Central modulators of human pain:

Effects of oxytocin, exam stress, breathing exercises and transcranial magnetic stimulation

Kumulative Inaugural-Dissertation zur Erlangung der Doktorwürde
der Philosophischen Fakultät II (Psychologie, Pädagogik und Sportwissenschaft)
der Universität Regensburg,

vorgelegt in englischer Sprache von

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aus Traunwalchen
am 28.05.2014.

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Regensburg 2014

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PREFACE

This cumulative dissertation comprises four studies investigating pain processing within the human brain. A short overview of these studies is provided on page 7. From here on, the four studies are referred to as Studies 1, 2, 3 and 4 in chronological order, starting with the latest. All studies have been published in peer-reviewed journals within the last four years. All manuscripts are reproduced in their last pre-print version with permission from the publishers. All manuscripts were adapted to the formatting and orthography recommended by the Publication Manual of the American Psychological Association (2010) for consistency.

The four separate reference sections were integrated into one bibliography at the end of the thesis. Tables, figures and appendices were renumbered. Otherwise the manuscripts were not changed.

A number of people have made this work possible. They are given due credit on page 5. The contributions of co-authors to the four studies are further detailed on page 8. My colleagues and I have completed a number of related studies during my doctoral studies, which are not included in this dissertation, but worth mentioning:

- In parallel with Study 1, we investigated the effects of oxytocin on emotional pain modulation and its brain correlates. The study is currently in preparation for publication.
- In parallel with Study 2, we assessed the effects of exam stress on sleep and legal drug consumption. The results have been submitted for publication, but do not match the topic of this dissertation and were therefore not included.
- Before Study 3, we completed and published an experiment testing the effects of acupuncture on motor system excitability (Zunhammer, Eichhammer, Franz, Hajak, & Busch, 2012). Main contributor was Dr. med. Johanna Franz, who also wrote her doctoral thesis on the topic.

EIDESSTATTLICHE VERSICHERUNG

Ich erkläre hiermit an Eides Statt, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Die aus anderen Quellen direkt oder indirekt übernommenen Daten und Konzepte sind unter Angabe der Quelle gekennzeichnet.
Bei der Auswahl und Auswertung des Materials haben mir die auf der Seite 8 aufgeführten Personen in der jeweils detaillierten Weise unentgeltlich geholfen. Weitere Personen waren an der inhaltlich-materiellen Erstellung der vorliegenden Arbeit nicht beteiligt. Insbesondere habe ich hierfür nicht die entgeltliche Hilfe von Vermittlungs- beziehungsweise Beratungsdiensten (Promotionsberater oder anderer Personen) in Anspruch genommen. Niemand hat von mir unmittelbar oder mittelbar geldwerte Leistungen für Arbeiten erhalten, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen.
Die Arbeit wurde bisher weder im In- noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt.
Ich versichere an Eides Statt, dass ich nach bestem Wissen die reine Wahrheit gesagt und nichts verschwiegen habe. Vor Aufnahme der obigen Versicherung an Eides Statt wurde ich über die Bedeutung der eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unrichtigen oder unvollständigen eidesstattlichen Versicherung belehrt.

Die Eidesstattliche Erklärung zu dieser Dissertation wurde in Regensburg am 14.05.2014 von Matthias Zunhammer gegeben und vom Fakultätsverwalter Peter Grimm aufgenommen. Die signierte Eidesstattlichen Versicherung wird vom Dekanat der Fakultäten für Sprach-, Literatur- und Kulturwissenschaft sowie Psychologie, Pädagogik und Sportwissenschaft verwahrt.

DANKSAGUNG

Über mehr als vier Jahre wurde ich von meinem Doktorvater Prof. Mark W. Greenlee und meinem Mentor am Klinikum Prof. Peter Eichhammer auf den Weg in die akademische Welt unterstützt und gefördert. Dafür möchte ich Ihnen aus ganzem Herzen danken. Für die ständige Erreichbarkeit, die schnellen Korrekturen, die Kongressteilnahmen und den vertrauensvollen Spielraum.
Berthold Langguth möchte ich für den Start in die Welt der Versuchsplanung, der akademischen Publikationen und der aufregenden Kongressreisen danken, genau so Volker Busch, Katharina Rosengart und Anton Beer für die vielen Diskussionen und die entscheidende Hilfe mit der verflixten Methodik. Besonderer Dank gilt Sandra Geis, Hanna Eberle, Sonja Blumenstock und Johanna Franz, die mir im Rahmen ihrer Abschlussarbeiten/Praktika geholfen und so viele Stunden Arbeitszeit geopfert haben. Für die bitter nötige Hilfe bei der Beantragung einer klinischen Studie nach Arzneimittelgesetz danke ich: Florian Zeman und dem Zentrum für Klinische Studien am Uniklinikum Regensburg, Ralph Heimke-Brinck von der Apotheke des Uniklinikums Erlangen, Jörg Pfeiffer von der Hausapotheke des Bezirksklinikums, Zaven Orfalian und Vincenco Giordano von der Firma Sigma Tau, sowie Prof. Dr. Thomas Baghai.
Dank an dieser Stelle auch den weit über 200 Probanden, die das Vertrauen aufgebracht haben im Rahmen dieser Arbeit an einer Schmerzstudie teilzunehmen. Der Studienstiftung des deutschen Volkes danke ich für das viele Geld; ohne sie wäre ich mittendrin ohne Finanzierung dagestanden und hätte viele inspirierende Menschen nicht kennengelernt. Zuletzt danke ich meinen Eltern für die Liebe und für die geduldigen Antworten auf das ständige „Warum?“.

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Status:	Accepted for publication by Psychosomatic Medicine, as of Oct 16th, 2014. © 2014 American Psychosomatic Society, Lippincott Williams & Wilkins. Reprinted with permission.	
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Authors:	Zunhammer, M, Eberle, H, Eichhammer, P, Busch, V.	
Status:	Published 2013 in PLOS ONE, 8(e84911). © Authors retain copyright.	
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Authors:	Zunhammer, M, Busch V, Griesbach, F, Landgrebe M, Hajak, G, Langguth, B.	
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ABSTRACT

The available means to control human pain are insufficient, novel mechanisms of pain modulation must be explored and understood. This cumulative dissertation comprises four studies, which explored potential means to modulate pain in the central nervous system. An overview on the current understanding of pain, its basic mechanisms, and its known modulators is provided.

Study 1 tested if a high intranasal dose of the neuro-hypophyseal hormone oxytocin affected perception and processing of thermal pain in 36 healthy male volunteers. Experimental pain thresholds were obtained and a functional Magnetic Resonance Imaging experiment with ratings of noxious heat was conducted. Oxytocin was found to reduce ratings of perceived heat intensity and amygdala activity. Both effects were small and independent of temperature. Although the hypothesis of an antinociceptive effect of oxytocin could not be confirmed, the study provides first evidence for effects of oxytocin on thermal stimulus processing.

