Cardiac effects 58
2.3.2.5 Adverse effects 59
2.3.3 Transcutaneous electrical nerve stimulation 60
2.3.4 Acupuncture 61
2.3.5 Physical training 62
2.3.6 Nutrition 63
2.4 Conclusio 64

3. EMPIRICAL STUDIES **65**

3.1 Vagal functionality as physiologic resource reducing stress-induced biopsychological responses **65**

3.1.1 Introduction 65

3.1.2 Materials and Methods 68

3.1.2.1 Participants 68

3.1.2.2 Procedure 68

3.1.2.3 Interventions 69

3.1.2.4 Measures 71

3.1.2.5 Data Analysis 72

3.1.3 Results 73

3.1.4 Discussion 81

3.2 Effects of auricular manual and electrical stimulation on vagal activity **87**

3.2.1 Introduction 87

3.2.2 Materials and Methods 91

3.2.2.1 Participants 91

3.2.2.2 Procedure 91

3.2.2.3 Interventions 92

3.2.2.4 Measures 94

3.2.2.5 Data Analysis 96

3.2.3 Results 96

3.2.4 Discussion 100

4. GENERAL DISCUSSION **106**

4.1 Summary of the results **106**

4.1.1 The vagus nerve functionality as indicator for biopsychological stress responsiveness 107

4.1.2 Effect of auricular electrostimulation on vagal activity 107

4.2 Discussion and embedding in the theoretical background **108**

4.3 Limitations and strengths **118**

4.4 Implications and directions for future studies **123**

5. REFERENCES **126**

Acknowledgements

First of all I would like to express my gratitude to my supervisor Prof. Dr. Ulrike Ehlert which made this work possible. She has supported and promoted me throughout the last three years with her expert knowledge and enthusiasm. I appreciate very much the confiance she gave me and the career advancement she offered me throughout all of these years. I am very thankful for all personal and professional skills I could develelop and acquire during my dissertation. In many aspects she was a role model to me. Additionally, I would like to thank also Prof. Dr. Markus Heinrichs for his evaluation of my thesis and his joy at science which was inspiring for me.

Furthermore I appreciate very much the excellent cooperation I had with my dear colleague Prof. Dr. Matthias Kliegel. Our professional and amicable contact was always an inspiration for me and made the cooperation a real pleasure. I appreciate the possibilites I was offered at my department to cooperate with excellent partners from whom I could acquire several skills and therefore contributed to my professional development (in alphabetical order): Dr. Bert Arnrich, Prof. Dr. Wolfgang Langhans, Prof. Dr. Dr. Andreas Maercker, Prof. Dr. Jens Pruessner, MSc Cornelia Setz and MSc Lizhuang Yuan.

This work would not have been possible without the professional and especially personal help and support from my friends and angels at the department (in alphabetical order): Elvira Abbruzzese, Beate Ditzen, Katja Erni, Tiziana Perini, Kerstin Suarez and Myriam Thoma. They have all contributed to the success of the present work and supported me in every step. A close friendship has developed between us and they have immensely contributed to the joy I had doing my projects. I am thankful for all experiences we made together in Zurich, Tenero, Bangkok, Tokyo and Kamakura. I look forward to many wonderful experiences in the future, here and abroad.

I would like to thank all my master students which all worked hard and with enthusiasm thus contributing to the excellent outcome of all my projects (in alphabetical order): Franziska Beck, Andrea Brand, Michael Hausherr, Marko Nedeljkovic, Thanh Thuy Nguyen, Silvan Steiner, Hanna Thörn, Mélanie Tripod and Patricia Waldvogel. I am greatful for all experiences gained with them challenging and boosting my enthusiasm and knowledge. Each of them in a very special and individual manner has contributed to this work and my development.

Furthermore this project was only possible thanks to the help of my research assistants which facilitated my work tremendously and contributed to the successful conduction of my studies through their loyality, selfless effort and often needed spontaneity (in alphabetical order): Simone Dörfler, Susanne Fischer, Michael Hausherr and Patricia Waldvogel.

I would like to thank also my proud parents, my beloved sister Elisabeth and my beloved brother Sandro which sustained and supported me all over the past years and offered me always a challenging and agreeable platform for developing my salesmanship. Special thanks to my sister who proof-readed my work.

A special thanks goes to my friends Roman Gräzer, Christoph Seiler, Chantal Baumann, Cornel, Bea und Martin Auf der Maur and all the Zuger who were an immense resource and had a big patience in the last years where I spent a lot of my time realizing my projects and living my professional dream.

In conclusion, I express my gratitude to the financial support of the Forschungs- und Nachwuchsförderungskommission der Universität Zürich which made possible the stress study presented in this work.

Abstract

The vagus nerve constitutes a major portion of the parasympathetic nervous system. It innervates several vital organs, among others the heart, where it elicits a decrease in HR, therefore minimizing metabolic costs and counterbalancing sympathetic influences. Low vagal activity is associated with several risk factors, morbidity and mortality, while vagal tone can be interpreted as a resource and index of the functional state of an organism. Stress, as a major risk factor, is known to decrease cardiovagal activity, but data are inconsistent, presumably due to methodological problems in association with the high sensitivity of the ANS. On the other hand, interventions increasing vagal activity show beneficial effects on health and well-being. The purpose of the present work was to prove the negative effect of acute stress and the positive effect of auricular stimulation on vagal activity. Additionally, we aimed to examine the role of vagal functionality as a resource during stress.

We therefore conducted two studies. Thirty-three healthy male subjects participated in random order in a psychosocial stress and control condition. Stress was induced by the Montreal Imaging Stress Task (MIST). To examine whether vagal functionality can be interpreted as resource during stress, a functional diagnostic test was further conducted (cold face test, CFT). In the stimulation study, manual and electroacupuncture were applied to the ear in 14 healthy male subjects while controlling for several confounding factors (among others placebo effect). Vagal activity was measured with the LifeShirt System 200 (Vivometrics, CA, USA).

Analyses identified a significant stress-induced decrease in vagal activity and mood, while provoking an increase in sympathetic and HPA axis activity. Vagal functionality was revealed to be inversely related to the biopsychological stress response. In contrast to stress, electric but not manual stimulation to the ear revealed a significant increase in vagal activity.

In the present work, we were able to underline the harmful character of stress on several body systems while boosting the role of vagal functionality for health. Additionally, electrical stimulation to the ear possibly constitutes a simple, mildly invasive method to improve individual health status, adaptability and flexibility.

Tables

Table 2.1	Functions of the ANS (Hamill & Shapiro, 2004)	8
Table 2.2	Established frequency-domain measures of HRV (adapted from Task Force, 1996)	18
Table 2.3	Selection of time-domain-measured of HRV often used to characterize autonomic activity (Task Force, 1996)	19
Table 2.4	Regulation of the heart as a function of vertebrate phylogeny (from Porges, 2001). '+' indicates a cardioexcitatory and '-' a cardioinhibitory influence	21
Table 3.1	Partial correlation coefficients between vagal reagibility and stress responses controlling for credibility of the stress task and when appropriate the MDBF baseline values	79

Figures

Figure 2.1	Interconnections of some structures associated with the CAN (adapted from Brown & Gerbarg, 2005)	7
Figure 2.2	Electrocardiogram (reproduced with permission from Porter, 2003, available: www.merck.com/media/mmhe2/figures/MMHE_03_021_01_eps.gif)	14
Figure 2.3	Association of RR inverval alterations (middle) from the ECG (bottom) with respiration (top)	16
Figure 2.4	The social engagement system: Social communication is determined by the cortical regulation of brainstem nuclei (adapted from Porges, 2007)	24
Figure 2.5	Schematic representation of the HPA axis	40
Figure 2.6	Simplified illustration of the autonomic innervation of the three great paired salivary glands (La Marca, 2005)	42
Figure 2.7	Effects of the CAN on heart rate (adapted from Thayer & Lane, 2009)	43
Figure 2.8	Cardiovascular events represent the main cause for mortality in Switzerland (43.3%; left) and hospitalization (53%; right) (adapted from Lüscher et al., 1999)	51
Figure 2.9	Vagus nerve stimulation (reproduced with permission from Bryan Christie Design LLC, available: www.bryanchristiedesign.com/uploadfiles/2840005_med_vns.jpg)	56
Figure 3.1	Salivary cortisol concentration (nmol/l) during the stress (solid line) and control condition (dashed line). Values represent mean +- standard error of the mean values	75

Figure 3.2 Salivary amylase activity (U/ml) during the stress (solid line) and control condition (dashed line). Values represent mean +- standard error of the mean values. 75

Figure 3.3 HR (bpm) during the stress (solid line) and control condition (dashed line). Values represent mean +- standard error of the mean values 76

Figure 3.4 RSA (ms) during the stress (solid line) and control condition (dashed line). Values represent mean +- standard error of the mean values 77

Figure 3.5 Stress reaction of salivary cortisol in subjects with fast (dashed line) and slow (solid line) vagal reagibility determined after median split. Values represent mean +- standard error of the mean values 80

Figure 3.6 Stress reaction of mood in subjects with fast (dashed line) and slow (solid line) vagal reagibility determined after median split. Values represent mean +- standard error of the mean values 81

Figure 3.7 Mean and SEM of ratings of pain sensation from a Lickert scale during the different interventions 97

Figure 3.8 Mean and SEM of AUCi of RSA during the different examinations 99

Abbreviations

AA	Alpha-amylase
ACC	Anterior cingular cortex
ACE	Angiotensin-converting enzyme
ACh	Acetylcholine
ACTH	Adrenocorticotropic hormone
ADS-L	Allgemeine Depressionsskala-Langversion
AN	Anorexia nervosa
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ANS	Autonomic nervous system
AUCg	Area under the curve with respect to the ground
AUCi	Area under the curve with respect to increase
AVP	Arginine vasopressin
BED	Binge eating disorder
BMI	Body mass index [$kg/(m^2)$]
bpm	beats per minute
CAN	Central autonomic network
cc	cubic centimeters
CeNA	Central nucleus of the amygdala
CES-D	Center for Emidemiologic Studies Depression Scale
CFT	Cold face test
$CFT_{latency}$	Time till reaching peak bradycardia
CFT_{max}	Difference between HR baseline prior to the CFT and peak bradycardia
CGRP	Calcitonin gene-related peptide

CNS	Central nervous system
CRH	Corticotropin-releasing-hormone
DMN	Dorsomotor nucleus / nucleus dorsalis nervi vagi
DVC	Dorsal vagal complex
eAP	Electroacupuncture
ECG	Electrocardiogram
EEG	Electroencephalogram
EPI	Epinephrine
fMRI	Functional magnetic resonance imaging
GAD	Generalized anxiety disorder
HF	Power in the high frequency range
HFnorm	HF power in the normalized units
HP	Heart period
HR	Heart rate
HRV	Heart rate variability
Hz	Hertz
IBI	Inter-beat-interval
IP	Inductive plethysmography
kg	Kilogramm
LC	Locus coeruleus
LF	Power in the low frequency range
LF/HF-ratio	Ratio LF[ms^2]/ HF[ms^2]
LFnorm	LF power in the normalized units
m	Meter
mAP	Manual acupuncture
MD	Major depression

MDBF	Mehrdimensionaler Befindlichkeitsfragebogen
MI	Myocardial infarction
MIST	Montreal Imaging Stress Task
MIST-C	Control condition of the MIST
MIST-S	Stress condition of the MIST
ms	milliseconds
N.	Nervus
NA	Nucleus ambiguous
NE	Norepinephrine
NN50count	Number of pairs of adjacent NN intervals differing by more than 50ms in the entire recording
NO_2	Nitrogen dioxide
nonAP	Control condition of the acupuncture study without needles
NPY	Neuropeptide Y
NSNT	Nucleus spinalis nervi trigemini
n.u.	Normalized units
NTS	Nucleus of the solitary tract
PAG	Periacqueductal grey
pAP	Placebo acupuncture with Streitberger needles
PASAT	Paced auditory serial addition test
PD	Panick disorder
PBN	Nucleus parabrachialis
PET	Positron emission tomography
PFC	Prefrontal cortex
$PM_{2.5}$	Particulate matter <2.5 µm in aerodynamic diameter
pNN50	NN50 count divided by the total number of all NN intervals
X	

PNS	Parasympathetic nervous system
PTSD	Posttraumatic stress disorder
PVN	Paraventricular nucleus
RAS	Renin-angiotensin system
rCBF	Regional cerebral blood flow
RMSSD	Square root of the mean of the sum of the squares of differences between adjacent NN intervals
RN	Raphe nucleus
rpm	Rounds per minute
RSA	Respiratory sinus arhythmia
RVLM	Rostral ventralateral medulla
SAM	Sympatho-adrenomedullary
SCN	Suprachiasmatic nucleus
SDNN	Standard deviation of all NN intervals
SES	Socio-economic status
SNS	Sympathetic nervous system
SO_2	Sulfur dioxide
SPECT	Single photon emission computed tomography
TENS	Transcutaneous electrical nerve stimulation
TSST	Trier Social Stress Test
tVNS	Transcutaneous vagus nerve stimulation
U	Units
ULF	Power in the ultra low frequency range
VAS	Visual analogue scale
VIP	Vasoactive intestinal peptide
VLF	Power in the very low frequency range

VN	Vagus nerve
VNS	Vagus nerve stimulation
VVC	Ventral vagal complex

1. INTRODUCTION

"If the pattern of the heart beat becomes as regular as the tapping of a woodpecker or the dripping of rain from the roof, the patient will be dead in four days"

(Wang, cited by Cheng, 2000, p. 2082)

The positive association between low variability of the heart rate (HRV) and morbidity and mortality was recognized early on, as demonstrated by the above quotation from the Chinese medical practitioner Wang Shuhe in the 3rd century AD. Since then, several disorders have been found to be associated with a reduced HRV as an index of the activity of the vagus nerve (VN) (for an overview, see Thayer & Brosschot, 2005). The VN constitutes a major portion of the parasympathetic nervous system, showing connections to a multitude of vital organs such as the heart. Interestingly, the VN is not only an output structure of the central autonomic network but reveals a huge portion of afferents, therefore influencing the activity of several central nervous structures. As mentioned above, the activity of the VN is reduced in several disorders such as depression, anxiety disorders, schizophrenia, eating disorders and cardiovascular diseases. Nowadays, it is additionally recognized that vagal activity is not only associated with morbidity but also with several risk factors present in (apparently) healthy subjects (e.g. Thayer & Lane, 2009). Some of these risk factors cannot be actively manipulated (e.g. age or gender), while others can (e.g. BMI, smoking, drinking or physical exercise). Therefore, the VN offers an explanation or mode of action regarding how risk factors harm our health.

A risk factor in everybody's life is stress. Stress is known to be an important factor in the development and progression of several disorders. Stress can elicit a multidimensional biopsychological stress response, such as an activity increase of the HPA axis and the SAM

system. The latter can be subsumed to the sympathetic nervous system, eliciting further responses to prepare the body in terms of the fight-or-flight response. Besides these two systems, which were discovered early on in stress research, stress is also known to elicit a decrease in parasympathetic activity (Porges, 1995). Nevertheless, data on this stress-induced effect are inconsistent. Several studies detected a decrease in parasympathetic activity, but in some of them, disturbing factors (e.g. motion, postural changes) might also, at least in part, explain the results (e.g. Klinkenberg et al., 2008). Furthermore, some results were presented showing no effect of acute stress on parasympathetic activity (e.g. Altemus et al., 2001). There are even data revealing an increase of parasympathetic activity during stress (e.g. Sahar et al., 2001). This inconsistency acts as a counterbalance to the clear theoretical assumption. Therefore, methodological aspects in stress research have to be reconsidered, especially with regard to the stress tasks. The Montreal Imaging Stress Task (MIST; Dedovic et al., 2005) is a newer, standardized computerized stress task combining challenging arithmetic problems with social-evaluative threat. It was originally developed for imaging purposes in stress research, and has revealed interesting insights into central nervous responses during acute stress (e.g. Pruessner et al., 2008). Due to the requirements regarding motionlessness in an fMRI environment, the MIST shows several characteristics that also make the task ideal for the examination of stress-induced autonomic activity alterations. While the task was shown to consistently excite the HPA axis and the sympathetic nervous system (Pruessner et al., 2004, Soliman et al., 2008), no study to date has examined its effect on the parasympathetic nervous system. Therefore, one purpose of the present work was the examination of the effectiveness of the MIST to elicit a parasympathetic decrease with a special focus on cardiovagal activity.

Stress in terms of the transactional stress model is present if the appraisal of demands is higher than the appraisal of the counterbalancing resources (Lazarus & Launier, 1981). In the framework of the neurovisceral integration model, the activity of the VN can be interpreted as

a physiological resource due to its reciprocal influences and associations with the central nervous system, especially the inhibitory prefrontal cortex (Thayer & Lane, 2009). Evidence exists showing the inverse relationship between HRV and cortisol in overnight urine (Thayer et al., 2006) and cortisol during stressful cognitive tasks (Johnsen et al., 2002). Since the findings supporting an association between cortisol and HRV are scarce, a second aim of the present study was to examine this relationship by measuring the cortisol response during the MIST on the one hand and resting vagal activity and vagal functionality during the cold face test on the other. The cold face test elicits a trigeminal-vagal-mediated bradycardia, therefore imitating the diving reflex (Khurana & Wu, 2006).

To summarize, the VN is thought to be an important physiological resource sensitive to several risk factors such as stress, and is negatively associated with morbidity and even mortality. Therefore, the question arises of whether the VN can also be used as a target for interventions. This question can be answered in the affirmative, since physical training, pharmacological and nutritional interventions can increase vagal activity, and one therapeutic method in particular has attracted attention in the past ten years: the invasive vagus nerve stimulation (VNS). The VNS is a highly invasive treatment entailing wires attached directly to and electrically stimulating the left VN. It is predominantly applied in therapy-resistant epilepsy and depression (Schachter & Saper, 1998). Its effect on primary symptomatology (number of epileptic seizures, depressive mood) was repeatedly confirmed, even though a placebo effect was also recently recognized in VNS therapy (Rush et al., 2005a). Furthermore, studies examining central and autonomic nervous activity alterations were conducted, showing positive effects on the activity of the structures subsumed in the central autonomic network (Benarroch, 1997; activations in structures positively associated with vagal activity), and inconsistent results on cardiovagal activity (Galli et al., 2003; Setty et al., 1998; Stemper et al., 2008). Altogether, the effects of the VNS are satisfactory, but due to its

high invasiveness, VNS is restricted to therapy-resistant patients only. An intervention increasing vagal activity applicable to all (sub)clinical patients or even in healthy subjects in terms of prevention would be appealing. Therefore, the interest in alternative methods has increased, with a focus on Asian medicine. Meditation, yoga, acupressure and acupuncture have been examined, revealing inconsistent results with regard to autonomic nervous effects. Some evidence exists suggesting vagoexcitatory effects of mildly invasive body and auricular acupuncture (e.g. Haker et al., 2000; Hübscher et al., 2007; Karst et al., 2007; Streitberger et al., 2008). Nevertheless, methods and interpretations from several acupuncture studies can be criticized, possibly contributing to the heterogeneous findings of these studies. The mode of action is often unclear. The examination of the effects of auricular stimulation (manual and electroacupuncture) on cardiovagal activity was the purpose of the second study presented in this thesis. The ear was chosen as the target organ due to the presence of vagal afferents (Fallgatter et al., 2003; Lang, 1992; Tekdemir et al., 1998), therefore providing a basis for the explanation of possible vagal effects. Additionally, several problems encountered in acupuncture research were controlled, such as placebo and pain effects. To summarize, the aim of this study was the examination of vagal activity during confrontation with a risk factor (stress), the role of the VN as a physiological resource during stress, and the appropriateness of auricular stimulation to induce an increase in vagal activity.

In the following, structural and functional properties of the VN, factors negatively associated with vagal activity and interventions increasing vagal activity are presented in the theoretical background (chapter 2). Following this, the two studies (stress and stimulation study) conducted as part of the present doctoral thesis are presented including the results (chapter 3), before concluding with a general discussion (chapter 4).

2. THEORETICAL BACKGROUND

The VN is often called the “health nerve” and has gained interest in clinical research in the last years. In the following, structural and functional properties are presented, culminating in two theories promoting the importance of the VN for (clinical) behavioural research (chapter 2.1). Following this, studies examining vagal functionality in association with several risk factors and disorders are reported, in an attempt to underline the importance of the thus far often neglected role of the VN in clinical research (chapter 2.2). A newer area of research focusing on interventions increasing the activity of the “health nerve” is then presented, with a focus on affective, central and autonomic effects (chapter 2.3).

2.1 The vagus nerve: structural and functional properties

The vagus nerve (VN) is part of the autonomic nervous system (ANS). Before going into detail about the VN, which is of special interest in this thesis, the ANS will be discussed.

2.1.1 The autonomic nervous system

The ANS, together with the somatic nervous system, constitutes the peripheral nervous system (Pinel & Pauli, 2007). In contrast to the somatic nervous system, the ANS is not subject to conscious and arbitrary control to the same extent and is therefore termed autonomic (Birbaumer & Schmidt, 2003). It consists of the sympathetic (SNS), parasympathetic (PNS), and enteric nervous system (Heim & Meinlschmidt, 2003). Sometimes, the enteric nervous system, which controls the gastrointestinal system, is considered independent and therefore ignored when referring to the ANS. Since the present work focuses mainly on the cardiac system, the enteric nervous system is left out when

referring to the ANS. From another perspective, the ANS can be divided structurally into the central and peripheral ANS.

2.1.1.1 The central autonomic nervous system

The central autonomic network (CAN) consists of several structures: the ventromedial prefrontal cortex (PFC), insular cortex, anterior cingular cortex (ACC), central nucleus of the amygdala (CeNA), paraventricular nucleus (PVN) and related nuclei of the hypothalamus, periacqueductal grey (PAG), nucleus parabrachialis (PBN), nucleus of the solitary tract (NTS), nucleus ambiguous (NA), ventromedial and ventrolateral medulla, and medullary tegmental field (Benarroch, 1997). These structures show complex and reciprocal, direct and indirect connections (fig. 2.1). The insular cortex, with its organotropic organization, constitutes the primary visceral sensory cortex (Saper, 2002). It shows, among others, connections with the NTS and PBN relaying visceral afferent information. The ventromedial PFC modulates emotional responses through connections with the amygdala and constitutes an important inhibitory control on the latter (Thayer & Lane, 2000). The amygdala possesses several connections and plays a critical role in emotional response, integrating autonomic, endocrine and motor responses with emotion (Amaral et al., 1992).

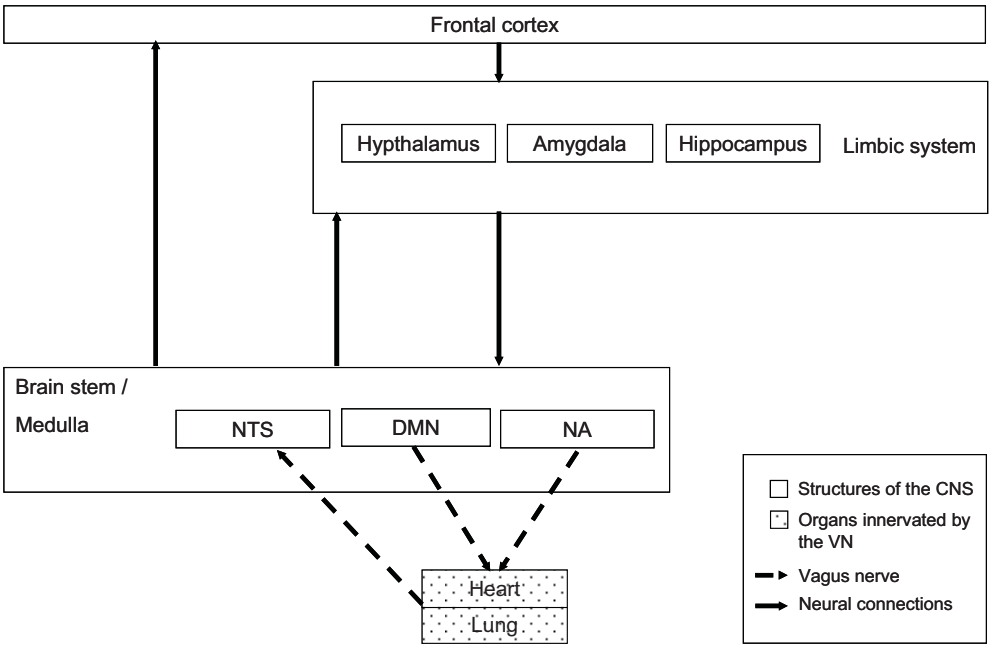


Figure 2.1 Interconnections of some structures associated with the CAN (adapted from Brown & Gerbarg, 2005)

The ACC is involved in the initiation, motivation, and execution of emotional and goal-directed behaviours. A magnitude of nuclei of the hypothalamus has various connections and functions. One of them, the PVN, contains a main portion of autonomic output of the hypothalamus (Benarroch, 2004). The PBN receives input from the NTS and projects to the thalamus, hypothalamus and amygdala, and plays an important role in cardiovascular activity and respiration, but also gastrointestinal activity (Benarroch, 2004). The NTS relays viscerosensory information to all central autonomic regions, including the PVN, PBN and medial orbitofrontal cortex. The premotor neurons of the ventrolateral medulla contain sympathetic output to preganglionic vasomotor neurons, and further control cardiac and respiratory functions. Sympathetic input to preganglionic neurons is further provided by neurons of the ventromedial medulla thought to play a role in arterial pressure (Benarroch, 2004). The dorsomotor nucleus (DMN) and NA contain parasympathetic efferents (Porges, 2003).

2.1.1.2 The peripheral autonomic nervous system

The cell bodies of preganglionic neurons of the peripheral ANS lie in the central nervous system (CNS). The short sympathetic preganglionic fibers leave the spinal cord to the near chain of sympathetic ganglia and activate long, unmyelinated postganglionic nerve fibers through chemical cholinergic synapses. Postganglionic neurons principally release norepinephrine (NE). By contrast, the PNS mediates its effects by leaving the CNS, among others, out of the brain stem through cranial nerves containing long and in part myelinated preganglionic fibers. The PNS releases acetylcholine (ACh) in the parasympathetic ganglions situated near to or in the walls of the target organs, where in contrast to the SNS, they release ACh (Birbaumer & Schmidt, 2003).

The ANS adapts the processes of the body to the rapidly changing demands of the environment and coordinates two major roles: homeostasis and the adaptive responses to stress (fight-or-flight response). To fulfil these two functions, it can fall back on the mostly antagonistic functions of the SNS and PNS on most of the innervated organs (tab. 2.1).

Table 2.1 Functions of the ANS (Hamill & Shapiro, 2004)

Organ	SNS	PNS
Eye (pupil)	Dilation	Constriction
Eye (ciliary muscle)	Relax (far vision)	Constrict (near vision)
Lacrimal gland	Slight secretion	Secretion
Parotid gland	Slight secretion	Secretion
Submandibular gland	Slight secretion	Secretion
Heart	Increased rate; pos. Inotropism	Slowed rate; neg. inotropism
Lungs	Bronchodilation	Bronchodilation
Gastrointestinal tract	Decreased motility	Increased motility
Kidney	Decreased output	-
Bladder	Relax detrusor; contract sphincter	Contract detrusor; relax sphincter
Penis	Ejaculation	Erection
Sweat gland	Secretion	Palmar sweating
Piloerection muscles	Contraction	-
Blood vessels: arterioles	Constriction	-
Muscle (arterioles)	Constriction or dilation	-
Muscle (metabolism)	Glycogenolysis	-

2.1.2 The vagus nerve

The VN (X. cranial nerve) constitutes the main portion of the PNS, innervates a multitude of organs and is vitally important (Benninghoff, 2008). It contains visceromotoric, viscerosensoric, and somatosensitive nerve fibers (Trepel, 2004), while the viscerosensitive afferents constitute 80% of the vagal fibers (Porges, 2003). The fibers of the VN are associated with four interconnected nuclei: the DMN contains visceromotor nerve fibers innervating among others the heart and lung, the NA contains visceromotor nerve fibers innervating organs superior to the thoracic diaphragm, the nucleus spinalis nervi trigemini (NSNT) receiving somatosensitive afferents and the NTS receiving viscerosensitive input and afferent information from the N. glossopharyngeus, N. facialis, and the NSNT (Chien et al., 1996; Trepel, 2004). The NTS, as mentioned above, possesses a multitude of direct and indirect connections to the other structures of the CAN. The VN leaves the brain stem behind the olive and extends through the foramen jugulare in the skull to the head, neck, chest and abdomen (Trepel, 2004) and divides into several branches. In general, the VN is involved in digestive and restorative somatic function (Swanson, 2003).

2.1.2.1 Vagal innervation of the heart

Two branches, the rami cardiaci cervicales superiores and inferiores, quit the DMN to innervate the plexus cardiacus of the heart. The right rami of the bilaterally organized VN mainly connects with the sinoatrial node, while the left rami innervate mainly the atrioventricular node. Conversely, viscerosensitive afferents reach the NTS through the same rami (Trepel, 2004). The heart is innervated by both branches of the ANS, which at the plexus cardiacus constitutes the major modulator of heart activity (Cacioppo, Tassinary & Berntson, 2007). The heart is active per se, since it contains cardiac myocytes and some of them are

subsumed in the sinoatrial node functioning as a pacemaker (Thews & Vaupel, 2005). The heartbeat of the denervated heart has a pace of about 100 beats per minute (bpm; Ganong, 2005; cited after Chai et al., 2008, p. 695), while sympathetic innervation produces an increase and vagal innervation a decrease of the heart rate (HR) (Cacioppo et al., 2007). However, an increase of HR can also be produced by a reduced vagal activation. As described by Thayer and Lane (2009), the increase of HR can be initiated in the CAN (fig. 2.8). The bidirectional and interacting forebrain structures can activate sympathoexcitatory neurons of the rostral ventrolateral medulla (RVLM), therefore increasing HR. But the structures of the CAN can simultaneously inhibit the NTS, thereafter eliciting a decreased activity of the visceromotor neurons in the DMN and NA, which results in a reduced vagal input to the heart leading to an increase in HR. Additionally, a CAN-mediated reduced activation in the NTS leads to a decrease of inhibition on the sympathoexcitatory neurons of the RVLM, again eliciting an increase in HR.

The normal contraction rate of 72 bpm at rest (Andrassy, 2007) compared to the 100 bpm in a denervated heart, underlines the dominance of the vagal input to the heart. In general, the VN possesses a negative chronotropic, dromotropic, and inotropic effect, resulting in a decrease in HR, an increase in time of intracardiac signal transmission, and a decrease in contractility (Lewis et al., 2001; Thews & Vaupel, 2005). An important difference between vagal and sympathetic innervation characteristics is the kinetics. While vagal inputs effect and decay rapidly and within a heartbeat, sympathetic inputs are much slower (Franchini & Cowley, 2004; Spear et al., 1979).

The bidirectional connection between the brain and the heart plays an important functional role in the fight-or-flight response but also in emotional, cognitive and attentional regulation. Vagal tone, further to be an output variable, can additionally be seen as a resource of the individual in a challenging environment (Thayer & Lane, 2009).

Although the main factor influencing cardiac activity is the impact of autonomic innervation described above, several other factors can influence the activity of the heart. Some of the most prominent factors controlling the heart besides the direct autonomic innervation are neuropeptides (for example neuropeptide Y (NPY), which is often present in autonomic synapses), indirect autonomic influence through catecholomines predominantly secreted after activation of the sympatho-adrenomedullary (SAM) system, activity alterations of the renin-angiotensin system (RAS), and the respiratory-related mechanical stretch of the sinoatrial node (Berntson et al., 1997).

2.1.2.2 Vagal innervation of the ear

The ear is an important organ, among other things in social interaction or in the detection of the direction of auditory threat signals. However, it is not a vital organ, but since the ear and its innervation plays a crucial role in the present work, the neuroanatomical basis of the ear is nevertheless presented. The ear is innervated among other things by the VN (Fallgatter et al., 2003; Lang, 1992; Tekdemir et al., 1998). The auricular branch of the VN, the ramus auricularis nervi vagi, is also called the Alderman's or Arnold's nerve. The Arnold's nerve contains vagal afferents leading from the skin of the external auricular canal (external acoustic meatus), through the fissura tympanomastoidea and the mastoidal canal into the superior ganglion, relaying the information through the foramen jugulare into the NSNT (Benninghoff, 2008). However, afferents are also reported in the concha (Lang, 1992), and there is additional evidence for vagal afferents in the helix and antihelix of the ear (Gao et al., 2008) and the presence of heterogeneous nerve fibers in one and the same area (Folan-Curran & Cooke, 2001).

The NSNT projects to the NTS (Chien et al., 1996), which leads bottom-up information to the structures of the CAN and might also influence affective state (Kraus et al., 2007). However,

together with the NSNT, the NTS can also interact with the visceromotor vagal nuclei, integrating these inputs with top-down information affecting cardiac function. The NSNT was shown to elicit a bradycardia and a hypotension in rabbits during electrical stimulation of the NSNT (Kumada et al., 1977). After several adjuvant pharmacological and chirurgical interventions, the authors came to the conclusion that the stimulation of the NSNT leads to a reflex activating cardiovagal efferents and an inhibition of cardiosympathetic nerve fibers, while baroreceptors do not seem to be too affected. The authors report that this reflex can be elicited through the stimulation of different cranial nerves such as the N. trigeminus (V.), the N. glossopharyngeus (IX.) and the somatosensitive nerve fibers of the NV (X.). Therefore, the stimulation of the ear, but also the reflex mediated through application of a cold stimulus like in the CFT, are thought to be mediated at least among other things through the stimulation of the NSNT. Aside from bradycardia as part of the diving reflex, stimulation of the ear can provoke different reflexes such as ear-palatal and the ear-vomiting reflexes attributable to vagal stimulation (Gupta et al., 1986; Majer, 1953).

2.1.2.3 Indicators of vagal activity: markers not referring to the heart

The PNS innervates several organs affecting their activity by some means or other. Normally, the innervated organs have several influencing factors and therefore their activity cannot uniquely be linked to parasympathetic input. Nevertheless, they provide some information on the activity of the PNS.

Pupillary responses are frequently used indicators of autonomic state. Alterations of the pupillary aperture are mediated through the ANS. While the SNS leads to a dilation of the pupil through activation of the radial fibers, the PNS leads to a pupillary constriction through innervation of circular fibers of the iris (Andreassi, 2007). This latter response is indicative of

the activity of the parasympathetic efferents along the III. cranial nerve (oculomotor nerve), but might not be associated with the activity of the VN.

The VN innervates, among others, the thyroid gland, thymus and pancreas. In these organs, vagal stimulation can alter the secretion of several substances. In the thyroid gland, vagotomy and stimulation studies suggest, among other things, a positive association between vagal activity and the secretion of calcitonin gene-related peptide (CGRP; Grunditz et al., 1986), substance P (Grunditz et al., 1988), and vasoactive intestinal peptide (VIP; Ito et al., 1987) and an inhibitory influence of the VN on thyroid hormone secretion (Melander et al., 1979). Referring to the thymus, the VN is thought to heighten lymphocyte release (Antonica et al., 1994). In the pancreas, the vagal stimulation can increase the secretion of amylase (Vega et al., 1977), while 2-3 months after a vagotomy, pancreatic gland weight and the amount of Langerhans islets were found to decrease (Tiscornia et al., 1981). The latter are important for the release of insulin and amylin.

These findings are only a small selection of the effects of the VN on the body. The problem about these parameters is that they can only be measured invasively and are susceptible to many influencing factors. Therefore, the markers above can indicate the state of the VN, but due to the many influencing factors cannot be seen as proper marker of vagal activity. Measuring vagal activity markers associated with cardiac and cardiorespiratory activity seems to be much more useful and reliable, since alterations of these markers allow a clearer attribution to vagal state and since a continuous measurement is possible.

2.1.2.4 Indicators of vagal activity: electrocardiographic markers

When considering the heart, several measures are used to indicate vagal activity. Electrocardiac markers thereby hold a great advantage compared to the determination of the non-cardiac markers mentioned above: the simplicity of determination, since electrocardiac

markers can be measured non-invasively on the body surface with stationary but also small and easy-to-wear ambulatory devices. Therefore, subjects can be examined in nearly every situation all day long.

All cardiac markers discussed in the following rely on the electrocardiogram (ECG). The early measurement of heart activity took place with galvanometers around 1900. The underlying phenomenon is based on the fact that a part of the electrical impulses passing the heart during its contraction spreads to the body's surface and can therefore be detected by electrodes on the skin. Thus, the ECG is the record of amplified cardiac electrical potentials on body surface. The ECG shows several signal alterations (fig. 2.2).

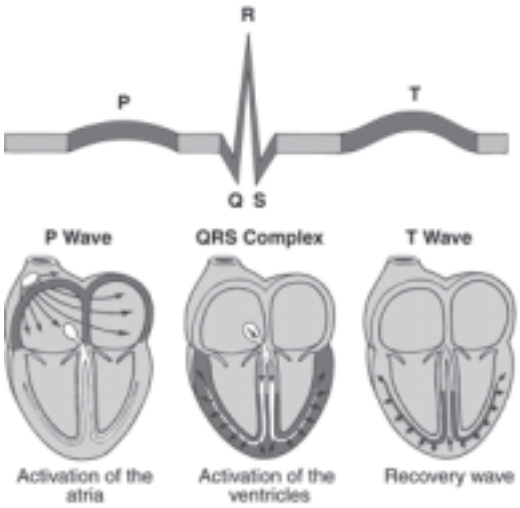


Figure 2.2 Electrocardiogram (reproduced with permission from Porter, 2003, available: http://www.merck.com/media/mmhe2/figures/MMHE_03_021_01_eps.gif)

The most important ones determine the QRS complex, representing currents generated in the ventricles (fig. 2.2) during depolarization prior to ventricular contraction. The P wave refers to the current generated before the contraction of the atria and the T wave the repolarisation of the ventricles (Andreassi, 2007).

For psychophysiological research, the most interesting characteristic extracted from the ECG is the RR interval (or NN interval; interval between two R waves). The time in-between two beats is called heart period (HP) or inter-beat-interval (IBI). Based on the HP, the heart rate (HR) can be reciprocally determined. In fact, the HR is based on the number of beats per unit of time (normally per one minute), and can therefore be determined with the following formula (Vossel & Zimmer, 1998): HR (bpm) = 60'000 (ms) / HP (ms).

Besides variations of HR called heart rate variability (HRV; see below), resting HR can be interpreted as an index of vagal activity, since under resting conditions, the heart is under predominant vagal inhibition (Thayer & Sternberg, 2006). Additionally, the HR change after cessation of physical exercise, called HR recovery, is used as an index of vagal activity (Thayer & Lane, 2007). An alternative and more complex variable is HRV, which offers the basis for several markers indicating the state of the ANS. At the beginning of cardiophysiological research, HR was thought to be a stable rhythm. As noted by Porges (2007), in the past, HRV was often misleadingly interpreted by several prominent psychophysiologists as an artefact due to poor experimental control. However, some authors noticed the simplest detectable marker of HRV early on: the alterations of HR in association with respiration. Hales (1733; cited following Berntson et al., 1997) was one of the first researchers to observe a respiratory pattern in the pulse and blood pressure of a horse. The alteration in the pulse associated with breathing is called respiratory sinus arrhythmia (RSA; fig. 2.3).