Study 2, a longitudinal questionnaire study, examined the effects of a period of academic exam stress on bodily symptoms in 150 students. Various symptoms of pain, as well as gastro-intestinal and autonomic complaints were found to increase during exam stress. Neuroticism, but not alexithymia, trait anxiety, or depression explained symptom increases under exam stress. Study two offers the first comprehensive, quantitative description of bodily symptoms under exam stress. Neuroticism was identified as a potential predisposing personality factor for the occurrence of bodily symptoms under stress.

Study 3 aimed at elucidating physiological mechanisms behind the proposed antinociceptive effects of slow breathing exercises in 20 healthy participants. Breathing frequency, heart rate variability, and hyperventilation were not found to predict changes in experimental pain thresholds or heat pain ratings. A correlation between heart rate at baseline and pain ratings could be observed, confirming that autonomic nervous system function and pain are intertwined.

Study 4 explored and tested if and how repetitive transcranial magnetic stimulation (rTMS) over the cerebellum affected thermal pain thresholds in two separate experiments. Although pain-relieving effects of cerebellar rTMS could be found in a first experiment, the second experiment showed that these effects were driven by peripheral mechanisms and/or the placebo effect. The study highlights the importance of proper experimental control conditions when investigating central modulators of pain.

In a concluding discussion, the current methodology of pain research is reviewed. The methods used for this dissertation are discussed and limitations are identified; findings are summarized and further directions of research are highlighted.

CONTRIBUTIONS

	Contributors
Study 1	**Effects of intranasal oxytocin on thermal pain in healthy males — a randomized fMRI study**
Type	Double-blind clinical trial; three sessions within 36 participants
Study idea:	Zunhammer, Eichhammer
Study design:	Zunhammer, Eichhammer, Greenlee
Data acquisition:	Zunhammer, Geis, Busch
Statistical analysis:	Zunhammer
Manuscript writing:	Zunhammer
Manuscript revision:	Geis, Busch, Eichhammer, Greenlee
Study supervision:	Eichhammer, Greenlee
Study 2	**Somatic symptoms evoked by exam stress in university students: the role of alexithymia, neuroticism, anxiety, and depression.**
Type	Longitudinal survey; three time-points within 150 participants
Study idea:	Zunhammer, Busch, Eichhammer
Study design:	Zunhammer, Busch
Data acquisition:	Zunhammer, Eberle
Statistical analysis:	Zunhammer
Manuscript writing:	Zunhammer
Manuscript revision:	Eberle, Busch, Eichhammer
Study supervision:	Busch, Eichhammer
Study 3	**Do cardiorespiratory variables predict the antinociceptive effects of deep and slow breathing?**
Type	Experimental study; four sessions within 20 participants
Study idea:	Zunhammer, Busch, Eichhammer
Study design:	Zunhammer, Busch
Data acquisition:	Zunhammer
Statistical analysis:	Zunhammer
Manuscript writing:	Zunhammer
Manuscript revision:	Busch, Eichhammer
Study supervision:	Busch, Eichhammer
Study 4	**rTMS over the cerebellum modulates temperature detection and pain thresholds through peripheral mechanisms**
Type	Experimental study; two experiments with 10 + 12 participants
Study idea:	Langguth
Study design:	Langguth, Griesbach (part one), Zunhammer (part two)
Data acquisition:	Griesbach (part one), Zunhammer (part two)
Statistical analysis:	Zunhammer, Langguth
Manuscript writing:	Zunhammer (~60%), Langguth (~40%)
Manuscript revision:	Langguth, Griesbach, Landgrebe, Hajak, Busch
Study supervision:	Langguth

ABBREVIATIONS

3-T	3-Tesla
AICc	Akaike's Information Criterion for finite sample sizes
ANS	autonomic nervous system
AR1	first-degree autoregressive model
ATP	adenosine triphosphate
BDI-II	Beck's Depression Inventory, revised version
BOLD	blood-oxygen dependent
Bonf.	Bonferroni (correction procedure for multiple comparisons)
°C	degree Celsius
CA	California
CCK	cholecystokinin
CDT	cold detection threshold
CGRP	calcitonin gene-related peptide
CI	confidence interval
cm	centimeter
CNS	central nervous system
CO_2	carbon-dioxide
CPT	cold detection threshold
CSF	cerebrospinal fluid
DIS	disorders reported
DLPFC	dorsolateral prefrontal cortex
DNIC	diffuse noxious inhibition controls
doi	digital online identifier
DSB	deep and slow breathing
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders 4th edition
e.g.	exempli gratia, for example
ECG	electrocardiogram
EPI	echo-planar imaging
EUDRA-CT	European Clinical Trials Database
F	F-value; standardized deviation from reference (Fisher-Snedecor distribution)
f	female
FIR	finite impulse response
fMRI	functional Magnetic Resonance Imaging
FoV	Field of View
FWE	family-wise error
FWHM	full-width at half maximum
GABA	gamma-aminobutyric acid
GLM	general linear model
h	hour
HPT	heat detection threshold
HRF	hemodynamic response function
Hz	Hertz
i.e.	id est, that is
I.U.	international unit (WHO standard unit for hormone dose based on a biological effect)

ICC	intra-class correlation coefficient
ICD-10	International Classification of Diseases 10th revision
IL	Illinois
KCNK	potassium channel subfamily K
L	left
LASSO-PCR	least absolute shrinkage and selection operator-regularized principal components regression
m	meter
m	male
MANOVA	multivariate analysis of variance
MES	medically explained symptom
mg	milligram
min	minute
ml	milliliter
mm	millimeter
MNI	Montreal Neurological Institute
MP-RAGE	Magnetization Prepared Rapid Gradient Echo
MR	magnetic resonance
MSO	maximum stimulator output
MUS	medically unexplained symptom
n	number of, (number of cases in subsample)
N	number of, (number of cases in total sample)
NEO-FFI	Big-Five Personality Interview
noDIS	no disorders reported (factor level of DIS in analysis)
noMES	no evidence for MES (factor level of MES in analysis)
NTS	nucleus of the solitary tract
p	p-value; probability of obtaining a statistic at least as extreme as the observed
p.	page
PAG	periaqueductal gray
pCO_2	carbon-dioxide partial pressure
PET	positron emission tomography
PFC	prefrontal cortex
pH	"power of hydrogen", unit for acidity/basicity (hydronium ion concentration)
PLOS	Public Library of Science
pp.	pages
PSQ-20	Perceived Stress Questionnaire 20-item version
PSQ-30	Perceived Stress Questionnaire 30-item version
QST	Quantitative Sensory Testing
R	right
r	Pearson correlation coefficient
rMS	repetitive magnetic stimulation (of peripheral tissue)
RMSSD	root mean square of successive differences (in R-R-interval)
ROI	region of interest
RR	R-wave-to-R-wave interval of the cardiac rythm
rTMS	repetitive transcranial magnetic stimulation
s	second
s.p.a.	Società per Azioni