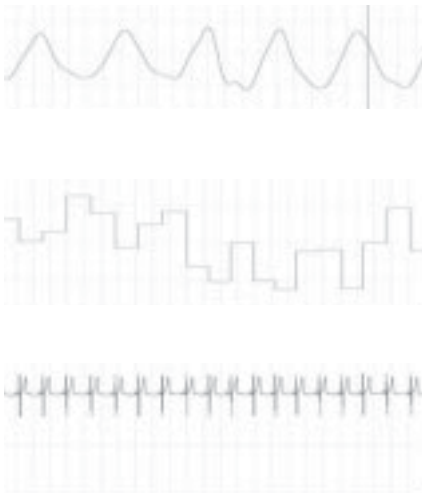


Figure 2.3 Association of RR interval alterations (middle) from the ECG (bottom) with respiration (top)

Hering (1910, cited following Berntson et al., 1997, p. 624) was one of the first researchers to support the association between the amplitude of RSA and vagal tone. In general, respiration-associated changes in HR are a sign of healthy autonomic state (Brotman et al., 2007) and more generally speaking of somatic and mental health (chapter 2.2.2). RSA is thought to be mediated predominantly by fluctuations of vagal-cardiac nerve traffic and therefore provides an index of vagal activity. Even if RSA is mediated predominantly by parasympathetic inputs on the sinoatrial node, the parasympathetic influence is not a simple one (Vaitl & Petermann, 2004), since several aspects of parasympathetic control are relevant (Berntson et al., 1997):

1. Respiration-associated phasic variation of vagal effects on the heart,
2. Central vagal output to the heart,
3. The mean level of vagal effect on the heart (meaning here a difference in mean RR interval between a resting intact baseline and a complete vagal blockade),

4. Dynamic vagal responses such as the parasympathetic baroreflex response affecting RR interval (by afferent carotic, aortic, and cardiopulmonary baroreceptor responses to short-term systemic blood pressure variations).

Therefore, the influence of the VN on the heart consists of different aspects of the VN and is not only unidimensional. Since the factors mentioned above are all dimensions of the parasympathetic control on the heart, they are normally correlated with each other. Nevertheless, lacking correlations have also been reported (for an overview, see Berntson et al., 1997). For example, respiratory patterns can have an influence on some of the aspects listed above; therefore, breathing alterations can minimize intercorrelations between the different parasympathetic factors. Thus, the consideration of respiratory patterns is normally recommended (e.g. Grossman & Taylor, 2007). In the present work respiratory parameters were not controlled. On the one hand, the additional analysis and statistical consideration of different respiratory parameters would go beyond the scope of this work, since it is very time consuming. On the other hand, the tasks and interventions were chosen to control for several disturbing factors influencing respiration (e.g. speaking, postural changes, motion).

As alternative variables of HRV, several time- and frequency-domain variables (besides rarer non-linear measures; see Task Force, 1996) are most often used in the literature indicative of several autonomic phenomena. Although they are more complex and newer indicators of autonomic control of the heart, in the following, the frequency-domain variables will be discussed before the time-domain variables due to their clearer association with RSA. Akselrod introduced power spectral analysis of HRV in order to evaluate beat-to-beat cardiovascular control (Akselrod et al., 1981). With spectral analysis, different frequency bands can be extracted out of the raw data (tab. 2.2).

Table 2.2 Established frequency-domain measures of HRV (adapted from Task Force, 1996)

Variable	Units	Description	Frequency range
Total Power	ms2	Variance of all NN intervals	Approximately ≤ 0.4 Hz
ULF	ms2	Power in the ultra low frequency range	≤ 0.003 Hz
VLF	ms2	Power in the very low frequency range	0.003-0.04Hz
LF	ms2	Power in the low frequency range	0.04-0.15Hz
LFnorm	n. u.	LF power in the normalized units LF/(Total Power-VLF)*100	
HF	ms2	Power in the high frequency range	0.15-0.4Hz
HFnorm	n. u.	HF power in the normalized units HF/(Total Power-VLF)*100	
LF/HF-ratio		Ratio LF[ms2]/ HF[ms2]	

Central frequency intervals were determined in association with autonomic interpretations.

The ULF has a slow period duration of more than 5.5 minutes (1/0.003=333), and the physiological interpretation is less clear, but it is thought that circadian, monthly and seasonal rhythms play a role. The VLF has a period duration of 25 seconds (1/0.04=25) up to about 5.5 minutes (1/.003). It is suggested that thermoregulatory processes and the effects of the RAS play a role in the VLF. The long periods of the ULF and VLF can be interpreted with increasing reliability with longer data acquisition times. When analyzing shorter segments of electrocardiographic data the LF and HF bands are more appropriate. The LF possesses period lengths of 6.6 (1/0.15) to 25 (1/.04) seconds. Sympathetic activity is thought to be the source of the LF, but there is also evidence for parasympathetic influences on this frequency band (Thayer & Lane, 2007). This is also evident from the high correlation of HF and LF (Liao et al., 2002). Therefore, the interpretation of alterations of this marker is not so clear. Thus, the ratio of LF to HF appears to be more promising. The HF band possesses the shortest periodic alterations of 2.5 (1/0.4) to 6.6 (1/0.15) seconds (Breitenbach, 2003). The peak is around 4 seconds and therefore parallels the respiration rate, with about 15 cycles per minute. Often, the HF is also used synonymously with RSA (Breitenbach, 2003; Kleiger et al., 2005) and is thought to be under parasympathetic control, as is apparent from stimulation and vagotomy

studies (see Task Force, 1996). The HF or HFnorm are very often used when authors are interested in determining parasympathetic activity. As noted above, an often used measure of HRV is the ratio of LF/HF. Due to the problems in the interpretation of LF, some authors use the ratio as an index of sympathovagal or sympathetic modulation (Berntson et al., 1997).

An alternative method to characterize HRV and the autonomic state of an individual is the determination of time-domain HRV measures. These are statistical and geometricmeasures (Task Force, 1996), but the latter are less frequently applied in the literature. They are easy to calculate from the ECG data. In the following, only a selection of the most common time-domain variables are discussed (tab. 2.3).

Table 2.3 Selection of time-domain measures of HRV often used to characterize autonomic activity (Task Force, 1996)

Variable	Units	Description
SDNN	ms	Standard deviation of all NN intervals
RMSSD	ms	Square root of the mean of the sum of the squares of differences between adjacent NN intervals
NN50 count		Number of pairs of adjacent NN intervals differing by more than 50ms in the entire recording. Three variants are possible, counting all such NN intervals pairs or only pairs in which the first or the second interval is longer.
pNN50	%	NN50 count divided by the total number of all NN intervals

Applied to a 24-hour ECG recording, these time-domain variables show approximate correspondence to frequency-domain variables. While the SDNN is associated with the total power, RMSSD, NN50 count and pNN50 are related to the HF measure of HRV (Task Force, 1996) and therefore constitute alternative variables determining the activity of the VN. Among the time-domain variables, pNN50 and RMSSD were shown to be highly correlated with each other (Kleiger et al., 2005), underlining their common parasympathetic derivation.

2.1.2.5. Determination of vagal functionality

The vagal markers mentioned above (chapter 2.1.2.4) can be determined during rest (normally named vagal tone) or during periods of phasic changes (e.g. during tests). Tests inducing phasic alterations of vagal activity (e.g. stress, exercise or reflexive tests) have different theoretical, anatomical and therefore different interpretative value. If the interest is in the functionality of the VN in terms of the reagibility of the "hardware" to react with an activity increase, an often used method is the cold face test (CFT; e.g. Khurana & Wu, 2006). In the CFT, a cold stimulus is applied to the face (the whole or a part of it), inducing the so-called diving reflex (Reyners et al., 2000). Besides a stimulation of the SNS, the CFT leads to an increase of the vagal activity through a trigeminal-vagal stimulation, provoking a bradycardia. The CFT is one of the most effective procedures to induce a stimulation of the VN (Arnold, 2000). Several studies used different cold stimuli such as the immersion of the face in cold water (e.g. LeBlanc et al., 1975), exposure to cold wind (LeBlanc et al., 2004) or the application of bags filled with iced water or cold gel (cold packs; e.g. Khurana & Wu, 2006). Methodological inconsistency also exists in terms of the temperature of the cold stimulus. Some authors use stimuli with a temperature between 0 degrees Celsius (Lin et al., 2004) and +6 degrees Celsius (Muth et al., 1998). Furthermore, the period of stimulation lasts between 1 (e.g. Hilz et al., 1999) and 4 minutes (e.g. Muth et al., 1998). Therefore, no standardization exists regarding the methodological characteristics of the CFT.

To summarize, following a neuroanatomical and functional description of the VN, several markers indicative of vagal activity were presented. These markers can be measured under different conditions (e.g. under rest or inhibiting or excitatory periods). The theoretical and methodological aspects reported above represent the basis upon which to understand findings regarding the VN (chapter 2.2 and 2.3). Two psychophysiologists, more then by the sum of their studies examining health variables associated with vagal activity, contributed to the

understanding of the role of the VN: Steven W. Porges and Julian F. Thayer. Both authors embedded the VN in physiologically higher-ranking theories of emotion and behaviour, which are supported by several (of their own) findings. In the following, the polyvagal theory (Porges, 1995) and the neurovisceral integration model (Thayer & Lane, 2000) are presented.

2.1.2.6 The polyvagal theory

The polyvagal theory by Porges (1995; 2001; 2003; 2007) constitutes a model that boosts and underlines the importance of the VN for different aspects of survival and well-being in general. Emotion, stress, orientation, attention and social engagement are central constructs for which the theory tries to deliver an explanation, and in return, the theory is also sustained by these constructs. As the name of the theory suggests, an important aspect of the model is based on the fact that humans, contrary to lower-developed species (tab. 2.4), possess more than one vagal system. Porges named the two vagal systems the dorsal (DVC) and ventral vagal complex (VVC).

Table 2.4 Regulation of the heart as a function of vertebrate phylogeny (Porges, 2001). '+' indicates a cardioexcitatory and '-' a cardioinhibitory influence

Animal group	Chromaffin tissue	Dorsal vagal complex	Spinal SNS	Adrenal medulla	Ventral vagal complex
Jawless fish	+	(+)			
Cartilaginous fish	+	-			
Bony fish	+	-	+		
Amphibians	+	-	+		
Reptiles	+	-	+	+	
Mammals	+	-	+	+	-

According to Porges, the two vagal systems possess structural and functional differences. From an evolutionary point of view, the DVC is the older, unmyelinated system, present even in more primitive life-forms, while the VVC is present only in mammals. Efferents of the DVC leave the DMN in the medulla oblongata to reach structures predominantly under the diaphragm, but also the heart. Vagal afferents of the DVC terminate in the NTS. By contrast, the myelinated vagal efferents of the VVC leave the NA via general visceral efferents to innervate structures mainly superior to the diaphragm such as the heart and the bronchi, and via special visceral nerve fibers to innervate striated muscles in the face and mouth. The functional output on the heart by the vagal pathways originating in the NA may be monitored by RSA (Porges, 2007). Dynamic changes in RSA and HR are suggested to be indicative of the status of the social engagement system (Porges, 2001). The afferents of the VVC are constituted via the NTS and the trigeminal and facial nerve.

In line with the more general dissolution theory of Jackson (1958), Porges postulates a hierarchical response of the organism when confronted with stressful challenges (2001). He proposes that distinct adaptive behavioural strategies lie under each stage. Older structures, which are still present in higher developed systems, are only activated in extreme cases. In mammals (including humans), the first choice of response is shown by engagement or disengagement from the environment (e.g. social communication, self-soothing, calming; Porges, 2003). In this case, the VVC can handle the demands of the environment. When the demands are too high to be met within the limits of the VVC, the older structures of the SNS, including the SAM system, are disinhibited to increase metabolic output to induce mobilization as part of the fight-or-flight response (active avoidance). Under extreme situations, the next phylogenetic older system, the DVC, can be mobilized and produces immobilization behaviours which are metabolically conservative and adaptive for primitive vertebrates (e.g. feigning death, defecation, apnoea and bradycardia; passive avoidance). The response does not have to be an all-or-nothing one, with only one system being activated or

disinhibited respectively. In mammals, it is possible for the responses of the three newest hierarchical stages (VVC, sympathetic-adrenal system, DVC) to co-occur. The hierarchical lower levels are not only present during (life-threatening) challenges, but are sometimes associated with dysfunctional behaviour and disorders.

To respond to the demands of the environment with an appropriate biological and behavioural response, the individual has to regularly evaluate whether a situation is safe or dangerous. This evaluation of risk does not require conscious awareness and is termed neuroception. It describes a neural process that can distinguish environmental (and visceral) features of safety and danger and matches neurophysiological state with actual risk evaluation of the environment (Porges, 2007). Neuroception enables the engagement in social behaviours, when it functions adequately and when the environment is safe. However, neuroception can also be maladaptive, as seen for example in social anxiety, inappropriately evaluating the environment as threatening and showing social disengagement. Neural circuits based in the temporal cortex are thought to be involved in neuroception (Porges, 2007).

Porges postulates a functional association of the VN and social engagement (Porges, 2001). This association is based on the neuroanatomical co-occurrence of the special and general visceral afferents of the VVC, whereas the cranial nerves regulate social engagement via facial expression and vocalization (fig. 2.5). The connection of the VN with facial expression emerged evolutionary, promoting, besides restoration and growth, spontaneous social engagement.

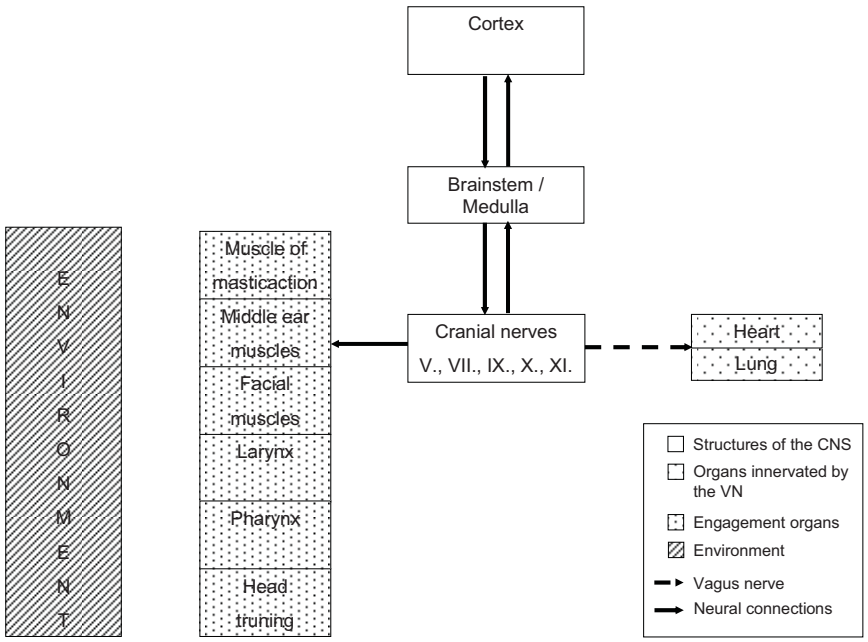


Figure 2.4 The social engagement system: Social communication is determined by the cortical regulation of brainstem nuclei (adapted from Porges, 2007)

Interestingly, Porges does not only argue that due to the association of the VN and social engagement a functional link between (low) vagal activity and (dysfunctional) behaviour exists. He also assumes a potential role of the functional and neuroanatomical association for therapeutic manipulations. Therefore, to stimulate the VN, one could intervene on different levels such as pharmacologically, with conditioning or psychotherapy, or by biological-behaviourally stimulating the neural regulation of the social engagement system (e.g. with auditory stimulation or rocking and swinging) (Porges, 2003). Results from invasive VN stimulation (VNS) also show beneficial effects on autistic-like behaviours associated with low social skills and communication (Murphy et al., 2000), offering further evidence for the association between the VN and social aspects.

In conclusion, the polyvagal theory boosts the role of the VN by highlighting the evolutionary development of the ANS with augmenting (poly-vagal) dimensions in higher-developed species. Therefore, the polyvagal theory underlines the benefits of the phylogenetic newer

neuroanatomical connection to a social network, allowing fine-tuning vegetative and behavioural responses in response to demands of the environment. Furthermore, the connection of the VN with the social engagement system offers an augmentation of possible therapeutic interventions to influence both systems. The polyvagal theory therefore offers a theoretical background in which the empirical studies presented in this thesis can be embedded, since the applied interventions are based on the interconnection of the different cranial nerves.

2.1.2.7 The neurovisceral integration model

The neurovisceral integration model was introduced by Thayer and colleagues (Thayer & Lane, 2000, 2009) and describes a link between neural structures involved in affective, cognitive, autonomic regulation and HRV, while highlighting and focusing on HRV. Interested in the associations between psychological phenomena and physiological properties, Thayer began the examination of the ANS with a special interest in HRV and studied this marker in animals, healthy subjects and patients, looking at normal and pathological phenomena associated with resting HRV. He also examined phasic or interventional changes in HRV.

Structurally, the neurovisceral integration model is based on the CAN (Benarroch, 1997), consisting of structures of the CNS (chapter 2.1.1.1). These reciprocally interconnected structures are not haphazardly pooled together but represent a functional unit controlling visceromotor, neuroendocrine and behavioural responses, which are critical for adaptability, goal-directed behaviour and health in general (Thayer, 2007; Thayer & Lane, 2000). The CAN is therefore associated with the process of response organisation and selection and serves to control psychophysiological resources in emotion and attention. Within the framework of the neurovisceral integration model, the CAN structures can additionally

flexibly revert to additional structures or networks when changing demands of the environment require it, therefore maximizing flexibility of the organism.

Autonomic output is mediated via preganglionic sympathetic and parasympathetic neurons. One direct output variable is therefore HRV, but since the VN also possesses afferents (to a greater extent than efferents), it can also feed back information from the heart to structures of the CAN. Thus, HRV can be interpreted as an indicator of CNS and ANS integration but also of central-peripheral neural feedback (Thayer, 2007). Individual differences in HRV can therefore be indicative of differences of the whole system. A low HRV can be symptomatic of a rigidly coupled or entirely uncoupled network unable to dynamically recruit specific, response-adequate structures.

The neurovisceral integration model points to the potential role of neural inhibition. One central component of inhibition is assured through the PFC, which during rest shows inhibitory control over subcortical structures (Thayer, 2006). There is evidence from pharmacological and neuroimaging studies showing a link between HRV and the PFC, but also some other structures of the CAN (left (mid-)insula, left amygdala-hippocampal complex, PAG, caudate nucleus, ventral ACC) (Ahern et al., 2001; Gianaros et al., 2004; Lane et al., 2009; Matthews et al., 2004). The inhibitory control of the PFC in controlling subcortical sympathoexcitatory circuits can therefore be indexed by HRV, as suggested by the neurovisceral integration model.

A functional loss of inhibition through the PFC is present when the environment requires allostatic processes to reach homeostasis, such as during stressful, fearful or threatening situations (≈ neuroception). However, subcortical disinhibition due to reduced PFC activity is also present in psychopathology (Thayer, 2007), and therefore findings of reduced HRV and rigid maladaptive behaviour in several disorders (chapter 2.2.2) are possibly associated with a loss of inhibition by the PFC.

Further evidence for the role of HRV as an index of inhibitory control of the PFC is provided by findings of associations of HRV with several constructs associated with inhibition ability and PFC activity as well as with emotional regulation. Associations between cognitive performance have been found, for example, in attentional, working and prospective memory tasks and HRV (Hanson et al., 2003, 2004, 2009; La Marca et al., in prep.), indicating better performance in subjects with high compared to low HRV. Interestingly, in contrast to the overall group, the low HRV group showed improved performance in a task during threat of shock (Hanson et al., 2009), a similar finding compared to the association found between low HRV and enhanced performance under conditions of high stress (Morgan et al., 2007). Associations of HRV with affective regulation were also found. In this regard, appropriate affective regulation promotes flexible adaptation to rapidly changing demands of the environment (Thayer & Lane, 2000). A failure to recognize safety signals by conscious perception or neuroception (Porges, 2007) can lead to prolonged, rigid readiness and heightened sympathetic output. Reduced differentiated emotion-modulated eyeblink startle responses to pleasant, neutral and unpleasant pictures were found in subjects with low HRV compared to subjects with high HRV (Ruiz-Padial et al., 2003). This finding supports the potential role of the PFC to inhibit amygdala output (Thayer, 2007).

To summarize, the neurovisceral integration model boosts the role of the VN by not only recognizing its characteristic as an index of state activity of the X. cranial nerve but also by promoting its relevance as part of a more complex and important network, the CAN. Within this complex network, HRV plays a more important role than merely an output variable. The VN and its activity are recognized to index inhibitory control predominantly of the PFC and to constitute an inhibitory control mechanism per se. Therefore, vagal activity can be interpreted as resource, since inhibition of subcortical structures is the best strategy to respond flexibly to demands. The neurovisceral integration model therefore constitutes an appropriate

basis for the interpretation of the results of the empirical studies presented in this thesis, since a special focus lies on the characteristic of the VN to form a resource.

2.2 Factors negatively associated with vagal activity

In the above chapters, a theoretical and methodological background was presented allowing an understanding HRV. HRV can be determined non-invasively on the body surface and offers a basis for the interpretation of the activity of the ANS and especially the VN, possessing a predominant influence on cardiac activity. On the one hand, the polyvagal theory and the neurovisceral integration model support the importance of the VN as an indicator of somatic and mental well-being, since flexibility, adaptability and social engagement are recognized to be structurally and functionally associated with HRV. On the other hand, morbidity is recognized to be associated with poor vagal activity. In the present empirical studies, the effect of stress, considered to be an important factor in the development and progression of numerous disorders, on vagal activity is examined. Disorders and stress, as risk factor, are reported to be associated with low HRV, but also other risk factors have also been studied in the past. In the following, generally known risk factors are discussed in terms of the association with HRV, before presenting possible evidence for reduced vagal activity in several disorders. On the one hand, this would support the role of the VN for health and well-being, and on the other hand an association would have several methodological implications, which should be considered in the empirical studies.

2.2.1 Risk factors

A multitude of factors are thought to be associated with decreased vagal activity and it would go beyond the scope of the present work to deliver a complete list of them. In the following, a

selection of prominent factors will therefore be discussed. The factors co-occur with decreased vagal activity and thus no direction of causality, underlying mechanism or independence of influence is suggested.

Besides the factors discussed in the following, other factors such as race, diet, social networks, job control, cholesterol, hypertension, diabetes and genetics have been found in the literature to be associated with HRV, but also cardiovascular morbidity and mortality (see chapter 2.2.2.5).

2.2.1.1 Age

Cardiovascular disorders are associated with age, and therefore alterations in ANS activity can be assumed. In a sample of 1,984 healthy subjects, Liao et al. (1995a) were able to demonstrate significant differences between a group of 45-54-year-old and a group of 55-64-year-old subjects in terms of cardiac activity. While the older group showed lower HF and LF values than the younger group, there were no clear differences in the LF/HF-ratio. In a younger sample of 1,780 subjects of both genders (age range 24-39 years), age was inversely related to all measured time- and frequency-domain values besides LF/HF-ratio (Koskinen et al., in press), indicating a decrease in autonomic control of the heart. As described below (chapter 2.2.1.3), Britton et al. (2007) found different changes over time depending on gender (5-year follow-up). Tasaki et al. (2000), who examined 10 women and 5 men in a 15-year follow-up, found significant decreases in SDNN, LF, LF/HF-ratio and significant increases in HR and NN50, whereas HF was unchanged. These data from a mixed-gender sample are difficult to interpret, but according to be authors might be interpreted as a decrease in sympathetic and a slight increase in vagal activity. Therefore, similar to the results concerning gender effects, findings on the relationship between age and HRV are also inconsistent.

2.2.1.2 Body mass index

In a current study (Koskinen et al., in press) examining young adults with ages ranging between 24 and 39 years, an inverse association between body mass index (BMI) and several HRV indices in the time- and frequency-domain (except LF/HF-ratio) has been found. Similarly, Hemingway et al. (2005) found a significant inverse relationship in 2,197 male subjects between waist circumference and quartiles of SDNN, LF and HF and a positive association with quartiles of HR. In a longitudinal study, Britton et al. (2007) found BMI (three groups: <25, 25-30, >30) to be a predictor of being in the worst quartile of HR and HRV (SDNN, LF, HF) at the follow-up five years later. In a study of 1,742 subjects aged ≥50 years (Felber-Dietrich et al., 2006), SDNN, LF and LF/HF-ratio on average decreased significantly by at least 0.7% per unit of BMI. Therefore, high BMI seems to be associated with a decreased autonomic control of the heart.

2.2.1.3 Gender

While some studies found no difference in HRV between women and men (e.g. Lampert et al., 2005), others found lower LF and higher HF in large samples of women compared to men (e.g. Britton et al., 2007; Liao et al., 1995a). In line with these findings, Koskinen et al. (in press) were able to demonstrate higher values of HF, lower values of LF and LF/HF-ratio in young women compared to young men. Therefore, it can be assumed that men show higher sympathetic and women higher parasympathetic activity.

2.2.1.4 Alcohol consumption

Subjects showing a high alcohol consumption (>28 units of alcohol per week; 1 unit=8g) revealed significantly higher HR and lower SDNN, LF and HF (Hemingway et al., 2005). This result was supported by Britton et al. (2007), who found an increased predictive value of high alcohol consumption (>21 U / week in men; >14 U / week in women) to be in the worst quartile of HR and HRV five years later. Felber Dietrich and colleagues (2006) found further evidence for decreased HRV values (SDNN, LF and VLF) associated with alcohol consumption. In conclusion, the latter seems to diminish autonomic control on the heart.

2.2.1.5 Smoking

Cigarette smoking is a well established risk factor, for example, for cardiovascular diseases. Therefore, a negative influence of smoking on vagal activity seems conceivable. Indeed, a multitude of studies have examined the effects of smoking.

Karakaya et al. (2007), for example, examined the effect of acute smoking on the ANS. Although we do not necessarily agree with the authors' interpretation of the presented raw data, the mean values of RMSSD and HF do seem to reveal a decrease in vagal activity in the first 5 and 10 minutes, respectively. Simultaneously, LF and LF/HF ratio increased as a sign of heightened sympathetic activity. This interpretation would be in line with the similar effects of chronic smoking on autonomic activity.

Foetuses from smoking mothers revealed significantly decreased HRV (Zeskind & Gingras, 2006), similar to data presented on boys, but not girls, in smoking compared to non-smoking breast-feeding mothers (Dahlström et al., 2008). HRV was negatively correlated with the number of smoked cigarettes and nicotine concentration in the milk. Similar results were also found in smoking adults. Habitual smokers show an increased LF and LF/HF-ratio and

simultaneously decreased SDNN, RMSSD and HF values compared to non-smokers (Alyan et al., 2008). In line with the findings in infants and foetuses, Alyan and colleagues found correlations between HRV parameters (LF, LF/HF-ratio) and the number of cigarettes smoked per day but not the duration of smoking. Burutcu et al. (2005) compared heavy smokers and non-smokers and found lower SDNN and RMSSD values and higher LF/HF-ratio values in smokers. HF and LF were not significantly different. Additionally, they found a positive correlation between the duration of smoking and LF/HF-ratio and a negative correlation between duration and RMSSD and HF. To summarize, these findings indicate that smoking leads to an autonomic imbalance by increasing sympathetic and decreasing parasympathetic activity.

2.2.1.6 Air pollution

Nowadays, a factor of increasing societal and political interest is air pollution, which has been associated with cardiovascular morbidity and mortality (Samet et al., 2000; Schwartz, 1999). Using different parameters of air pollution and gaseous pollutants (particulate matter <2.5 μm in aerodynamic diameter (PM2.5), particle number concentration, black carbon, ozone, sulfur dioxide (SO2), nitrogen dioxide (NO2), carbon monoxide), Park et al. (2005) examined their relation to HRV in 497 male subjects by matching the effects of different duration (4hr, 24hr, 48hr) and associated moving averages of air pollution to the time of ECG measurement for each subject. The authors reported a significant inverse relationship predominantly between PM2.5 and HF and a positive relationship with LF and LF/HF-ratio. Min et al. (2008) examined the effects of air pollution on HRV in 1,349 subjects and found, similar to Park et al. (2005), a negative association between PM10, SO2 and NO2 and HRV. SO2 further showed stronger effects in smokers compared to non-smokers (Min et al., in press). In contrast, Felber Dietrich et al. (2008) examined the effect of long-term exposure to NO2 and found a negative

association with SDNN, LF and LF/HF-ratio but not with HF or total power in women but not in men. These results, albeit inconsistent, indicate that an increase in cardiovascular morbidity and mortality associated with air pollution might be at least in part attributed to harmful alterations of ANS activity.

2.2.1.7 Little or no exercise

Exercise is recognized to be beneficial for health and well-being. Indeed, Hemingway et al. (2005) found significantly higher values of SDNN, LF and HF and a lower HR in a group of subjects who exercised (n=714) compared to a group that did little or no vigorous exercise (<1h/week; n=1329). Accordingly, Lampert and colleagues (2005) compared subjects who exercised on more than or less than three days per week in a sample of 360 outpatients. They found higher values of SDNN and all frequency-domain variables in subjects who did more exercise, but differences reached significance only when SDNN and ULF were taken into consideration. Felber Dietrich et al. (2006) found, in a large sample (n=1,742), higher HRV values with increasing amount of exercise. For every weekly hour of exercise ("get out of breath or sweat"), SDNN, HF, LF, VLF and ULF, but not LF/HF-ratio increased significantly by at least 2%. The beneficial effects of sport with health might therefore be mediated by increased cardiac control by the ANS.

2.2.1.8 Low socio-economic status

As part of the Whitehall II study, Hemingway et al. (2005) examined the association of HRV and, among other things, socio-economic status (SES) in 2,197 male subjects. The latter was measured using salary and work role (termed employment grade) and divided into a low, medium and high group. The employment grade was inversely associated with HR and

positively associated with HF, LF and SDNN. Lampert et al. (2005) examined the association of HRV and social class defined as three dichotomous scales (education, occupation, income) in 360 outpatients undergoing ambulatory ECG monitoring. Similar to the findings of the Whitehall II study, HF was significantly higher in the high education group and higher, but not statistically significant, with regard to occupation and income. Additionally, LF, ULF, VLF and SDNN were significantly higher within the high education, occupation and income group compared to the low social class group.

These findings support the assumption of a chronically impaired autonomic function in subjects with low SES and might therefore offer an explanation for increased coronary risk in subjects with lower SES.

2.2.1.9 Circadian rhythm

Normally, circadian rhythm is not a usual risk factor. However, since it has important methodological implications when examining vagal activity, and there is a relationship between cardiovascular disorders and time of day, a short overview is warranted.

The body adapts to environmental factors in order to maximize functionality and promote well-being. The activity of all cells and organs of the body have a circadian rhythm. The so-called master clock of the body is the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN synchronizes the circadian rhythm of all other cells of the body. The main Zeitgeber affecting the SCN and thus also the rest of the body by inducing circadian oscillations is light. Additional Zeitgeber are, for example, food intake, sleep habits and work (Panda et al., 2002). When not disturbed, for instance by shift work, the circadian rhythm is not a risk factor per se, but acute cardiovascular events are not distributed homogeneously over the day. In fact, pathological events are most prominent between 6 a.m. and 12 noon (Muller et al., 1989).

The activity of the heart also shows circadian rhythms. Vagal activity is highest during sleeping hours (Burgess et al., 1997; Furlan et al., 1990), while sympathetic activity is lowest during this time (Furlan et al., 1990). In line with the reduction of sympathetic activity and the increase in vagal parasympathetic activity, the sympathovagal balance is deepest during the night and highest during waking hours. According to Fallen and Kamath (1995), the rise in HRV during the night is predominantly due to the vagal increase, whereas changes in HRV during the day are due to more complex interactions between sympathetic and vagal modulation of sinus node activity. An association of low HRV and cardiovascular diseases is also recognized (chapter 2.2.2.5). Nevertheless, the circadian distribution of acute cardiac events cannot solely be associated with the circadian variation of cardiac activity, since other important health-influencing variables also show circadian alterations, such as cortisol, catecholamines, fibrinogen activity, platelet aggregability and alpha adrenergic tone (Fallen & Kamath, 1995), therefore making interpretation of causality difficult.

2.2.1.10 Psychological factors

Several psychological factors have been found to be negatively associated with HRV and health in healthy subjects. In the following, a short overview of some factors showing an association with vagal activity is presented.

Bleil et al. (2008) found an inverse association between the construct negative affect and HF HRV. The construct was built on depression, anxiety and anger questionnaires, with the former two also revealing a negative association with vagal activity. Similarly, subjects with a comparable health constitution and increased ratings of anxiety showed a heightened baseline HR and reduced SDNN in a study by Kawachi et al. (1995). Hughes and Stoney (2000) found an increased reduction of HF during mental stress and a smaller increase of HF during CFT in subjects with higher compared to lower depressive symptoms. These findings indicate a

dysfunctional vagal imbalance in more depressed subjects. In fact, this is in line with results in depressed patients (chapter 2.2.2.1). Further support was presented by Schwarz et al. (2003), who found a negative association between rated hopelessness/helplessness and HF HRV in subjects playing chess.

Furthermore, hostility was also shown to be negatively associated with vagal activity. Negative correlations were reported among other things between hostility ratings and HF and LF (Sloan et al., 2001). A study examining HR during a CFT revealed reduced capacity to induce a bradycardia in healthy subjects within the high compared to the low hostile group (Ruiz et al., 2006). This finding supports a reduced vagal functionality in hostile subjects, therefore offering an explanation for the association between coronary heart diseases and hostility. Additionally, in a large population of 2,197 apparently healthy men, Hemingway et al. (2005) found lower social networks to be negatively associated with resting HF HRV. In conclusion, several psychosocial factors seem to be related to low vagal (re-)activity and should therefore be considered when examining vagal activity.

2.2.1.11 Stress

Stress is thought to have a negative influence on vagal activity. Since in the empirical part of the present work, the effect of stress on cardiac markers, but also the potential role of vagal functionality as a physiological resource in buffering the effects of stress, was focused upon, a deeper insight into the phenomenon of stress is offered in the following. First, an attempt is made to provide a definition of stress. Second, effects of stress on different body systems are described, with a special focus on responses of the hypothalamic-pituitary-adrenal (HPA) axis, the SNS and PNS.

2.2.1.11.1 Definition

The definition of stress as used in health sciences has changed considerably over the years. Stress was associated with different terms such as activation, emotion, fear, conflict and frustration (Lazarus, 1966). Depending on the purpose, stress was defined in a more stimulus-, reaction-, situation- or relation-oriented manner (Lazarus & Launier, 1981). Although stress possesses a negative connotation, another differentiation was introduced in association with the valence: pleasant stress was termed eustress and unpleasant stress distress (Selye, 1981).

Since the focus of the present work lies on biological aspects of stress, one would be tempted to use a reaction-oriented definition of stress. Selye, for example, defined stress as an unspecific response of the organism to each demand (Selye, 1981). Nowadays, it is known that interindividual differences in responses exist (Steptoe, 1991). For this reason, the unspecificity concept of stress was abandoned. Mason (1971, 1975a, 1975b) was one of the first authors to call the concept of unspecificity into question. In animal experiments, he was able to demonstrate that a physical stressor (change in temperature) elicited an increase of glucocorticoid metabolite only when the increase in temperature was conducted quickly and unexpectedly, but not when implemented gradually (Mason, 1971). Therefore, appraisal played an important role in the stress concept used by Mason.

This notion was integrated into a new stress concept: the transactional stress model. The authors presumed that stress results from a transaction between a person and its environment (Lazarus & Folkman, 1984). In this still established model, two central concepts play an important role: appraisal and coping. Internal and external events therefore elicit stress by being processed by primary and secondary appraisal and in connection with the selected coping. To simplify, stress emerges when perceived demands of a situation (primary appraisal) exceed the perceived resources (secondary appraisal) and therefore activation persists (Lazarus & Launier, 1981). The notion of coping refers to the processes of handling the

external and internal demands, which are evaluated by the organism as exceeding one's own resources. The transactional model of stress additionally considers individual as well as environmental factors. Furthermore, personality traits and learning history constitute important influencing factors on cognitive appraisal as well as coping (Lazarus & Folkman, 1984). In conclusion, a transaction is stressful depending on the (im)balance between the demands and resources (Lazarus, 1966).

Additional factors to those considered in the transactional stress model have also been discussed: the emotional and motivational factors. The latter refers to the importance of being able to cope with a demand (McGrath, 1970). Emotions were discussed by Zajonc (1981, 1984), who suggested that emotions can precede cognitions and that the two are based on different (physiological) modes. Additional factors were discussed thought to modulate the impact of stress. The most important of these are time pressure, work load, novelty, control, predictability, social evaluation and ego involvement (e.g. Cannon, 1915; Dickerson & Kemeny, 2004; Mason, 1968).

A further concept in association with stress was promoted by McEwen (1998), although the term was first introduced by Sterling (1988): allostasis. Differently to homeostasis, which describes a process of keeping vital body systems (milieu interieur) in a stable range (Noll, 2002), allostasis defines the process applied actively to maintain homeostasis. It supports the adaptation to stress and the coping with stress. When the demands on the organism are too high or the allostatic process has to be maintained for too long, allostatic load develops, referring to the costs of the body arising from adapting to the demands (McEwen, 1998, 2000). McEwen therefore described four types of allostatic load: (i) repeated normal stress response, (ii) response without adaptation, (iii) prolonged reactivity, and (iv) inadequate response to stress.

2.2.1.11.2 Stress response

The systematic biological stress research began with the experiments of Cannon at the beginning of the 20th century, who focused on the somatic basis of emotions (Cannon, 1914a) and the differentiation of the biological concept of equilibrium under the term homeostasis (Cannon, 1929). Cannon propagated the importance of the SAM system and the secretion of epinephrine (EPI) as an unspecific stress response. This adrenergic emergency reaction as part of the fight-or-flight response was interpreted by Cannon as mobilization of energy to re-establish homeostasis (Cannon, 1914b).

In animal experiments, Selye (1936a,b) found a triad of morphological indicators associated with stress: adrenal enlargement, thymolymphatic involution and gastrointestinal ulcer. To describe the time response of this morphological syndrome and the underlying physiological process, Selye introduced the general adaptation syndrome, consisting of three stages (Selye, 1937, 1946): alarm, resistance and exhaustion. In contrast to Cannon, Selye focused on the HPA axis and the secreted glucocorticoid. Therefore, the SAM system and the HPA axis are the oldest and best examined biological stress systems. Nevertheless, a variety of additional biological stress responses not (directly) associated with these two prominent stress systems are known. In the following, an overview of the biological stress reactivity of the markers examined in the present work is given.