SD	standard deviation
SDRR	standard deviation of the RR-interval
SEM	standard error of mean
SI, SII	somatosensory cortex I and II, also: primary and secondary somatosensory cortex
SOMS-7d	Screening for Somatoform Symptoms 7-day version
SPECT	single photon emission computed tomography
SPM	statistical parametric mapping
STAI-G-X1	State-Trait Anxiety Inventory – State Form, German Version
STAI-G-X2	State-Trait Anxiety Inventory – Trait Form, German Version
t	t-value; standardized deviation from reference (Student's t-distribution)
T1	longitudinal (spin-lattice relaxation time
T2*	transverse (spin-spin) relaxation time, including magnetic field inhomogeneity
TAS-20	Toronto-Alexithymia Scale 20-item version
TE	echo-time
TENS	transcutaneous electrical nerve stimulation
TGF-beta	tumor growth factor beta
TMS	transcranial magnetic stimulation
TR	time of repetition
TRAAK	outdated acronym; synonym for: potassium channel subfamily K
TREK	outdated acronym; synonym for: potassium channel subfamily K
TrkA	Tropomyosin related kinase A
TRP	transient receptor potential (channel)
TRPA1	transient receptor potential channel ankyrin subtype 1
TRPM8	transient receptor potential channel melastatin subtype 8
TRPV1-4	transient receptor potential vanilloid channel subtype 1-4
TSA	thermo-sensory analyzer
UK	United Kingdom
USA	United States of America
VAS	Visual Analog Scale
WDT	warmth detection threshold
WMA	World Medical Association
yesDIS	stable past or chronic disorders (factor level of DIS in analysis)
yesMES	evidence for MES (factor level of MES in analysis)
Z	Z-value; standardized deviation from reference (normal distribution)
α	Type-I (or alpha) error level. see: p
β	unstandardized parameter estimate (in regression analysis), but *also*: Type-II error level (in power analysis)
Δ	deviation in
τ	Kendall's τ-b coefficient of non-parametric correlation

INTRODUCTION

Rationale, aims and hypotheses

All studies of this thesis have a common motivation: The World Health Organization (2011) ranks "back and neck pain" as the 4th leading cause of disability adjusted life years in Europe. Chronic pain is a burden for the individual sufferer and the social welfare systems. Most patients are diagnosed with idiopathic pain, i.e. pain of unknown physiological cause (McMahon & Wall, 2013, pp. 233–247). A considerable proportion of surgery patients suffer from chronic pain long after the acute recovery phase (Perkins & Kehlet, 2000). The available pharmacological and non-pharmacological treatments are limited in their effectiveness and leave many non-responders with insufficient relief of pain (Moore, Derry, & Wiffen, 2013). Despite evidence suggesting that chronic pain is influenced by behavior, beliefs, emotions, motivation, and stress (Gatchel, Polatin, & Kinney, 1995; Wiech & Tracey, 2013) the psychological aspects of pain are complex and insufficiently understood. In this situation the study of central pain modulators can help to comprehend the mechanisms of pain and thus help to develop new and better treatments. The present dissertation is concerned with mechanisms of pain modulation from a neuropsychiatric perspective and a focus on stress-related phenomena. Four means of pain modulation were investigated:

- **Study 1**, the main study of this thesis, was employed to test a novel pharmacological approach: Intranasal oxytocin has been shown to modulate stress, anxiety, and social cognition in a multitude of studies (Guastella & MacLeod, 2012; Kubzansky, Mendes, Appleton, Block, & Adler, 2012). Oxytocin was repeatedly found to affect amygdala activity (Bethlehem et al., 2013). Moreover, Rash, Aguirre-Camacho, & Campbell, (2013) recently suggested that oxytocin might have antinociceptive actions, based on a review of animal and human results.

  Study 1 tested if the hormone oxytocin, given as a nasal spray has antinociceptive effects. Effects of oxytocin within the brain were investigated by functional magnetic resonance imaging (fMRI). We hypothesized that intranasal oxytocin should:

  a) increase noxious thermal pain thresholds;
  b) decrease ratings of noxious heat intensity and/or unpleasantness;
  c) alter blood oxygen dependent (BOLD) signal changes related to the processing of the thermal stimulus on whole brain level;
  d) alter stimulus processing in the amygdala.
- **Study 2** focused on the phenomenon of somatization, i.e. bodily complaints in response to psychosocial stress: This questionnaire survey investigated how academic exam stress impacts on reports of pain and other everyday symptoms in university students. In addition, personality factors contributing to the development of symptoms were assessed.

  Our aim was to provide a detailed quantitative description of somatic symptom increases under exam stress. We hypothesized that symptoms of pain and other bodily complaints would increase during an exam period and return to baseline after a period without exams.

  We further aimed to compare the personality traits alexithymia, neuroticism, trait anxiety and depression with respect to their ability to explain increases in somatization under exam stress.

According to the stress-alexithymia hypothesis, a stronger association of somatization symptoms with alexithymia than with neuroticism, trait anxiety and depression was expected.

- **Study 3** followed up hypotheses on the mechanisms of slow breathing, a widespread alternative treatment for stress and pain control (Martin et al., 2012; Zautra et al., 2010): It was experimentally tested if breathing exercises modulate pain perception via breathing frequency, hyperventilation, or cardiac autonomic control, as suggested by Chalaye, Goffaux, Lafrenaye, & Marchand (2009). According to this hypothesis, measures of heart rate variability, such as the standard deviation of heart rate, were expected to explain a significant amount of variance in thermal pain thresholds and/or pain ratings.
- **Study 4** aimed to modulate pain perception experimentally by stimulating the cerebellum with repetitive Transcranial Magnetic Stimulation (rTMS). This non-invasive brain stimulation technique was previously shown to modulate thalamo-cortical excitability (Fierro et al., 2007).

  A two-step study was performed: Cerebellar target regions and frequencies of rTMS were explored for potential antinociceptive effects in a first experiment. Based on the results of the first experiment, it was hypothesized that 1 Hz stimulation over the lateral cerebellum would significantly decrease thermal detection and pain thresholds, compared to sham stimulation over the cerebellum and 1 Hz verum stimulation over the peripheral neck. A second experiment was then performed to test this hypothesis.

Before these four studies are presented in detail, background information on the current knowledge on the processing of acute pain is provided. Information with relevance to the four studies of this dissertation will be highlighted in grey boxes.

Background

The concept of pain anno 2014

Pain is defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (International Association for the Study of Pain, 2002, p. 210). This definition implies that pain is not only a sensory but also an affective phenomenon with multiple dimensions, to which motivation, memory, and other cognitive processes must be added (Wiech & Tracey, 2013). The definition further underscores the subjective nature of pain, "which is always a psychological state, even though we may well appreciate that pain most often has a proximate physical cause" (International Association for the Study of Pain, 2002, p. 210). This definition pays regard to the fact that many disorders of pain are characterized by the lack of a detectable physiological cause and that "resultant pain is not necessarily related linearly to the nociceptive drive or input" (Tracey & Mantyh, 2007). Pain ultimately is a product of the brain and its function.

Peripheral nociception

The current definition of pain emphasizes the psychological nature of pain, but also acknowledges its usual bottom-up causes. Especially the understanding of the latter has advanced within the last decade:

Transducer proteins are the sensors detecting harmful mechanical, thermal, and chemical signals on cellular level (for review see: Dubin & Patapoutian, 2010). These proteins are ion channels, located in the cellular membranes of so-called nociceptors, i.e. neurons dedicated to the perception of pain (for review see: Woolf & Ma, 2007). Transducer proteins are specifically located in the highly branched unmyelinated endings of nociceptors (Dubin & Patapoutian, 2010). When activated, transducer proteins generate transient membrane potentials, which can cause neuronal discharge of the nociceptor (Dubin & Patapoutian, 2010). Accordingly, the main family of pain transducers was named "transient receptor potential" (TRP) channel family (Julius, 2013). The existence of a machinery specific to pain detection rebuts the historical "intensity theory of pain" (Prescott & Ratté, 2012), which claimed that pain results from an "overstimulation" of somatosensory neurons rather than a distinct sensory machinery (e.g. Darwin, 1796; for review see: Mendell, 2013).

Studies 1, 3 and 4 involved experimental heat and cold stimulation in the painful range. While the stimulation procedures were used to examine central pain processes, the following known TRP-family members were likely involved in mediating the observed effects in the periphery (for review see: McMahon & Wall, 2013, pp. 1–47):

The "vanilloid" TRP channel subtypes TRPV3 and TRPV4 show optimal response characteristics below 43 °C and therefore might be responsible for the detection of non-noxious heat (Schepers & Ringkamp, 2010). Heat above this temperature is known to activate the subtypes TRPV1 and TRPV2, which are acknowledged transducers of heat pain (Julius, 2013; McMahon & Wall, 2013, pp. 1–47). For non-noxious cold the candidate transducers are the TRPV4 (again), and the "melastatin" TRP subtype TRPM8 (Schepers & Ringkamp, 2010). The "ankyrin" subtype TRPA1 has been suggested as the transducer for cold pain temperatures below 17 °C (Schepers & Ringkamp, 2010). Other proteins may play a role in noxious hot and

cold transduction as well, but are less understood, e.g. the transducer proteins of the potassium channel subfamily K (KCNK, also known as TREK/TRAAK), who have been shown to contribute to thermal pain perception, (Dubin & Patapoutian, 2010).

Injuries and burns induce local sensitization within the skin. Immune cells, mast cells, keratinocytes, as well as nociceptors (Dubin & Patapoutian, 2010) of affected tissues release a noxious "soup" (Woolf & Ma, 2007), consisting of substances such as: hydroxonium ions (low pH), Adenosine triphosphate (ATP), glutamate, serotonin, bradykinin, interleukins, neurotrophins and prostaglandins (Mense, 2009). While some of these chemicals can directly activate transducers proteins and elicit nociceptor activation, most of them have a local sensitizing effect, especially when acting in combination (Julius, 2013; McMahon & Wall, 2013, pp. 1–47). The repeated and prolonged experimental thermal stimulation in **Studies 1, 3 and 4** might have caused such sensitization.

Primary nociceptive neurons

Nociceptors are pseudo-unipolar afferent neurons with soma located in the dorsal-root ganglia of the spinal cord that detect noxious signals within their receptive field and convey them to the central nervous system (CNS, Dubin & Patapoutian, 2010; McMahon & Wall, 2013, pp. 2–11). Two morphologically different nociceptive fiber classes can be found in humans: Small, myelinated nociceptive axons are classified as "Aδ-type"; they have conduction velocities between 5 and 30 m/s. Nociceptors with unmyelinated axons are classified as "C-type" and have relatively slow conduction velocities between 0.4 and 1.4 m/s (Dubin & Patapoutian, 2010). Both fiber types have been subject to several sub-classifications by researchers, according to target, function, and molecular features (McMahon & Wall, 2013, pp. 2–11). For brevity only the most prevalent sub-classifications are presented: Cutaneous nociceptors innervating the skin and visceral nociceptors innervating internal organs are target-specific sub-classifications (McMahon & Wall, 2013, pp. 2–11). Examples of function-specific sub-classifications are "mechano-insensitive", "heat-sensitive", "heat-insensitive", "cold-sensitive", "cold-insensitive" and "polymodal" Aδ- and C-nociceptors (Ringkamp et al., 2001; Schepers & Ringkamp, 2010). Importantly, a mechano-sensitive C-fiber subtype not involved in nociception, but in the detection of gentle/social touch has been described (Olausson, Wessberg, Morrison, McGlone, & Vallbo, 2010). The main molecular classification divides Aδ-, as well as C-fibers, into "peptidergic" and "non-peptidergic" subgroups (Woolf & Ma, 2007). The peptidergic neurons contain neuropeptides like Substance P, calcitonin gene-related peptide (CGRP), and somatostatin; they are ontogenetically dependent of the Tropomyosin related kinase A (TrkA) pathway (Woolf & Ma, 2007). The non-peptidergic neurons do not produce neuromodulatory signaling-peptides in significant amounts and are ontogenetically dependent on the expression of a receptor tyrosine kinase "Ret" (Woolf & Ma, 2007). Differences of peptidergic and non-peptidergic nociceptors in terms of target tissue innervation, signal relay in the spine, and transducer protein expression have been described, but are still a matter of ongoing research. (McMahon & Wall, 2013, pp. 6–7; Woolf & Ma, 2007) The fact that these classifications overlap and that these overlaps differ between model organisms (mouse, rat, cell culture, human), tissue types (glabrous skin, hairy skin, muscles vessels, viscerae), and even stimulation protocols (fast and slow ramping stimuli, high and low stimulus intensities) leads to a confusing picture (McMahon & Wall, 2013, pp. 2–11). An illustrative example is the often cited notion that Aδ-fibers convey the "fast and sharp early pain" and C-fibers convey the "slow and burning pain" (Campbell & LaMotte, 1983): It is much less noted that the original report found this distinction

only for the hairy skin of the lower arm, but not for the glabrous skin of the hand (Campbell & LaMotte, 1983). Further, the distinction of "fast and early" and "slow and burning" was based on the transduction velocity of the fibers (Campbell & LaMotte, 1983). However, there are both slow- and fast-adapting C-fibers *and* Aδ-fibers, as well as Aδ-fibers with delayed-onset. (McMahon & Wall, 2013, pp. 2–11; Schepers & Ringkamp, 2010; Treede, Meyer, Raja, & Campbell, 1995)

The experimental thermal stimulation in **Studies 1, 3 and 4** most likely activated both Aδ- and C-fibers. Nociceptive signaling during the thresholding procedures may have been dominated by fibers with no latency, and low activation thresholds, since the stimuli involved were of short duration and low intensity. Slow-adapting, late onset fibers might have contributed to the pain rating procedures used in **Studies 1 and 3**, which involved prolonged stimulation. As discussed above, the relative contributions of Aδ- and C-fiber (sub-)types to the observed effects are difficult to judge. However, the use of repeated stimuli and of pre-conditioning stimuli for the pain rating procedures might have promoted C-fiber signaling over Aδ-fiber signaling according to Hashmi & Davis (2008).