2.2.1.11.2.1 Cortisol

Cortisol is a hormone secreted from the adrenal cortex and is an index of the neuroendocrine HPA axis (fig. 2.6). Corticotropin-releasing-hormone (CRH) is synthesized in the whole body. The highest impact on the HPA axis is exerted by CRH, originating in the PVN of the hypothalamus, which activates the secretion of adrenocorticotropic hormone (ACTH) at the

anterior lobe of the pituitary gland. ACTH for its part reaches the adrenal cortex over the blood stream, eliciting the secretion of the glucocorticoid cortisol in humans (cortisol in most mammals, corticosterone in rodents). Biologically meaningful, unbound cortisol in terms of a negative feedback loop can inhibit the activity of the HPA axis (Mendel, 1989; Kirschbaum & Hellhammer, 1999, 2000).

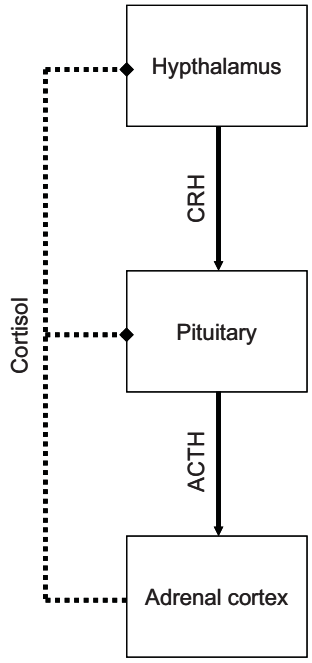


Figure 2.5 Schematic representation of the HPA- axis

The level of cortisol can nowadays be detected not only in blood but also in saliva, since the diffusion of unbound cortisol into saliva happens passively and is independent of salivary flow rate (Kirschbaum & Hellhammer, 1994). The correlation of the cortisol levels in both fluids is high (r=.71 to r=.96; Gunnar et al., 1989; Harris et al., 1990; McCracken & Poland, 1989; Reid et al., 1992; Tunn et al., 1992; Woodside et al., 1991). Similar to plasma, the level of cortisol in saliva peaks about 20-30 minutes after termination of acute stress exposure (Kirschbaum & Hellhammer, 1994; O'Connor & Corrigan, 1987).

Several studies have examined the influence of physical and psychological stressors on the activity of the HPA axis. Physical stressors normally induce an increase of ACTH and cortisol (Luger et al., 1987; Mason et al., 1973; Wittert et al., 1991), often with no changes in CRH, but possibly explained by the changes in arginine vasopressin (AVP), coexisting in some neurons with CRH (Whitnall, 1990). While cortisol shows a delayed peak, ACTH reaches a maximum about 15-30 minutes earlier (Kirschbaum & Hellhammer, 1999). An increase of both HPA indicators is also evoked by psychological stressors. In particular, psychosocial stressors that provoke social evaluative threat induce the strongest increases (Dickerson & Kemeny, 2004).

2.2.1.11.2.2 Alpha-amylase

Alpha-amylase (AA) is an enzyme synthesized in and secreted from the salivary glands, with the paired parotid gland being the most important with regard to AA (Buddecke, 1981; Morse et al., 1983). AA plays a central role in the predigestion of food by splitting starch into glucose and maltose, but also possesses several protective characteristics (Edgar, 1992; Scannapieco et al., 1993). The activity of the salivary glands is controlled by the ANS (Malfertheiner & Kemmer, 1987; fig. 2.7). Stress, through an increase in SNS activity, is thought to induce a viscous salivary fluid (Malfertheiner & Kemmer, 1987; Morse et al., 1981).

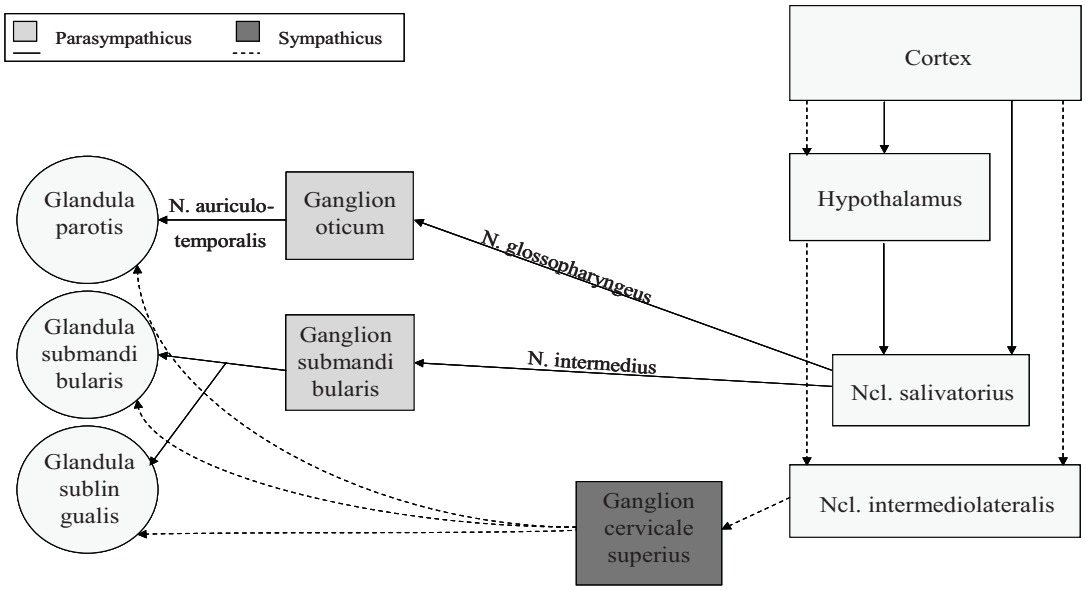


Figure 2.6 Simplified illustration of the autonomic innervation of the three major paired salivary glands (La Marca, 2005)

When observing the activity of AA, physical stress studies (e.g. Chatterton et al., 1996) reveal a clear result: AA concentration is increased during physical stress. The effects provoked by psychological stressors on AA concentration are indeed not as consistent, possibly due to some methodological problems, although more recent studies with established standardized stressors seem to provide similar results to those provided by physical stressors (e.g. Nater et al., 2006; Nierop et al., 2006; Rohleder et al., 2006). Therefore, AA is assumed to show an increase in concentration during psychological stress.

Furthermore, an interesting role of AA as an indicator of SAM activity during stress was suggested due to correlations with NE (Chatterton et al., 1996; Rohleder et al., 2006). Due to the small amount of evidence and the weak association of the two markers, the indicative role of AA for SAM activity has to be considered with caution and needs to be examined further.

2.2.1.11.2.3 Heart rate

The heart is innervated by the ANS, which constitutes the main influencing factor. As described above (chapter 2.1.2.1), a denervated human heart would show an HR of about 100 bpm but is decreased predominantly by vagal activity. Traditionally, an increase in HR due to stress would be explained by an increase in sympathetic activity, but an increase in HR can also be explained by reduced vagal activity or a combination of both. As described by Thayer and Lane (2009), the increase in HR is normally initiated in the CAN.

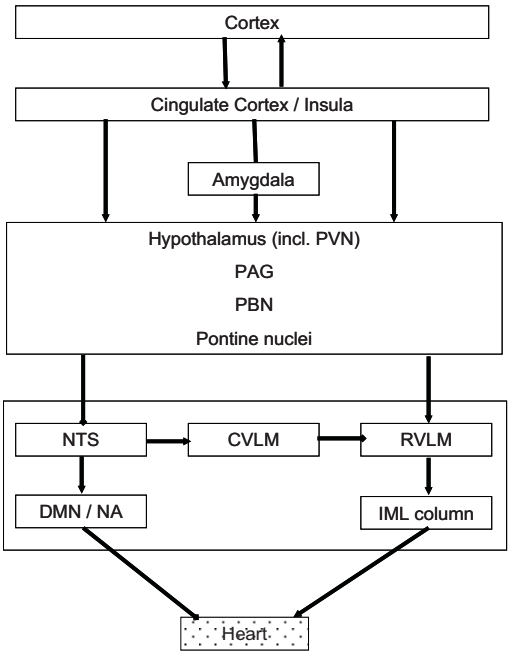


Figure 2.7 Effects of the CAN on heart rate (adapted from Thayer & Lane, 2009)

The interacting forebrain structures can activate sympathoexcitatory neurons of the RVLM, therefore increasing HR. The tonic inhibition from the PFC on subcortical structures is weakened under stress, meaning that the latter structures show increased activity resulting in excitation of the RVLM and leading to the final increase in HR. At the same time, under stress, the structures of the CAN can simultaneously inhibit the NTS through excitation of

inhibiting subcortical structures. The inhibited NTS elicits a decrease in activity of the visceromotor neurons in the DMN and NA due to inhibition of the excitatory pathway. The reduced activation in the DMN and NA results in a decreased vagal input to the heart, finally increasing HR also. In addition to these parallel pathways, an interaction of the two pathways exists. A CAN-mediated reduced activation of the NTS during stress leads to a decrease of inhibition on the sympathoexcitatory neurons of the RVLM, again augmenting the excitatory effect of the direct pathway, resulting in an increase in HR.

Several studies examining the effect of physical stressors on HR show consistent outcomes. Physical stress leads to an increase of the HR (e.g. Theurel et al., 2008), which seems obvious considering the main function of the heart: to supply blood, with all its nutriments and oxygen, to the tissues of the body. An increase of the HR is therefore a result of an increase in energy expenditure and metabolic requirements. Findings of psychological stress effects on HR are also consistent, showing higher values during stress (see above). For example, a cognitive stress task (paced auditory serial addition test, PASAT) induced a significant increase in HR (Carroll et al., 2007). Stress tasks combining a cognitive task with psychosocial stress were also shown to provoke significantly heightened HR (e.g. Klinkenberg et al., 2008).

2.2.1.11.2.4 Heart rate variability

The pathway regarding how HRV is decreased during stress was explained above (chapter 2.2.1.11.2.3) and is the same as for HR (fig. 2.8). Therefore, at this point, only a short overview of results referring to the effects of stress is provided.

Physical stress was shown to produce a decrease of vagal activity. Hatfield et al. (1998) found that RSA decreased during exercise and increased progressively during recovery. This effect is seen during acute exercise stress. By contrast, chronic exercise training produces an

increase of resting HF (Sandercock et al., 2005) and is in line with the known beneficial effects of exercise training on health and well-being (chapter 2.3.5).

Psychological stress shows similar effects to physical stress in terms of acute effects, but differs when it comes to chronic stress exposure, as chronic psychological stress leads to allostatic load (chapter 2.2.1.11.1). With regard to acute psychological stress, several laboratory and real-life stressors were examined in their capacity to alter vagal activity. It is recognized that the PNS is inhibited, while the SNS is normally activated under stress. Therefore, a decrease of vagal activity can be assumed (Porges, 1995), although results are inconsistent. Several studies found a decrease of vagal activity (Carrere et al., 2005; Hall et al., 2004; Isowa et al., 2006; Klinkenberg et al., 2008; Li et al., 2005; Mezzacappa et al., 2001; Sato et al., 2004; Sloan et al., 1994; 2001; Steptoe & Marmot, 2005; Yin et al., 2004), while some were unable to find a significant decrease (Altemus et al., 2001; Hjortskov et al., 2004; Lin et al., 2001; Yoshino et al., 2005). Others found alterations only in a subgroup of the whole sample (dependent on hostility: Shapiro et al., 2000; dependent on verbalization of answers: Bernardi et al., 2000; Sloan et al., 1991).

As the results are inconsistent, two methodological problems should be considered in future studies addressing stress effects on vagal activity: orthostatic challenges (postural changes, motion) and verbalization of answers. Postural changes and motion are required by some stressors due to changes of control and examination rooms (e.g. in the Trier Social Stress Test, TSST, Kirschbaum et al., 1993). In fact, postural changes and walking were shown to alter the highly sensitive ANS (Chan et al., 2007; Nater et al., 2006). Therefore, changes of HRV might be attributable at least in part to orthostatic challenges and not solely to the stressor. The influence of verbalization of the answers was examined by Sloan et al. (1991), who found that HRV during mental arithmetic decreased only when the subjects' answers were not verbalized. Similar findings were reported by Bernardi and colleagues (2000), who similar to

Sloan examined mental arithmetic with and without verbalization. HF was reduced compared to spontaneous breathing only when the answers were written on a blackboard, but not when given aloud. The TSST, in which answers are verbalized, shows inconsistent results. While Altemus et al. (2001) found no effect on RSA, Klinkenberg et al. (2008) reported significant time effects on HF. Mental arithmetic is thought to be an appropriate stressor to induce HRV alterations (Pagani et al., 1995). Due to the disturbing factors mentioned above, an appropriate stress task should not require verbalization of answers and postural changes or motion. Therefore, computerized mental arithmetic might be an ideal stressor, at least when the focus lies on vagal activity alterations.

Another problem when examining stress responses of the vagal nerve, but also stress responses in general, relates to interpretation. Different vagal stress responses might be explained differently. For instance, a reduced response might be interpreted such that a subject does not feel stressed, or that he possesses a high stress resistance, or that he cannot respond adequately to a demand. Therefore, further information, such as additional dimensions of the stress response, resources, personality or functionality, should be evaluated in order to appropriately interpret a result.

2.2.2 Morbidity and mortality

The association between the activity of the VN and morbidity was recognized early in VN research. Eppinger and Hess (1910) suggested the importance of changes in vagal tone when they investigated RSA and bradycardia. Based on their observations, they suggested that the individual character of vagal tone was connected to psychiatric pathology (e.g. neuroses) and therefore emphasized a possible pharmacological influence by cholinergic agents (Eppinger & Hess, 1910; Porges, 2007). Nowadays, vagal dysfunction is recognized in a huge amount of different somatic and psychiatric disorders through evidence of altered resting vagal activity

or phasic vagal activity alterations (Birkhofer et al., 2005; Thayer & Brosschot, 2005). Furthermore, an association between the activity of the VN and mortality, especially associated with cardiovascular disease, was identified and discussed (e.g. Birkhofer et al., 2005; Carney et al., 2005; Kudaiberdieva et al., 2007; Singh et al., 2002; Thayer & Lane, 2007). It is recognized that autonomic alterations are associated with hemodynamic, metabolic, trophic and rheologic abnormalities constituting a risk for cardiovascular morbidity and mortality (Brook & Julius, 2000). Additionally, autonomic or vagal activity alterations are associated with physiological systems and processes of importance for allostasis such as the HPA axis, inflammation and glucose regulation (Thayer & Sternberg, 2006). Therefore, the possible findings of associations between low HRV and morbidity and mortality might not be explainable by a direct linkage.

It would go beyond the scope of this work to deliver a full list of existing evidence for associated disorders; thus, a selection of disorder-specific studies are listed below in order to provide an overview of the role of the VN in morbidity and mortality. Besides the discussed disorders (depression, anxiety disorders, eating disorders, schizophrenia, cardiovascular disease), several other disorders were examined regarding autonomic dysfunctions (e.g. alcoholism (Thayer et al., 2006); chronic fatigue syndrome (Boneva et al., 2007; Stewart et al., 1998; Yamamoto et al., 2003; Yoshiuchi et al., 2004); hypertension (Pavithran et al., 2008); gastroesophageal reflux disorder (Lee et al., 2004, 2006); irritable bowel syndrome (Jarrett et al., 2008; Mazur et al., 2007); Down syndrome (Baynard et al., 2004; Figueroa et al., 2005); human immunodeficiency virus (HIV)-positive patients (Mittal et al., 2004; Sakhuja et al., 2007)). Evidence and interpretations for an association between vagal dysfunction and symptoms / disorders is restricted due to small numbers of studies and medical treatment of some study samples. In the following, a selection predominantly of studies with unmedicated subjects was aimed at.

2.2.2.1 Depression

Depression is assumed to be associated with a reduced vagal activity. Agelink et al. (2002), for example, found significant group differences between non-depressed healthy subjects, patients with a moderate major depression (MD) and patients with a severe MD referring among others to HF. The decreased values in the patients were also confirmed when examining only the pharmacologically untreated patients. Interestingly, the authors also found a negative correlation between depression scores and HF. This association reached a trend level when considering the whole group and a significant level when regarding only the pharmacologically untreated patients. Similarly, van der Kooy et al. (2006) found decreased vagal activity values (RMSSD, HF) in patients with an MD compared to non-depressed controls. Additionally, a decrease in LF power was apparent. Phasic changes of HF in healthy subjects were studied by Hughes and Stoney (2000) during two tests: a stress task and a CFT. Subjects in the high compared to the low depressed mood group revealed no difference in baseline values, but a higher decrease in HF during stress and a smaller increase during the CFT. These findings reveal decreased vagal baseline and altered reagibility values associated with depression or depressive mood.

2.2.2.2 Anxiety disorders

Several studies examined different anxiety disorders with regard to tonic activity or phasic changes during different tasks. The topic has been examined by some of the most well-known authors with regard to the VN such as Sloan, Porges, Thayer, Grossman and Wilhelm.

Thayer et al. (1996) examined clients with a generalized anxiety disorder (GAD) compared to non-anxious controls during rest, relaxation and worry periods. Among other things, they found lower HF across all task conditions in the GAD clients compared to the controls.

Additionally, worry was associated with lower HF, indicating less cardiac parasympathetic activity. The same group (Friedman & Thayer, 1998) examined the autonomic cardiac control in panickers (PD), blood phobics and controls. Controls showed significantly higher HF than PD and blood phobics and a lower LF/HF-ratio than PD but not blood phobics. PD subjects for their part showed lower HF and higher LF/HF-ratio than blood phobics. Sahar et al. (2001) studied the association of VN activity and posttraumatic stress disorder (PTSD). They found no significant difference in resting RSA between outpatients with PTSD compared to controls who had no current or past PTSD but had experienced a trauma in the past. Differently, in a mental arithmetic task, the PTSD group revealed no significant increase of RSA, while the non-PTSD group showed a significantly higher increase in RSA. This is astonishing, since a decrease in RSA would be expected during mental arithmetic (chapter 2.2.1.11.2.3). Nevertheless, in the non-PTSD group, changes in HR and RSA were highly correlated, but no significant correlation was found within the PTSD group. This suggests that HR changes are influenced more by vagal influences in the subjects from the non-PTSD group. The coupling between HR and vagal activity is normally reported in healthy subjects. Another study compared PTSD patients, PD patients and healthy controls (Blechert et al., 2007). In contrast to Sahar et al. (2001), they found resting differences. PTSD patients showed elevated HR and lower RSA than the other two groups. In contrast to Friedman and Thayer (1998), PD showed no decreased vagal activity compared to the controls. To summarize, anxiety disorders show predominantly decreased activity of the VN and dysfunctional alterations under challenge, but the data are not entirely consistent.

2.2.2.3 Eating disorders

Patients with chronic anorexia nervosa (AN) were compared with non-anorexic women during 24-hour Holter monitoring, revealing decreased HF, RMSSD, pNN50, SDNN and LF,

while HR and LF/HF-ratio did not differ between groups (Melanson et al., 2004). The same values were all non-significant during rest with metronomic breathing. In contrast, Ishizawa and colleagues (2008) found contrary results. They examined patients with AN compared to healthy controls and found decreased HR and LF/HF-ratio during rest with spontaneous breathing, while HF and total power were significantly higher compared to the control group, indicating an enhanced parasympathetic activity, which is astonishing considering the increased risk of cardiac sudden death in AN patients. The authors suggest that this change might be seen as an adaptive response to caloric deprivation in AN. The opposite findings underline the need for further examination. Another study examining binge eating disorder (BED) in obese women compared to obese women without BED revealed no differences in HR and HF during rest, but an enhanced HF decrease in the BED group during mental stress, therefore showing an increased vagal suppression during stress (Friederich et al., 2006). This could be indicative of higher stress vulnerability and/or (associated) decreased vagal reagibility.

2.2.2.4 Schizophrenia

Schizophrenia was shown to be negatively associated with vagal activity. Bär et al. (2005) examined untreated schizophrenic patients and healthy controls. Patients showed an increased HR and reduced HF and RMSSD values compared to the controls. This effect was not significant after the start of medication therapy. Additionally, the authors found an association between several HRV variables and the duration of the illness. After multiple regressions, a parasympathetic variable (mean circular resultant; measured in a deep breathing test and a measure of the synchronization between HR variation and respiration) significantly explained the variation of duration of illness, indicating lower parasympathetic activity in patients suffering from the disease over a longer period of time. In an additional study (Bär et al.,

2008), the author and his colleagues were able to replicate their findings of significantly decreased HF and RMSSD values in unmedicated patients compared to controls. Additionally, they found a negative trend correlation between severity of psychotic symptoms and HF and a positive trend correlation between severity and LF/HF-ratio. Similar to the results in depressed subjects, schizophrenic patients show reduced activity of the VN, and an association with the duration and severity of the symptoms is suggested.

2.2.2.5 Cardiovascular events, disorders and mortality

Cardiac and vascular diseases are among the most prevalent disorders and constitute major reasons of mortality in western countries, including Switzerland (Lüscher et al., 1999; fig. 2.9).

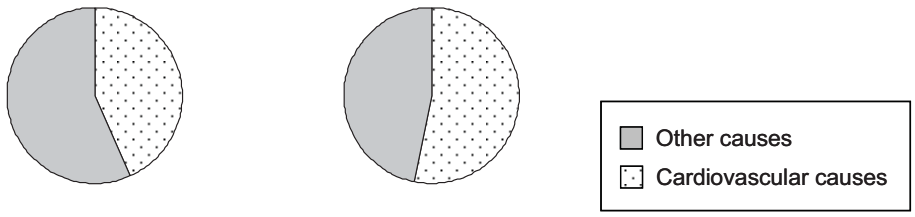


Figure 2.8 Cardiovascular events represent the main cause for mortality (43.3%; left) and hospitalization (53%; right) in Switzerland (adapted from Lüscher et al., 1999)

The examination of associated and predictive factors are therefore of great interest. Since the ANS constitutes the most important influencing factor on the heart, it follows that indicators of autonomic activity should be examined in terms of their potential predictive value for possible autonomic imbalance (for reviews, see Kudaiberdieva et al., 2007; Singh, 2003; Thayer & Lane, 2007). Below, the focus of interest is placed on the predictive value of HRV, especially of the indicators for VN activity. Several studies have focused on this topic in

longitudinal studies. Before reporting these data, it must be mentioned that even if a statistical prediction of HRV for cardiovascular morbidity and mortality is found, a vagal dysfunction as a unique and deterministic cause cannot be unequivocally postulated, since it could still be an epiphenomenon. It could still be argued that another factor influencing vagal functionality might be the prime cause of cardiovascular morbidity and mortality, for example altered cardiac demands for oxygen (Routledge et al., 2002).

Risk of all-cause death increases in tandem with resting HR. Habib (1999) demonstrated a threefold increase in mortality in subjects with a resting HR over 90 bpm compared to the group with a HR below 60 bpm, while resting HR can be seen as an indicator of vagal activity (chapter 2.1.2.4). As part of the Framingham Heart Study (Tsuji et al., 1996), the association between HRV and the incidence of new cardiac events in the follow-up (3.5 years later) was studied in 2,501 subjects with no clinically apparent coronary heart disease and congestive heart failure by history and clinical examination. After controlling for age, sex and clinical risk factors (e.g. smoking), several time- and frequency-domain variables (SDNN, pNN50, rMSSD, HF, LF, VLF, but not LF/HF-ratio) were significantly associated with risk for a new cardiac event. The lower the tertile of the respective HRV variables, the higher the incidence of the events was. Similar results were reported by Gerritsen et al. (2001), who examined a glucose-tolerance-stratified sample from a general population (n=605) during 9 years of follow-up, examining the predictive value of HRV (SDNN, HF, LF, LFnorm) for mortality. The authors found significantly lower values of SDNN, HF and LF for all-cause mortality but also specifically for cardiovascular mortality.

Patients after myocardial infarction (n=1284) rather than healthy subjects were studied in a 21-month follow-up in order to examine the predictive role of HRV on cardiac mortality (La Rovere et al., 1998). The only HRV variable examined was SDNN. The findings revealed that low values were significantly related to increased risk for mortality.

HR recovery after exercise, as mentioned above, is an index of vagal functionality (chapter 2.1.2.4). Nishime et al. (2000) examined 9,454 patients, who had been referred specifically for exercise ECG to a clinic, regarding the predictive value of abnormal HR recovery (a decrease of less than 12 bpm during the first minute after peak exercise) for all-cause mortality (follow-up: 5.2 years). They found an increased hazard ratio of 4.16 in subjects with abnormal HR recovery compared to patients with normal HR recovery. In a sample of 132 emergency department patients with sepsis (Chen et al., 2008), non-survivors were compared to survivors, revealing significantly lower SDNN, VLF, LF and LF/HF-ratio. With regard to the indicators of the VN, the results are not entirely clear. While RMSSD did not differ between the two groups (p=.983), HF tended to be lower (p=.070), while HFnorm (p=.012) was significantly increased in the non-survivors. Multiple regressional models identified only SDNN and HFnorm as significant independent variables predicting the in-hospital mortality. Therefore, autonomic imbalance indicated by HRV could predict mortality, while the findings of vagal activity are not as clear and are not in line with the other data found regarding the prediction of mortality by HRV. Given the importance of the VN in inflammatory processes (Tracey, 2002), the possibly heightened VN activity reported by Chen might be associated with immunological alterations in sepsis. The VN constituting the cholinergic anti-inflammatory pathway is also discussed as a therapeutic intervention site in sepsis (Parrish et al., 2008).

These findings suggest decreased autonomic and more specific vagal functionality to be predictive of predominantly cardiovascular events and mortality in healthy subjects and patients.

2.3 Interventions increasing vagal activity

As seen above, low vagal activity is associated with several risk factors, disorders and mortality. Therefore, the emerging idea of vagal stimulation is corollary in preventing morbidity and mortality or improving health and well-being in general. If one were to assume that vagal stimulation is possible, what would be the explanation for the underlying mode of action? On the one hand, beneficial effects are suggested on the heart: through a direct influence on cardiovagal activity and through indirect inhibitory effects on the SNS, cardiac effort and oxygen consumption would decrease. On the other hand, in terms of the neurovisceral integration model, a vagal stimulation could be interpreted as an increase in a physiological resource affecting the balance of the whole CAN, possibly boosting the neuroinhibitory influence of the PFC on subcortical areas. Both explanations would promote health outcome. In the following, a selection of more and less invasive interventions is discussed.

2.3.1 Pharmacological interventions

When searching for interventions to influence the ANS, pharmacological options have to be mentioned. Besides animal studies, evidence comes predominantly from studies examining the effects of medication in patients with cardiovascular diseases. Therefore, a large proportion of research was conducted with beta-blockers, a typical pharmacotherapeutic intervention. Negative effects (cardiotoxic, cytotoxic, hypertrophic effects, apoptosis) of high levels of NE are recognized (Routledge et al., 2002). An increase of vagal activity would thus not only elicit beneficial direct effects on the sinoatrial node, but could also indirectly prevent negative health outcomes by inhibiting sympathetic activity through pre- and postsynaptic interactions of the PNS and SNS. Betablockers are thought not only to have an antiadrenergic

action in the periphery, but also a centrally mediated increase in cardiac vagal activity. In fact, in a placebo-controlled study, Vaile et al. (1999) demonstrated that beta-blockers (atenolol, metoprolol) were able to increase HF HRV under different conditions in healthy subjects.

Additionally, studies were conducted examining the effects of angiotensin-converting enzyme (ACE) inhibitors, another drug often used in cardiovascular disorders. Kontopoulos et al. (1996) were able to demonstrate that in patients with uncomplicated myocardial infarction, an ACE inhibitor (quinapril) produced higher pNN50 and RMSSD values compared to a placebo group after 35 days. Vagal activity increased to a similar degree to with an examined beta-blocker (metoprolol). The two pharmacological interventions did not differ statistically from each other, therefore underlining the effectiveness of both drugs to stimulate the VN.

2.3.2 Invasive vagus nerve stimulation

Vagus nerve stimulation is a newer medical intervention with interesting effects. Before discussing the effects and typical disorders treated with VNS, a short introduction to the device and its neuroanatomical basis is presented.

2.3.2.1 General introduction

A newer medical intervention targeting the activity of the VN is vagus nerve stimulation (VNS). VNS is the electrical stimulation of the VN. The term is normally attributed to a subcutaneously implanted device producing electrical stimuli transmitted by wires to the left branch of the VN (fig. 2.10). Surgery is needed for the implantation of the device, therefore holding some risks.

The left branch is chosen since it possesses more afferents (approximately 80%; Walsh & Kling, 2004) compared to the right branch and the primary aim of the VNS is activity

alterations in the CNS, while cardiac arrhythmia is to be avoided. The device can be programmed regarding the intensity, frequency and impulse width (Schachter & Saper, 1998).

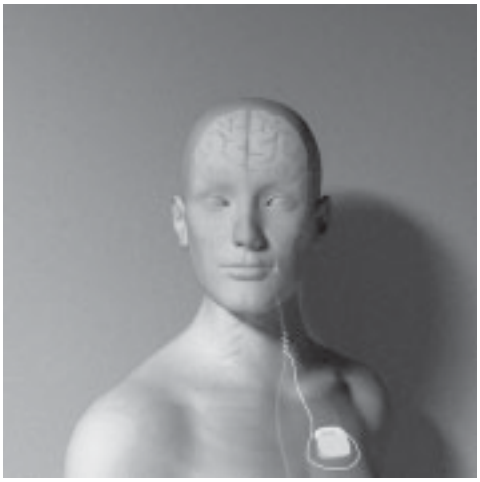


Figure 2.9 Vagus nerve stimulation (reproduced with permission from Bryan Christie Design LLC, available: www.bryanchristiedesign.com/uploadfiles/2840005_med_vns.jpg)

2.3.2.2 Neuroanatomical basis of VNS action

The exact mode of action is still not completely clarified (Dorr & Debonnel, 2006; Milby et al., 2008). It seems that the elicited physiological effects are dependent on the examined clinical population. While in epileptic patients, the activity of the thalamus was shown to be increased (Henry et al., 1999), no activity changes were found in depressed patients (Lomarev et al., 2002). In the latter, functional magnetic resonance imaging (fMRI) study, the authors were able to demonstrate dose-dependent significant alterations in several CAN areas associated with emotional regulation such as the orbitofrontal cortex, hypothalamus and left amygdala. In a single photon emission computed tomography (SPECT) study (Zobel et al., 2005), regional cerebral blood flow (rCBF) changes were assessed from before VNS to after 4 weeks of VNS treatment. An activity increase was found in the middle frontal gyrus, while activity decreased in the amygdala, left hippocampus, left subgenual cingulate cortex, bilateral ACC, right thalamus and brain stem. Similarly, a positron emission tomography

(PET) study was able to show increased rCBF in the orbitofrontal cortex, ACC and right superior and medial frontal cortex, and decreased rCBF in the temporal cortex and right parietal area during VNS activity (on-phase) compared to an off-phase. In an animal model, Dorr and Debonnel (2006) examined short-term (1 hour to 3 days) and long-term (14 to 90 days) effects of VNS on electrophysiological firing rates in the dorsal raphe nucleus (RN) containing 5-HT neurons and the locus coeruleus (LC) containing NE neurons. During the first 3 days, the VNS resulted in a significantly heightened activity in the LC but not dorsal RN. The long-term intervention revealed significantly increased activity in both structures of the rats. These results are in line with the recognized neuroanatomical connections of the VN as part of the CAN.

2.3.2.3 Treated disorders

In the initial phase, the VNS was developed for application in therapy-resistant epilepsy, where the VNS shows beneficial effects (Bernstein et al., 2006; Rychlicki et al., 2006). The first VN stimulator was implanted in a human subject in 1989 (Vonck et al., 2001). Since several authors reported a concomitant amelioration of mood, VNS was also introduced in treatment-resistant depression (Milby et al., 2008). In a recently published multicenter study (Schlaepfer et al., 2008), a beneficial effect of VNS on treatment-resistant depression was reported with a response rate of 37% (decrease of depression scores ≥50%) and a remission rate of 17% after 3 months. After a total of 12 months, the values were 53% and 33%, respectively, while 44% of patients showed no relapse in the first 12 months. Similar results to this European study were found in an American examination (Rush et al., 2000, 2005b). In a sham-controlled trial, Rush and colleagues (2005a) found a symptom reduction in depression. Interestingly, the improvement was higher than in a sham intervention, but the difference did not reach statistical significance. Therefore, like other interventions, VNS too

seems to possess a placebo effect, and results referring to VNS need to be interpreted with caution.

In terms of epilepsy and depression, which constitute the two disorders most frequently treated with VNS, this invasive treatment form is also applied with interesting results in terms of other (comorbid) symptoms or disorders such as migraine (Hord et al., 2003; Mauskop, 2005), Alzheimer's disease (Merrill et al., 2006; Sjögren et al., 2002) and anxiety (George et al., 2003; Rush et al., 2000).

2.3.2.4 Cardiac effects

Several studies examined the effect of VNS on cardiac activity. The results are quite inconsistent, which might be attributable to methodological problems such as small sample sizes, medication (in epilepsy, VNS is normally applied together with pharmacotherapy), different VNS characteristics and different study designs (comparison from pre- and peri-intervention period or on- and off-phases). Nevertheless, data supporting a positive effect on autonomic characteristics of cardiac activity is prevalent.

Kamath et al. (1992a) explored early on the effects of VNS on cardiac activity. The authors compared a high (30Hz, 500ms pulse) with a low stimulation group (2Hz, 130ms pulse) regarding cardiac activity before and 2 weeks after VNS implantation. They found a significant decrease in LF/HF-ratio in the high stimulation group after implantation, while the low stimulation group showed no change in HR, HF and LF/HF-ratio over time. During the intervention, the high stimulation group showed a significantly higher HF value than the low stimulation group. In the same year, the authors (Kamath et al., 1992b) published data referring to the difference between on- and off-phase of stimulation in a medium stimulation group (20Hz, 300ms pulse). During the on-phase, HF HRV was increased, while LF HRV and LF/HF-ratio were reduced compared to the off-phase. Beneficial effects were also reported in

more recent examinations. Stemper et al. (2008) found an increase of HF and LF during on-phases of VNS compared to off-phases. Similarly, Koenig et al. (2008) reported an increase in HF, LF and VLF during the on-phase of VNS in a case report of Lennox-Gastaut Syndrome with severely impaired HRV.

As already noted, findings are inconsistent. In contrast to Kamath (1992a), Barone et al. (2007) were unable to find any changes in HRV after three months of VNS. Setty et al. (1998), who compared short-term baseline, stimulation and post-stimulation periods, did not find differences either in the time domain or in the frequency domain. Contrary to most findings and expectations, Galli et al. (2003) found a trend towards a nocturnal decrease in HF from pre- to peri-intervention of 36 months. Concurrently, they found a significant flattening of day-to-night changes of sympathovagal balance. Due to these inconsistencies, further examinations of the effects of acute stimulation (on-phase) and long-term effects during off-phases should be conducted. A potential role of VNS in cardiovascular disorders could be a consequence of further supporting evidence for a beneficial cardiac effect, even though a direct, efferent stimulation through VNS is not assumed, but rather a primary bottom-up effect of VNS with subsequent top-down regulation of the activity of the heart (Frei & Osorio, 2001; Kamath, 1992b; Ronkainen et al., 2006).

2.3.2.5 Adverse effects

Besides the beneficial effects of VNS, some adverse effects were also documented such as hoarseness, voice alterations, cough, dyspnoea, dyspepsia, vomiting, pain and insomnia (Ansari et al., 2007; Schlaepfer et al., 2008). However, since these effects are present only while the VN stimulator is active, the authors note that in the worst case scenario, the device can be turned off with a magnet. Additionally, the adverse effects seem to be mild and tend to diminish over time (Ansari et al., 2007). Additionally, some adverse effects can occur due to

the surgery needed for the implantation and maintenance of the device or battery exchange (Smyth et al., 2003).

Due to these adverse effects, and since VNS is reserved to treatment-resistant patients, alternative non- or mild-invasive interventions should be examined with regard to a potential therapeutic or preventive benefit. Some alternative methods, which are associated with increased vagal activity and can be applied to nearly all patients, are dealt with in the following.

2.3.3 Transcutaneous electrical nerve stimulation

Transcutaneous electrical nerve stimulation (TENS) is a non-invasive method which is easy to apply. Predominantly, the non-invasive electrostimulation is used for pain disorders. The device is similar to a VNS, but the point of application is different. The TENS is applied on the skin and stimulates nerves transcutaneously. The stimulation characteristics are not standardized (Sulka & Walsh, 2003). Since vagal afferents are recognized in the ear (chapter 2.1.2.2), TENS has also been applied to stimulate the VN. Some authors therefore named the intervention also transcutaneous VNS (tVNS: Dietrich et al., 2008; Kraus et al., 2007), which might be critical, as reported evidence for the effective stimulation is still scarce. Activation of the NSNT, which is thought to be elicited in the stimulation of the vagal afferents in the ear, was shown to elicit a bradycardia and an increase in blood pressure when stimulated electrically or pharmacologically in rabbits (Kumada et al., 1977).

After measuring far-field potentials in the electroencephalogram (EEG) associated with precedent stimulation of the ear, Fallgatter et al. (2003) proposed the ear to be a target organ to stimulate vagal afferents. Recently, Kraus et al. (2007) applied TENS in the left outer auditory canal in healthy subjects and demonstrated affective and central activity alterations similar to those seen in VNS (decreases in the amygdala, hippocampus, parahippocampal

gyrus, middle and superior temporal gyrus and increases in the insula, precentral gyrus and thalamus). These effects were not seen in a sham condition applied in the ear lobe. Additionally, they were unable to find any alterations in HR and blood pressure. Similarly, Johnson et al. (1991) were unable to find any effects of TENS in the concha on HR and blood pressure. Therefore, to our knowledge, no studies have been able to prove a cardiovagal effect of TENS in humans.

2.3.4 Acupuncture

An increasing number of interventions from the Asiatic continent are reaching Western countries: meditation (Phongsuphap et al., 2008; Wu & Lo, 2008), yoga (Khattab et al., 2007; Raghuraj et al., 1998; Shapiro et al. 2007), Tai Chi (Audette et al., 2006), acupressure (Arai et al., in press) and acupuncture have all been studied regarding their effects on the so-called health nerve. In the following, only acupuncture is discussed referring to its potential role to alter ANS activity.

Studies examining the effects of acupuncture on the activity of the ANS were conducted in animals and humans, with inconsistent results. The inconsistency is possibly due to the high heterogeneity of the applied methods. Animal and human studies (Agelink et al., 2003; Bäcker et al., 2008; Imai et al., 2008; Hsu et al., 2006; Huang et al., 2005; Imai & Kitakoji, 2003; Li et al., 2003, 2005; Nishijo et al., 1997; Ouyang et al., 2002; Sakai et al., 2007; Shinohara, 1997; Sparrow, 2007; Streitberger et al., 2008; Wang et al., 2002; Wu et al., in press; Zhang, 2006; 2007) found augmenting effects of body acupuncture on vagal activity indicated by cardiac and/or gastric activity. Only a small number of studies failed to find an effect of body acupuncture on the activity of the VN (Hübscher et al., 2007), which is suggested to be at least in part due to publication bias. Similar inconsistency was also found during auricular acupuncture, with some studies showing a positive effect on vagal activity

(Gao et al., 2008; Haker et al., 2000; Hsu et al., 2007; Saxena et al., 1976), while others found no effect (Karst et al., 2007; Wang & Kain, 2001; White & Ernst, 1999). In acupuncture research, the high heterogeneity of applied methods (chapter 3.2) and the often unclear mode of action of the heterogeneous interventions represent a problem. Some authors argue that the effects of acupuncture are mediated by ANS, placebo or pain effects.

2.3.5 Physical training

Physical training is recognized as promoting health and well-being. An association with the activity of the VN can be assumed, even though training presumably leads to a multitude of changes not solely restricted to the VN. An association between physical training, cardiac vagal control and mortality was also discussed (Buch et al., 2002). Several studies have examined the potential role of physical training to increase vagal activity.

Sztajzel et al. (2008) examined differences in ambulatory 24-hour ECG during all-day activity in three different groups: untrained controls, hockey players and endurance athletes. Both trained groups revealed significantly higher levels of RMSSD, pNN50, HF, LF and lower LF/HF-ratio compared to the controls. Additionally, endurance athletes but not hockey players showed statistically higher SDNN levels than untrained controls. These findings support a beneficial effect of physical training with a slightly better outcome of endurance athletic activity on ANS activity. Similar results were presented by Martinmäki et al. (2008) in a longitudinal study examining the effects of 14-week endurance training in untrained subjects. Subjects showed no pre-post changes in HR, HF and LF during rest, but showed a decrease in HR and an increase in HF and LF at submaximal exercise intensities, underlining the beneficial effect of physical training. Further evidence for a beneficial effect of training is provided by Gamelin et al. (2007), who examined the effects of training and detraining in healthy young men. The 12-week-long intensive training resulted in an increase of HF, LF

and “total power” (HF + LF), while only LF and “total power” reached statistical significance. After 8 weeks of detraining, the differences were no longer significant. This negative effect of detraining was also found by Hansen et al. (2004), who examined a group that continued physical training for 4 weeks after an initial training period of 8 weeks. They compared cognitive function and HF HRV with a second group who stopped training after the initial 8 weeks. They found a significant difference in HF after detraining, with the detrained group showing lower values, while HF did not differ before the detraining. Additionally, they found changes in cognitive functioning of both groups only in the post-detraining test but not at baseline, which they interpreted as support for the neurovisceral integration model.

2.3.6 Nutrition

Fish oil containing omega-3 polyunsaturated fatty acids is thought to have a beneficial outcome on health. Therefore, the association between omega-3 fatty acids and HRV was recently studied and reviewed (Christensen, 2003; Christensen & Schmidt, 2007). Christensen reports antiarrhythmic, antiatherogenic, antithrombotic, and antiinflammatory properties of the fatty acids, therefore offering an explanation for the beneficial effects on cardiovascular morbidity and mortality. A positive correlational association between cellular levels of omega-3 fatty acid and HRV in post-myocardial infarction (MI) patients was presented. Additionally, a significant dose-dependent increase of HRV was documented when distributing marine omega-3 fatty acids daily over 12 weeks to post-MI patients. Christensen (2003) suggests a sodium or calcium channel, adrenergic receptors or central mediated effect on vagal activity. In a recently published study (Mozaffarian et al., 2007), the authors found a positive association between SDNN and RMSSD and dietary consumption of omega-3 fatty acids assessed by a questionnaire evaluating the frequency of consumption among other things of tuna and other broiled or baked fish. Subjects with a higher consumption revealed

higher baseline SDNN and RMSSD values, while 24-hour Holter recording revealed significantly higher HFnorm and reduced LFnorm values and a reduced LF/HF-ratio. HRV markers in association with the frequency of consumption furthermore revealed a lower relative risk for coronary heart disease after a mean follow-up of 10.8 years (comparison of subjects with the highest compared to the lowest quintile of consumption).

2.4 Conclusions

The VN is often called the health nerve, since on the one hand, as a major constituent of the PNS, it innervates several vital organs such as the heart and the lung, functionally promoting "rest and digest" contrary to "fight or flight " promoted by the SNS. On the other hand, several associations with common risk factors, morbidity and mortality are recognized, suggesting a mediating role of the VN in the development and progression of disorders. Stress is also recognized as an important risk and etiological factor in several disorders. In this regard, it stands to reason that the VN possibly plays a mediating role also in stress. Therefore, we aimed to examine the effect of stress on vagal activity indicated by HRV and the potential role of vagal functionality to buffer the stress response in terms of a physiological resource. If stress were to elicit a loss of inhibition indexed by vagal activity and the VN were to constitute a resource, the question of the potential role of the VN as a therapeutic target emerges. This suggestion is supported by beneficial effects of invasive VNS. Therefore, a further aim of this thesis was the evaluation of an alternative, mildly invasive method to increase vagal activity. In a second study, we examined the effects of manual and electrical stimulation by applying acupuncture at the vagally innervated ear. A stimulating effect could possibly be used in prevention and therapy in terms of a resource-cumulative intervention. In the next chapter, the empirical studies are presented, controlling for several of the disorders, disturbing factors and risk factors mentioned in this chapter.

3. EMPIRICAL STUDIES

Below, the two studies underlying this doctoral thesis are presented consecutively. First, the stress study examining the protective role of vagal functionality is presented, and second, the stimulation study is reported.

3.1 Vagal functionality as a physiological resource reducing stress-induced biopsychological responses[1]

A short introduction is provided, before reporting the applied methods and the results. A study-specific discussion follows, which embeds the results in a theoretical background.

3.1.1 Introduction

Vagal activity has been proposed as an index for the ability to adapt to changing demands from the environment (Thayer, 2007). However, an association between vagal functionality and acute biopsychological stress responses has not been systematically investigated. A theoretical framework to integrate these systems is provided through the neurovisceral integration model, which is based upon the central autonomous network (CAN; Benarroch, 1997; Thayer and Lane, 2000). The structures underlying the CAN are the ventromedial prefrontal cortex (PFC), insular cortex, anterior cingular cortex (ACC), central nucleus of the amygdala (CeNA), paraventricular nucleus (PVN) and related nuclei of the hypothalamus, periacqueductal grey (PAG), nucleus parabrachialis, nucleus of the solitary tract, nucleus ambiguus, ventromedial and ventrolateral medulla, and medullary tegmental field (Benarroch,

[1] Parts of this study are submitted or in preparation for submission (La Marca, Waldvogel, Thörn, Tripod, Pruessner & Ehlert, 2009; La Marca, Thörn, Waldvogel, Tripod, Pruessner, Arnold & Ehlert, 2009).

1997). These structures are complexly interconnected by parallel, reciprocal, direct and indirect connections, and modulate psychophysiological resources in emotion (Thayer and Friedman, 2002). Additional to the CAN structures, the neurovisceral integration model describes the possibility to flexibly revert to other neuronal networks when demands of the environment require it (Thayer, 2007). Thereby, in this dynamical model, neuronal inhibitory processes represent an important basis for the ability to rapidly adapt to internal or external demands (Thayer and Friedman, 2002). A breakdown of inhibitory processes is associated with a reduced and rigid behavioral repertory, as indicated, for example, by a reduced differentiated emotion-modulated startle response in subjects with low heart rate variability (HRV) (Ruiz-Padial *et al,* 2003). Similar to the PFC eliciting an inhibitory control on subcortical structures, HRV as an indicator of cardiovagal activity is associated with this inhibitory process (Thayer and Friedman, 2002), and therefore represents an additional indicator for the negative feedback mechanisms that are important for the self-regulation of behavior (Thayer and Lane, 2000). The vagal nuclei contain direct and indirect connections to the PVN (Benarroch, 1997; Palkovits, 1999; Porges, 2001), where vagal afferents are thought to have an inhibitory influence. In actual fact, Thayer et al. (2006) reported an inverse relationship between HRV and overnight urinary cortisol in a sample of 542 healthy men. Further evidence was also reported by Johnsen et al. (2002), who found a higher salivary cortisol response to stressful tasks in subjects with low HRV compared to those with high HRV. However, to the best of our knowledge, to date, there has been no examination of the association between cardiovagal reagibility and biopsychological stress responses to a standardized stressor.

When examining this relationship, psychosocial stressors might be the first choice. On the one hand, social evaluation is a potent contributor to the secretion of cortisol during stress (Dickerson and Kemeny, 2004). On the other hand, a link exists between neuronal structures underlying the social engagement system and vagal structures, but also stress reactivity,

(Porges, 2001). Besides social evaluation, further important factors such as novelty, uncontrollability, unpredictability, and ego involvement are capable of inducing an acute stress response (Mason, 1968; Wirtz *et al,* 2007). Different psychological stressors are applied in stress reactivity research. While stressors containing both uncontrollable and social-evaluative elements emerged as the most effective to increase the activity of the HPA axis (Dickerson & Kemeny, 2004), mental arithmetic is thought to be appropriate to decrease vagal activity (Pagani *et al*, 1995). Typical acute psychosocial laboratory stressors containing these elements are public speaking tasks (e. g. Trier Social Stress Test, TSST, Kirschbaum *et al*, 1993). Thus, in the current study, we employed the Montreal Imaging Stress Task (MIST; Dedovic *et al,* 2005), a recently published standardized computerized stress task combining challenging arithmetic problems with social-evaluative threat. We chose the MIST because it allows potentially disturbing factors such as postural change or motion to be controlled for, as the MIST was designed for application in an fMRI environment. Pruessner and colleagues were repeatedly able to show the ability of the MIST to increase cortisol concentration (Dedovic *et al,* 2005; Pruessner *et al,* 2004, 2008; Soliman *et al,* 2008), sympathetic activity (Pruessner *et al,* 2004, Soliman *et al,* 2008), and induce electromyographic responses (Soliman *et al,* 2008). Neuronal activity alterations in several brain structures related to the CAN have also been identified within healthy subjects using functional magnetic resonance imaging such as deactivations in the medio-orbitofrontal cortex, ACC, amygdala, hypothalamus, and hippocampus (Pruessner *et al,* 2008). Heightened striatal activity was further found during the MIST in students reporting negative symptom schizotypy, possibly indicative of reduced frontal lobe function (Soliman *et al,* 2008).

Thus, we aimed to investigate the association between acute biopsychological stress responses and vagal functionality using the MIST in healthy young men.

3.1.2 Materials and Methods

3.1.2.1 Participants

Subjects were recruited by advertisement at the Universities of Zurich. Inclusion criteria included male sex and an age range of 18 to 40 years and dexterity. Exclusion criteria included depression, self-reported acute and chronic somatic or psychiatric disorders, medication in the last two months, the consumption of psychoactive substances, and excessive consumption of alcohol (>2 alcohol beverages / day) or tobacco (>5 cigarettes / day). Participants received monetary compensation for their participation. The study was conducted in accordance with the Declaration of Helsinki and was approved by the cantonal ethics committee. Subjects provided written informed consent prior to participation.

3.1.2.2 Procedure

After arriving at the laboratory, subjects were fitted with the cardiorespiratory ambulatory device, and were seated in a comfortable chair. All tests were conducted in the same room at a constant temperature (21° celsius), while subjects were sitting in front of a table with a computer and several available magazines. After a rest period of thirty minutes, subjects were asked to fill out mood questionnaires. This was followed by the MIST. Subjects were exposed in random order to the stress (MIST-S) and control condition (MIST-C), on two separate occasions two weeks apart. Mood questionnaires were again handed out after the termination of the task. To include cortisol stress recovery, the examination lasted for an additional 60 minutes. At the end of the control examination, the CFT was conducted, while at the end of the stress examination, subjects were debriefed and informed about the cover story (examination of interaction between cognitive skills and physiological marker). Subjects who

first participated in the stress condition were reassured that on the next occasion, a cognitive but not a stress task would take place. At the end of the second examination, participants gave a second written informed consent allowing the further use of their data.

3.1.2.3 Interventions

Montreal Imaging Stress Task

To induce a stress response, a slightly modified version of the MIST (Dedovic *et al,* 2005) was used, since the task was originally developed for fMRI environments. The MIST is a standardized computerized stress task combining challenging arithmetic problems with social-evaluative threat and can be carried out with or without time pressure and social evaluation (MIST-S vs. MIST-C). During the MIST-S, the program adapts the difficulty and time provided to solve the problems impeding a good performance (45 to 50 percent of correct answers). During the MIST-C, the difficulty of the arithmetic problems is randomly chosen and neither time pressure nor social evaluation is applied. In both conditions, three blocks of 4 minutes each were run, with feedback of 2-3 minutes provided by the examiner in-between blocks. The first feedback of the MIST-S consisted of informing the participants that their performance was poor, with the examiner inquiring whether the subjects were experiencing any methodical problems (e.g. with the keyboard). A fictitious study leader then informed the examiner by telephone to repeat the test. In the second feedback of the MIST-S, the study leader entered the examination room and interrogated the participants about individual problems (e.g. about school performance). Participants were then informed about the high costs due to a possible exclusion if they did not achieve a better performance. The last block was then started, while the study leader remained in the examination room during the first 3.5 minutes. Before leaving the room, he instructed the examiner to continue with the normal procedure. At the very end of the stress examination, participants were debriefed and asked

how much they believed that their bad performance was due to a bug in the program (credibility of the stress task: 10-point Likert scale). During the first and second feedback of the MIST-C, participants were provided with a neutral feedback. They were asked to perform a second and third block, respectively, in order to evaluate the time course of interactions between cognitive and physiological characteristics.

Cold Face Test

The Cold Face Test (CFT) mimics the diving reflex by inducing a trigeminal-vagal-mediated bradycardia and can be used to determine cardiovagal function (Khurana, 2007; Khurana, Watabiki *et al,* 1980; Khurana and Wu, 2006). Bradycardia was induced by using a full-face mask (Dr. Winkler GmbH, Ainring-Mitterfelden, Germany) covering wide parts of the face, with openings for the eyes avoiding an occulocardiac reflex, and for the nose and mouth allowing normal breathing. An additional cold pack (Nexcare™, 3M, Health Care, MN, USA) was affixed to the full-face mask to augment the cold mass. While the room temperature was kept constant (21° celsius) the temperature of the cold stimulus was 1° celsius and was applied for two minutes, while subjects were sitting in a comfortable chair. Subjects were instructed in advance not to move or talk and to continue breathing normally during the CFT. Six visual analogue scales (VAS; feeling stressed, exhausted, queasy, relaxed, good humoured, and pain sensation) were additionally handed out two minutes before and after the CFT to assess any changes in subjective sensation. The effects of the CFT were measured as alterations in HR and RSA from one minute before until one minute after the CFT. The HR over the one-minute period preceding the CFT was set as baseline. Maximum response of HR was determined as the difference between the baseline and peak response due to the CFT (CFT_{max}), and the latency of response was defined as the time interval from the first instance of three successively slowing beats below baseline until peak bradycardia ($CFT_{latency}$) (Khurana and Wu, 2006).

3.1.2.4 Measures

Biochemical measures

Examinations started in the afternoon between 1:30 and 4:15 p.m. to control for circadian fluctuations of the different markers (e.g. Burgess *et al*, 1997). Salivary samples were repeatedly collected with salivettes (Sarstedt, Sevelen, Switzerland) before, during and after the interventions by collecting unstimulated whole saliva: immediately before the MIST instruction (baseline), during the third block, and at +10, +20, +30, +45 and +60 minutes. Subjects placed a salivette under the tongue and kept the head slightly inclined for two minutes. Salivary flow rate was determined by weighing each salivette before and after collection. Afterwards, the samples were stored at -20°C before the biochemical analysis took place.

After thawing, saliva was centrifuged at 3000 rpm for 5 min before free *cortisol* was analyzed using an immunoassay with time-resolved fluorescence detection (Dressendorfer *et al,* 1992). The activity of *salivary alpha-amylase* was analyzed with a kinetic colorimetric test using assay kits and the automatic analyser Cobas Mira (Roche, Switzerland). The procedure is described elsewhere (Nater *et al,* 2006).

Electrophysiological measures

The LifeShirt system 200 (Vivometrics, Ventura, CA, USA) was used to measure *HR* and *RSA*. This ambulatory cardiopulmonary measurement device consists of a garment with two integrated inductive plethysmography (IP) bands surrounding the midthorax and midabdomen and a connected palm, which additionally saves electrocardiographic data measured by three electrodes. The device was recently evaluated and shown to possess a good accuracy of detection and timing of beat-to-beat values (Heilman and Porges, 2007). After volume calibration of the IP bands by the subjects breathing repeatedly into a fixed volume bag

(800cc) data recording started. The record was then examined for artifacts and edited manually to correct for ectopic beats and arrhythmias by using linear interpolation.

The corrected inter-beat-interval allowed the calculation of HR and RSA, which were determined for 5 minute intervals during the MIST and 1 minute intervals during the CFT. Both markers were determined using the VivoLogic 3.1 software package (Vivometrics, Ventura, CA, USA). The CFT response was further analyzed by visual examination of the HR trace.

Psychological measures

Depressive mood was measured with a German version (Allgemeine Depressionsskala-Langform, ADS-L: Hautzinger and Bailer, 1992) of the Center for Epidemiologic Studies Depression Scale (CES-D: Radloff, 1977) in order to exclude subjects with a possible depressive disorder (cut-off score >23). *State mood* was determined repeatedly, immediately before and after the MIST. To assess different aspects of mood, subjects filled out the Multidimensional Mood State Questionnaire (German original version: Multidimensionaler Befindlichkeitsfragebogen, MDBF; Steyer *et al,* 1997) consisting of three dimensions: 'good-bad mood', 'calmness-nervousness', and 'wakefulness-tiredness'. Furthermore, a visual analogue scale (VAS) was handed out asking subjects to rate how 'stressed' they felt at that moment. The distributed questionnaires have been broadly used and have shown satisfactory internal consistency and validity.

3.1.2.5 Data Analysis

Analyses were performed using SPSS (15.0) software packages (SPSS, Chicago, IL, USA). Homogeneity of variance was assessed using the Levene test. Besides raw data, the trapezoid formula for total response (area under the curve with respect to the ground AUC_G) and total

change of response in consideration of individual baseline (area under the curve with respect to increase, AUC_I) were computed (Pruessner *et al,* 2003).

For comparisons between groups, the Student's t-test was used, while repeated-measures analyses of variance (ANOVA) were computed after Greenhouse–Geisser corrections to reveal possible time, condition and interaction effects. Partial correlations controlling for the credibility of the stress task were calculated to examine associations between the MIST responses and vagal indicators. Linear regression analyses were used to examine the relationship between predictors and dependent variables, adjusting for the credibility of the stress task and baseline values where appropriate. Explained variance of the whole regression model is reflected by R^2. In condition x time interactions, the effect size was determined by partial eta-square (partial eta2) using the possibly conservative cutoffs of .01 (small), .06 (medium), and .14 (large) (Green *et al,* 2000). All analyses were two-tailed, with the level of significance set at $p<.05$.

3.1.3 Results

Sample characteristics

Of the initially 34 healthy subjects who agreed to participate, one subject was excluded because of acute illness during the examination period. Low saliva amounts in some samples further reduced the available number of data points for some measures (cortisol AUC: N=28, alpha-amylase-AUC: N=24). The mean age of participants was M=24.06 (SD=4.56, range 19-34) and the mean BMI was M=23.63 (SD=2.94, range 19.47-29.70). Depression scores were in a normal range of values (M=7.84, SD=4.14, range 2-21). Randomization resulted in two groups, with 16 subjects undergoing the control condition first and 17 subjects undergoing the stress condition first. The two groups did not differ with respect to demographic (BMI, age) or physiological (salivary flow rate, cortisol, alpha-amylase, HR, RSA) or mood changes

(MDBF) during the stress condition (all p=n.s.). Referring to the control condition, the corresponding variables did not differ, with the exception of the wakefulness-tiredness dimension of the MDBF, whereas subjects participating in the control condition in the second session became less tired compared to subjects who started with the control condition (t_{31}=-2.93, p=.006). The randomization was therefore successful.

Responses to the stress task

Biochemical responses

The stress condition of the MIST resulted in a significant decrease in *salivary flow rate* (F(4.94/148.27)=13.36; p<.001), while the control condition revealed no significant fluctuations (F(5.15/154.35)=.97; p=.44). The interaction of condition and time was highly significant (F(5.23/156.89)=4.96; p<.001; partial eta2=.14), while this finding was not supported by the total scores of the AUC (AUC_G: t_{30}=1.06, p=.296; AUC_I: t_{30}=.42, p=.677), although a significantly lower salivary flow rate was evident during the MIST (t_{30}=-4.94, p<.001).

C*ortisol* levels over the course of the stress task showed a significant increase over time (F(2.02/54.51)=16.59; p<.001), while under the control condition, a significant decrease typical for the circadian course of cortisol was observed (F(1.83/45.65)=24.55; p<.001). Thus, conditions differed significantly, with the cortisol response over time during the stress condition showing a significantly higher response (F(2.27/56.69)=20.94; p<.001; partial eta2=.46; Fig.3.1). This result was also reflected by significant differences in the AUC_G and AUC_I of cortisol (AUC_G: t_{25}=-4.98, p<.001; AUC_I: t_{25}=-5.58, p<.001). The MIST resulted in a peak increase of 164% compared to the equivalent time point in the rest condition (+20: M_{MIST-S}=9.04, SD_{MIST-S}=5.86; M_{MIST-C}=3.43, SD_{MIST-C}=1.39).

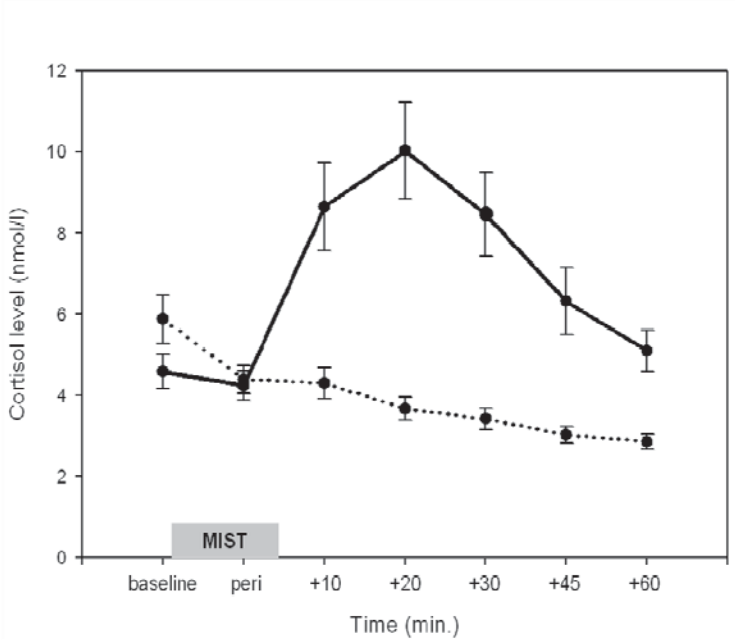


Figure 3.1 Salivary cortisol concentration (nmol/l) during the stress (solid line) and control condition (dashed line). Values represent mean +- standard error of the mean values

The concentration of salivary *alpha-amylase* rose significantly during the stress (F(3.28/75.49)=3.77; p=.012) and control condition (F(4.17/112.59)=2.676; p=.033).

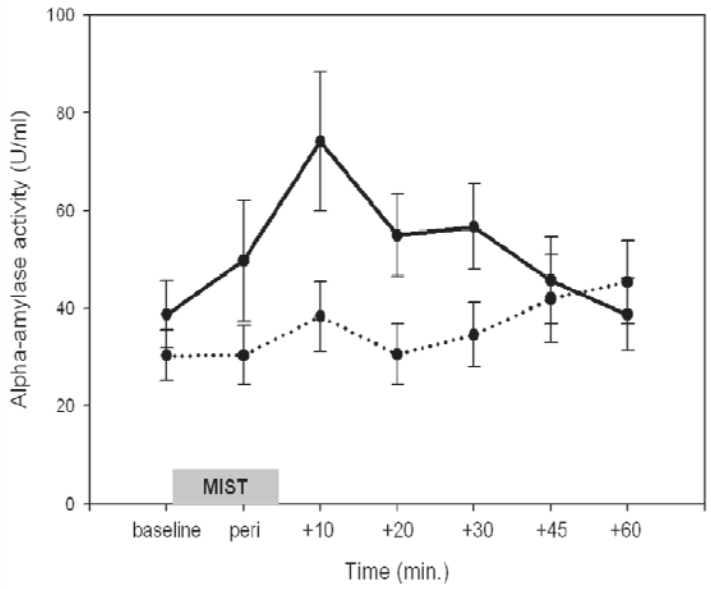


Figure 3.2 Salivary amylase activity (U/ml) during the stress (solid line) and control condition (dashed line). Values represent mean +- standard error of the mean values

The interaction of condition and time was significant, with higher levels during the stress condition (F(3.34/73.55)=4.64; p=.004; partial eta2=.17; Fig. 3.2). A significant difference could also be observed for salivary alpha-amylase AUC_G (t_{22}=-2.15, p=.043) but not AUC_I levels (t_{22}=-1.49, p=.15).

Electrophysiological responses

HR changed significantly during the stress as well as the control condition (MIST-S: F(2.34/75.04)=53.07, p<.001; MIST-C: F(3.10/99.23)=17.36, p<.001). The interaction was significant, with higher values during the stress condition (F(2.53/80.94)=39.93, p<.001, f2=.23; partial eta2=.56; Fig. 3.3). AUC_G and AUC_I differences further supported this finding (AUC_G: t_{33}=-3.53, p=.001; AUC_I: t_{33}=-5.33, p<.001).

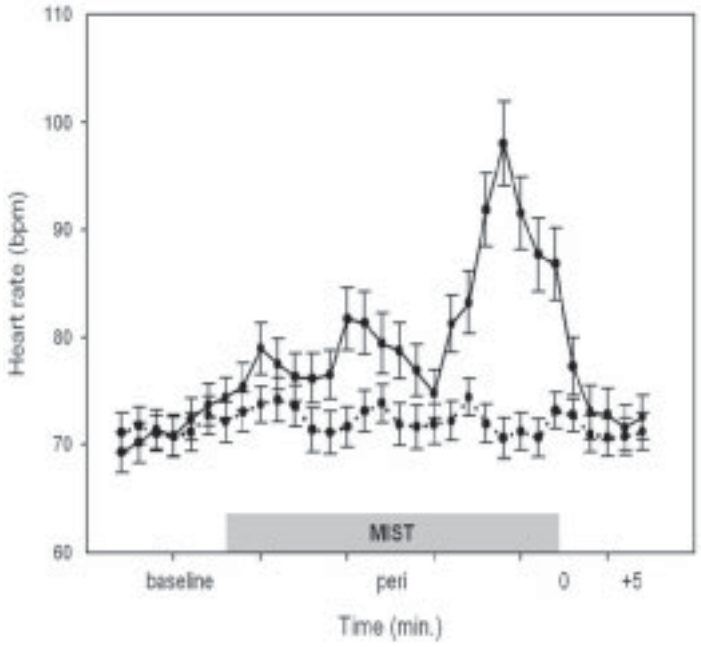


Figure 3.3 HR (bpm) during the stress (solid line) and control condition (dashed line). Values represent mean +- standard error of the mean values

Under the control condition, *RSA* increased significantly during the MIST-C (F(3.48/111.32)=2.81, p=.035) and decreased significantly during the MIST-S

(F(3.49/111.57)=7.16, p<.001). Both conditions differed significantly over time (F(5.01/180.34)=8.80, p<.001; partial eta2=.22; Fig. 3.4). The trapezoid formulas revealed a significant difference of the total change of response (AUC_I: t_{33}=2.21, p=.034) but not of total response (AUC_G: t_{33}=1.05, p=.30).

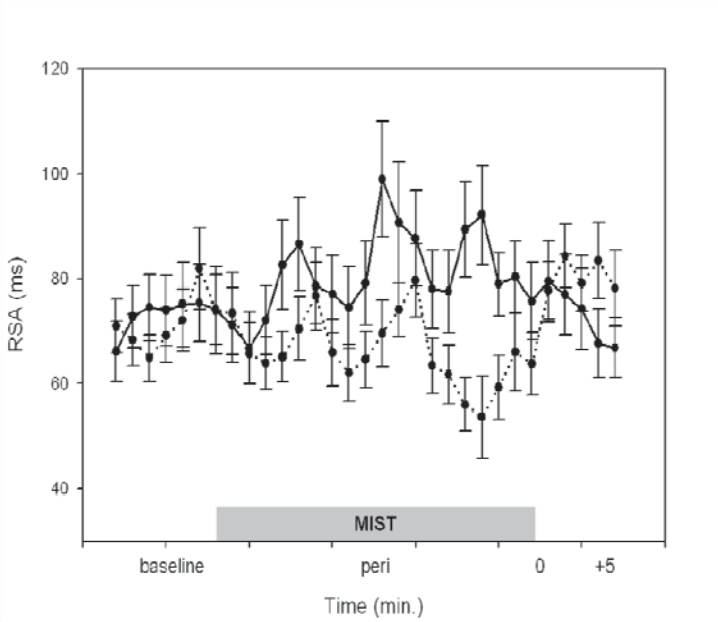


Figure 3.4 RSA (ms) during the stress (solid line) and control condition (dashed line). Values represent mean +- standard error of the mean values

Mood responses

During the MIST-S*, mood* assessed by the MDBF decreased significantly from immediately before to immediately after the stress task (F(1/32)=27.80, p<.001), while there was no significant alteration during the control condition (F(1/32)=.52, p=.476). The interaction condition x time was highly significant (F(1/32)=25.51, p<.001, partial eta2=.44) due to lower values following the MIST-S (before MIST: t_{32}=.83, p=.413; after MIST: t_{32}=-5.494, p<.001). *Calmness* (MDBF) decreased significantly under the stress condition but not under the control condition (MIST-S: F(1/32)=64.90, p<.001; MIST-C: F(1/32)=1.27, p=.27), and the interaction was highly significant (F=(1/32)=52.30, p<.001, partial eta2=.62) due to heightened nervousness after the MIST-S (before MIST: t_{32}=1.823, t=.078; after MIST: t_{32}=-

6.814, p<.001). There were no significant changes concerning *wakefulness* (MDBF) under both conditions. Indeed, both conditions showed an inverse course over time (F(1/31)=4.41, p=.044, partial eta2=.12), although the values did not differ either before or after the MIST (before MIST: t_{31}=1.652, p=.11; after MIST: t_{32}=-1.02, p=.314).

Finally, the MIST-S elicited a significant increase in the subjective rating of how *stressed* participants felt (VAS: F(1/32)=28.53, p<.001), while MIST-C showed no significant effect (F(1/32)=3.25, p=.081). The interaction condition x time was highly significant (F(1/32)=25.97, p<.001, partial eta2=.45) due to higher values of rated stress immediately after the MIST-S (before MIST: t_{32}=-.32, p=.753; after MIST: t_{32}=8.19, p<.001).

Responses to the Cold Face Test

The CFT induced a significant decrease in *HR* (F 2.17/69.48)=39.94, p<.001, partial eta2=.56) and accordingly, a significant rise of *RSA* (F(2.34/74.97)=8.25, p=.001, partial eta2=.20). The bradycardia during the CFT peaked after M($CFT_{latency}$)=29.85sec (SD=19.95sec) and resulted in a maximum decrease of M(CFT_{max})=24.80% (SD=7.64%). The CFT influenced neither subjective mood ratings (VAS: feeling stressed, exhausted, queasy, relaxed, good humoured: all p>.1) nor pain sensation (VAS: F(1/32)=.008, p=.930).

Associations between Vagal Functionality and Biopsychological Stress Response

Analysis of Correlation

Partial correlations controlling for the credibility of the stress task revealed no significant associations between the baseline of RSA and mood and biological responses to the stress task (all p=n.s.), with the exception of the AUC_G of RSA during the MIST-S (r=.852, p<.001; table 3.1) due to the mathematical determination of the trapezoid formula.

Table 3.1 Partial correlation coefficients between vagal reagibility and stress responses controlling for credibility of the stress task and, where appropriate, the MDBF baseline values

		RSA_{BL}	CFT_{max} (relative)	$CFT_{latency}$
SFR	**AUCg**	.184	-.162	-.123
	AUCi	-.223	-.052	-.093
Cortisol	**AUCg**	.140	-.091	.326
	AUCi	-.015	-.035	.491**
Amylase	**AUCg**	.222	.130	.077
	AUCi	.208	.366	-.009
HR	**AUCg**	-.320	.018	.270
	AUCi	-.095	-.292	.140
RSA	**AUCg**	.852***	.382*	-.085
	AUCi	-.033	.104	-.081
MDMQ	**Good/ bad mood**	.123	-.085	-.628***
	Wakefulness/ Tiredness	.303	.375*	-.307
	Calmness/ Nervousness	.174	.018	-.290
VAS	**Stressed**	-.200	.000	.273

*p<.05, **p<.01, ***p<.001

Similarly CFT_{max} correlated significantly with the AUC_G of RSA (r=.382, p=.028), but also with the baseline of RSA (r=.399, p=.021), underlining the vagal participation in the CFT. Furthermore, CFT_{max} significantly correlated with the wakefulness-tiredness dimension of the MDBF (r=.409, p=.020), even when additionally considering individual baseline (r=.375, p=.038). Furthermore, $CFT_{latency}$ significantly correlated with cortisol AUCi (r=.491, p=.009), and the mood dimension of the MDBF without controlling for baseline values (r=-.615, p<.001) and with controlling for baseline values (r=.628, p<.001).

Regression Analysis

We calculated linear regression analyses with AUCi of cortisol, mood or wakefulness as the dependent variables. As independent variable, we entered the credibility of the stress task, and with regard to psychological responses, the respective baseline values. Finally, based on the analysis of correlation, we entered CFT_{max} or $CFT_{latency}$ as independent variable. CFT_{max} as a predictor explained 12.7% (β=.36, p=.038) out of 22.6% (R^2=.23) of the dimension wakefulness-tiredness of the MDBF. The model with $CFT_{latency}$ as independent variable predicted 27.7% of cortisol AUCi in the stress condition (R^2=.28), of which $CFT_{latency}$ independently predicted 23.0% (β=.49, p=.009; Fig. 3.5).

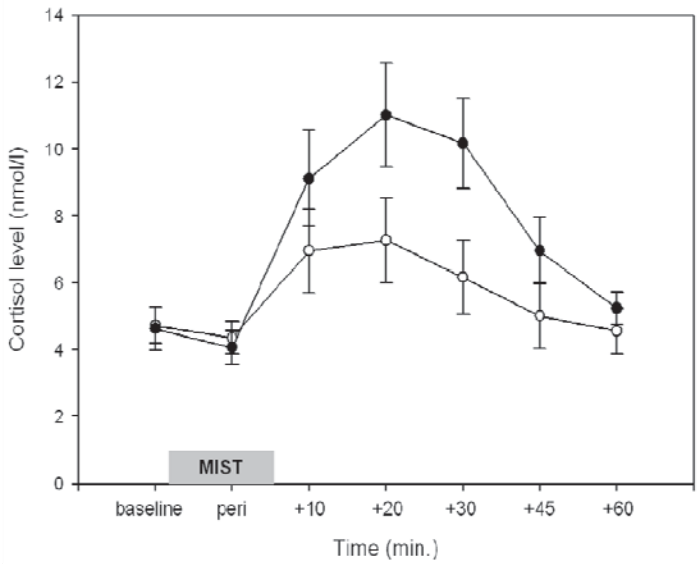


Figure 3.5 Stress reaction of salivary cortisol in subjects with fast (white circles) and slow (black circles) vagal reagibility determined after median split. Values represent mean +- standard error of the mean values

When referring to the mood dimension of the MDBF, the regression model was able to explain 40.2% (R^2=.40), 38.9% attributable to $CFT_{latency}$ (β=-.63, p<.001; Fig. 3.6).

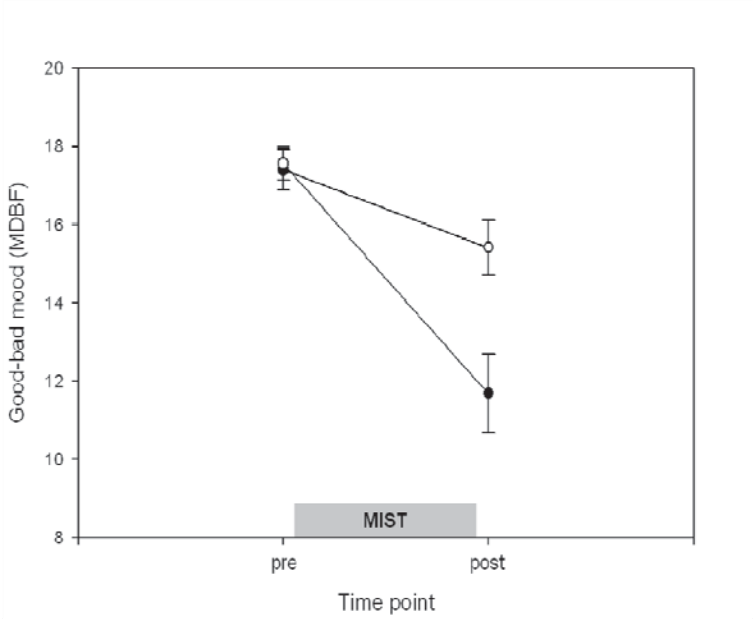


Figure 3.6 Stress reaction of mood in subjects with fast (white circles) and slow (black circles) vagal reagibility determined after median split. Values represent mean +- standard error of the mean values

3.1.4 Discussion

One of the objectives of the present study was a multidimensional biopsychological evaluation of a recently published standardized psychosocial stress task in a laboratory setting taking into account parasympathetic (vagal) effects. Replicating earlier studies, the stress condition of the Montreal Imaging Stress Test induced a significant increase in salivary cortisol levels, salivary alpha-amylase levels, and HR, and a significant decrease in salivary flow rate and RSA, when compared to the control condition. With regard to subjective ratings, participants reported a decline in mood and a rise in nervousness, tiredness and stress in response to the stress task. Interactions of time and condition revealed large effect sizes for all variables. The second objective of the present study was to test the hypothesis that the vagus nerve constitutes a resource that can be utilized during acute stress. In this context, we measured vagal tone and additionally vagal reagibility during the CFT. The latter test induced

a significant short-term fall in HR and rise in RSA. Maximal response latency during the CFT ($CFT_{latency}$) but not vagal tone significantly predicted the cortisol and mood stress response, while CFT_{max} predicted the grade of tiredness in response to the stress task.