Spinal pain processing and descending brainstem control

Aδ-fibers and C-fibers from the periphery do not project directly to the brain via axonal collaterals, like non-nociceptive afferents (McMahon & Wall, 2013, pp. 77–84), but connect with neurons in the dorsal horn of the spine[1]: Nociceptive C-fibers mainly terminate in laminae I and II of the dorsal horn, while nociceptive Aδ-fibers mainly terminate in laminae I, II and V; visceral nociceptors tend to connect to deeper laminae (McMahon & Wall, 2013, pp. 77–84). Within the dorsal horn, primary nociceptors pass their signals on to interneurons and/or projection neurons (secondary nociceptive neurons) by releasing the excitatory neurotransmitter glutamate and signaling peptides such as Substance P (Millan, 2002; Todd, 2010). The interneurons accomplish an immediate, spinal-level pre-processing of the incoming nociceptive information (Todd, 2010). Around 30 % of them release inhibitory (GABA, glycine) neurotransmitters, while the others are thought to release excitatory (glutamate) neurotransmitters (Todd, 2010). The interneurons modulate the activity of primary nociceptive neurons, projection neurons, and other interneurons (McMahon & Wall, 2013, pp. 87–88). The axons of the projection neurons decussate to the contralateral spinal hemisphere and then project to the brain via the anterolateral system (Kandel, Schwartz, Jessell, Siegelbaum, & Hudspeth, 2013, pp. 493–495). It is still a matter of dispute if the information from different nociceptor subtypes is relayed to the brain in a "labeled lines" fashion (specificity theory), or if information from different nociceptors is combined at spinal level first (combinatory theory, for a discussion see: Prescott & Ratté, 2012)

A number of brainstem nuclei, such as the periaqueductal gray (PAG), the nucleus of the solitary tract (NTS), and the nucleus cuneiformis receive direct input from secondary nociceptive neurons (for review see: Todd, 2010; Tracey & Mantyh, 2007). These and other brainstem regions can provide modulatory feedback to the spinal level, e.g. modulation of spinal presynaptic primary and postsynaptic secondary nociceptor activity can be achieved by descending

[1] Cranial nerves terminate in homologous regions of the trigeminal nucleus of the brainstem.

projections from the nucleus raphe (serotonin), the locus coeruleus (norepinephrine), and most importantly the rostral ventro-medial medulla (GABA, for review see: Millan, 2002).
The majority of projection neurons relay their information to the ventral posterio-lateral thalamus and other posterior thalamic nuclei (Millan, 2002; Gauriau & Bernard, 2004). These thalamic nuclei have been suggested to convey the sensory-discriminative domain of pain (Gauriau & Bernard, 2004). Further, intra-laminar nuclei of the thalamus receive nociceptive input and were suggested to mediate arousal and motivation (Gauriau & Bernard, 2004). Besides the thalamus, two other forebrain structures receive direct input from secondary nociceptive neurons: The amygdala and hypothalamus (Millan, 2002). These connections are thought to mediate pain-related emotional and autonomic reactions, consistent with the central roles of these regions in anxiety and bodily homeostasis (Millan, 2002).

The magno- and parvo-cellular nuclei of the paraventricular hypothalamus are the main source of oxytocin in the body (Lee, Macbeth, Pagani, & Young, 2009). From here, oxytocinergic neurites project to the posterior pituitary gland, diverse brain regions and the spinal cord (Lee, Macbeth, Pagani, & Young, 2009). The projections to the spinal cord have been suggested to be involved in anti-nociception in animal studies (Millan, 2002; Rash et al., 2013). Following intranasal application, oxytocin and other neuropeptides were found in increased amounts within the cerebrospinal fluid at spinal level (Born et al., 2002; Striepens et al., 2013). The modulation of spinal nociceptive signalling therefore poses one potential mechanism of action of intranasal oxytocin in **Study 1**.

Stress is partly mediated by the autonomic nervous system (ANS), with its antagonistic sympathetic and parasympathetic branches. Especially the sympathetic branch is known as a potent modulator of pain perception (Millan, 2002). Of the brain regions known to control the sympathetic branch, almost all receive direct or indirect nociceptive input from spinal levels: The hypothalamus, the NST, the parabranchial nucleus, the locus coeruleus, and the ventrolateral medulla (compare: Kandel, Schwartz, Jessell, Siegelbaum, & Hudspeth, 2013, pp. 1069–1076 and Millan, 2002). These regions can directly modulate pain perception via descending noradrenergic fibers (locus coeruleus) and via controlling the spinal intero-mediolateral cell column, which in turn modulates spinal pain processing via noradrenergic signaling (Millan, 2002). Further, sympathetic effectors can impact on pain via systemic effectors, e.g. epinephrine release from the adrenal medulla, which can modulate inflammatory processes at peripheral and visceral sites (Dhabhar, 2009). These and other interactions of ANS control and pain processing might underlie the symptoms reported by our participants in **Study 2**.

The nervus vagus is a main source of pre-ganglionic parasympathetic effector neurons (Kandel, Schwartz, Jessell, Siegelbaum, & Hudspeth, 2013, pp. 1069–1076). Its pre-ganglionic axons originate from a system of brainstem nuclei: (1) the dorsal vagal motor nucleus (2) the nucleus ambiguus, (3) the NTS, and (4) the spinal part of the trigeminal nucleus (Kandel et al., 2013, p 1025). The NTS not only receives secondary nociceptor input, but is also part of the dorsal respiratory group and gathers information from pulmonary stretch receptors (Kandel et al., 2013, p 1032). Due to the anatomical co-localization a modulatory effect of breathing on pain-related processes in the NTS seems plausible. Moreover, breathing exercises were hypothesized to modulate ANS activity through cardiac vagal control (Chalaye et al., 2009), which is coordinated centrally in the dorsal vagal motor nucleus and the nucleus ambiguus. **Study 3** was

designed to test whether breathing frequency, or autonomic cardiac control are related to the proposed efficacy of breathing exercises as a behavioral intervention for pain relief.