The MIST possesses all stress elements believed to induce changes in the activity of the HPA axis, the sympathetic and parasympathetic nervous system, and affect (Dickerson and Kemeny, 2004). In fact, in the present study, we found a significant increase in rated stress, nervousness and tiredness, and a significant worsening of mood during the stress condition compared to the control condition. These ratings correspond to the results of prior studies (Pruessner *et al,* 2004, Soliman *et al,* 2008) demonstrating an increase of stress rating and state anxiety due to the MIST. Furthermore, our findings replicate those of prior investigations showing an increase in cortisol concentration (Dedovic *et al,* 2005; Pruessner *et al,* 2008; Soliman *et al,* 2008) and markers mainly associated with sympathetic activity (Pruessner *et al,* 2004; Soliman *et al,* 2008). The stress task in the present study in fact induced a significant increase in HR and salivary alpha-amylase concentration, indicating an increase in sympathetic activity. While HR increased continuously throughout the stress condition, suggesting a successive increase in stress load from block to block, which recovered immediately after termination of the MIST, salivary alpha-amylase levels peaked ten minutes after the end of the stress task. We suggest that this delay could be explained by the reduction in salivary flow rate during the MIST-S, which was possibly too low to transport the salivary enzyme through the glandular canal system in real time, causing a peak delay. Supporting this, and contrary to Rohleder et al. (2006), we found a positive correlation between salivary alpha-amylase concentration and salivary flow rate in the stress condition (data not presented). Significant alterations in salivary flow rate during the stress task could be expected because salivary glands are innervated by both branches of the autonomic nervous system (Malfertheiner and Kemmer, 1987). Therefore, a decrease in salivary flow

rate can be interpreted as an increase in sympathetic or decrease in parasympathetic activity, or both. Nevertheless, reported stress responses of salivary flow rate are inconsistent, often reporting no influence of acute stress (e.g. Nater *et al,* 2006). This is perhaps to be explained by measurement time, since we collected saliva during the period of maximum stress exposure without interrupting the stress procedure.

This is the first study to examine the effects of the MIST on parasympathetic, in particular cardiovagal activity. In accordance with theoretical assumptions (Pagani et al., 1995; Porges, 1995), RSA decreased during the stress task, with a peak depression during the final block followed by a rapid recovery after the termination of the task. In contrast to other psycho-social stress tasks, the cardiorespiratory responses in the present study can be exclusively attributed to the stress task, since disturbing factors like postural changes or walking (Chan *et al,* 2007; Nater *et al,* 2006), which influence the highly sensitive autonomic nervous system, were controlled for. When examining vagal activity, any vocalization is considered a significant confound (Bernardi *et al*, 2000; Sloan *et al,* 1991). In the current study, this was partially controlled for, since during the MIST, speaking is limited to the feedback periods in-between the blocks. Thus, the MIST is particularly useful to investigate the relationship between different response variables not equally susceptible to the disturbing factors.

Another aim of the current study was to investigate vagal functionality as index of adaptability of the organism to stress. Structural and functional assumptions would suggest a protective role of high vagal functionality during stress, although evidence for this is sparse. In fact, we found an inverse relationship between the cortisol response elicited by the MIST and vagal reagibility during the CFT but not vagal tone. With regard to the CFT, subjects with a slow bradycardia showed a larger cortisol stress response. Similarly, Johnsen et al. (2002) reported a significant difference in cortisol response to stressful cognitive tasks between a

group with high compared to a group with low vagal tone. Thayer and Sternberg (2006) found an association between the overnight urinary cortisol concentration and vagal tone in healthy subjects. Further support for this inverse association is provided by O'Keane et al. (2005), who examined responses to the corticotropin-releasing-hormone (CRH) challenge test in eleven patients with chronic depression before and after three months of treatment with vagus nerve stimulation (VNS). They reported significantly increased responses of adrenocorticotropic hormone (ACTH) and cortisol before treatment compared to intraindividual posttreatment responses, and in comparison to a control group. The negative correlation might point to direct and indirect bidirectional connections between vagal nuclei in the medulla oblongata and the hypothalamus (Benarroch, 1997; Palkovits, 1999). Furthermore, evidence from imaging studies supports interconnections between CAN structures, the hypothalamus and the vagus nerve. For example, the salivary cortisol in response to stress has been shown to be positively associated with hypothalamic activity (Ahs *et al,* 2006), and negatively correlated with medial PFC (Ahs *et al,* 2006; Kern *et al,* 2008), suggesting a possible phasic loss of inhibitory control of the medial PFC over subcortical regions during stress. Similarly, under stressful conditions, associations between High Frequency (HF) HRV (Task Force, 1996) as an index of cardiovagal activity and the activity of several structures of the CAN were demonstrated (Gianaros *et al*, 2004; Lane *et al,* 2009; Matthews *et al,* 2004).

With regard to our CFT results, it is proposed as a method to test cardiac vagal function (Khurana and Wu, 2006). Although unfortunately, no study has examined the neural associations of latency and maximum bradycardia, the CFT was found to elicit cerebral blood flow alterations (Brown *et al,* 2003). More specifically, Harper et al. (2003) were able to demonstrate in an fMRI study that the CFT affects CAN activity in healthy subjects. Significant activity increases during the application of the cold stimulus were found among other things in the insula, ventral midbrain, and dorsal, medial, and ventral medulla, while

activity decreased significantly in the hippocampus. Interestingly, the CFT was reported to be associated also with psychological variables known to be associated with HPA axis and vagal dysregulations. Hughes et al. (2000) categorized healthy subjects as having high or low depressed mood following a median split and found a higher increase in HF HRV in response to the CFT in the latter group. This finding indicates an impaired vagal regulation in subjects with higher depressive mood, while depression was often reported to show increased cortisol concentrations (e.g. Wang *et al*, 2000) and decreased vagal tone (e.g. van der Kooy *et al*, 2006). Similarly, more hostile students showed less HR deceleration during the CFT when compared to low hostile peers (Ruiz *et al,* 2006), while an inverse association between anger control and cortisol reactivity was also determined (e.g. Gouin *et al*, 2008). The CFT might thus be a methodological addition to the measurement of vagal tone in order to determine the functionality of the vagus nerve and, moreover, the whole network underlying the neurovisceral integration model and therefore to examine the (in-)ability of individuals to flexibly respond to rapidly changing demands of the environment.

Analyses of the role of vagal functionality during acute stress revealed a protective role not only with regard to cortisol but also to mood. Subjects with a slower bradycardia during the CFT showed a more negative affect in response to the stressor. This is in line with the assumption of vagal activity as being a type of resource when demands of the environment request emotional regulation (Thayer and Lane, 2009). Evidence for this was provided among others by Ruiz-Padial et al. (2003), who demonstrated a more differentiated emotional startle response to emotional pictures in subjects with high HRV compared to the group with low HRV. Additionally, HRV was shown to be inversely correlated with negative mood but positively with positive mood in a population of alcoholics and healthy controls (Ingjaldsson *et al*, 2003). In the present study, we found that the maximum response to the CFT predicted tiredness after the stress task. Subjects with smaller relative bradycardia were therefore more

tired after the stress task compared to subjects with stronger bradycardia. This is in line with the reported positive association between HRV and efficient attentional regulation (Johnsen *et al,* 2003), which might offer an explanation for the present findings. Additional support for this interpretation is provided by the findings of an inverse course of effort and HRV in burnout patients and healthy controls during a repeated Stroop color word task over one day (Zanstra *et al,* 2006). Controls showed a decrease in HRV and effort over the day, while at the same time, burnout patients revealed an increase in HRV, effort and tiredness.

Although our hypotheses were mostly supported, the current study holds a number of limitations. First, we examined a small sample size, including only healthy and medication-free subjects. Therefore, results are restricted to a group of healthy, well-educated, middle-aged men and can not be generalized to the general population. Referring to the MIST, it cannot be excluded that subjects felt bored in the control task, and angry or anxious in the stress task, and thus findings might not solely be attributed to stress. Moreover, the CFT in this study was used to determine vagal functionality, suggested by us to be indicative of the flexibility of the whole CAN. It should be mentioned on a critical note that CFT responses are initially mediated by the trigeminal nerve and therefore responses could also be influenced by the latter responsiveness. However, due to its role in emotion and social engagement and its structural and functional associations with the CAN, the trigeminal nerve might also be allocated to the neurovisceral integration model in terms of a flexibly reverted structure to meet demands of the environment. Despite these limitations, however, our study does have a number of methodological strong points. First of all, the present study is the first to examine the role of vagal functionality for cortisol stress response using a standardized psychosocial stress task. Second, several disturbing factors were controlled for, thus leading to a cleaner study design. Third, we examined several indicators of vagal activity by examining not only vagal tone but also phasic responses to the CFT. Therefore, the line-up of vagal characteristics

was enhanced to examine different levels of the underlying associations between the vagus nerve and the HPA axis.

In summary, our results underline the capacity and validity of the MIST to provoke a stress response of affect, the HPA axis, the sympathetic and parasympathetic nervous system. The inverse relationship between the vagal reagibility and biopsychological stress responses suggest that the former can be applied as a test to examine the stress adaptability of the neuronal network underlying the neurovisceral integration model. We speculate that vagal tone might reflect the tonic state of the interconnected network, while the CFT response might reflect the dynamic capacity of the organism to respond to rapidly changing environmental demands. However, the mechanisms underlying latency and maximum of bradycardia during the CFT and its relationship with different brain regions are not entirely understood. Further research is therefore needed to study functional and structural correlates of the CFT response. Moreover, the reported association should be examined in subjects with (sub-)clinical somatic and psychiatric disorders with primarily or assumed comorbid autonomic dysfunctions.

3.2 Effects of auricular manual and electrical stimulation on vagal activity[2]

After a short introduction the methods and results of the stimulation study are reported, before a discussion embedding the results in a theoretical background is provided.

3.2.1 Introduction

The activity of the vagus nerve (VN) is associated with health and well-being, and questions concerning its role for therapeutic manipulation are emerging (Routledge et al., 2002). The

[2] This study is submitted for publication (La Marca, Nedeljkovic, Yuan, Maercker & Ehlert, 2009)

VN constitutes the main part of the parasympathetic branch of the autonomic nervous system (ANS), which plays an important role in regeneration. Its action is associated with "rest and digest" (Rohen, 1994). The VN consists of afferents and efferents, and it controls, among other things, respiration and heart rate. Heart rate fluctuations are called heart rate variability (HRV), which can be measured non-invasively (Task Force, 1996). HRV indicates the regulatory capacity of the ANS (Berntson et al., 1997) and moreover the ability of the whole organism to respond to rapidly changing demands of the environment (Thayer & Friedman, 2002). Low vagal activity or responsiveness is associated with specific personality factors such as hostility (Ruiz et al., 2006; Sloan et al., 2001), type A behavior (Sato *et al*. 1998), and several risk factors (Thayer & Lane, 2009). Furthermore, stressful events can promote a phasic decrease of HRV (Klinkenberg et al., 2008; La Marca, Waldvogel et al., 2009; Nater et al., 2006; Pagani et al., 1995; Porges, 1995), and chronic stress leads to allostatic load accompanied by dampened vagal activity (Lucini et al., 2005; Thayer & Sternberg, 2006). In addition to these risk factors, evidence shows a link between low vagal activity and somatic or psychiatric morbidity and mortality (Thayer & Brosschot, 2005; Thayer & Lane, 2007), possibly mediated by associations between vagal activity and glucose regulation, hypothalamic-pituitary-adrenal (HPA) axis functioning, and inflammatory processes (Thayer & Sternberg, 2006). All these negative associations are paralleled by an augmenting interest in intervenetions targeting the VN. In recent years invasive vagus nerve stimulation (VNS) emerged as a treatment applied predominantly in epilepsy and depression (Milby et al., 2008). Since the body of data especially referring to its long-term effects is still insufficient and VNS shows several limitations due to its invasivity and restriction to therapy-resistant patients (Rijkers et al., 2008; Schlaepfer et al., 2008; Smyth et al., 2003), the focus has been on alternative and less invasive interventions, such as acupuncture, with regard to their effectiveness in influencing vagal activity. Studies examining the effects of acupuncture on the activity of the ANS were conducted in animals and humans, with inconsistent results at least in part due to the

high heterogeneity of the applied methods. Imai and colleagues (2008) found increases in gastric motility and cardiovagal activity and a decrease in sympathovagal balance in rats during and after electroacupuncture (stomach channel, ST36), indicating an overall increase in vagal activity. A similar result regarding gastric and cardiac activity was found in dogs during but not after electroacupuncture on ST36 and PC6 (pericardium meridian) (Ouyang et al., 2002). In line with these animal studies, several reports from human studies support an increase in vagal activity and/or a decrease in sympathovagal balance during acupuncture on PC6 (Huang et al., 2005; Li et al., 2003; Wu et al., 2008), whereas others found no effects (Hübscher et al., 2007) or found an effect predominantly on sympathetic activity (Chang et al., 2008). Similar results supporting heightened cardiovagal and/or reduced sympathetic activity were found also during acupuncture on other body points (Agelink et al., 2003; Bäcker et al., 2008; Hsu et al., 2006; Imai & Kitakoji, 2003; Li et al., 2005; Nishijo et al., 1997; Sakai et al., 2007; Shinohara, 1997; Sparrow, 2007; Streitberger et al., 2008; Wang et al., 2002; Zhang, 2006; 2007).

Auricular acupuncture is a special form of acupuncture, and somatotopic organization of the ear is postulated as containing 168 acupuncture points (Ulett et al., 1998). Differently, from an anatomical point of view, just a few areas are defined, due to the occurrence of different neuronal afferents (Lang, 1992). Some authors even restrict the mode of action of auricular acupuncture to just vagal manipulation in the concha (Ulett et al., 1998). A recent study examining the influence of manual and electroacupuncture on different ear points (inferior concha, helix, antihelix) of the rat found the best effect on the ANS (heart rate, mean arterial pressure, intragastric pressure) when stimulating the inferior concha (Gao et al., 2008). Since effects were also evident in those latter areas, the authors suggest that there is no specific functional map but rather a variable intensity depending on the area of stimulation. White and Ernst (1999) conducted a similar study in humans and examined manual acupuncture in the

concha and a control area of the helix. They found a marginal decrease in heart rate (HR) during stimulation of the concha but not the helix. Because the findings were not statistically significant, White and Ernst concluded that they did not find evidence supporting the representation of the body in the ear. Similarly, Kraus et al. (2007) studied the effect of transcutaneous electrical nerve stimulation (TENS) on the outer auditory canal, which is thought to be vagally innervated, while stimulation of the ear lobe served as a sham intervention. They found central activity alterations in the fMRI similar to the ones induced by VNS but could not find a significant effect on HR. Two other studies examining the effects of auricular acupuncture found no effect on HR, as well (Karst et al., 2007; Wang & Kain, 2001). Nevertheless, some studies found evidence that auricular acupuncture increases vagal activity and/or induces a shift in sympathovagal balance indexed by HR, HRV, and/or gastric variables (Haker et al., 2000; Hsu et al., 2007; Saxena et al., 1976), but interpretations of the these results are not unambiguous.

The inconsistent results on the effects of acupuncture on the ANS might be explained by the high degree of freedom regarding the methodological aspects of the different studies. These concern participants (healthy subjects vs. patients), point selection, type of stimulation (sham, magnetic, laser, manual, electrical), amount of interventions (singular vs. repeated), duration of stimulation, and interval between interventions, control condition (none, intervention using placebo needles, subcutaneous acupuncture, stimulation of presumably ineffective insertion points), statistical analyses (verum vs. control intervention, pre vs. peri/post intervention), blinding, interpretation of results (effects on the ANS, placebo effect, effects mediated through pain), exclusion criteria, and controlled disturbing factors.

The main purpose of the present study was to evaluate the effects of auricular manual and electroacupuncture on the activity of the VN in healthy men in a three-armed randomized trial. Furthermore, we wanted to examine factors supposed to influence dependent variables such as effects of time, placebo, pain sensation, and belief in the effectiveness of acupuncture.

3.2.2 Materials and Methods

3.2.2.1 Participants

Participants were recruited by advertisement at two universities in Zurich. Inclusion criteria were male sex and age ranging from 20 to 40 years, while exclusion criteria were depression, self-reported acute and chronic somatic or psychiatric disorders, medication in the last two months, consumption of psychoactive substances and excessive consumption of alcohol (>2 alcohol beverages / day) or tobacco (>5 cigarettes / day). Of the initial 15 men who volunteered to participate in the present study, 14 met the study criteria. One person was excluded due to medication. To control for disturbing factors, participants were instructed not to drink caffeinated beverages 48 h, to avoid excessive physical exercise and smoking 24 h, and to avoid eating in the last 2 h prior to the examination.

Participants received no monetary compensation, but they were given individual feedback on their responses. The study design was in accordance with the declaration of Helsinki and approved by the ethics committee of the Canton of Zurich.

3.2.2.2 Procedure

The participants came to the laboratory on four occasions, always in the afternoon between 1:30 p.m. and 4:00 p.m. to minimize possible circadian fluctuations of the dependent variable (Burgess et al., 1997; Fallen & Kamath, 1995). After arriving, participants signed written informed consent forms, and the cardiorespiratory ambulatory device (LifeShirt system, VivoMetrics, Ventura, CA, USA) was connected and calibrated. The participants then sat on a comfortable chair. Each examination lasted 90 minutes, consisting of 30 minutes of habituation and baseline measurement, 30 minutes of intervention, and 30 minutes of post intervention. After baseline measurement the acupuncturist (LY) entered the room,

disinfected the participant's ear, and then opened an instruction envelope placed in the examination room. Depending on the instructions, the acupuncturist placed no, placebo, or verum acupuncture needles before leaving the room. In the electroacupuncture condition the acupuncturist additionally attached the wires of the electronic acupunctoscope. After 30 minutes of intervention the acupuncturist entered the room for removal of the needles and disinfection in all conditions but the control condition. During all examinations, the participants were allowed to read popular magazines (such as *National Geographic* and *Anima*) before, during, and after the acupuncture intervention; this was to keep participants active to a minimum. The control condition was identical to the different acupuncture conditions, with the exception no needle was inserted after disinfection.

3.2.2.3 Interventions

Acupuncture Interventions

All participants came to our laboratory on four occasions, each one week apart. They took part in random order in a control condition with no needling (nonAP), a condition with placebo acupuncture (pAP), manual acupuncture (mAP), and electroacupuncture (eAP). Randomization was controlled by the author (RL) by writing all of the possible combinations on slips of paper, which were then put into a box, before drawing one for each participant. Each participant thus underwent a different sequence of conditions across the four occasions.

Placebo and verum needles were inserted into the left cavum conchae inferior, a region that is known to be innervated by vagal afferents (Lang, 1992; Schnorrenberger, 1994). Additionally, this area corresponds to lung and heart points according to auricular acupuncture theory (Rubach, 2000). In all acupuncture sessions two needles were placed 5 mm apart to allow an electrical flow in the eAP and to keep the number of needles equal in all interventions.

After disinfection of the participant's ear and application of the adhesive plaster, the acupuncturist applied the appropriate needles (see *Blinding*). In the nonAP condition no needles were set after disinfection. For the pAP two Streitberger placebo needles (0.3 x 30mm, asiamed, Germany) were used (see Streitberger & Kleinhenz, 1998). For the mAP and eAP visually identical but smaller verum needles were used (special needle nr. 12, 0.2 x 15mm, asiamed, Germany), while participants in all conditions were shown the same needles by an examiner during disinfection (special needle nr. 16, 0.3 x 30mm, asiamed, Germany). In the meantime the acupuncturist, who was situated to the left and behind the participants and was therefore outside of their visual fields, placed the placebo or the verum needles. In the eAP condition the acupuncturist additionally attached an electronic acupunctoscope (WQ-6F, Beijing Xindonghua Electronic Instrument Company Ltd., China). The frequency of stimulation was 10 Hz (Rubach, 2000), and the intensity of the current was adjusted by the examiner by asking the participants to let him know when the current passed the detection threshold without being painful.

Blinding

The study was partially blind in design in accordance with recommendations (White et al., 2001). For one, the participants were blinded regarding the application of placebo needles. Similar to Bäcker et al. (2008) the participants in the present study were told that different kinds of acupuncture interventions would be examined. For another, they were not told about the dependent variables or about the expected effects of acupuncture. Additionally, the examiner was completely blinded until the end of the habituation and baseline period to avoid any differences in the conducting of the experiment. When the acupuncturist applied the needles with or without electrical stimulation, the examiner was no longer blinded, but he was instructed to behave identically during all conditions and to leave the examination room together with the acupuncturist immediately after needle insertion. This reduced the contact

time between participants and examiner and acupuncturist to a minimum. Further, the acupuncturist was blinded as long as possible. After randomization of the order of conditions, the author noted the interventions (control, placebo, manual, electrical) on separate pieces of paper and placed them in closed, opaque envelopes in the examination room before the participants arrived. The acupuncturist opened the envelope only after disinfecting the participant's ear and was then no longer blinded to the condition. Additionally, physiological data was coded so that data analysis was also blind.

3.2.2.4 Measures

Assessment of Subjective Judgments

A German version of the Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff, 1977) was distributed at the beginning of the first examination (Allgemeine Depressionsskala, Langform, ADS-L: Hautzinger & Bailer, 1992) to exclude participants with a possible *depressive mood* (values >23). The CES-D questionnaire was developed to measure depressive symptoms and is often used for non-clinical populations and has shown satisfactory internal consistency and validity. Next, before the first intervention the participants were given two items asking them to estimate on a five-point Likert scale (from *not at all* to *very strong*) their *belief in effectiveness* of acupuncture to induce physical effects (phE). To validate the *effectiveness of the sham intervention*, the participants were asked immediately after the intervention whether they sensed the insertion of one or two needles. Afterwards, they answered on a seven-point Likert scale (from *not at all* to *very strong*) how *painful* the insertion of the needles was and how painful they found the whole intervention. To further check the placebo manipulation using the Streitberger placebo needles, the participants were asked three questions at the very end. First, they were asked if they had noticed anything special during the four examinations; here nobody suspected the use of a sham procedure.

Second, they were told that some participants had received a placebo intervention and were asked whether they believed that they themselves were one of those participants; no participant stated that he was sure that he had received a placebo intervention ("yes, sure"); all participants chose the answers "no, not at all" (n=6) or "not sure, maybe" (n=8). Next, the participants were told how the Streitberger placebo needles work and were asked if they thought they had received a Streitberger placebo intervention; all of the participants answered "no, not at all" (n=7) or "not sure, maybe" (n=7).

Electrophysiological measures

RSA was measured using the LifeShirt system 200 (VivoMetrics, USA). This ambulatory cardiopulmonary measurement device consists of a wearable garment with two integrated inductive plethysmography (IP) bands surrounding the midthorax and midabdomen and piezoelectrical signals are recorded on a palm. Electrocardiographic data are collected through three electrodes. The device was recently evaluated and showed accurate detection and timing of beat-to-beat values (Heilman & Porges, 2007). Before the data collection was begun, the participant breathed repeatedly into a fixed volume bag (800cc) for calibration of the IP bands. Following the last examination of the study, cardiorespiratory data were examined for artefacts and edited manually to correct for ectopic beats and arrhythmias. To do this, linear interpolations were applied. The corrected inter-beat-interval (IBI) allows the determination and extraction of RSA. Five-minute intervals were determined using the VivoLogic 3.1 software package (Vivometrics, Ventura, CA, USA). After importing data to a statistical program, the five-minute segments were averaged for the time during the intervention and the post-intervention period.

3.2.2.5 Data analysis

All analyses were performed using the SPSS (15.0) software package (SPSS, Chicago, IL, USA). Homogeneity of variance was assessed using the Levene test. In addition to raw data for five minutes, averages for the intervention and post-intervention period, the absolute increase of RSA from baseline to intervention and post-intervention, respectively, and the trapezoid formula for total change of response in consideration of individual baseline (area under the curve with respect to increase, AUCi) were computed to detect effects due to the different interventions (Pruessner et al., 2003; Scholz et al., 2009).

For comparisons between intervention-specific alterations, repeated measures analysis of variance (ANOVA) was computed after Greenhouse–Geisser corrections to reveal possible time, condition, and interaction effects. If appropriate, covariates (pain sensation, belief in effectiveness) were considered (ANCOVA). All analyses were two-tailed, with the level of significance set at $p<.05$.

3.2.3 Results

Sample Characteristics

The participants' mean age was 28.14 (SD=4.50) and mean body mass index (BMI) was 23.43 (SD=3.8). Mood scores were in a normal range of values (M=7.36, SD=4.91). The participants took part in the four conditions in random order, with no order of condition assigned more than once. To control for successful randomization, levels of RSA were tested and revealed no significant differences between the different conditions (RSA: $.280<p<.889$). The randomization can thus be considered as successful.

Effects of Pain

Pain sensation did not differ significantly when the needles were inserted, but it was revealed to be significantly elevated during the half-hourly eAP as compared to the pAP (p=.007) and mAP (p=.034), with the latter two conditions not differing (Figure 3.7). Therefore, differences in pain ratings during the interventions were considered as covariates when analyzing effects of eAP on vagal activity.

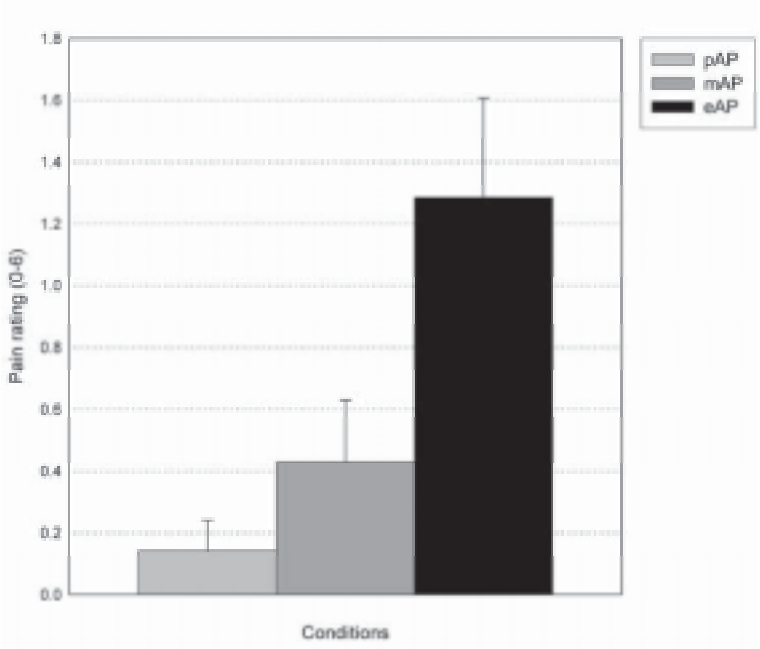


Figure 3.7 Mean and SEM of ratings of pain sensation from a Lickert scale during the different interventions

Effect of Belief in Effectiveness of Acupuncture

Belief in the effectiveness of acupuncture to induce physical effects (phE) was divided by a median split (phE-low: n=6, phE-high: n=8), since some of the five answer alternatives were chosen by only one or two participants. Univariate ANOVAs revealed a significant effect of phE on RSA increases during eAP (F(1/14)=5.32, p=.040) and a trend after eAP (1/14)=3.92, p=.071). Overall increase indexed by AUCi revealed a significant effect of phE (F(1/14)=6.06, p=.03) with phE-low showing higher RSA values. No effect on RSA increase was evident

during or after the other interventions or in AUCi. Therefore, belief in the ability of acupuncture to induce physical effects was further considered when analyzing effects of eAP.

Placebo Effect

Regarding the placebo intervention, all participants stated that they had sensed the insertion of one or two needles. None of them affirmed having received a placebo. Streitberger placebo needles thus seem to be applicable also on the ear. pAP did not differ from nonAP regarding RSA (during intervention: p=.954; post-intervention: p=.615; AUCi: p=.861), indicating no significant placebo effect during the pAP, and it was therefore neglected in the following analyses.

Time Effect

To detect changes of RSA over time ANOVAs with repeated measures were conducted for all four conditions. With the exception of the mAP ($F(1.73/22.43)=2.28$, p=.132), all of the conditions revealed a significant effect of time (nonAP: $F(1.65/18.17)=6.026$, p=.013; pAP: $F(1.12/13.41)=6.09$, p=.025; eAP: $F(1.71/22.25)=10.80$, p=.001; Figure 3.8). During the nonAP some 5min segments differed significantly or on trend level as compared to baseline, whereas during the whole intervention period RSA was not significantly heightened (p=.103). In contrast, the post-intervention period was significantly increased (p=.012). Similarly, during the pAP the last 5min segment was significantly elevated, whereas there was no significant difference regarding the whole half-hourly pAP intervention (p=.127). Instead, the post-intervention again revealed a significant increase in RSA as compared to the baseline (p=.021). With the exception of one significant heightened 5min segment in the post-intervention period, both the intervention and the post-intervention period missed significance (p=.125, p=.101). The strongest effect was obvious in the eAP, where the intervention and post-intervention periods revealed a significant increase when compared to baseline (p=.008 and p=.001 respectively). All 5min segments with one exception (p=.058) showed a

significant heightened RSA (.001≤p≤.018). RSA seems to increase over time, independent of the chosen intervention. Therefore, increases in RSA were analyzed pairwise to detect acupuncture specific effects.

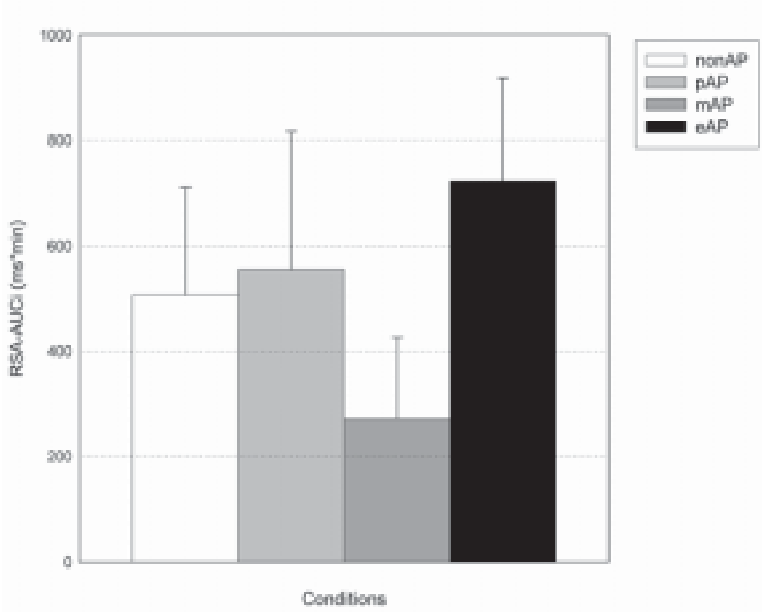


Figure 3.8 Mean and SEM of AUCi of RSA during the different examinations

Effect of Manual Acupuncture

When regarding the RSA increase from before to during the intervention the mAP revealed no significant difference from the nonAP and pAP (p=.954, p=.719), but lower values during the post-intervention period of the mAP were obvious compared to the nonAP and pAP (p=.060, p=.043). The missing effect of mAP on RSA was also evident when using the AUCi (mAP-nonAP: $F(1/11)=1.09$, p=.319; mAP-pAP: $F(1/12)=1.24$, p=.287).

Effect of Electroacupuncture

With regard to the eAP condition, the pain rating during the eAP and belief in the effectiveness of acupuncture to elicit physical effects (phE) were controlled as covariates (see above). The RSA increase was significantly higher during the eAP than during the nonAP

(F(1/9)=13.25, p=.005), pAP (F(1/10)=9.13, p=.013), and marginally higher than during mAP (F(1/11)=3.46, p=.090). Similarly, the increase was higher after termination of the eAP as compared to the post-intervention period during the other conditions but did not reach significance in all comparisons (eAP-nonAP: F(1/10)=1.45, p=.257; eAP-pAP: F(1/11)=3.87, p=.075; eAP-mAP: F(1/11)=5.51, p=.039). AUCi indicating the overall increase due to the interventions from baseline to the intervention and post-intervention period revealed clearer results (eAP-nonAP: F(1/9)=7.64, p=.022; eAP-pAP: F(1/10)=7.00, p=.024; eAP-mAP: F(1/11)=4.82, p=.051; see Figure 3.8).

3.2.4 Discussion

The main purpose of this study was to evaluate the capacity of auricular acupuncture to increase vagal activity. This was examined by conducting a three-armed randomized trial with a control condition without intervention (nonAP) and a condition with sham acupuncture, manual acupuncture (mAP), and electroacupuncture (eAP). eAP but not mAP was effective in increasing respiratory sinus arrhythmia (RSA). This result was found after the control of several variables that are supposed to influence acupuncture effects, which represented a second purpose of the present study. In fact, we found differences in pain sensation during the various interventions, cardiorespiratory differences associated with belief in the effectiveness of acupuncture, and significant time-associated increases of RSA. This second purpose will be discussed first.

Since pain is known to elicit autonomic nervous responses (Burton et al., 2009) and is discussed as a mode of action of acupuncture (Irnich & Beyer, 2002), the participants were asked to estimate their pain sensation at insertion of the needles and during the half-hourly intervention. Results indicated differences in pain sensation during the various interventions, with the highest pain rating during the eAP. Due to the potential role of pain to elicit

physiological alterations, this difference should be taken into account in further studies. In the present study pain sensation during the interventions was considered as covariate in the statistical analyses regarding effects of acupuncture.

Furthermore, participants rated their belief in the effectiveness of acupuncture to provoke physical effects (phE). High vs. low responders revealed a significant difference in RSA increases during the eAP. Contrary to our hypothesis of stronger effects in participants with higher belief, we found that the low-belief group showed higher increases in RSA. One possible explanation could be that participants having higher expectations were more cognitively loaded, whereas the low-belief group could perhaps show a higher degree of relaxation, as they did not expect the interventions to have any effect.

Different sham interventions have been discussed in acupuncture literature. There is no ideal method (Li et al., 2005), because all sham interventions show advantages and disadvantages. Whereas some studies used placebo needles (e.g., Kong et al., 2005, 2008; Streitberger & Kleinhenz, 1998; Streitberger et al., 2008), others applied real needles on points that are thought to have no meaningful effects (e.g., Chang et al., 2008; Wang et al., 2002; White & Ernst, 1999), subcutaneously on acupuncture point sides without reaching them (e.g., Haker et al., 2000; Li et al., 2005), or even using a combination of both methods (e.g., Agelink et al., 2003; Bäcker et al., 2008). Furthermore, when examining electrical stimulation some studies used an electronic device that is turned off, although participants are told that there is stimulation subliminal to conscious perception (Kong et al., 2005, 2008). To our knowledge this is the first study to apply Streitberger placebo needles on the ear, whereas a comparable device was reported elsewhere (Karst et al., 2007). Since all of the participants reported having felt the needle insertion, the use of this device seems appropriate to control for any placebo effects in auricular acupuncture. In the present study we were not able to find any difference between the pAP and the nonAP, even though the placebo effect is known to play

an important role in acupuncture (Bäcker et al., 2006; Ernst, 2007). Ernst (2007) names different components contributing to the perceived placebo effects in clinical trials: the true placebo effect, clinician-patient interaction, natural history, regression towards the mean, social desirability, concomitant therapies, and other effects. A possible explanation of the missing effect in the present study is that participants were blinded regarding the dependent variables, and the expected effects on these variables therefore possibly minimized the placebo effect. A further explanation could be that participants in the nonAP were more relaxed and less alert, since they could not expect any effects due to the lack of intervention.

Interestingly, when analyzing the pAP a significant increase in RSA was evident over time, respectively at the end and after termination of the intervention. Similar results were found also in previous studies and interpreted as effects due to the intervention (e.g., Haker et al., 2000). As we conducted a three-armed trial, we were able to attribute this effect not to the intervention itself, since the increase was obvious even in the nonAP. Therefore, several studies seem to neglect possible phenomena associated with time, such as habituation to the examination environment (room, setting, examiner), anticipatory stress due to the expectation of pain or novelty especially in singular interventions, and relaxation due to the absence of activity. Therefore, the use of an adequate control condition seems to determine placebo effects on the one hand, and on the other hand to interpret alterations of dependent variables that might be attributable to variables associated with time rather than to the intervention itself. Without a control condition in the present study one might also have been tempted to interpret the results of increasing RSA as effects due to the various interventions.

Because we found time-associated changes during all examinations, we analyzed specific effects of verum acupuncture by comparing alterations in verum and nonAP and pAP, respectively. Results indicate a beneficial effect of eAP but not mAP on RSA. Since from a methodological point of view we wanted to apply comparable conditions, we decided to conduct the mAP by just placing the needles and leaving them in place for 30 minutes, and

therefore not providing further stimulation by twisting. Even if our result is in line with literature suggesting a higher effect of electrical than manual stimulation (Gao et al., 2008; Lux et al., 1994), it can not be excluded that a repeated manual stimulation would have induced similar effects as the eAP. The increase of RSA during the eAP underlines the capacity of electrical auricular stimulation to influence the activity of vagal afferents. After controlling for pain sensation and individual belief in the effectiveness of acupuncture there was an additional augmenting effect of eAP as compared to the increases during the other three conditions. This eAP-specific increase reached significant or at least trend level with regard to RSA.

In the present study we did not measure central nervous activity, but the literature offers evidence for different activity alterations also in the central nervous system. Kraus et al. (2007) found similar (de-)activations as provoked during VNS, therefore underlining the capability of singular auricular stimulation to affect the activity of vagal afferents. Whether or not repeated auricular TENS or eAP can be used as an intervention to induce long-lasting beneficial effects on vagal activity, and therefore the activity of the reciprocally interconnected structures of the central autonomic network (Benarroch, 1997), can not be answered based on the present findings and is notional (Routledge et al., 2002). A long-lasting increase of HRV, which constitutes an index of adaptability of the whole organism to respond to rapidly changing demands of the environment and furthermore can constitute a type of resource when these demands request emotional regulation (Thayer & Lane, 2009), should be examined in future studies. Effects of the invasive VNS are being examined in an increasing field of mental, somatic, and cognitive disorders (Ansari et al., 2007), but the intervention is normally restricted to therapy-resistant patients. Therefore, auricular electroacupuncture might constitute an interesting, mildly invasive, adjuvant intervention in clinical and subclinical populations or even a preventive intervention in healthy subjects.

The current study has several limitations. First, we examined a small sample including only healthy and medication-free subjects. Therefore, the present results are restricted to a group of healthy, well-educated, middle-aged men and cannot be generalized to the male population as a whole or to women. Second, an effect on RSA due to skin irritation cannot be excluded in the placebo condition, but it seems implausible due to the lack of difference between the nonAP and the pAP conditions. Additionally, we conducted mAP without stimulation during the intervention, therefore perhaps minimizing any possible specific effect. Furthermore, our sham intervention was ideal with regard to the mAP, since in consideration of the results of Gao et al. (2008) the choice of a different point in the ear might have also elicited some effects. But possibly there are better sham interventions when focusing on the eAP, since it cannot be excluded that mAP and eAP elicits different placebo effects (Kaptchuk et al., 2000). A sham eAP session with an attached electrical device without current but telling participants that they are receiving low subliminal stimulation (Kong et al., 2005, 2008) might be the best possible sham intervention in the evaluation of eAP.

One strength of the present study – besides the control of pain sensation, the collection of data on belief in the effectiveness, and the use of a three-armed randomized trial – is the fact that the participants were provided with sufficient time to calm down, and therefore, time effects within a condition were minimized due to a sympathovagal balance in favour of vagal activity. In previous studies there was often no clear evidence for habituation periods before the initiation of the intervention, and therefore, a higher increase in vagal activity during the examination can be expected.

In conclusion, our results show that electrical stimulation in the concha seems to be an interesting method for the stimulation of the VN in a mildly invasive manner. To our knowledge this is the first study revealing a stimulating effect of auricular electrical stimulation on cardiovagal activity in humans. Our results further underline the necessity to control for pain and for belief in the effectiveness of acupuncture and to use potentially less

painful interventions such as TENS. Future studies should evaluate the effects of eAP in (sub-)clinical populations or possible beneficial effects even in healthy subjects.

4. GENERAL DISCUSSION

One aim of this doctoral thesis was to examine the NV as a physiological resource during a major health-threatening state, namely stress. Studies have demonstrated that the majority of the population in Western countries feel stressed, which leads to individual and societal burdens and provokes high financial costs. During stress, resources constitute an important buffering and health-protecting factor. While, to our knowledge, only one study has examined the potential role of resting vagal activity as a resource for the HPA axis stress response (Johnsen et al., 2002), no replication has been conducted so far. Additionally, no study has been conducted to examine the role of dynamic characteristics of the NV as a component of the neurovisceral integration model on the stress response. The second aim of this thesis was the examination of an alternative mildly invasive intervention to induce an acute, short-term increase of vagal activity. The ear is recognized to possess vagal, somatosensory afferents. While invasive VNS was shown to have healthful effects on some disorders, first results of auricular stimulation in animals and humans show interesting results on cardiovagal, affective and central nervous activity. Thus, we examined the potential role of a single session of auricular (electro-)acupuncture to induce an increase in vagal activity.

In the following, the results of the two empirical studies are summarized, before discussing the findings in the framework of the theoretical background. Limitations and strengths of the present studies, with a special focus on the methodological aspects, are presented, followed by suggestions for implications and directions for future studies.

4.1 Summary of the results

Below, a brief overview is provided of the findings of the stress and the stimulation studies.

4.1.1 Vagal functionality as physiological resource reducing stress-induced biopsychological responses

In the present examination, the slightly modified MIST proved to be an appropriate laboratory task to induce a multidimensional biopsychological stress response, additionally offering an adequate control condition. In fact, the MIST, measured by comparing the responses during the stress and control condition, elicited a significant increase in the cortisol level. Similarly, the MIST resulted in a rise of SNS activity, indicated by significant increases of HR, salivary alpha-amylase and EDA (EDA data not presented in this work; see Setz et al., submitted). Furthermore, the stressor produced a significant decrease of PNS activity indicated by a decline in RSA and salivary flow rate. To test the role of vagal functionality in terms of a physiological resource, the VN was tested under different conditions: resting vagal activity and vagal reagibility during the CFT. While the finding of Johnsen et al. (2002), indicating a protective effect of vagal tone on the stress response of the HPA axis, could not be replicated, vagal reagibility was shown to be negatively correlated with the stress-induced cortisol secretion and worsening of mood. All effect sizes for the biopsychological stress and CFT responses (indicated by partial eta square) were large.

4.1.2 Effects of auricular manual and electrical stimulation on vagal activity

To detect stimulating effects of auricular acupuncture and electrostimulation on vagal activity, a randomized, partially blind, three-armed trial with a crossover design was chosen, controlling for several disturbing factors. Auricular electroacupuncture, but not manual acupuncture, was effective in increasing RSA. This effect emerged after controlling for time-associated changes, pain ratings and the belief in the effectiveness of acupuncture. At the

same time, placebo acupuncture with Streitberger needles revealed no difference regarding RSA compared to the control condition without intervention, which might be explained by the blinding of the subjects with regard to the possible parasympathoexcitatory effect. Interestingly, the examinations all revealed an effect of time, therefore indicating the necessity to apply an additional control condition without intervention. An alternative would be to use appropriate statistical analyses, searching for differences between verum and sham conditions. Furthermore, pain sensation during the interventions differed significantly, speaking in favour of its consideration in future studies.

4.2 Discussion and embedding in the theoretical background

In the following the findings of phasically reduced vagal activity during acute stress, the health-protecting role of fast vagal reagibility for the biopsychological stress response and the possibility to stimulate the VN are discussed.

The effects of the MIST on the affective ratings are in line with previous studies (Pruessner et al., 2004, Soliman et al., 2008) and indicate a flexible and dynamic emotional adaptation of the organism to changing demands. According to previous studies by Pruessner and his work group (Dedovic et al., 2005; Pruessner et al., 2008; Soliman et al., 2008), the slightly modified version of the MIST adapted to a non-fMRI environment was effective in increasing cortisol levels.

The increase seems to be higher compared to previous studies, which is suggested to be attributable to the differences in the trial characteristics. In contrast to prior studies, the MIST was conducted outside the fMRI, therefore allowing the subjects to be permanently confronted with the examiners. Even though there is some evidence that stress-induced cortisol increases are independent of the visual presence of a panel (in or outside a room) and the number of judges (one or two) (Andrews et al., 2007), we suggest that the social-

evaluative threat in sum was higher in the present study compared to previous ones. Social evaluation is known to be a powerful stimulus boosting cortisol secretion (Dickerson & Kemeny, 2004). Andrews et al. (2007) examined subjects during the TSST by introducing one or two examiners to the subjects, and during the stress task, the investigators were present in the examination or in an adjacent room with a one-way mirror. Subjects verbalized their answers. By verbalizing their answers, the social-evaluative threat was permanently present in the subjects, since verbalization is probably the most important form of maintaining social interaction. In contrast, in the original MIST in the fMRI environment, the answers are given via a keyboard without verbalization. Therefore, social-evaluative threat might not be sustained over the whole stress exposure, since the awareness of it might be interrupted by the arithmetic problems. By contrast, in the present study with the slightly modified MIST, the investigators were continuously present in the examination room, thus highlighting the social-evaluative component of the task.

Additional to the cortisol response, the MIST was able to induce an activation of the SNS. The increase in sympathetic activity is indicated by the rise in HR, salivary alpha-amylase and EDA (data not reported; Setz et al., submitted) measured in this study, and is in accordance with the results of previous studies applying the MIST (Pruessner et al., 2004; Soliman et al., 2008). HR showed a rapid and progressive increase for the duration of the stressor, and decreased immediately after cessation of the task. From a biological and health-promoting point of view, this pattern, starting with stress exposure and finishing with its cessation, is reasonable, since energy is needed as part of the fight-or-flight response but becomes health-threatening when unnecessarily prolonged.

In contrast, salivary alpha-amylase peaked only ten minutes after cessation of the stressor and was still significantly increased 60 minutes after the task. It is assumed that the peak is delayed due to a significantly decreased salivary flow rate during the task, therefore possibly

impeding the transport of the whole enzyme amount secreted in the acini of the salivary glands. Contrary to the HR, where a slow or delayed decrease after the end of a stressor could be harmful, alpha-amylase decreases slowly. So far, no health-threatening consequence of a delayed decrease of alpha-amylase is known.

Besides the activation of the HPA axis and the SNS, the MIST resulted in a dampening of the PNS activity. This was indicated by decreased RSA and salivary flow rate. Theoretically, a decrease in salivary flow rate can be assumed even if evidence of stress-induced alterations are seldom found (e.g. Nater et al., 2006). The successful detection of the decrease in our study might be due to the measurement of salivary flow rate during maximal stress confrontation, therefore allowing the theoretically expected decrease to be found. Furthermore, we measured unstimulated whole saliva, possibly avoiding confounding effects attributable to mastication. The latter affects salivary flow rate to a high extent, thus possibly overlapping an effect of stress.

From a theoretical point of view, a decrease in RSA can also be expected (Porges, 1995), and was successfully detected in the present study, therefore showing for the first time an inhibitory effect of the MIST on PNS. Nevertheless, several studies failed to find such a decrease, possibly due to the high sensitivity of the ANS and the VN in particular for a huge amount of factors. This is not surprising, since the ANS / VN permanently try to adapt the organism to the huge amount of internal and external demands in order to improve functioning of the organism while keeping costs low. Factors known to be associated with autonomic adaptations are, for example, respiration and vocalization, changes in body posture and motion (Bernardi et al., 2000; Chan et al.,2007; Nater et al., 2006; Sloan et al., 1991).

Several stress examination paradigms contain one or more of these disturbing factors, therefore challenging the detection of decreased PNS activity during stress. In real-life settings, for example, subjects normally show several factors complicating the measurement and interpretation of stressor-associated alterations. However, even popular laboratory stress

tests often contain ANS disturbing factors. The TSST, for example, is such a laboratory test. It was proved to effectively induce an HPA axis stress response (Dickerson & Kemeny, 2004) and was repeatedly recognized to affect also the ANS (e.g. Nater et al., 2006). Nevertheless, subjects in the original TSST have to move between different rooms, therefore entailing changes in body posture and motion. Furthermore, subjects have to verbalize their answers. Thus, changes occurring during the TSST are not solely attributable to the stressor. Nater et al. (2006), for example, identified an effect of standing on NE while no stressor was applied. Similarly, Chan et al. (2007) were able to demonstrate an effect of postural changes on HRV, which is the consequence of muscle activation and changes in hemodynamic demands. It is evident that motion in terms of physical stress elicits changes of ANS activity (chapter 2.2.1.11.2).

Additionally, it was recognized that vocalization of answers can impede the detection of cardiovagal decrease under stress (Bernardi et al., 2000; Sloan et al., 1991). In fact, the applied stress tasks elicited a reduction of HRV only when subjects gave their answers without verbalization, but not when verbalizing them.

From a methodological point of view, an effective stress task to elicit changes in cardiovagal activity should therefore control for major motion requests, postural changes and verbalization. Moreover, since mental arithmetic was proposed to be an effective stressor influencing cardiovagal activity (Pagani et al., 1995), the MIST seems to be an appropriate choice to detect a stress response free of several well-known disturbing factors. The successful detection of a vagal brake during stress in the present study is in line with theory and was probably found at least in part due to the controlling of several disturbing factors.

The controlling of disturbing factors is not only important in terms of finding a stress-induced response of vagal activity, but is of major importance when the aim is to find an association between different variables. Since in the present study, the aim was to examine the

relationship between vagal characteristics and the biopsychological stress response, controlling for disturbing factors was of major importance. With regard to the vagal characteristics, resting baseline values but also reagibility to a proposed diagnostic test was measured. Resting vagal activity (vagal tone) was therefore assessed in all subjects prior to the MIST-S while subjects sat quietly in a comfortable chair filling out some questionnaires. Therefore, the disturbing factors mentioned before were all excluded. Additionally, the chair, posture and room were the same for the whole examination and all interventions.

The vagal diagnostic test (the CFT; Khurana, 2007; Khurana, Watabiki et al., 1980; Khurana & Wu, 2006) was applied at the end of the control condition, revealing an association between vagal reagibility and biopsychological stress response during the MIST. In the context of the neurovisceral integration model, the VN, indexed by HRV, is interpreted as a physiological resource, among other things for emotional regulation. Furthermore, a connection between the VN and the HPA axis is recognized (Benarroch, 1997; Palkovits, 1999; Porges, 2001) and a correlational finding at least between the CFT and the HPA axis stress response was hypothesized. As mentioned before, disturbing factors are assumed to play an important role when examining existing associations. Therefore, the CFT was conducted in the same set-up as the baseline and the MIST. Additionally, subjects were asked to behave passively during the minutes of the CFT, i.e. not to talk to the examiner and not to move during the whole test. Furthermore, they were asked to breathe regularly during the whole test.

The CFT in the present study was effective in eliciting a significant bradycardia through vagal excitation. This was apparent by the finding of increased vagal activity (RSA) and a phasic decrease of HR. Immediately after cessation of the cold stimulation, HR and RSA again reached pre-test levels. Peak bradycardia was successfully induced during the CFT, while not affecting mood or pain ratings measured via visual analogue scales (VAS) two minutes prior

to and after the CFT. Therefore, cardiorespiratory alterations were not induced by changes in mood or pain.

Contrary to Johnsen et al. (2002), who found a significant difference in cortisol response to stressful cognitive tasks between the high and low vagal tone groups determined by a median split, we found no association between resting vagal tone and biopsychological stress responses. The finding by Johnsen et al. (2002) and the inverse association between urinary cortisol and vagal tone found in a large sample of apparently healthy subjects (n=542; Thayer et al., 2006) can be interpreted as evidence for the role of the VN as a resource for stress response or level. The latter is also in line with the structural evidence of negative inhibitory connections between the VN and the hypothalamus, especially the PVN, which constitutes the most important hypothalamic nucleus for CRF influence on the HPA axis (Benarroch, 1997; Palkovits, 1999; Porges, 2001). Despite the evidence for an association, to our knowledge, only Johnsen and colleagues (2002) found a negative relationship between vagal tone and acute cortisol stress response. Considering resting vagal activity, our results could not underline this association, independent of statistical method (median split, correlation).

Nevertheless, we were able to find a clear association between vagal functionality and the cortisol stress response when considering the CFT response. In fact, the speed with which a maximal bradycardia was reached was able to statistically predict cortisol values during the stress condition. The faster the maximal bradycardia was reached, the smaller the stress response of the HPA axis was.

Furthermore, vagal speed predicted mood changes during the stress task, while the magnitude of bradycardia was predictive for alterations in wakefulness due to the MIST-S. Subjects with a fast or strong bradycardia thus showed significantly less worsening of mood and less increased fatigue due to the stress task. Therefore, good vagal reagibility during the CFT can be interpreted as a physiological resource during stress.

This is in accordance with the interpretation of Thayer and his group in the context of the neurovisceral integration model. Within this model of structural and functional associations between its components, especially the structures of the CAN, the VN plays a central role as an indicator of inhibitional capacity of predominantly the PFC (Thayer & Lane, 2000). The neurovisceral integration model is a dynamic model highlighting the importance of the capacity of an individual to respond to rapidly changing demands of the (internal and external) environment (Thayer & Friedman, 2002). If the structures underlying the CAN and the reverted networks as components of the neurovisceral integration model are not able to rapidly and flexibly "respond" to the demands of the environment, a rigid behaviour is assumed. This rigidity in response repertoire is seen in several disorders, while some of these were recognized to possess reduced vagal tone or altered task responsiveness compared to healthy subjects (chapter 2.2.2). It can be hypothesized therefore that the response shown during the CFT is diminished in patient populations showing a reduced or inappropriate repertoire (e.g. Hughes et al., 2000).

The question that emerges is why we found an association between the biopsychological stress response and vagal reagibility but not vagal tone. Vagal tone describes resting activity of the VN and thus indicates the resting activity of the structures underlying the neurovisceral integration model, or more specifically the resting state of inhibitional control of predominantly the PFC on subcortical structures. It can be assumed that there is an association between tonic, resting HRV and inhibition on the one hand and phasic alterations of HRV and inhibition on the other hand. By contrast, the response during the CFT, which was initially propagated as a vagal diagnostic test (Khurana & Wu, 2006), is shown to influence several structures subsumed in the neurovisceral integration model, as was recognized among other things in imaging studies (Brown et al., 2003; Harper et al., 2003). This is of course also the case under rest. However, differently to vagal tone, the CFT demands a (fast) response of the body to an external demand (cold stimulus eliciting the

diving reflex) and therefore might constitute a better variable to indicate the flexibility of the whole CAN discussed in the neurovisceral integration model. A vagal test of a phasic response to a stimulus / demand might be a better indicator for a system which is postulated to be important in terms of flexibility to respond to demands of the environment.

In conclusion, vagal tone and vagal reagibility to the CFT are presumably two related qualities of the VN and of the whole system underlying the neurovisceral integration model respectively, while we suggest the CFT response to be a more appropriate indicator for the system flexibility. In terms of the present study, we were at least able to find a predictive value of the response to the CFT for the HPA axis and mood responses to a standardized laboratory stress task, therefore underlining the important role of the VN in handling stressful demands.

HRV as an index of the VN cannot only be seen as an output variable, but also constitutes an important feedback system to the structures of the CAN (Thayer, 2007). In fact, the importance of the bidirectional connections between the brain and the heart were recognized early on (Thayer & Lane, 2009). The VN does not only possess efferents; on the contrary, it possesses more afferents (Walsh & Kling, 2004). Therefore, good vagal characteristics, such as indicated by a fast and strong response to the CFT, can be assumed to be a physiological resource during stress. The question of the second examination was as follows: Is it possible to stimulate the VN non- or mild-invasively in healthy subjects? A stimulation of the VN could be interpreted as an increase in physiological resources, and a positive vagal effect of such an intervention could have implications for prevention and therapy. Several methods stimulating the VN were discussed above (chapter 2.3). We examined the effect of manual acupuncture and electroacupuncture to induce a vagal activity increase and found a significant effect on RSA by electrical stimulation.

From a theoretical point of view, the VNS constitutes an interesting method to examine the role of the VN in health, since it directly stimulates the cranial nerve, while other methods (physical training, nutrition) are non-directly linked to the VN. The VNS was repeatedly shown to possess beneficial effects, best established in epilepsy and depression. Evidence for positive outcome, albeit sometimes scarce and inconsistent, is provided among other things for affective, cognitive and cardiovascular measures. In epilepsy, a decrease in the amount of seizures is also recognized. The problem with regard to the VNS is the highly invasive character of this intervention. Therefore, the VNS is often restricted to application in therapy-resistant patients, who have been suffering for many years or who showed no beneficial effect of different pharmacotherapies in the past (Rijkers et al., 2008; Schlaepfer et al., 2008; Smyth et al., 2003). One problem in examining the effects of the VNS is, on the one hand, the fact that VNS is normally an adjuvant therapeutic intervention, and therefore effects are not solely attributable to the VNS. Often, the medication of the patients is continued after the device is implanted. On the other hand, medication is often reduced after first benefits are shown, therefore complicating the attribution of symptom alterations to the VNS. Some studies examining medication-free patients reveal promising results, even though placebo effects also seem to be present in this newer therapy form (Rush et al., 2005a). Some side effects were also reported, and together with the high-invasive character of this intervention, make VNS appropriate only for a restricted spectrum of subjects.

Acupuncture was repeatedly studied in terms of its effectiveness in altering the activity of the ANS. The mode of action regarding how acupuncture elicits positive effects is not clear. Different explanations have been discussed and examined scientifically. Evidence for pain-, placebo- and ANS-mediated effects were repeatedly reported, but the methods used are not always ideal and the interpretations offered are sometimes not sufficiently adequate or critical. When a stimulation of the VN is focused upon, there is virtually no possibility to do this directly, since the VN predominantly innervates organs not reachable from the body surface.

Evidence is provided for some areas of the ear, where vagal afferents were recognized (e.g. Benninghoff, 2008; Folan-Curran & Cooke, 2001; Gao et al., 2008; Lang, 1992). Therefore, the ear seems to be a good target organ to stimulate the VN. The difficulty in acupuncture interventions is the attribution of effects to the suggested mode of action. As mentioned before, a detected vagal effect could be explained by placebo, pain or direct stimulation. Even if some authors name applications at the ear VNS (transcutaneous VNS, tVNS: Dietrich et al., 2008; Kraus et al., 2007), possible effects could be explained in alternative ways. Therefore, appropriate study designs are a challenging problem.

Some recommendations were published referring to sham interventions and information that has to be published to adequately interpret and compare studies and their results (MacPherson et al., 2001; White et al., 2001). Referring to placebo and acupuncture recommendations, in the present work a three-armed trial (Ernst, 2007) was chosen in combination with a maximum of possible blinding (White et al., 2001). With the combination of a partially-blind three-armed trial, containing besides the verum also a control and a placebo condition, and a questionnaire asking subjects to estimate their belief in the effectiveness of acupuncture, we aimed to control for possible placebo effects. To further minimize interindividual differences between the control, placebo and verum conditions, subjects were invited to participate in random order in all conditions in a crossover design.

To further control for pain effects, singular items were distributed at the end of each intervention. Furthermore, we examined two verum interventions: a manual acupuncture without repeated stimulation and an electroacupuncture to examine different effects of manual and electrical stimulation.

The stimulation study revealed several interesting findings. On the one hand, we found significant differences in pain ratings during the three acupuncture interventions (placebo, manual, electro), a significant vagal association of the rating regarding the belief in the

effectiveness, while the placebo intervention per se was not able to elicit a significant vagal increase, and significant increases of RSA over time in all conditions, therefore not attributable to the stimulation.

On the other hand, the main outcome of the stimulation study was the successful increase of vagal activity considering the influencing / disturbing factors (rating of belief of effectiveness, rating of pain, time-associated alterations). Electroacupuncture, but not placebo or manual acupuncture, was able to significantly elicit a vagal stimulation. Therefore, it seems that the vagal afferents were stimulated directly by the electrical stimulation, while the insertion of needles alone in the area of interest resulted in no effect. To our knowledge, this is the first human study to reveal an excitatory effect of auricular electrical stimulation on the VN.

To summarize, we found a vagal decrease during acute psychosocial stress, while identifying trait vagal reagibility determined with a CFT as a physiological resource during stress. Furthermore, we found a positive effect of auricular electrical stimulation on vagal activity, therefore possibly offering interesting implications in the treatment of several disorders associated with low vagal functionality and stress.

4.3 Limitations and strengths

Two different studies were reported in the present work, both possessing some limitations and strengths. The latter are discussed after the limitations.

First, in both studies, only healthy male subjects were examined, therefore restricting the findings to that particular group. The applied tasks and interventions (stress task, CFT, electrical stimulation) are thought, in accordance with the literature, to show similar but presumably altered effects in patients (e.g. Hughes & Stoney, 2000). Nevertheless, the association between the CFT response and the HPA axis stress response might not be found in

patients due to a breakdown of connectivity. Thayer et al. (2006) reported an association between urinary cortisol and vagal tone in apparently healthy subjects. However, the inverse relationship was not significant in the high alcohol-consumption group, suggesting a breakdown of the "functional connectivity" between CAN structures (Thayer & Sternberg, 2006). Therefore, the results of the present study might not be generalisable and consequently need further examination.

Second, the diving reflex imitated by the CFT is first mediated by the trigeminus nerve. Differences in responses induced by a cold stimulus thus might be attributable to interindividual trigeminal differences and not (solely) to vagal differences or CAN differences indexed by vagal characteristics. Nevertheless, the trigeminus nerve can possibly also be integrated in the neurovisceral integration model in terms of a flexibly reverted structure, which might be helpful in dealing with social demands of the environment. Additionally, the trigeminus nerve shows structural and functional connections to the VN, both recognized and incorporated by Porges in the social engagement system (Porges, 2001). Since the response during the CFT is suggested to be an index that is not merely restricted to vagal functionality but presumably to the functionality of several participating CAN structures with a focus on the VN, it does not seem so problematic that the trigeminus nerve is integrated in the reflex. Furthermore, subjects with a history of facial and dental pain or neuropathy were excluded from the study in order to rule out known pathological differences.

Third, sample sizes with respect to some questions were small. The association between vagal tone and cortisol baseline or stress response would perhaps reach significance only when examining large sample sizes. In fact, Johnsen et al. (2002) examined 56 healthy male subjects while Thayer et al. (2006) examined 542 apparently healthy men. Additionally, with respect to the intervention-specific responses, great interindividual differences were shown.

Some differences in the present study reached only the trend level or lacked significance, possibly attributable to the small sample size.

Fourth, since the subjects in the stimulation study were all healthy, vagal activity might was in an ideal range. Therefore, stimulation might be possible only to a small extent. Subjects with known low vagal tone (e.g. patients with cardiovascular disorders) could perhaps benefit to a higher extent from stimulation.

Fifth, the MIST-S may not only produce stress but also aggressive responses, which might elicit a different physiological response. With regard to the control condition, a feeling of boredom can arise, presumably resulting in different interindividual responses.

Sixth, even though we were unable to find any difference between the control and placebo condition in the stimulation study, it cannot be excluded that the irritation of the skin by the tip of the Streitberger placebo needles per se could elicit an effect. Furthermore, since different nerves are presumed to be existent in the ear (Folan-Curran & Cooke, 2001), the elicited effect might also have been mediated by the activation of other nerves interacting with vagal structures in the midbrain.

Seventh, the placebo condition was appropriate regarding the manual acupuncture session and less adequate for the electroacupuncture intervention. Since a different placebo effect of the two verum interventions cannot be denied, it would be possible that the vagal effect during electroacupuncture was elicited by a higher placebo effect. An ideal placebo condition for the electroacupuncture intervention would have been the application of the same device without electrical stimulation but with an appropriate cover story (e.g. different stimulus intensity, amplitude and frequency with similar expected effects, but not consciously perceivable).

Eighth, the items used to evaluate pain perception and the belief in the effectiveness of acupuncture might not be the ideal method to capture both constructs. The measurement of beta-endorphin in blood secreted due to pain would possibly reflect a more objective variable for the determination of pain-induced effects. The belief in the effectiveness should

furthermore have been assessed in more detail, asking about intervention-specific effectiveness.

Besides all of these limitations, several strengths are present in the discussed studies, resulting in the successful confirmation of all major hypotheses. Moreover, to our knowledge, both studies are the first to provide such results with regard to some of the assumptions.

First, several disturbing factors were controlled for. Besides the verbalization, motion and postural influences minimized during the examinations and especially during the interventions (MIST, CFT, acupuncture), several influencing factors were kept constant by selecting a homogenous sample and instructing participants to adhere to some rules. Inclusion and exclusion criteria considered several factors known to be associated with vagal activity and discussed previously (chapter 2.2): age, gender, neuropathy (due to the CFT), BMI, depression, smoking, alcohol consumption, consumption of psychoactive substances, acute and chronic somatic or psychiatric disorders, acute medication, colour blindness (due to the MIST) and handedness (due to the MIST). Furthermore, subjects were asked to comply with some instructions: no consumption of beverages containing caffeine 48 hours prior to the study, no excessive physical exercise 24 hours prior to the examination, cessation of smoking in non-habitual smokers at least 24 hours prior to the examination, no physical exercise or food intake 2 hours prior to the examination.

Second, the stress and acupuncture interventions were evaluated and controlled for using resting and placebo conditions for comparison. Therefore, the different conditions could be statistically tested each against each other. Statistical analyses revealed significant effects of the interventions. On the one hand, the MIST was developed so that it can be used as a stress and control task, thus constituting an ideal tool for stress research. On the other hand, in the stimulation study, in contrast to most previous studies, a three-armed trial was chosen,

therefore providing the opportunity to distinguish between verum, placebo and control conditions. In this respect, it was possible to recognize that vagal activity can significantly increase over time while no intervention is applied. This result is of huge importance since evidence from several previous studies is based on comparisons between pre- and peri- or rather post-values, and therefore the latter have to be reconsidered.

Third, subjects from both studies were blinded to a possible maximal extent, therefore trying to avoid any effects not attributable to the measured construct or intervention. For example, a cover story was told to the subjects in the stress study ("study about the relationship between cognitive skills and physical and mental variables") in order to avoid anticipation of stress or any influence on the stress responses due to early identification of the task as stress manipulation. In the acupuncture study, subjects were blinded regarding the use of a placebo and the expected effects.

Fourth, subjects from both studies participated in a crossover design in random order in all conditions, therefore minimizing interindividual differences, while randomization was revealed to be effective.

Fifth, in the acupuncture study, several staff members were also blinded in line with recommendations (White et al., 2001), such as the data analyst, the acupuncturist and examiner, at least until the moment immediately after the disinfection of the ear.

Sixth, even if not definitively attributable to an effective stimulation of the VN, the findings seem to be directly associated with the VN, since structural evidence, and evidence from stimulation studies reveal the presence of the VN at the place of stimulation. Alternative explanations (placebo, pain) were controlled for.

Seventh, although measured only with single items, we asked subjects to rate their pain during the interventions. Therefore, it was possible to detect significant intervention-specific differences, which could be statistically considered as covariates.

Eighth, some authors suggest the control of respiration when examining HRV. Analytical procedures considering breathing patterns would probably contribute to more accurate data. Due to the huge amount of data and the timeframe of this work, it was not possible to consider this recommendation. In the present study, a control of respiration and verbalization was undertaken by using control conditions comparable to the interventions (stress task, acupuncture sessions) and by asking subjects to breathe normally and to avoid speaking if possible. Furthermore, the stress task was chosen due to its advantages, including a comparable control condition and minimizing verbalization. Additionally, the CFT was analyzed with a special focus on bradycardia, meaning that breathing patterns can be neglected.

4.4 Implications and directions for future studies

First of all, it must be underlined that the major aims of the presented studies were successfully achieved. The stressor induced a significant stress response, while the CFT response was able to determine a predictive value for mood and HPA axis responses during stress. Furthermore, electrical stimulation at the ear was able to elicit a significant excitatory effect on vagal activity. Therefore, all of this evidence provides support for the theory of neurovisceral integration model, and speaks in favour of a potential preventive and therapeutic role of electrical stimulation.

In the first study, the CFT was shown to statistically predict the stress response. This association was evident when considering the magnitude of, but more importantly the speed until, maximal bradycardia. We suggest that the CFT constitutes an indicator for the flexibility and adaptability of the structures underlying the neurovisceral integration model to respond to demands of the environment. Nevertheless, only a few studies were conducted to

examine structures, constructs or variables associated with the CFT, especially regarding its speed in inducing bradycardia. Therefore, further studies are needed to detect possible associations with neuronal structures and processes but also different psychosocial constructs. Furthermore, the CFT should be examined in patient populations with associated vagal imbalance and should further be considered as a diagnostic tool and indicator of behavioural disturbances. In basic sciences, the CFT should further be examined to detect its possible predictive value for other markers known to respond to stress such as NE, EPI and chromogranin A or markers of the immune system. Furthermore, other laboratory and daily stressors, but also the cortisol awakening response (CAR), should be examined in association with the CFT response.

The influence of interventions known to have an increasing effect on vagal activity should be evaluated in terms of beneficial effects not only on vagal tone and symptoms but also on the CFT response. It would be interesting to examine invasive VNS for possible increasing effects on speed and magnitude of bradycardia and whether such increases are associated with symptom improvements.

An advantage of the CFT is the low methodological costs. With regard to materials, only a freezer, cold stimulus and ECG device are needed. Subjects can be examined after a short adaptation period since the ANS adapts very quickly to new situations. Additionally, consistency between intraindividual responses on different days, different times of day and different seasons has been proven (Heath & Downey, 1990).

Low vagal activity shows an inverse relationship with several risk factors, morbidity and mortality (chapter 2.2). Since invasive VNS seems to possess a positive effect on symptoms and the activity of the CNS and ANS, vagal stimulation might show beneficial effects also in healthy subjects and in subclinical or clinical populations, and not only in therapy-resistant patients. Some common interventions such as physical training show beneficial effects on mental and somatic health and an increase in vagal activity. Nevertheless, causality is not so

clear compared to invasive VNS. Effects are directly attributable to the electrical stimulation of the VN, even though a placebo effect was also recognized (Rush et al., 2005a). Nevertheless, restrictions are given by the high cost, adverse effects and high invasive character, therefore limiting the intervention to patients in whom other therapies did not work or pathology is severe. Some findings with TENS together with the evidence of the present stimulation study indicate a potential role of auricular electrical stimulation to induce an increase in vagal activity. Therefore, future studies should investigate the effects of auricular electrical stimulation in healthy subjects but also in several somatic and psychiatric patients. Furthermore, an application during (chronic) stress, when vagal activity is reduced, might be of interest. Electrical stimulation might be effective in confronting allostatic load in terms of an external resource.

Even though auricular electrical stimulation has several interesting implications for the application in non-, sub- and clinical populations, some further basic research is needed. First of all, the findings need to be replicated, since to our knowledge, this is the first human study to show an excitatory effect of auricular electrical stimulation on cardiovagal activity. Furthermore, the effects need to be really attributable to a real stimulation of the VN. Therefore, future studies should examine the effects of verum- and sham-electrical stimulation on different ear points. Additionally, with regard to the methods used by Fallgatter et al. (2003), far-field potentials during electrical stimulation with different stimulation characteristics can be applied to detect the best individual stimulation parameters. Furthermore, a beneficial effect of repeated, long-term electrical stimulation should be evaluated multidimensionally. What effect would repeated electrical stimulation induce with regard to ANS, CNS, affect and cognition? Furthermore, since subjects reported pain during the interventions with electroacupuncture, the use of a device without needles (e.g. TENS) would possibly elicit less disturbing effects.

5. REFERENCES

Agelink, M. W., Boz, C., Ullrich, H. & Andrich, J. (2002). Relationship between major depression and heart rate variability: clinical consequences and implications for antidepressive treatment. *Psychiatry Research, 113,* 139-149.

Agelink, M. W., Sanner, D., Eich, H., Pach, J., Bertling, R., Lemmer, W., Klieser, E. & Lehmann, E. (2003). Beeinflusst die Akupunktur die autonom kardiale Regulation bei Patienten mit leichten depressiven Episoden oder Angststörungen? *Fortschritte der Neurologie - Psychiatrie, 71,* 141-149.

Ahern, G. L., Sollers, J. J., Lane, R. D., Labiner, D. M., Herring, A. M., Weinand, M. E., Hutzler, R. & Thayer, J. F. (2001). Heart rate and heart rate variability changes in the intracarotid sodium amobarbital test. *Epilepsia, 42,* 912-921.

Åhs, F., Furmark, T., Michelgård, Å., Långström, B., Appel, L., Wolf, O. T., Kirschbaum, C. & Fredrikson, M. (2006). Hypothalamic blood flow correlates positively with stress-induced cortisol levels in subjects with social anxiety disorder. *Psychosomatic Medicine, 68,* 859-862.

Akselrod, S., Gordon, D., Ubel, F. A., Shannon, D. C., Berger, A. C. & Cohen, R. J. (1981). Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science, 213,* 220-222.

Altemus, M., Redwine, L. S., Leong, Y. M., Frye, C. A., Porges, S. W. & Carter, C. S. (2001). Response to laboratory psychosocial stress in postpartum women. *Psychosomatic Medicine, 63,* 814-821.

Alyan, O., Kacmaz, F., Ozdemir, O., Maden, O., Topaloglu, S., Ozbakir, C., Metin, F., Karadede, A. & Ilkay, E. (2008). Effects of cigarette smoking on heart rate variability and plasma N-terminal pro-B-type natriuretic peptide in healthy subjects: is there the relationship between both markers? *Annals of Noninvasive Electrocardiology, 13,* 137-144.

Amaral, D. G., Price, J. I., Pitkanen, A. & Charmichael, S. T. (1992). Anatomical organization of the primate amygdaloid complex. In J. P. Aggleton (Ed.), *The Amygdala: Neurobiological aspects of emotion* (pp. 1-66). New York: Wiley-Lyss.

Andreassi, J. L. (2007). *Psychophysiology - Human behavior & physiological response.* Hillsdale, NJ: Lawrence Erlbaum Associates.

Andrews, J., Wadiwalla, M., Juster, R. P., Lord, C., Lupien, S. J. & Pruessner, J. C. (2007). Effects of manipulating the amount of social-evaluative threat on the cortisol stress response in young healthy men. *Behavioral Neuroscience, 121*: 871-876.

Ansari, S., Chaudhri, K. & Al Moutaery, K. A. (2007). Vagus nerve stimulation: indications and limitations. *Acta Neurochirurgica Supplementum, 97,* 281-286.

Antonica, A., Magni, F., Mearini, L. & Paolocci, N. (1994). Vagal control of lymphocyte release from rat thymus. *Journal of the Autonomic Nervous System, 48,* 187-197.

Arai, Y. C., Ushida, T., Matsubara, T., Shimo, K., Ito, H., Sato, Y., Wakao, Y. & Komatsu, T. (in press). The influence of acupressure at extra 1 acupuncture point on the spectral entropy of the EEG and the LF/HF ratio of heart rate variability. *Evidence-based complementary and alternative medicine.*

Arnold, R. W. (2000). The human heart rate response profiles to five vagal maneuvers. *Yale Journal of Biology and Medicine, 72*, 237-244.

Audette, J. F., Jin, Y. S., Newcomer, R., Stein, L., Duncan, G. & Frontera, W. R. (2006). Tai Chi versus brisk walking in elderly women. *Age and Ageing, 35,* 388-393.

Bäcker, M., Grossman, P., Schneider, J., Michalsen, A., Knoblauch, N., Tan, L., Niggemeyer, C., Linde, K., Melchart, D. & Dobos, G. J. (2008). Acupuncture in migraine: investigation of autonomic effects. *The Clinical Journal of Pain, 24,* 106-115.

Bäcker, M., Tao, I. & Dobos, G. J. (2006). Akupunktur - quo vadis? *Deutsche Medizinische Wochenschrift, 131,* 506-511.

Bär, K. J., Letzsch, A., Jochum, T., Wagner, G., Greiner, W. & Sauer, H. (2005). Loss of efferent vagal activity in acute schizophrenia. *Journal of Psychiatric Research, 39,* 519-527.

Bär, K. J., Wernich, K., Boettger, S., Cordes, J., Boettger, M. K., Löffler, S., Kornischka, J. & Agelink, M.-W. (2008). Relationship between cardiovagal modulation and psychotic state in patients with paranoid schizophrenia. *Psychiatry Research, 157,* 255-257.

Barone, L., Colicchio, G., Policicchio, D., Di Clemente, F., Di Monaco, A., Meglio, M., Lanza, G. A. & Crea, F. (2008). Effect of vagal nerve stimulation on systemic inflammation and cardiac autonomic function in patients with refractory epilepsy. *Neuroimmunomodulation, 14,* 331-336.

Barutcu, I., Esen, A. M., Kaya, D., Turkmen, M., Karakaya, O., Melek, M., Esen, O. B. & Basaran, Y. (2005). Cigarette smoking and heart rate variability: dynamic influence of parasympathetic and sympathetic maneuvers. *Annals of Noninvasive Electrocardiology, 10,* 324-329.

Baynard, T., Pitetti, K. H., Guerra, M. & Fernhall, B. (2004). Heart rate variability at rest and during exercise in persons with down syndrome. *Archives of Physical Medicine and Rehabilitation, 85,* 1285-1290.

Beauchaine, T. P. (2001). Vagal tone, development, and Gray`s motivational theory: toward an integrated model of autonomic nervous system functioning in psychopathology. *Development and Psychopathology, 13,* 183-214.