Pain processing in the forebrain

Corresponding to the multidimensional nature of pain, not one, but several cerebral regions are thought to underlie pain processing (Tracey, 2008). The thalamus distributes nociceptive information to the cortex. Main targets of nociceptive input from the thalamus are the insula, the somatosensory cortices I and II (SI, SII), as well as the cingulate cortex (McMahon & Wall, 2013, pp. 111–128). Further, prefrontal areas–e.g. the dorsolateral prefrontal cortex (DLPFC)– supplementary motor areas, basal ganglia, and the cerebellum (Duerden & Albanese, 2013) are thought to play a role in pain processing. All of these regions could be identified by neuroimaging studies (for review see: Apkarian, Bushnell, Treede, & Zubieta, 2005; Duerden & Albanese, 2013) and were termed in the past as the "pain matrix" (May, 2007). Attempts have been made to establish functional subdivisions of the pain matrix (May, 2007; Iannetti & Mouraux, 2010): e.g. brain regions have been divided into a "medial pain system" (medial/intralaminar thalamic nuclei, anterior cingulate cortex, amygdala, and hypothalamus) and a "lateral pain system" (ventral posterolateral thalamus, SI, and SII). The medial system has been suggested to mediate affective, motivational and somatic aspects of pain, while the lateral system has been suggested to process discriminative and sensory aspects of pain (May, 2007; Iannetti & Mouraux, 2010). The insula was proposed to be part of both systems (May, 2007). However, the term pain matrix has been criticized: According to Iannetti & Mouraux (2010) the concept is ill defined and has been interpreted differently by different authors. Iannetti & Mouraux (2010) particularly question if all brain regions identified in neuroimaging studies are pain-specific and necessary for the pain experience, as implied by the term "pain matrix". This view is supported by a meta-analysis of Cauda et al. (2012).

Nevertheless, for most regions commonly associated with pain in neuroimaging studies specific functional hypotheses have been proposed: SI and SII are thought to process location, controllability, and intensity of painful and non-painful somatosensation (Helmchen, Mohr, Erdmann, Binkofski, & Büchel, 2006; Mazzola, Isnard, & Mauguière, 2006; McMahon & Wall, 2013, pp. 112-118). The insula has been suggested to serve as a monitor of bodily states and to be involved in magnitude estimation per se (Sterzer & Kleinschmidt, 2010). The insula has also been suggested to specifically code pain intensity and contribute to affective pain processing (Baliki, Geha, & Apkarian, 2009). The posterior cingulate cortex has been suggested to coordinate immediate motoric responses to pain via the supplementary motor area, whereas the anterior cingulate cortex is thought to be involved in affective pain processing and pain-related decisions (McMahon & Wall, 2013, pp. 112-118). Finally, higher cognitive modulation of the pain experience has been located in the DLPFC and the orbitofrontal cortex (Krummenacher et al., 2010; Wiech & Tracey, 2013).

Modulators of central pain processing

"Central pain modulation", in the sense of this thesis, is an umbrella term covering any internal and external mechanism affecting the processing of pain in the CNS. The term includes external and internal pharmacological, as well as cognitive, affective, and autonomic mechanisms. Since the means of central pain modulation are tantamount, only a few examples for established effectors on central pain processing are provided here.

The most prominent pharmacological modulators of central pain may be opiates, cannabinoids and substance P, which have been shown to play a relatively specific role in pain processing (McMahon & Wall, 2013, pp. 375–401). However, many "classic" neurotransmitters, like glutamate, GABA, glycine, dopamine, serotonin, and acetylcholine are also an integral part of the pain signaling pathways (McMahon & Wall, 2013, pp. 375–401). Further, hormones, neuromodulators and immune signals, such as vasopressin, oxytocin, cholecystokinin (CCK), TGF-beta, and histamine have been shown to have a modulatory effect on pain (Millan, 2002). Most of the substances have been found to influence pain processing at multiple levels of the nociceptive pathway: the periphery, the spine, the brainstem, and/or the forebrain (McMahon & Wall, 2013, pp. 375–401; Millan, 2002).

Examples for cognitive modulators of pain experience are attention, expectation (Buhle, Stevens, Friedman, & Wager, 2012), the placebo effect (Krummenacher, Candia, Folkers, Schedlowski, & Schönbächler, 2010), hypnotic suggestions (Rainville, Carrier, Hofbauer, Bushnell, & Duncan, 1999), (perceived) control over pain (Helmchen, Mohr, Erdmann, Binkofski, & Büchel, 2006; Salomons, Johnstone, Backonja, Shackman, & Davidson, 2007), social context (Aslaksen, Myrbakk, Høifødt, & Flaten, 2007; Jackson, Iezzi, Chen, Ebnet, & Eglitis, 2005), catastrophizing, memory, motivations, and goals (Read & Loewenstein, 1999). Prefrontal areas, especially the anterior cingulate cortex, DLPFC (Krummenacher et al., 2010), and the orbitofrontal cortex are considered to be involved in these higher order, top-down modulatory influences on pain (for review see: Wiech & Tracey, 2013).

The affective modulation of pain has been extensively studied, e.g. by using facial pictures (Heckel et al., 2011), scenes (Meagher, Arnau, & Rhudy, 2001), film sequences (Loggia, Mogil, & Bushnell, 2008), odors (Villemure & Bushnell, 2009), music (Roy, Peretz, & Rainville, 2008), and emotional narratives (Mayer, Allen, & Beauregard, 1995). Generally emotional content of negative valence has been found to increase pain, while positive emotional material has been found to decreased pain (Wiech & Tracey, 2009). However, when occurring in high intensities, negative emotions, anxiety, and stress have also been reported to exert an antinociceptive effect, which might be an advantageous adaption for fight-or-flight situations (Wiech & Tracey, 2009).

Oxytocin has been shown to modulate emotional processing (Di Simplicio, Massey-Chase, Cowen, & Harmer, 2009; Domes et al., 2009) and emotional processing is known to be a central component of pain (Wiech & Tracey, 2009). Oxytocin has repeatedly been shown to be a modulator of pain in animals (Rash et al., 2013). Spinal mechanisms, as well as opioid effects, have been hypothesized (Rash et al., 2013). Interestingly, oxytocin receptors are found in high densities within several pain processing regions in human brain samples (compare: Duerden & Albanese, 2013 and: http://human.brain-map.org, last seen: 01.03.2014, for reference see: Hawrylycz et al. 2012). Main motivation for **Study 1** was that more controlled human trials investigating the role of oxytocin in pain are necessary, since there are only a few preliminary human studies on the topic (for review see: Rash et al., 2013).