Benarroch, E. E. (2004). Central autonomic control. In D. Robertson (Ed.), *Primer on the autonomic nervous system* (pp. 17-19). Amsterdam: Elsevier Academic Press.

Benarroch, E. E. (1997). *Central autonomic network: functional organization and clinical correlations.* Armonk, NY: Futura Publishing Company, Inc..

Benninghoff, A. (2008). *Taschenbuch Anatomie*. München: Elsevier, Urban & Fischer.

Bernardi, L., Wdowczyk-Szulc, J., Valenti, C., Castaldi, S., Passino, C., Spadaccini, G. & Sleight, P. (2000). Effects of controlled breathing, mental activity and mental stress with or without verbalization on heart rate variability. *Journal of the American College of Cardiology, 35,* 1462-1469.

Bernstein, A. L., Barkan, H. & Hess, T. (2006). Vagus nerve stimulation therapy for pharmacoresistant epilepsy: effect on health care utilization. *Epilepsy & Behavior, 10,* 134-137.

Berntson, G. G., Bigger, J. T., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., Nagaraja, H. N., Porges, S. W., Saul, J. P., Stone, P. H. & van der Molen, M. W. (1997). Heart rate variability: origins, methods and interpretive caveats. *Psychophysiology, 34*, 623-648.

Birbaumer, N. & Schmidt, R. F. (2003). *Biologische Psychologie* (5. Aufl.). Berlin: Springer.

Birkhofer, A., Schmidt, G. & Förstl, H. (2005). Herz und Hirn - Die Auswirkungen psychischer Erkrankungen und ihrer Therapie auf die Herzfrequenzvariabilität. *Fortschritte der Neurologie - Psychiatrie, 73,* 192-205.

Blechert, J., Michael, T., Grossman, P., Lajtman, M. & Wilhelm, F. H. (2007). Autonomic and respiratory characteristics of posttraumatic stress disorder and panic disorder. *Psychosomatic Medicine, 69,* 935-943.

Bleil, M. E., Gianaros, P. J., Jennings, J. J., Flory, J. D. & Manuck, S. B. (2008). Trait negative affect: toward an integrated model of understanding psychological risk for impairment in cardiac autonomic function. *Psychosomatic Medicine, 70,* 328-337.

Boneva, R. S., Decker, M. J., Maloney, E. M., Lin, J.-M., Jones, J. F., Helgason, H. G., Heim, C. M., Rye, D. B. & Reeves, W. C. (2007). Higher heart rate and reduced heart rate variability persist during sleep in chronic fatigue syndrome: a population-based study. *Autonomic Neuroscience: Basic and Clinical, 137,* 94-101.

Breitenbach, C. (2003). *Die gesundheitsbezogene Lebensqualität und das kardiovasukuläre Regulationsverhalten - Eine Pilotstudie bei diabetischer autonomer Neuropathie* [On-line]. Available: http://www.diss.fu-berlin.de/diss/receive/FUDISS_thesis_000000001044.

Britton, A., Shipley, M., Malik, M., Hnatkova, K., Hemingway, H. & Marmot, M. (2007). Changes in heart rate and heart rate variability over time in middle-aged men and women in the general population (from the Whitehall II cohort study). *The American Journal of Cardiology, 100,* 524 –527.

Brook, R. D. & Julius, S. (2000). Autonomic imbalance, hypertension, and cardiovascular risk. *American Journal of Hypertension, 13,* 112S-122S.

Brotman, D. J., Golden, S. H. & Wittstein, I. S. (2007). The cardiovascular toll of stress. *Lancet, 370,* 1089-1100.

Brown, R. P. & Gerbarg, M. D. (2005). Sudarshan Kriya yogic breathing in the treatment of stress, anxiety, and depression: part 1 - neurophysiologic model. *The Journal of Alternative and Complementary Medicine, 11,* 189-201.

Brown, C. M., Sanya, E. O. & Hilz, M. J. (2003). Effect of cold face stimulation on cerebral blood flow in humans. *Brain Research Bulletin, 61,* 81-86.

Buch, A. N., Coote, J. H. & Townend, J. N. (2002). Mortality, cardiac vagal control and physical training - what's the link? *Experimental Physiology, 87,* 423-435.

Buddecke, E. (1981). *Biochemische Grundlagen der Zahnmedizin*. Berlin: Walter de Gruyter.

Burgess, H. J., Trinder, J., Kim, Y. & Luke, D. (1997). Sleep and circadian influences on cardiac autonomic nervous system activity. *American Journal of Physiology. Heart and Circulatory Physiology, 273,* H1761-H1768.

Burton, A. R., Birznieks, I., Bolton, P. S., Henderson, L. A. & Macefield, V. G. (2009). Effects of deep and superficial experimentally-induced acute pain on muscle sympathetic nerve activity in human subjects. *Journal of Physiology, 587,* 183-193.

Cacioppo, J. T., Tassinary, L. G. & Berntson, G. G. (2003). *Handbook of Psychophysiology* (2nd edition). New York, NY: Cambridge University Press.

Cannon, W. B. (1914a). The interrelations of emotions as suggested by recent physiological researches. *American Journal of Psychology, 25*, 256-282.

Cannon, W. B. (1914b). The emergency function of the adrenal medulla in pain and the major emotions. *American Journal of Psychology, 33*, 356-372.

Cannon, W. B. (1915). *Bodily changes in pain, hunger, fear and rage: an account of recent researches into the function of emotional excitement*. New York: Appleton.

Cannon, W. B. (1929). Organization for physiological homeostasis. *Physiological Revue, 9*, 399-431.

Carney, R. M., Freedland, K. E. & Veith, R. C. (2005). Depression, the autonomic nervous system, and coronary heart disease. *Psychosomatic Medicine, 67,* S29-S33.

Carrère, S., Yoshimoto, D., Mittmann, A., Woodin, E. M., Tabares, A., Ullman, J., Swanson, C. & Hawkins, M. (2005). The roles of marriage and anger dysregulation in biobehavioral stress responses. *Biological Research for Nursing, 7,* 30-43.

Carroll, D., Phillips, A. C., Hunt, K. & Der, G. (2007). Symptoms of depression and cardiovascular reactions to acute psychological stress: evidence from a population study. *Biological Psychology, 75,* 68-74.

Chai, C. L., Tu, Y. K. & Huang, S. J. (2008). Can cerebral hypoperfusion after sympathetic storm be used to diagnose brain death? A retrospective survey in traumatic brain injury patients. *The Journal of Trauma, 64,* 688-697.

Chan, H. L., Lin, M. A., Chao, P. K. & Lin, C. H. (2007). Correlates of the shift in heart rate variability with postures and walking by time-frequency analysis. *Computer Methods and Programs in Biomedicine, 86,* 124-130.

Chang, S., Chao, W. L., Chiang, M. J., Li, S. J., Lu, Y. T., Ma, C. M., Cheng, H. Y. & Hsieh, S. H. (2008). Effects of acupuncture at Neiguan (PC 6) of the pericardial meridian on blood pressure and heart rate variability. *The Chinese Journal of Physiology, 51,* 167-177.

Chatterton, R. T. Jr., Vogelsong, K. M., Lu, Y.-C., Ellman, A. B. & Hudgens, G. A. (1996). Salivary alpha-amylase as a measure of endogenous adrenergic activity. *Clinical Physiology, 16*, 433-448.

Chen, W.-L., Chen, J.-H, Huang, C.-C., Kuo, C.-D., Huang, C.-I. & Lee, L.-S. (2008). Heart rate variability measures as predictors of in-hospital mortality in ED patients with sepsis. *American Journal of Emergency Medicine, 26,* 395-401.

Cheng, T. O. (2000). Decreased heart rate variability as a predictor for sudden death was known in China in the third century A. D.. *European Heart Journal, 21*, 2081-2082.

Chien, C. H., Shieh, J. Y., Ling, E. A., Tan, C. K. & Wen, C. Y. (1996). The composition and central projections of the internal auricular nerves of the dog. *Journal of Anatomy, 189*, 349-362.

Christensen, J. H. (2003). N-3 fatty acids and the risk of sudden cardiac death. Emphasis on heart rate variability. *Danish Medical Bulletin, 50,* 347-367.

Christensen, J. H. & Schmidt, E. B. (2007). Autonomic nervous system, heart rate variability and n-3 fatty acids. *The Journal of Cardiovascular Medicine, 8,* S19-S22.

Conway, C. R., Sheline, Y. I., Chibnall, J. T., George, M. S., Fletcher, J. W. & Mintun, M. A. (2006). Cerebral blood flow changes during vagus nerve stimulation for depression. *Psychiatry Research, 146*, 179-184.

Czura, C. J. & Tracey, K. J. (2005). Autonomic neural regulation of immunity. *Journal of Internal Medicine, 257,* 156-166.

Dahlström, A., Ebersjö, C. & Lundell, B. (2008). Nicotine in breast milk influences heart rate variability in the infant. *Acta Paediatrica, 97,* 1075-1079.

Dedovic, K., Renwick, R., Khalili-Mahani, N., Engert, V., Lupien, S. J. & Pruessner, J. C. (2005). The Montreal Imaging Stress Task: using functional imaging to investigate the effects of perceiving and processing psychosocial stress in the human brain. *Journal of Psychiatry and Neuroscience, 30,* 319-325.

Dickerson, S. S. & Kemeny, M. E. (2004). Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychological Bulletin, 130,* 355-391.

Dietrich, S., Smith, J., Scherzinger, C., Hofmann-Preiss, K., Freitag, T., Eisenkolb, A. & Ringler, R. (2008). A novel transcutaneous vagus nerve stimulation leads to brainstem and cerebral activations measured by functional MRI. *Biomedical Engineering, 53,* 104-111.

Dorr, A. E. & Debonnel, G. (2006). Effect of vagus nerve stimulation on serotonergic and noradrenergic transmission. *The Journal of Pharmacology and Experimental Therapeutics, 318*, 890-898.

Dressendorfer, R. A., Kirschbaum, C., Rohde, W., Stahl, F. & Strasburger, C. J. (1992). Synthesis of a cortisol-biotin conjugate and evaluation as a tracer in an immunoassay for salivary cortisol measurement. *The Journal of Steroid Biochemistry and Molecular Biology, 43,* 683-692.

Edgar, W. M. (1992). Saliva: its secretion, composition and functions. *British Dental Journal*, *172*, 305-312.

Eppinger, H. & Hess, L. (1910). *Vagotonie: Klinische Studie*. Berlin: Hirschwald.

Ernst, E. (2007). Placebo: new insights into an old enigma. *Drug Discovery Today, 12,* 413-418.

Fallen, E. L. & Kamath, M. V. (1995). Circadian rhythms of heart rate variability. In M. Malik & A. J. Camm (Eds.), *Heart rate variability* (pp. 21-30). Armonk, NY: Futura Publishing Company.

Fallgatter, A. J., Neuhauser, B., Herrmann, R. J., Ehlis, A. C., Wagener, A., Scheuerpflug, P., Reiners, K. & Riederer, P. (2003). Far field potentials from the brain stem after transcutaneous vagus nerve stimulation. *Journal of Neural Transmission, 110*, 1437-1443.

Felber Dietrich, D., Gemperly, A., Gaspoz, J.-M., Schindler, C., Liu, L.-J. S., Gold, D. R., Schwartz, J., Rochat, T., Barthélémy, J.-C., Pons, M., Roche, F., Probst Hensch, N. M., Bridevaux, P.-O., Gerbase, M. W., Neu, U., Ackermann-Liebrich, U. & SAPALDIA-team (2008). Differences in heart rate variability associated with the long-term exposure to NO2. *Environmental Health Perspectives, 16*, 1357-1361.

Felber Dietrich, D., Schindler, C., Schwartz, J., Barthelemy, J.-C., Tschopp, J.-M., Roche, F., von Eckardstein, A., Brändli, O., Leuenberger, P., Gold, C. R., Gaspoz, J. M., Ackermann-Liebrich, U. & SAPALDIA-team (2006). Heart rate variability in an ageing population and its association with lifestyle and cardiovascular risk factors: results of the SAPALDIA study. *Europace, 8,* 521-529.

Felber Dietrich, D., Schwartz, J., Schindler, C., Gaspoz, J. M., Barthelemy, J.-C., Tschopp, J.-M., Roche, F., von Eckardstein, A., Brändli, O., Leuenberger, P., Gold, D. R., Ackermann-Liebrich, U. & SAPALDIA-team (2007). Effects of passive smoking on heart rate variability, heart rate and blood pressure: an observational study. *International Journal of Epidemiology, 36,* 834-840.

Figueroa, A., Collier, S. R., Baynard, T., Giannopoulou, I., Goulopoulou, S. & Fernhall, B. (2005). Impaired vagal modulation of heart rate in individuals with Down syndrome. *Clinical Autonomic Research, 15*, 45-50.

Folan-Curran, J. & Cooke, F. J. (2001). Contributions of cranial nerve ganglia to innervation of the walls of the rat external acoustic meatus. *Journal of the Peripheral Nervous System, 6*, 28-32.

Franchini, K. G. & Cowley, A. W. (2004). Autonomic control of cardiac function. In D. Robertson (Ed.), *Primer on the autonomic nervous system* (pp. 134-138). Amsterdam: Elsevier Academic Press.

Frei, M. G. & Osorio, I. (2001). Left vagus nerve stimulation with the neurocybernetic prosthesis has complex effects on heart rate and on its variability in humans. *Epilepsia, 42*, 1007-1016.

Friederich, H. C., Schild, S., Schellberg, D., Quenter, A., Bode, C., Herzog, W. & Zipfel, S. (2006). Cardiac parasympathetic regulation in obese women with binge eating disorder. *International Journal of Obesity, 30*, 534-542.

Friedman, B. H. & Thayer, J. F. (1998). Anxiety and autonomic flexibility: a cardiovascular approach. *Biological Psychology*, 47, 243-263.

Furlan, R., Guazzetti, S., Crivellaro, W., Dessi, S., Tinelli, M., Baselli, G., Cerutti, S., Lombardi, F., Pagani, M. & Malliani, A. (1990). Continuous 24-hour assessment of the neural regulation of systemic arterial pressure and RR variabilities in ambulant subjects. *Circulation, 81,* 537-547.

Gamelin, F. X., Berthoin, S., Sayah, H., Libersa, C. & Bosquet, L. (2007). Effect of training and detraining on heart rate variability in healthy young men. *International Journal of Sports Medicine, 28,* 564-570.

Galli, R., Limbruno, U., Pizzanelli, C., Giorgi, F. S., Lutzemberger, L., Strata, G., Pataleo, L., Mariani, M., Iudice, A. & Murri, L. (2003). Analysis of RR variability in drug-resistant epilepsy patients chronically treated with vagus nerve stimulation. *Autonomic Neuroscience, 107*, 52-59.

Ganong, W. F. (2005). *Review of Medical Physiology* (22nd edition). New York:The McGraw-Hill Companies.

Gao, X. Y., Zhang, S. P., Zhu, B. & Zhang, H. Q. (2008). Investigation of specificity of auricular acupuncture points in regulation of autonomic function in anesthetized rats. *Autonomic Neuroscience*, *138,* 50-56

George, M. S., Rush, A. J., Sackeim, H. A. & Marangell, L. B. (2003). Vagus nerve stimulation (VNS): utility in neuropsychiatric disorders. *The International Journal of Neuropsychopharmacology, 6,* 73-83.

Gerritsen, J., Dekker, J. M., Ten Voorde, B. J., Kostense, P. J., Heine, R. J., Bouter, L. M. & Heethaar, R. M. (2001). Impaired autonomic function is associated with increased mortality, especially in subjects with diabetes, hypertension, or a history of cardiovascular disease - the Hoorn study. *Diabetes Care, 24*, 1793-1798.

Gianaros, P. J., Van Der Veen, F. M. & Jennings, J. R. (2004). Regional cerebral blood flow correlates with heart period and high-frequency heart period variability during working-memory tasks: implications for the cortical and subcortical regulation or cardiac autonomic activity. *Psychophysiology, 41,* 521-530.

Gouin, J. F., Kiecolt-Glaser, J. K., Malarkay, W. B. & Glaser, R. (2008). The influence of anger expression on wound healing. *Brain, Behavior, and Immunity, 22*, 699-708.

Green, S. B., Salkind, N. J. & Akey, T. M. (2000). *Using SPSS for Windows* (2nd edition). Englewood Cliffs, NJ: Prentice Hall.

Grossman, P. & Taylor, E. W. (2007). Toward understanding respiratory sinus arrhythmia: Relations to cardiac vagal tone, evolution and biobehavioral functions. *Biological Psychology, 74*, 263-285.

Grunditz, T., Ekman, R., Håkanson, R., Rerup, C., Sundler, F. & Uddman, R. (1986). Calcitonin gene-related peptide in thyroid nerve fibers and C cells: effects on thyroid hormone secretion and response to hypercalcemia. *Endocrinology, 119,* 2313-2324.

Grunditz, T., Håkanson, R., Sundler, F. & Uddman, R. (1988). Neuronal pathways to the rat thyroid revealed by retrograde tracing and immunocytochemistry. *Neuroscience, 24,* 321-335.

Gruppo Italiano per lo Studio della Sopravvivenza nell'infarto Miocardico (1994). GISSI-3: effects of lisinopril and transdermal glyceryl trinitrate singly and together on 6-week mortality and ventricular function after acute myocardial infarction. *Lancet, 343,* 1115-1122.

Gunnar, M. R., Connors , J. & Isensee, J. (1989). Lack of stability in neonatal adrenocortical reactivity because of rapid habituation of the adrenocortical response. *Developmental Psychobiology, 22*, 221-233.

Gupta, D., Verma, S. & Vishwakarma, S. K. (1986). Anatomic basis of Arnold's ear- cough reflex. *Surgical and Radiologic Anatomy, 8*, 217-220.

Gyurak, A. & Ayduk, Ö. (2008). Resting respiratory sinus arrhythmia buffers against rejection sensitivity via emotional control. *Emotion, 8,* 458-467.

Habib, G. B. (1999). Reappraisal of heart rate as a risk factor in the general population. *European Heart Journal Supplements, 1*, H2-H10.

Haker, E., Egekvist, H. & Bjerring, P. (2000). Effect of sensory stimulation (acupuncture) on sympathetic and parasympathetic activities in healthy subjects. *Journal of the Autonomic Nervous System,* 79, 52-59.

Hales, S. (1733). *Statical essays: Containing haemastaticks; or, An account of some hydraulick and hydrostatical experiments made on the blood and blood-vessels of animals.* London: W. Innys, R. Manhy, and T. Woodward,

Hall, M., Vasko, R., Buysse, D., Ombao, H., Chen, Q., Cashmere, J. D., Kupfer, D. & Thayer, J. F. (2004). Acute stress affects heart rate variability during sleep. *Psychosomatic Medicine, 66,* 56-62.

Hamill, R. W. & Shapiro, R. E. (2004). Peripheral autonomic nervous system. In D. Robertson (Ed.), *Primer on the autonomic nervous system* (pp. 20-28). Amsterdam: Elsevier Academic Press.

Hansen, A. L., Johnsen, B. H., Sollers III, J. J., Stenvik, K. & Thayer, J. F. (2004). Heart rate variability and its relation to prefrontal cognitive function: the effects of training and detraining. *European Journal of Applied Physiology, 93,* 263-272.

Hansen, A. L., Johnsen, B. H. & Thayer, J. F. (2003). Vagal influence on working memory and attention. *International Journal of Psychophysiology, 48,* 263-274.

Hansen, A. L., Johnsen, B. H. & Thayer, J. F. (2009). Relationship between heart rate variability and cognitive function during threat of shock. *Anxiety, Stress, and Coping, 22,* 77-89.

Harper, R. M., Macey, P. M., Henderson, L. A., Woo, M. A., Macey, K. E., Frysinger, R. C., Alger, J. R., Nguyen, K. P. & Yan-Go, F. L. (2003). fMRI responses to cold pressor challenges in control and obstructive sleep apnea subjects. *Journal of Applied Physiology, 94,* 1583-1595.

Harris, B., Watkins, S., Cook, N., Walker, R. F., Read, G. F. & Riad-Fahmy, D. (1990). Comparison of plasma and salivary cortisol determinations for the diagnostic efficacy of the dexamethasone suppression test. *Biological Psychiatry, 27*, 897-904.

Hatfield, B. D., Spalding, T. W., Santa Maria, D. L., Porges, S. W., Potts, J. T., Byrne, E. A., Brody, E. B. & Mahon, A. D. (1998). Respiratory sinus arrhythmia during exercise in aerobically trained and untrained men. *Medicine and Science in Sports and Exercise, 30,* 206-214.

Hautzinger, M. & Bailer, M. (1992). *Allgemeine Depressions Skala. Manual.* Konstanz und Mainz: Beltz.

Heath, M. E. & Downey, J. A. (1990). The cold face test (diving reflex) in clinical autonomic assessment: methodological considerations and repeatability of responses. *Clinical Sciences, 78*: 139-147.

Heilman, K. J. & Porges, S. W. (2007). Accuracy of the LifeShirt® (Vivometrics) in the detection of cardiac rhythms. *Biological Psychology, 75,* 300-305.

Heim, C. & Meinlschmidt, G. (2003). Biologische Grundlagen. In U. Ehlert (Hrsg.), *Verhaltensmedizin* (S. 17-88). Berlin: Springer.

Hemingway, H., Shipley, M., Brunner, E., Britton, A., Malik, M. & Marmot, M. (2005). Does autonomic function link social position to coronary risk? The Whitehall II study. *Circulation, 111,* 3071-3077.

Henry, T. R., Votaw, J. R., Pennell, P. B., Epstein, C. M., Bakay, R. A., Faber, T. L., Grafton, S. T. & Hoffman, J. M. (1999). Acute blood flow changes and efficacy of vagus nerve stimulation in partial epilepsy. *Neurology, 52*, 1166-1173.

Hering, H. E. (1910). A functional test of heart vagi in man. *Menschen München Medizinische Wochenschrift, 57,* 1931-1933.

Hilz, M. J., Stemper, B., Sauer, P., Haertl, U., Singer, W. & Axelrod, F. B. (1999). Cold face test demonstrates parasympathetic cardiac dysfunction in familial dysautonomia. *The American Journal of Physiology, 276*, R1833-R1839.

Hord, E. D., Evans, M. S., Mueed, S., Adamolekun, B. & Naritoku, D. K. (2003). The effect of vagus nerve stimulation on migraines. *The Journal of Pain, 4,* 530-534.

Hsu, C. C., Weng, C. S., Liu, T. S., Tsai, Y. S. & Cang, Y. H. (2006), Effects of electrical acupuncture on acupoint BL15 evaluated in Terms of heart rate variability, pulse rate variability and skin conductance response. *The American Journal of Chinese Medicine, 34*, 23-36

Hsu, C. C., Weng, C. S., Sun, M. F., Shyu, L. Y., Hu, W. C. & Chang, Y. H. (2007). Evaluation of scalp and auricular acupuncture on EEG, HRV, and PRV. *The American Journal of Chinese Medicine, 35,* 219-230.

Huang, S. T., Chen, G. Y., Lo, H. M., Lin, J. G., Lee, Y. S. & Kuo, C. D. (2005). Increase in the vagal modulation by acupuncture at Neiguan point in the healthy subjects. *The American Journal of Chinese Medicine, 33,* 157-164.

Hübscher, M., Vogt, L. & Banzer, W. (2007). Laser needle acupuncture at Neiguan (PC6) does not mediate heart rate variability in young, healthy men. *Photomedicine and Laser Surgery, 25,* 21-25.

Hughes, J. W. & Stoney, C. M. (2000). Depressed mood is related to high-frequency heart rate variability during stressors. *Psychosomatic Medicine, 62,* 796-803.

Hjortskov, N., Rissén, D., Blangsted, A. K., Fallentin, N., Lundberg, U. & Søgaard, K. (2004). The effect of mental stress on heart rate variability and blood pressure during computer work. *European Journal of Applied Physiology, 92,* 84-89.

Imai, K., Ariga, H., Chen, C., Mantyh, C., Pappas, T. N. & Takahashi, T. (2008). Effects of electroacupuncture on gastric motility and heart rate variability in conscious rats. *Autonomic Neuroscience, 138,* 91-98.

Imai, K. & Kitakoji, H. (2003). Comparison of transient heart rate reduction associated with acupuncture stimulation in supine and sitting subjects. *Acupuncture in Medicine, 21,* 133-137.

Ingjaldsson, J. T., Laberg, J. C. & Thayer, J. F. (2003). Reduced heart rate variability in chronic alcohol abuse: relationship with negative mood, chronic thought suppression, and compulsive drinking. *Biological Psychiatry, 54,* 1427-1436.

Irnich, D. & Beyer, A. (2002). Neurobiologische Grundlagen der Akupunkturanalgesie. *Schmerz, 16,* 93-102.

Ishizawa, T., Yoshiuchi, K., Takimoto, Y., Yamamoto, Y. & Akabayashi, A. (2008). Heart rate and blood pressure variability and baroreflex sensitivity in patients with anorexia nervosa. *Psychosomatic Medicine, 70,* 695-700.

Isowa, T., Ohira, H. & Murashima, S. (2006). Immune, endocrine and cardiovascular responses to controllable and uncontrollable acute stress. *Biological Psychology, 71,* 202-213.

Ito, H., Matsuda, K., Sato, A. & Tohgi, H (1987). Cholinergic and VIPergic vasodilator actions of parasympathetic nerves on the thyroid blood flow in rats. *The Japanese Journal of Physiology, 37,* 1005-1017.

Jackson, J.H. (1958). Evolution and dissolution of the nervoussystem. In J. Ž. Taylor (Ed.), *Selected Writings of John Hughlings Jackson (*pp. 45–118). London: Stapes Press.

Jarrett, M. E., Burr, R. L., Cain, K. C., Rothermel, J. D., Landis, C. A. & Heitkemper, M. M. (2008). Autonomic nervous system function during sleep among women with irritable bowel syndrome. *Digestive Diseases and Sciences, 53,* 694-703.

Johnsen, B. H., Hansen, A. L., Sollers III, J. J., Murison, R. & Thayer, J. F. (2002). Heart rate variability is inversely related to cortisol reactivity during cognitive stress. *Psychosomatic Medicine, 64,* 148.

Johnsen, B. H., Thayer, J. F., Laberg, J. C., Wormnes, B., Raadal, M., Skaret, E., Kvale, G. & Berg, E. (2003). Attentional and physiological characteristics of patients with dental anxiety. *Journal of Anxiety Disorders, 17,* 75-87.

Johnson, M. I., Hajela, V. K., Ashton, C. H. & Thompson, J. W. (1991). The effects of auricular transcutaneous electrical nerve stimulation (TENS) on experimental pain threshold and autonomic function in healthy subjects. *Pain, 46*, 337-342.

Kamath, M. V., Upton, A. R., Talalla, A. & Fallen, E. L. (1992a). Effect of vagal nerve electrostimulation on the power spectrum of heart rate variability in man. *Pacing and Clinical Electrophysiology, 15,* 235-243.

Kamath, M. V., Upton, A. R., Talalla, A. & Fallen, E. L. (1992b). Neurocardiac responses to vagoafferent electrostimulation in humans. *Pacing and Clinical Electrophysiology, 15,* 1581-1587.

Kaptchuk, T. J., Goldman, P., Stone, D. A. & Stason, W. B. (2000). Do medical devices have enhanced placebo effects? *Journal of Clinical Epidemiology, 53,* 786-792.

Karakaya, O., Barutcu, I., Kaya, D., Esen, A. M., Saglam, M., Melek, M., Onrat, E., Turkmen, M., Esen, O. B. & Kaymaz, C. (2007). Acute effect of cigarette smoking on heart rate variability. *Angiology, 58,* 620-624.

Karst, M., Winterhalter, M., Münte, S., Francki, B., Hondronikos, A., Eckardt, A., Hoy, L., Buhck, H., Bernateck, M. & Fink, M. (2007). Auricular acupuncture for dental anxiety: a randomised controlled trial. *Anesthesia & Analgesia, 104,* 295-300.

Kawachi, I., Sparrow, D., Vokonas, P. S. & Weiss, S. T. (1995). Decreased heart rate variability in men with phobic anxiety (data from the Normative Aging Study). *The American Journal of Cardiology, 75,* 882-885.

Kern, S., Oakes, T. R., Stone, C. K., McAuliff, E. M., Kirschbaum, C. & Davidson, R. J. (2008). Glucose metabolic changes in the prefrontal cortex are associated with HPA axis response to a psychosocial stressor. *Psychoneuroendocrinology, 33,* 517-529.

Khattab, K., Khattab, A. A., Ortak, J., Richardt, G. & Bonnemeier, H. (2007). Iyengar yoga increases cardiac parasympathetic nervous modulation among healthy yoga practitioners. *Evidence-based Complementary and Alternative Medicine, 4,* 511-517.

Khurana, R. K. (2007). Cold face test: adrenergic phase. *Clinical Autonomic Research, 17,* 211-216.

Khurana, R. K., Watabiki, S., Hebel, J. R., Toro, R. & Nelson, E. (1980). Cold face test in the assessment of trigeminal-brainstem-vagal function in humans. *Annals of Neurology, 7,* 144-149.

Khurana, R. K. & Wu, R. (2006). The cold face test: a non-baroreflex mediated test of cardiac vagal function. *Clinical Autonomic Research, 16,* 202-207.

Kirschbaum, C. & Hellhammer, D. H. (1994). Salivary cortisol in psychoneuroendocrine research: recent developments and applications. *Psychoneuroendocrinology, 19*, 313-333.

Kirschbaum, C. & Hellhammer, D. H. (1999). *Psychoendokrinologie und Psychoimmunologie. Sonderdruck aus Enzyklopädie der Psychologie, Themenbereich C - Theorie und Forschung, Serie I - Biologische Psychologie*. Göttingen: Hogrefe.

Kirschbaum, C. & Hellhammer, D. H. (2000). Salivary cortisol. In G. Fink (Ed.), *Encyclopaedia of stress* (Vol. 3, pp. 379-383). San Diego, CA: Academic Press.

Kirschbaum, C., Pirke, K. M. & Hellhammer, D. H. (1993). The 'Trier Social Stress Test'-a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology, 28,* 76-81.

Kleiger, R. E., Stein, P. K. & Bigger, J. T. Jr. (2005). Heart rate variability: measurement and clinical utility. *Annals of Noninvasive Electrocardiology, 10,* 88-101.

Klinkenberg, A. V., Nater, U. M., Nierop, A., Bratsikas, A., Zimmermann, R. & Ehlert, U. (2008). Heart rate variability changes in pregnant and non-pregnant women during standardized psychosocial stress. *Acta Obstetricia et Gynecologica Scandinavica, 20,* 1-6.

Koenig, S. A., Longin, E., Bell, N., Reinhard, J. & Gerstner, T. (2008). Vagus nerve stimulation improves severly impaired heart rate variability in a patient with Lennox-Gastaut-Syndrome. *Seizure, 17,* 469-472.

Kong, J., Fufa, D. T., Gerber, A. J., Rosman, I. S., Vangel, M. G., Gracely, R. H. & Gollub, R. L. (2005). Psychophysical outcomes from a randomized pilot study of manual, electro, and sham acupuncture treatment on experimentally induced thermal pain. *The Journal of Pain, 6,* 55-64.

Kong, J., Gollub, R. L., Polich, G., Kirsch, I., Laviolette, P., Vangel, M., Rosen, B. & Kaptchuk, T. J. (2008). A functional magnetic resonance imaging study on the neural mechanisms of hyperalgesic nocebo effect. *The Journal of Neuroscience, 28,* 13354-13362.

Kontopoulos, A. G., Athyros, V. G., Papageorgiou, A. A., Papadopoulos, G. V., Avramidis, M. J. & Boudoulas, H. (1996). Effect of quinapril or metoprolol on heart rate variability in post-myocardial infarction patients. *The American Journal of Cardiology, 77,* 242-246.

Koskinen, T., Kähönen, M., Jula, A., Laitinen, T., Keltikangas-Järvinen, L., Viikari, J., Välimäki, I. & Raitakari, O. T. (in press). Short-term heart rate variability in healthy young adults The Cardiovascular Risk in Young Finns Study. *Autonomic Neuroscience: Basic and Clinical*.

Kraus, T., Hösl, K., Kiess, O., Schanze, A., Kornhuber, J. & Forster, C. (2007). BOLD fMRI deactivation of limbic and temporal brain structures and mood enhancing effect by transcutaneous vagus nerve stimulation. *Journal of Neural Transmission, 114*, 1485-1493.

Kudaiberdieva, G., Görenek, B. & Timuralp, B. (2007). Heart rate variability as a predictor of sudden cardiac death. *Anadolu Kardiyoloji Dergisi, 7,* 68-70.

Kumada, M., Dampney, R. A. & Reis, D. J. (1977). The trigeminal depressor response: a novel vasodepressor response originating from the trigeminal system. *Brain Research, 119*, 305-326.

La Marca, R. (2005). *Alpha-Amylase im Speichel als Indikator für autonome Aktivierung unter Stress*. Unveröff. Lizentiatsarbeit, Universität Zürich, Psychologisches Institut, Abt. Klinische Psychologie & Psychotherapie.

La Marca, R., Abbruzzese, E., Kliegel, M., Beck, F. B., Hausherr, M., Brand, A. & Ehlert, U. (2009). *Associations between resting RSA and working and prospective memory.* Manuscript in preparation.

La Marca, R., Nedeljkovic, M., Yuan, L., Maercker, A. & Ehlert, U. (2009). *Effects of auricular electrical stimulation on vagal activity in healthy men: Evidence from a three-armed randomized trial.* Manuscript submitted for publication.

La Marca, R., Thörn, H., Waldvogel, P., Tripod, M., Pruessner, J. Arnold, M. & Ehlert, U. (2009). *Autonomic responses to the Montreal Imaging Stress Task*. Manuscript in preparation.

La Marca, R., Waldvogel, P., Thörn, H., Tripod, M., Pruessner, J. & Ehlert, U. (2009). *Cold face test response indicates biopsychological adaptability to acute stress.* Manuscript submitted for publication.

Lampert, R., Ickovics, J., Horwitz, R. & Lee, F. (1995). Depressed autonomic nervous system function in African Americans and individuals of lower social class: a potential mechanism of race- and class-related disparities in health outcomes. *American Heart Journal, 150,* 153-160.

Lane, R. D., McRae, K., Reiman, E. M., Chen, K., Ahern, G. L. & Thayer, J. F. (2009). Neural correlates of heart rate variability during emotion. *NeuroImage, 44,* 213-222.

Lang, J. (1992). *Klinische Anatomie des Ohres*. Wien: Springer.

La Rovere, M. T., Bigger, J. T., Marcus, F. I., Mortara, A. & Schwartz, P. J. (1998). Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. *Lancet, 351*, 478-484.

Lazarus, R. S. (1966). *Psychological stress and the coping process*. New York: McGraw-Hill.

Lazarus, R. S. & Folkman, S. (1984). *Stress, appraisal and coping.* New York: Springer.

Lazarus, R. S. & Launier, R. (1981). Stressbezogene Transaktionen zwischen Person und Umwelt. In: J. R. Nietsch (Hrsg.), *Stress - Theorien, Untersuchungen, Massnahmen* (S. 213-260). Bern: Verlag Hans Huber.

LeBlanc, J., Ducharme, M. B. & Thompson, M. (2004). Study on the correlation of the autonomic nervous system responses to a stressor of high discomfort with personality traits. *Physiology & Behavior, 82*, 647-652.

LeBlanc, J., Dulac, S., Côté, J. & Girard, B. (1975). Autonomic nervous system and adaptation to cold in man. *Journal of Applied Physiology, 39*, 181-186.

Lee, Y.-C., Wang, H.-P., Lin, L.-Y., Lee, B.-C., Chiu, H.-M., Wu, M.-S., Chen, M.-F. & Lin, J.-T. (2004). Heart rate variability in patients with different manifestations of gastroesophageal reflux disease. *Autonomic Neuroscience: Basic and Clinical, 116,* 39-45.

Lee, Y.-C., Wang, H.-P., Lin, L.-Y., Chuang, K.-J., Chiu, H.-M., Wu, M.-S., Chen, M.-F. & Lin, J.-T. (2006). Circadian change of cardiac autonomic function in correlation with intra-esophageal pH. *Journal of Gastroenterology and Hepatology, 21,* 1302-1308.

Lewis, M. E., Al-Khalidi, A. H., Bonser, R. S., Clutton-Brock, T., Morton, D., Paterson, D., Townend, J. N. & Coote, J. H. (2001). Vagus nerve stimulation decreases left ventricular contractility in vivo in the human and pig heart. *Journal of Physiology, 534*, 547-552.

Li, Z., Jiao, K., Chen, M. & Wang, C. (2003). Effect of magnitopuncture on sympathetic and parasympathetic nerve activities in healthy drivers - assessment by power spectrum analysis of heart rate variability. *European Journal of Applied Physiology, 88,* 404-410.

Li, Z., Wang, C., Mak, A. F. & Chow, D. H. (2005). Effects of acupuncture on heart rate variability in normal subjects under fatigue and non-fatigue state. *European Journal of Applied Physiology, 94,* 633-640.

Liao, D., Barnes, R. W., Chambless, L. E., Simpson, R. J., Sorlie, P., & Heiss, G. (1995a). Age, race, and sex differences in autonomic cardiac function measured by spectral analysis of heart rate variability - the ARIC Study. *American Journal of Cardiology, 76,* 906-912.

Liao, D., Carnethon, M., Evans, G. W., Cascio, W. E. & Heiss, G. (2002). Lower heart rate variability is associated with the development of coronary heart disease in individuals with diabetes - the atherosclerosis risk in communities (ARIC) study. *Diabetes, 51,* 3524-3531.

Lin, Z., Chen, M. L., Keens, T. G., Ward, S. L. D. & Khoo, M. C. K. (2004). Noninvasive assessment of cardiovascular autonomic control in congenital central hypoventilation snydrome. *Conference proceedings : 26th Annual International Conference of the IEEE Engineering in Medicine and Biology Society, 5,* 3870-3873.

Lin, L.-Y., Wu, C.-C., Liu, Y.-B., Ho, Y.-L., Liau, C.-S. & Lee, Y.-T. (2001). Derangement of heart rate variability during a catastrophic earthquake: a possible mechanism for increased heart attacks. *Pacing and Clinical Electrophysiology, 24,* 1596-1601.

Lomarev, M., Denslow, S., Nahas, Z., Chae, J. H., George, M. S. & Bohning, D. E. (2002). Vagus nerve stimulation (VNS) synchronized BOLD fMRI suggests that VNS in depressed adults has frequency/dose dependent effects. *Journal of Psychiatry Research, 36,* 219-227.

Lucini, D., Di Fede, G., Parati, G. & Pagani, M. (2005). Impact of chronic psychosocial stress on autonomic cardiovascular regulation in otherwise healthy subjects. *Hypertension, 46,* 1201-1206.