Psychosocial stress has been shown to exacerbate experimental pain (Crettaz et al., 2013) and might be an important factor in the development of chronic pain (Van Houdenhove, 2000). Besides the effects of stress on the ANS, higher brain functions like stress coping (Koh, Choe, Song, & Lee, 2006), attention to bodily states (for review see: Van Damme, Legrain, Vogt, & Crombez, 2010), and personality traits (Pud, Eisenberg, Sprecher, Rogowski, & Yarnitsky, 2004) might be involved. Therefore one aim of **Study 2** was to identify personality traits predicting the

occurrence of symptoms under stress.

Study 3 aimed to determine if and how breathing exercises affect pain processing at brainstem level. However, alternative hypotheses had to be considered, which involved higher brain function: Distraction and attention-related processes, the placebo effect (Buhle et al., 2012), and relaxation (Busch et al., 2012) had to be considered as alternative explanations for the effects of breathing exercises on pain perception.

The cerebellum has been found to be involved in pain processing, but its exact role is still cryptic (Moulton, Schmahmann, Becerra, & Borsook, 2010). **Study 4** explored whether repetitive rTMS over the cerebellum could modulate experimental pain thresholds. A preceding study showed that cerebellar rTMS affects measures of thalamo-cortical excitability (Langguth et al., 2008). As the thalamo-cortical connections are an integral part of the pathway for pain, it was hypothesized that rTMS over the cerebellum might influence pain perception.

STUDY 1

Effects of intranasal oxytocin on thermal pain in healthy males — a randomized fMRI study

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This is a pre-copy-editing, author-produced version of an article accepted for publication in Psychosomatic Medicine (16.10.2014) after peer review.

Abstract

OBJECTIVE: Intranasal oxytocin has been shown to affect human social and emotional processing, but its potential to affect pain remains elusive. This randomized, placebo-controlled, double-blind, crossover trial investigated the effect of intranasal oxytocin on the perception and processing of noxious experimental heat in 36 healthy male volunteers.

METHODS: Thermal thresholds were determined according to the Quantitative Sensory Testing (QST) protocol. A functional Magnetic Resonance Imaging (fMRI) experiment including intensity and unpleasantness ratings of tonic heat was used to investigate the effects of oxytocin within the brain.

RESULTS: Thirty participants entered analysis. Intranasal oxytocin had no significant effect on thermal thresholds, but significantly (t = -2.06, p = .046) reduced heat intensity ratings during fMRI. The effect on intensity ratings was small (-3.46 points on a 100-point visual analog scale, 95% *CI* [-6.86; -0.07]) and independent of temperature. No effects of oxytocin on stimulus- or temperature-related processing were found at the whole-brain level at a robust statistical threshold. A ROI analysis indicated that oxytocin caused small but significant decreases in left (-0.045 %, 95% *CI* [-0.087, -0.003], t = -2.19, p = .037) and right (-0.051 %, 95% *CI* [-0.088, -0.014], t = -2.82, p = .008) amygdala activity across all temperatures.

CONCLUSIONS: The present study provides evidence for a significant, but subtle inhibitory effect of oxytocin on thermal stimulus ratings and concurrent amygdala activity. Neither of the two effects significantly depended of temperature, therefore the hypothesis of a pain-specific effect of oxytocin could not be confirmed.

Introduction

Anti-nociceptive effects of oxytocin have been reported by nearly 30 non-human, but only a few human studies (Rash, Aguirre-Camacho, & Campbell, 2013). Only four studies have investigated the effects of oxytocin on human pain using the convenient nasal application route, which has been established as a safe (MacDonald et al., 2011) and effective method to increase oxytocin concentrations in the central nervous system (Born et al., 2002; Neumann, Maloumby, Beiderbeck, Lukas, & Landgraf, 2013; Striepens et al., 2013). Singer et al. (2008) were the first to test the potential of intranasal oxytocin to alter empathic responses to pain. Although this hypothesis could not be confirmed, they found that oxytocin reduced amygdala reactivity in response to pain in a small sub-sample. Using an experimental model of placebo analgesia, Kessner et al. (2013) showed that intranasal oxytocin enhances the placebo effect. However, no general anti-nociceptive effect of intranasal oxytocin was evident within their large healthy sample. Mameli et al. (2014) explored the potential of oxytocin as an adjunctive analgesic in a small sample of fibromyalgia patients with negative results. Rash & Campbell (2014) recently found that intranasal oxytocin reduces behavioral and physiological reactions in response to cold-pressor pain.

Various effects and neural correlates of intranasal oxytocin on the processing of stress and emotion have been identified, yet most neuroimaging studies reported oxytocin effects on the amygdala, a key region for anxiety processing (Bethlehem, Baron-Cohen, van Honk, Auyeung, & Bos, 2014; Bethlehem, van Honk, Auyeung, & Baron-Cohen, 2013). For example, intranasal oxytocin was found to attenuate amygdala responses related to threatening scenes (Kirsch et al., 2005) and to conditioned fear of faces (Petrovic, Kalisch, Singer, & Dolan, 2008). Interestingly, a recent meta-analysis found 17 neuroimaging studies that report increased amygdala activity in response to experimental pain, which suggests that the amygdala plays a role in acute pain processing (Simons et al., 2014).

The potential of neuropeptides such as oxytocin as an adjunctive treatment of chronic pain is of considerable interest. Nevertheless, the potential of intranasal oxytocin to affect pain processing is understudied (Rash et al., 2013). The present trial aimed to investigate the effect of oxytocin on experimental pain perception and processing. The established Quantitative Sensory Testing (QST) protocol was used to test effects of oxytocin on noxious and non-noxious thermal thresholds. Further, a functional Magnetic Resonance Imaging (fMRI) experiment with tonic heat pain stimulation and Visual Analog Scale (VAS) ratings of heat intensity and unpleasantness was employed to measure oxytocin effects on experimental pain perception and processing.

Based on the above-mentioned studies we hypothesized that intranasal oxytocin should

A) increase noxious thermal pain thresholds;

B) decrease VAS ratings of noxious heat intensity and/or unpleasantness;

C) cause detectable blood oxygen level dependent (BOLD) signal changes related to thermal stimulus processing on whole brain level;

D) alter painful stimulus processing in the amygdala.

Methods

Trial information

The present study was approved by the ethics committee of the University of Regensburg (Approval Number: 11-111-0322) and the responsible federal medical agency. It was registered in a clinical trial registry (EUDRA-CT Number: 2009-015115-40) and conforms to the Declaration of Helsinki (59th WMA General Assembly, 2009). Written informed consent was obtained from every participant. All measures took place at the MRI facilities of the University of Regensburg at the Bezirksklinikum Regensburg between November 2012 and April 2013.

Study design

This is a placebo-controlled, double-blind, crossover trial. The random allocation sequence was generated by the Center for Clinical Trials at the University of Regensburg. According to this sequence, the Hospital Pharmacy of the University of Erlangen labeled and numbered the nasal sprays and corresponding emergency wrappers. At study inclusion, participant were assigned sequential participant numbers by author PE and hereby allocated to Group A or B at random. Group A received placebo and Group B received oxytocin at Visit 1; Group A received oxytocin and Group B received placebo at Visit 2. Both groups completed an additional training visit (Visit 0) at study inclusion. A period of ≥ 7 days between all visits was observed to minimize carry-over effects.