Luger, A., Deuster, P. A., Kyle, S. B., Gallucci, W. T., Montgomery, L. C., Gold, P. W., Loriaux, D. L. & Chrousos, G. P. (1987). Acute hypothalamic-pituitary-adrenal responses to the stress of treadmill exercise. Physiologic adaptations to physical training. *The New England Journal of Medicine, 316*, 1309-1315.

Lüscher, T. F., Noll, G, Candinas, R., Jenni, R. & Yang, Z. (1999). *Block Kardiologie*. Vorlesung im SS 1999 am Institut für Kardiologie, Universitätsspital Zürich und am Institut für Physiologie, Kardiovaskuläre Forschung, Universität Zürich-Irchel, Zürich.

Lux, G., Hagel, J., Bäcker, P., Bäcker, G., Vogl, R., Ruppin, H., Domschke, S. & Domschke, W. (1994). Acupuncture inhibits vagal gastric acid secretion stimulated by sham feeding in healthy subjects. *Gut, 35,* 1026-1029.

MacPherson, H., White, A., Cummings, M., Jobst, K., Rose, K. & Niemtzow, R. (2001). Standards for reporting interventions in controlled trials of acupuncture: the STRICTA recommendations. *Complementary Therapies in Medicine, 9*: 246-249.

Majer, E. H. (1953). Die vegetativen Regulationen im Bereiche von Hals, Nase und Ohr. *European Archives of Oto-Rhino-Laryngology and Head & Neck, 163,* 235-249.

Malfertheiner, P. & Kemmer, T. (1987). Nervale Regulation der Speicheldrüsen. *Zeitung für Gastroenterologie, 25*, 15-20.

Martinmäki, K., Häkkinen, K., Mikkola, J. & Rusko, H. (2008). Effect of low-dose endurance training on heart rate variability at rest and during an incremental maximal exercise test. *European Journal of Applied Physiology, 104,* 541–548.

Mason, J. W. (1968). A review of psychoendocrine research on the pituitary-adrenal cortical system. *Psychomatic Medicine, 30,* 576-607.

Mason, J. W. (1971). A re-evaluation of the concept of "non-specifity" in stress theory. *Journal of Psychiatric Research, 8*, 323-333.

Mason, J. W. (1975a). A historical view of the stress field. Part I. *Journal of Human Stress, 1*, 7-12.

Mason, J. W. (1975b). A historical view of the stress field. Part II. *Journal of Human Stress, 1*, 22-36.

Mason, J. W., Hartley, L. H., Kotchen, T. A., Mougey, E. H., Ricketts , P. T. & Jones, L. G. (1973). Plasma cortisol and norepinephrine responses in anticipation of muscular exercise. *Psychosomatic Medicine, 35*, 406-414.

Matthews, S. C., Paulus, M. P., Simmons, A. N., Nelesen, R. A. & Dimsdale, J. E. (2004). Functional subdivisions within anterior cingulate cortex and their relationship to autonomic nervous system function. *Neuroimage, 22,* 1151-1156.

Mauskop, A. (2005). Vagus nerve stimulation relieves chronic refractory migraine and cluster headaches. *Cephalalgia, 25,* 82-86.

Mazur, M., Furgala, A., Jablonski, K., Madroszkiewicz, D., Ciecko-Michalska, I., Bugajski, A. & Thor, P. J. (2007). Dysfunction of the autonomic nervous system activity is responsible for gastric myoelectric disturbances in the irritable bowel syndrome patients. *Journal of Physiology & Pharmacology, 58,* 131-139.

McCracken , J. T. & Poland, R. E. (1989). Saliva and serum cortisol dynamics following intravenous dexamethasone in normal volunteers. *Life Sciences, 45*, 1781-1785.

McEwen, B. S. (1998). Protective and damaging effects of stress mediators. *New England Journal of Medicine, 338,* 171-179.

McEwen, B. S. (2000). Allostasis and allostatic load: implications for neuropsychopharmacology. *Neuropsychopharmacology, 22,* 108-124.

McGrath, J. E. (1970). *Social and psychological factors in stress.* England: Holt, Rineheart & Winston.

Melander, A. & Sundler, F. (1979). Presence and influence of cholinergic nerves in the mouse thyroid. *Endocrinology, 105,* 7-9.

Melanson, E. L., Donahoo, W. T., Krantz, M. J., Poirier, P. & Mehler, P. S. (2004). Resting and ambulatory heart rate variability in chronic anorexia nervosa. *The American Journal of Cardiology, 94*, 1217-20.

Mendel, C. M. (1989). The free hormone hypothesis: a physiologically based mathematical model. *Endocrine Reviews, 10* (3), 232-274.

Merrill, C. A., Jonsson, M. A., Minthon, L., Ejnell, H., C-son Silander, H., Blennow, K., Karlsson, M., Nordlund, A., Rolstad, S., Warkentin, S., Ben-Menachem, E. & Sjögren, M. J. (2006). Vagus nerve stimulation in patients with Alzheimer's disease: additional follow-up results of a pilot study through 1 year. *The Journal of Clinical Psychiatry, 67,* 1171-1178.

Mezzacappa, E. S., Kelsey, R. M., Katkin, E. S. & Sloan, R. P. (2001). Vagal rebound and recovery from psychological stress. *Psychosomatic Medicine, 63,* 650-657.

Milby, A. H., Halpern, C. H. & Baltuch, G. H. (2008). Vagus nerve stimulation for epilepsy and depression. *Neurotherapeutics, 5*, 75-85.

Min, K.-B., Min, J.-Y., Cho, S.-I., & Paek, D. (2008). The relationship between air pollutants and heart-rate variability among community residents in Korea. *Inhalation Toxicology, 20,* 435-444.

Min, J.-Y., Min, K.-B., Cho, S.-I. & Paek, D. (in press). Combined effect of cigarette smoking and sulfur dioxide on heart rate variability. *International Journal of Cardiology*.

Mittal, C. M., Wig, N., Mishra, S. & Deepak, K. K. (2004). Heart rate variability in human immunodeficiency virus-positive individuals. *International Journal of Cardiology, 94,* 1-6.

Morgan, C. A. 3rd, Aikins, D. E., Steffian, G., Coric, V. & Southwick, S. (2007). Relation between cardiac vagal tone and performance in male military personnel exposed to high stress: three prospective studies. *Psychophysiology, 44,* 120-127.

Morse, D. R., Schacterle, G. R., Esposito, J. V., Chod, S. D. Furst, M. L., Di Ponziano, J. & Zaydenberg, M. (1983). Stress, meditation and saliva: a study of separate salivary gland secretions in endodontic patients. *Journal of Oral Medicine, 38*, 150-160.

Morse, D. R., Schacterle, G. R., Esposito, J. V., Furst, M. L. & Bose, K. (1981). Stress, relaxation and saliva: a follow-up study involving clinical endodontic patients. *Journal of Human Stress, 7,* 19-26.

Mozaffarian, D., Stein, P. K., Prineas, R. J. & Siscovick, D. S. (2008). Dietary fish and omega-3 fatty acid consumption and heart rate variability in US adults. *Circulation, 117,* 1130-1137.

Muller, J. E., Tofler, G. H. & Stone, P. H. (1989). Circadian variation and triggers of onset of acute cardiovascular disease. *Circulation, 79,* 733-743.

Murphy, J. V., Wheless, J. W. & Schmoll, C. M. (2000). Left vagal nerve stimulation in six patients with hypothalamic hamartomas. *Pediatric Neurology, 23,* 167-168.

Muth, E. R., Thayer, J. F., Stern, R. M., Friedman, B. H. & Drake, C. (1998). The effect of autonomic nervous system activity on gastric myoelectrical activity: Does the spectral reserve hypothesis hold for the stomach? *Biological Psychology, 71*, 265-278.

Nater, U. M., La Marca, R., Florin, L., Moses, A., Langhans, W., Koller, M. M. & Ehlert, U. (2006). Stress-induced changes in human salivary alpha-amylase activity - associations with adrenergic activity. *Psychoneuroendocrinology, 31,* 49-58.

Nierop, A., Bratsikas, A., Klinkenberg, A., Nater, U. M., Zimmermann, R. & Ehlert, U. (2006). Prolonged salivary cortisol recovery in second-trimester pregnant women and attenuated salivary alpha-amylase responses to psychosocial stress in human pregnancy. *The Journal of Clinical Endocrinology & Metabolism, 91,* 1329-1335.

Nishijo, K., Mori, H., Yosikawa, K. & Yazawa, K. (1997). Decreased heart rate by acupuncture stimulation in humans via facilitation of cardiac vagal activity and suppression of cardiac sympathetic nerve. *Neuroscience Letters, 227,* 165-168.

Nishime, E. O., Cole, C. R., Blackstone, E. H., Pashkow, F. J. & Lauer, M. S. (2000). Heart rate recovery and treadmill-exercise score as predictors of mortality in patients referred for exercise ECG. *JAMA: The Journal of the American Medical Association, 284, 1392-1398*.

Noll, A. W. (2002). *Validation of the concept of allostatic load in a working context*. Unpubl. Ph. D. thesis, Universität Zürich, Psychologisches Institut, Abt. Klinische Psychologie II.

O'Connor, P. J. & Corrigan, D. L. (1987). Influence of short-term cycling on salivary cortisol levels. *Medicine and Science in Sports and Exercise, 19*, 224-228.

O'Keane, V., Dinan, T. G., Scott, L. & Corcoran, C. (2005). Changes in hypothalamic-pituitary-adrenal axis measures after vagus nerve stimulation therapy in chronic depression. *Biological Psychiatry, 58,* 963-968.

Ouyang, H., Yin, J., Wang, Z., Pasricha, P. J. & Chen, J. D. (2002). Electroacupuncture accelerates gastric emptying in association with changes in vagal activity. *American Journal of Physiology: Gastrointestinal and Liver Physiology, 282,* 390-396.

Pagani, M., Lucini, D., Rimoldi, O., Furlan, R., Piazza, S. & Biancardi, L. (1995). Effects of physical and mental exercise on heart rate variability. In M. Malik & A. J. Camm (Eds.), *Heart Rate Variability* (pp. 245-265). Armonk, NY: Futura Publishing Company.

Palkovits, M. (1999). Interconnections between the neuroendocrine hypothalamus and the central autonomic system. *Frontiers in Neuroendocrinology, 20,* 270-295.

Panda, S., Hogensch, J. B. & Kay, S. A. (2002). Circadian rhythms from flies to human. *Nature, 417*, 329-335.

Pavithran, P., Mithun, R., Jomal, M. & Nandeesha, H. (2008). Heart rate variability in middle-aged men with new-onset hypertension. *Annals of Noninvasive Electrocardiology, 13,* 242-248.

Park, S. K., O'Neill, M. S., Vokonas, P. S., Sparrow, D. & Schwartz, J. (2005). Effects of air pollution on heart rate variability: the VA normative aging study. *Environmental Health Perspectives, 113,* 304-309.

Parrish, W. R., Gallowitsch-Puerta, M., Czura, C. J. & Tracey, K. J. (2008). Experimental therapeutic strategies for severe sepsis: mediators and mechanisms. *Annals of the New York Academy of Sciences, 1144,* 210-236.

Peltola, M., Tulppo, M. P., Kiviniemi, A., Hautala, A. J., Seppänen, T., Barthel, P., Bauer, A., Schmidt, G., Huikuri, H. V. & Mäkikallio, T. H. (2008). Respiratory sinus arrhythmia as a predictor of sudden cardiac death after myocardial infarction. *Annals of Medicine, 40,* 376-382.

Phongsuphap, S., Pongsupap, Y., Chandanamattha, P. & Lursinsap, C. (2008). Changes in heart rate variability during concentration meditation. *International Journal of Cardiology, 130,* 481-484.

Pinel, J. P. J. & Pauli, P. (2007). *Biopsychologie* (6. Aufl.). München: Pearson Studium.

Porges, S. W. (1995). Cardiac vagal tone: a physiological index of stress. *Neuroscience and Biobehavioral Reviews, 19*, 225-233.

Porges, S. W. (2001). The polyvagal theory: phylogenetic substrates of a social nervous system. *International Journal of Psychophysiology, 42,* 123-146.

Porges, S. W. (2003). The polyvagal theory: phylogenetic contributions to social behavior. *Physiology & Behavior, 79,* 503-513.

Porges, S. W. (2007). The polyvagal perspective. *Biological Psychology, 74,* 116-143.

Porges, S. W., Doussard-Roosevelt, J. A. & Maiti, A. K. (1994). Vagal tone and the physiological regulation of emotion. *Monographs of the Society for Research in Child Development, 59,* 167-186.

Porter, R. S. (2003). *The merck manual of medical information* (2nd home edition, p. 122). [Online]. Available: http://www.merck.com/media/mmhe2/figures/MMHE_03_021_01_eps.gif

Pruessner, J. C., Champagne, F., Meaney, M. J. & Dagher, A. (2004). Dopamine release in response to a psychological stress in humans and its relationship to early life maternal care: a positron emission tomography study using [11C]Raclopride. *The Journal of Neuroscience, 24,* 2825-2831.

Pruessner, J. C., Dedovic, K., Khalili-Mahani, N., Engert, V., Pruessner, M., Buss, C., Renwick, R., Dagher, A., Meaney, M. J. & Lupien, S. (2008). Deactivation of the limbic system during acute psychosocial stress: evidence from positron emission tomography and functional magnetic resonance imaging studies. *Biological Psychiatry, 63,* 234-240.

Pruessner, J. C., Hellhammer, D. H. & Kirschbaum, C. (1999). Low self-esteem, induced failure and the adrenocortical stress response. *Personality and Individual Differences, 27,* 477-489.

Pruessner, J. C., Kirschbaum, C., Meinlschmid, G. & Hellhammer, D. H. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology, 28,* 916-931

Radloff, L. S. (1977). The CES-D scale: a self-report depression scale for research in the general population. *Applied Psychological Measurement, 3,* 385-401.

Raghuraj, P., Ramakrishnan, A. G., Nagendra, H. R. & Telles, S. (1998). Effect of two selected yogic breathing techniques of heart rate variability. *Indian Journal of Physiology and Pharmacology, 42,* 467-72.

Reid, J. D., Intrieri, R. C., Susman , E. J. & Beard, J. L. (1992). The relationship of serum and salivary cortisol in a sample of healthy elderly. *Journal of Gerontology, 47*, P176-P179.

Reyners, A. K. L., Tio, R. A., Vlutters, F. G., van der Woude, G. F., Reitsma, W. D. & Smit, A. J. (2000). Re-evaluation of the cold face test in humans. *European Journal of Applied Physiology, 82*, 487-492.

Rijkers, K., Berfelo, M. W., Cornips, E. M. J. & Majoie, H. J. M. (2008). Hardware failure in vagus nerve stimulation therapy. *Acta Neurochirurgica, 150,* 403-405.

Rohen, J. W. (1994). *Funktionelle Anatomie des Nervensystems: Lehrbuch und Atlas* (5. Aufl.). Stuttgart, New York: Schattauer.

Rohleder, N., Wolf, J. M., Maldonado, E. F. & Kirschbaum, C. (2006). The psychosocial stress-induced increase in salivary alpha-amylase is independent of saliva flow rate. *Psychophysiology, 43,* 645-652.

Ronkainen, E., Korpelainen, J. T., Heikkinen, E., Myllylä, V. V., Huikuri, H. V. & Isojärvi, J. I. T. (2006). Cardiac autonomic control in patients with refractory epilepsy before and during vagus nerve stimulation treatment: a one-year follow-up study. *Epilepsia, 47*, 556-562.

Routledge, H. C., Chowdhary, S. & Townend, J. N. (2002). Heart rate variability - a therapeutic target? *Journal of Clinical Pharmacy and Therapeutics, 27,* 85-92.

Rubach, A. (2000). *Propädeutik der Ohrakupunktur* (2. Aufl.). Stuttgart: Hippokrates.

Ruiz, J. M., Uchino, B. N. & Smith, T. W. (2006). Hostility and sex differences in the magnitude, duration, and determinants of heart rate response to forehead cold pressor: parasympathetic aspects of risk. *International Journal of Psychophysiology*, *60,* 274-283.

Ruiz-Padial, E., Sollers III, J. J., Vila, J. & Thayer, J. F. (2003). The rhythm of the heart in the blink of an eye: emotion-modulated startle magnitude covaries with heart rate variability. *Psychophysiology, 40,* 3006-3013.

Rush, A. J., George, M. S., Sackeim, H. A., Marangell, L. B., Husain, M. M., Giller. C., Nahas, Z., Haines, S., Simpson, R. K. & Goodmann, R. (2000). Vagus nerve stimulation (VNS) for treatment-resistant depression: a multicenter study. *Biological Psychiatry, 47*, 276-286.

Rush, A. J., Marangell, L. B., Sackeim, H. A., George, M. S., Brannan, S. K., Davis, S. M., Howland, R., Kling, M. A., Rittberg, B. R., Burke, W. J., Rapaport, M. H., Zajecka, J., Nierenberg, A. A., Husain, M. M., Ginsberg, D. & Cooke, R. G. (2005a). Vagus nerve stimulation for treatment-resistant depression: a randomized, controlled acute phase trial. *Biological Psychiatry, 58,* 347-354.

Rush, A. J., Sackeim, H. A., Marangell, L. B., George, M. S., Brannan, S. K., Davis, S. M., Lavori, P., Howland, R., Kling, M. A., Rittberg, B., Carpenter, L., Ninan, P., Moreno, F., Schwartz, T., Conway, C., Burke, M. & Barry, J. J. (2005b). Effects of 12 months of vagus nerve stimulation in treatment-resistant depression: a naturalistic study. *Biological Psychiatry, 58,* 355-363.

Rychlicki, F., Zamponi, N., Trignani, R., Ricciuti, R. A., Iocoangeli, M. & Scerrati, M. (2006). Vagus nerve stimulation: clinical experience in drug-resistant pediatric epileptic patients. *Seizure, 15*, 483-490.

Sahar, T., Shalev, A. Y. & Porges, S. W. (2001). Vagal modulation of responses to mental challenge in posttraumatic stress disorder. *Biological Psychiatry, 49,* 637-643.

Sakai, S., Hori, E., Umeno, K., Kitabayashi, N., Ono, T. & Nishijo, H. (2007). Specific acupuncture sensation correlates with EEGs and autonomic changes in human subjects. *Autonomic Neuroscience, 133,* 158-169.

Sakhuja, A., Goyal, A., Jaryal, A. K., Wig, N., Vajpayee, M., Kumar, A. & Deepak, K. K. (2007). Heart rate variability and autonomic function tests in HIV positive individuals in India. *Clinical Autonomic Research, 17,* 193-196.

Samet, J. M., Dominici, F., Curriero, F. C., Coursac, I. & Zeger, S. L. (2000). Fine particulate air pollution and mortality in 20 U.S. cities, 1987-1994. *The New England Journal of Medicine, 343,* 1742-1749.

Sandercock, G. R., Bromley, P. D. & Brodie, D. A. (2005). Effects of exercise on heart rate variability: inferences from meta-analysis. *Medicine and Science in Sports and Exercise, 37,* 433-439.

Saper, C. B. (2002). The central autonomic nervous system: conscious visceral perception and autonomic pattern generation. *Annual Review of Neuroscience, 25,* 433-469.

Sato, N., Kamada, T., Miyake, S., Akatsu, J., Kumashiro, M. & Kume, Y. (1998). Power spectral analysis of heart rate variability in type A females during psychomotor task. *Journal of Psychosomatic Research, 45,* 159-169.

Sato, N. & Miyake, S. (2004). Cardiovascular reactivity to mental stress: relationship with menstrual cycle and gender. *Journal of Physiological Anthropology and Applied Human Science, 23,* 215-223.

Saxena, S. R., Solanki, D. & Kataria, M. S. (1976). Ear, janeu, and heart. *Lancet, 1,* 1415.

Scannapieco, F. A., Torres, G. & Levine, M. J. (1993). Salivary alpha-amylase: role in dental plaque and caries formation. *Critical Reviews in Oral Biology and Medicine, 4,* 301-307.

Schachter, S. C. & Saper, C. B. (1998). Vagus nerve stimulation. *Epilepsia, 39*, 677-686.

Schlaepfer, T. E., Frick, C., Zobel, A., Maier, W., Heuser, I., Bajbouj, M., O'Keane, V., Corcoran, C., Adolfsson, R., Trimble, M., Rau, H., Hoff, H.-J., Padberg, F., Müller-Siecheneder, F., Audenaert, K., Van den Abbeele, D., Matthews, K., Christmas, D., Stanga, Z. & Hasdemir, M. (2008). Vagus nerve stimulation for depression: efficacy and safety in a European study. *Psychological Medicine, 38*, 651-661.

Schnorrenberger, C. C. (1994). *Die topographisch-anatomischen Grundlagen der chinesischen Akupunktur und Ohrakupunktur* (6. Aufl.). Stuttgart: Hippokrates.

Scholz, U., La Marca, R., Nater, U. M., Aberle, I., Ehlert, U., Hornung, R., Martin, M., & Kliegel, M. (2009). Go no-go performance under psychosocial stress: Beneficial effects of implementation intentions. *Neurobiology of Learning and Memory, 91*, 89-92.

Schwartz, J. (1999). Air pollution and hospital admissions for heart disease in eight U.S. counties. *Epidemiology, 10,* 17-22.

Schwarz, A. M., Schächinger, H., Adler, R. H. & Goetz, S. M. (2003). Hopelessness is associated with decreased heart rate variability during championship chess games. *Psychosomatic Medicine, 65,* 658-661.

Selye, H. (1936a). A syndrome produced by diverse nocuous agents. *Nature, 138*, 32.

Selye, H. (1936b). Thymus and adrenals in the response of the organism to injuries and intoxications. *British Journal of Experimental Pathology, 17*, 234-248.

Selye, H. (1937). Studies on adaptation. *Endocrinology, 21*, 169-188.

Selye, H. (1946). The general adaptation syndrome and the diseases of adaptation. *Journal of Clinical Endocrinology, 6*, 117-230.

Selye, H. (1981). Geschichte und Grundzüge des Stresskonzepts. In: J. R. Nietsch (Hrsg.), *Stress - Theorien, Untersuchungen, Massnahmen* (S. 163-187). Bern: Verlag Hans Huber.

Setty, A. B., Vaughn, B. V., Quint, S. R., Robertson, K. R. & Messenheimer, J. A. (1998). Heart period variability during vagal nerve stimulation. *Seizure, 7*, 213-217.

Setz, C., Arnrich, B., Schumm, J., Troester, G., La Marca, R. & Ehlert, U. (2009). Discriminating Stress from Cognitive Load Using a Wearable EDA Device. Manuscript submitted for publication.

Shapiro, D., Cook, I. A., Davydov, D. M., Ottaviani, C., Leuchter, A. F. & Abrams, M. (2007). Yoga as complementary treatment of depression: effects of traits and moods on treatment outcome. *Evidence-based Complementary and Alternative Medicine, 4*, 493-502.

Shapiro, P. A., Sloan, R. P., Bagiella, E., Kuhl, J. P., Anjilvel, S. & Mann, J. J. (2000). Cerebral activation, hostility, and cardiovascular control during mental stress. *Journal of Psychosomatic Research, 48,* 485-491.

Shinohara, M. (1997). Decreasing heart rate and shortening of the arterial pulse propagation time by acupuncture in the spectral analyses. *Masui, 46,* 213-221.

Singh, B. N. (2003). Increased heart rate as a risk factor for cardiovascular disease. *European Heart Journal Supplements, 5,* G3-G9.

Singh, J. P., Larson, M. G. & O'Donnell, C. J. (2002). Association of hyperglycemia with reduced heart rate variability (The Framingham Heart Study). *American Journal of Cardiology, 86,* 309-312.

Sjögren, M. J., Hellström, P. T., Jonsson, M. A., Runnerstam, M., Silander, H. C. & Ben-Menachem, E. (2002). Cognition-enhancing effect of vagus nerve stimulation in patients with Alzheimer's disease: a pilot study. *The Journal of Clinical Psychiatry, 63,* 972-980.

Sloan, R. P., Bagiella, E., Shapiro, P. A., Kuhl, J. P., Chernikhova, D., Berg, J. & Myers, M. M. (2001). Hostility, gender, and cardiac autonomic control. *Psychosomatic Medicine, 63,* 434-440.

Sloan, R. P., Korten, J. B. & Myers, M. M. (1991). Components of heart rate reactivity during mental arithmetic with and without speaking. *Physiology and Behavior, 50,* 1039-1045.

Sloan, R. P., Shapiro, P. A., Bigger, J. T. Jr, Bagiella, E., Steinman, R. C. & Gorman, J. M. (1994). Cardiac autonomic control and hostility in healthy subjects. *The American Journal of Cardiology, 74,* 298-300.

Smyth, M. D., Tubbs, R. S., Bebin, E. M., Grabb, B. A. & Blount, J. P. (2003). Complications of chronic vagus nerve stimulation for epilepsy in children. *Journal of Neurosurgery, 99,* 500-503.

Smith, S. M. & Vale, W. W. (2006). The role of the hypothalamic-pituitary-adrenal axis in the neuroendocrine response to stress. *Dialogues in Clinical Neuroscience, 8,* 383-397.

Soliman, A., O'Driscoll, G. A., Pruessner, J., Holahan, A. V., Boileau, I., Gagnon, D. & Dagher, A. (2008). Stress-induced dopamine release in humans at risk of psychosis: a [^{11}C]Raclopride PET study. *Neuropsychopharmacology, 33,* 2033-2041.

Sparrow, K. (2007). Analysis of heart rate variability in acupuncture practice: can it improve outcomes? *Medical Acupuncture, 19,* 37-41.

Spear, J. F., Kronhaus, K. D., Moore, E. N. & Kline, R. P. (1979). The effect of brief vagal stimulation on the isolated rabbit sinus node. *Circulation Research, 44,* 75-88.

Steptoe, A. (1991). Invited review. The links between stress and illness. *Journal of Psychosomatic Research, 35*, 633-644.

Stemper, B., Devinsky, O., Haendl, T., Welsch, G. & Hilz, M. J. (2008). Effects of vagus nerve stimulation on cardiovascular regulation in patients with epilepsy. *Acta Neurologica Scandinavia, 117*, 231-236.

Steptoe, A. & Marmot, M. (2005). Impaired cardiovascular recovery following stress predicts 3-year increases in blood pressure. *Journal of Hypertension, 23,* 529-536.

Sterling, P. & Eyer, J. (1988). Allostasis: a new paradigm to explain arousal pathology. In S. R. Fisher (Ed.), *Handbook of life stress, cognition and health* (pp. 629-649). New York: John Wiley & Sons.

Stewart, J., Weldon, A., Arlievsky, N., Li, K. & Munoz, J. (1998). Neurally mediated hypotension and autonomic dysfunction measured by heart rate variability during head-up tilt testing in children with chronic fatigue syndrome. *Clinical Autonomic Research, 8,* 221-230.

Steyer, R., Schwenkmezger, P., Notz, P. & Eid, M. (1997). *Der Mehrdimensionale Befindlichkeitsfragebogen (MDBF). Handanweisung*. Göttingen: Hogrefe.

Streitberger, K. & Kleinhenz, K. (1998). Introducing a placebo needle into acupuncture research. *Lancet, 352,* 364-365.

Streitberger, K., Steppan, J., Maier, C., Hill, H., Backs, J. & Plaschke, K. (2008). Effects of verum acupuncture compared to placebo acupuncture on quantitative EEG and heart rate variability in healthy volunteers. *Journal of Alternative and Complementary Medicine, 14,* 505-513.

Sulka, K. A. & Walsh, D. (2003). Transcutaneus electrical nerve stimulation: basic science mechanisms and clinical effectiveness. *The Journal of Pain, 4*, 109-121.

Swanson, L. W. (2003). *Brain architecture, understanding the basic plan*. New York: Oxford University Press.

Swanson, N. & Swanson, L. W. (1990). *New ideas on the structure of the nervous system in man and vertebrates.* Cambridge: MIT.

Sztajzel, J., Jung, M., Sievert, K., Bayes De Luna, A. (2008). Cardiac autonomic profile in different sports disciplines during all-day activity. *The Journal of Sports Medicine and Physical Fitness, 48,* 495-501.

Tasaki, H., Serita, T., Irita, A., Hano, O., Iliev, I., Ueyama, C., Kitano, K., Seto, S., Hayano, M. & Yano, K. (2000). A 15-year longitudinal follow-up study of heart rate and heart rate variability in healthy elderly persons. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences, 55,* M744-M749.

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996). Heart rate variability - standards and measurement, physiologcial interpretation an cinical use. *Circulation, 93,* 1043-1065.

Tekdemir, I., Aslan, A., & Elhan, A. (1998). A clinico-anatomic study of the auricular branch of the vagus nerve and Arnold's ear-cough reflex. *Surgical and Radiologic Anatomy, 20,* 253-257.

Thayer, J. F. (2006). On the importance of inhibition: central and peripheral manifestations of nonlinear inhibitory processes in neural systems. *Dose Response, 4,* 2-21.

Thayer, J. F. (2007). What the heart says to the brain (and vice versa) and why we should listen. *Psychological Topics, 16,* 241-250.

Thayer, J. F. & Brosschot, J. F. (2005). Psychosomatics and psychopathology: looking up and down from the brain. *Psychoneuroendocrinology, 30,* 1050-1058.

Thayer, J. F. & Friedman, B. H. (2002). Stop that! Inhibition, sensitization, and their neurovisceral concomitants. *Scandinavian Journal of Psychology, 43,* 123-130.

Thayer, J. F., Friedman, B. H. & Borkovec, T. D. (1996). Autonomic characteristics of generalized anxiety disorder and worry. *Biological Psychiatry, 39,* 255-266.

Thayer, J. F., Hall, M., Sollers III, J. J. & Fischer, J. E. (2006). Alcohol use, urinary cortisol, and heart rate variability in apparently healthy men: evidence for impaired inhibitory control of the HPA axis in heavy drinkers. *International Journal of Psychophysiology, 56,* 244-250.

Thayer, J. F. & Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *Journal of Affective Disorders, 61,* 201-216.

Thayer, J. F. & Lane, R. D. (2007). The role of vagal function in the risk for cardiovascular disease and mortality. *Biological Psychology, 74*, 224-242.

Thayer, J. F. & Lane, R. D. (2009). Claude Bernard and the heart-brain connection: further elaboration of a model of neurovisceral integration. *Neuroscience and Biobehavioral Reviews, 33,* 81-88.

Thayer, J. F. & Sternberg, E. (2006). Beyond heart rate variability: vagal regulation of allostatic systems. *Annals of the New York Academy of Sciences, 1088,* 361-372.

Theurel, J., Offret, M., Gorgeon, C. & Lepers, R. (2008). Physiological stress monitoring of postmen during work. *Work, 31,* 229-236.

Thews, G. & Vaupel, P. (2005). *Vegetative Physiologie* (5. Aufl.). Stuttgart: Thieme.

Tiscornia, O. M., Perec, C. J., Celener, D., de Lehmann, E. S., Caro, L., De Paula, J., Baratti, C. & Martínez, J. L. (1981). Trophic and functional changes of the pancreas induced by chronic truncal vagotomy. *Acta Gastroenterologica Latinoamericana, 11,* 67-86.

Tracey, K. J. (2002). The inflammatory reflex. *Nature, 420,* 853-859.

Trepel, M. (2004). *Neuroanatomie - Struktur und Funktion* (3.Aufl.). München: Urban & Fischer.

Tsuji, H., Larson, M. G., Venditti, F. J., Manders, E. S., Evans, J. C., Feldman, C. L. & Levy, D. (1996). Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. *Circulation, 94*, 2850-2855.

Tunn, S., Mollmann, H., Barth, J., Derendorf, H. & Krieg, M. (1992). Simultaneous measurement of cortisol in serum and saliva after different forms of cortisol administration. *Clinical Chemistry, 38*, 1491-1494.

Ulett, G. A., Han, S. & Han, J. S. (1998). Electroacupuncture: mechanisms and clinical application. *Biological Psychiatry, 44,* 129-138.

Vaile, J. C., Fletcher, J., Al-Ani, M., Ross, H. F., Littler, W. A., Coote, J. H. & Townend, J. N. (1999). Use of opposing reflex stimuli and heart rate variability to examine the effects of lipophilic and hydrophilic beta-blockers on human cardiac vagal control. *Clinical Science, 97,* 585-593.

Vaitl, D. & Petermann, F. (2004). *Entspannungsverfahren - Das Praxishandbuch* (3. Aufl.). Weinheim: Beltz.

van der Kooy, K. G., van Hout, H. P. J., van Marwijk, H. W. J., de Haan, M., Stehouwer, C. D. A. & Beekman, A. T. F. (2006). Differences in heart rate variability between depressed and non-depressed elderly. *International Journal of Geriatric Psychiatry, 21,* 147-150.

Van Eekelen, A. P., Houtveen, J. H. & Kerkhof, G. A. (2004). Circadian variation in base rate measures of cardiac autonomic activity. *European Journal of Applied Physiology, 93,* 39-46.

Vega, M. D., Esteller, A., Varela, G. & Murillo, A. (1977). Some aspects of vagal control of exocrine pancreatic secretion in rabbits (author's transl). *Revista Española de Fisiología, 33,* 211-215.

Vonck, K., Van Laere, K., Dedeurwaerdere, S., Caemaert, J., De Reuck, J. & Boon, P. (2001). The mechanism of action of vagus nerve stimulation for refractory epilepsy - the current status. *Journal of Clinical Neurophysiology,18,* 394-401.

Vossel, G. & Zimmer, H. (1998). *Psychophysiologie*. Stuttgart: Kohlhammer Urban.

Walsh, S. P. & Kling, M. A. (2004). VNS and depression: current status and future directions. *Expert Review of Medical Devices, 1,* 155-160.

Wang, S. & Kain, Z. N. (2001). Auricular acupuncture: a potential treatment for anxiety. *Anesthesia & Analgesia, 92,* 548-553.

Wang, J. D., Kuo, T. B. J. & Yang, C. C. H. (2002). An alternative method to enhance vagal activities and suppress sympathetic activities in humans. *Autonomic Neuroscience, 100,* 90-95.

White, A. & Ernst, E. (1999). The effect of auricular acupuncture on the pulse rate: an exploratory randomised controlled trial. *Acupuncture in Medicine, 17,* 86-88.

White, A. R., Filshie, J. & Cummings, T. M. (2001). Clinical trials of acupuncture: consensus recommendations for optimal treatment, sham controls and blinding. *Complementary Therapies in Medicine, 9,* 237-245.

Whitnall, M. H. (1990). Subpopulation of corticotrophin-releasing hormone neurosecretory cells distinguished by presence or absence of vasopressin: confirmation with multiple corticotrophin-releasing hormone antisera. *Neuroscience, 36*, 201-205.

Wirtz, P. H., Elsenbruch, S., Emini, L., Rüdisüli, K., Groessbauer, S. & Ehlert, U. (2007). Perfectionism and the cortisol response to psychosocial stress in men. *Psychosomatic Medicine, 69,* 249-255.

Wittert, G. A., Stewart, D. E., Graves, M. P., Ellis, M. J., Evans, M. J., Wells, J. E., Donald, R. A. & Espiner, E. A. (1991). Plasma corticotrophin releasing factor and vasopressin responses to exercise in normal man. *Clinical Endocrinology, 35*, 311-317.

Wong, M. L., Kling, M. A., Munson, P. J., Listwak, S., Licinio, J., Prolo, P., Karp, B., McCutcheon, I. E., Geracioti, T. D. Jr., DeBellis, M. D., Rice, K. C., Goldstein, D. S., Veldhuis, J. D., Chrousos, G. P., Oldfield, E. H., McCann, S. M. & Gold, P. W. (2000). Pronounced and sustained central hypernoradrenergic function in major depression with melancholic features: relation to hypercortisolism and corticotropin-releasing hormone. *Proceedings of the National Academy of Sciences of the United States of America, 97*:325–30.

Woodside, D. B., Winter, K. & Fisman, S. (1991). Salivary cortisol in children: correlations with serum values and effect of psychotropic drug administration. *Canadian Journal of Psychiatry / Revue Canadienne de Psychiatrie, 36*, 746-748.

Wu, J. H., Chen, H. Y., Chang, Y. J., Wu, H. C., Chang, W. D., Chu, Y. J. & Jiang, J. A. (2008). Study of autonomic nervous activity of night shift workers treated with laser acupuncture. *Photomedicine and Laser Surgery, 27*, 273-279.

Wu, S. D. & Lo, P. C. (2008). Inward-attention meditation increases parasympathetic activity: a study based on heart rate variability. *Biomedical Research, 29,* 245-250.

Yamamoto, Y., Lamanca, J. J. & Natelson, B. H. (2003). A measure of heart rate variability is sensitive to orthostatic challenge in women with chronic fatigue syndrome. *Experimental Biology and Medicine, 228,* 167-174.

Yin, J., Levanon, D. & Chen, J. D. Z. (2004). Inhibitory effects of stress on postprandial gastric myoelectrical activity and vagal tone in healthy subjects. *Neurogastroenterology and Motility, 16,* 737-744.

Yoshino, K. & Matsuoka, K. (2005). Causal coherence analysis of heart rate variability and systolic blood pressure variability under mental arithmetic task load. *Biological Psychology, 69,* 217-227

Yoshiuchi, K., Quigley, K. S., Ohashi, K., Yamamoto, Y. & Natelson, B. H. (2004). Use of time-frequency analysis to investigate temporal patterns of cardiac autonomic response during head-up tilt in chronic fatigue syndrome. *Autonomic Neuroscience: Basic and Clinical, 113,* 55-62.

Zajonc, R. B. (1981). A one factor mind about mind and emotion. *American Psychologist, 36*, 102-103.

Zajonc, R. B. (1984). Emotion and facial efference: a theory reclaimed. *Science, 228*, 15-21.

Zanstra, Y. J., Schellenkens, J. M. H., Schaap, C. & Kooistra, L. (2006). Vagal and sympathetic activity in burnouts during a mentally demanding workday. *Psychosomatic Medicine, 68,* 583-590.

Zeskind, P. S. & Gingras, J. L. (2006). Maternal cigarette-smoking during pregnancy disrupts rhythms in fetal heart rate. *Journal of Pediatric Psychology, 31,* 5-14.

Zhang, W. P. (2006). Effects of acupuncture for dispersing fei, invigorating pi and reinforcing shen on heart rate variability and pulmonary function in bronchial asthma patients. *Zhongguo Zhong xi yi jie he za zhi Zhongguo Zhongxiyi jiehe zazhi, 26,* 799-802.

Zhang, W. P. (2007). Effects of acupuncture on the pulmonary function and heart rate variability in different state of bronchial asthma. *Zhen Ci Yan Jiu, 32,* 42-48.

Zobel, A., Joe, A., Freymann, N., Clusmann, H., Schramm, J., Reinhardt, M., Biersack, H.J., Maier, W. & Broich, K. (2005). Changes in regional cerebral blood flow by therapeutic vagus nerve stimulation in depression: an exploratory approach. *Psychiatry Research, 139*, 165-179.