Participants

Healthy right-handed male volunteers between 18 to 50 years of age were eligible for trial participation. Participants were recruited by advertisement at the University of Regensburg, aiming at a sample size of 36. The sample size was chosen to optimize statistical power within the limits of the devoted resources. Participants received a compensation of 10 Euros per hour. Exclusion criteria were surveyed in a structured interview at inclusion. Exclusion criteria were: Allergies against any ingredients of the trial medication; past or present cardiac, major internal, neurological, psychiatric, hormonal, or chronic conditions; acute infections; recent surgery; recent use of illicit drugs, psychotropics, or analgesics; alcohol addiction; conditions incompatible with fMRI safety. Participants were required to abstain from alcohol and caffeinated beverages at least 24 hours and 12 hours before visits, respectively.

Procedure

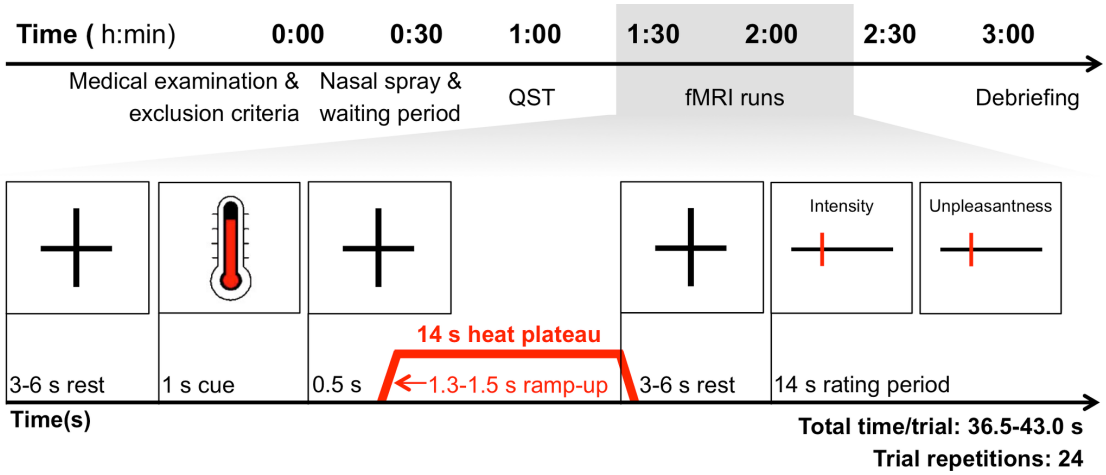


Figure 1.1: Time schedule of experimental procedures (upper row) and schematic overview of a typical trial within the fMRI block design (lower row). The temperature applied during the 14-second heat plateau ranged from 44.7 °C to 47.5° C in steps of 0.4 °C. Abbreviations: fMRI: functional Magnetic Resonance Imaging, QST: Quantitative Sensory Testing.

An overview of the experimental procedures is provided in Figure 1.1. Visits were scheduled between 8:00 h and 20:00 h, always at the same time of day (± 1h) within participants. Each visit started with a medical examination and a re-evaluation of inclusion and exclusion criteria. Subsequently, participants received a dose of 32 IU oxytocin, or placebo, applied as four puffs of 0.1 ml per nostril. The dose was chosen according to Singer et al., (2008). The oxytocin and the placebo spray only differed in the absence of oxytocin in the placebo. Both sprays had the formulation of Syntocinon Spray (Sigma Tau, Rome, Italy). Participants self-administered the nasal spray under supervision by a physician, according to recommendations by Guastella et al. (2013). Testing began after a waiting period of 40 minutes, consistent with the expected peak CSF concentration of nasally applied neuropeptides (Born et al., 2002). During the waiting period, participants completed questionnaires and were given standardized instructions for the following testing procedures. Then, thermal thresholding according to the QST protocol (20 min) was performed outside of the MRI scanner, followed by two fMRI runs. At debriefing, the occurrence and severity of 18 typical side effects was recorded on a 5-point numeric rating scale ranging from 0 (side effect absent) to 4 (severe). Additionally, each participant was asked to guess if he received placebo or oxytocin.

Monitoring mood

The Profile of Mood States (POMS, Pollock, Cho, Reker, & Volavka, 1979), an adjective rating scale instrument with 65 items, was used to monitor mood changes over the course of the experiment. The POMS total score reflects mood disturbance in general, while sub-scales allow for the monitoring of "tension-anxiety", "depression-dejection", "anger-hostility", "fatigue-inertia", "vigor-activity", and "confusion-bewilderment". The POMS was administered three times: At the beginning of each session, after nasal spray application (near the end of the waiting period), and at the end of each session. At each time-point the participants were asked to rate their "momentary" state.

Quantitative sensory testing (QST)

Participants were seated in upright position outside the MRI chamber with their arms comfortably resting on a cushioned tabletop. Thermal thresholding was performed on the volar surface of the left lower arm, 5 cm proximal from the wrist crease. Thermal stimuli were applied using a Thermosensory Analyzer II (Medoc, Israel) and a MR-safe 30 x 30 mm thermode, kept in place by an elastic strap. All thresholding procedures were performed in the presence of the same experimenter (MZ), with no other person or distractors present. Visual, auditory, or social clues indicating the onset of stimulation were precluded by the experimental setup. Written instructions were read aloud to the participant at each session. Cold (CDT) and warmth (WDT) detection thresholds, as well as cold (CPT) and heat (HPT) pain thresholds were retrieved in this order, according to the established QST protocol (Magerl et al., 2010; Rolke et al., 2006). For each measure five repetitions were obtained. The first stimulus of each measure was defined as a trial stimulus and discarded from analysis; thresholds were defined as the mean of the last four stimuli.

fMRI—general information:

After QST, participants underwent two separate fMRI experiments. Again, stimulation was applied to the volar surface of the left lower arm. Now the thermode location was 10 cm, or 15 cm proximal from the wrist crease. The thermode was moved in-between the fMRI runs to avoid skin damage. The order of fMRI runs and the order of thermode locations were balanced across participants and medication conditions. Only one of the two fMRI experiments was part of the present study, the other experiment will be reported elsewhere (Zunhammer et al., unpublished observations).

A 3-Tesla Allegra Head Scanner (Siemens, Germany) equipped with a single channel head coil was used for MRI. Functional volumes were obtained with a T2*-weighted Echo-Planar Imaging (EPI) sequence (TR = 2000 ms, TE = 30 ms, interleaved slicing, flip angle = 90 °, 3 × 3 x 3.5 mm voxel size, including a 16 % slice-gap, FoV = 192 × 192 mm), covering the full brain in 34 horizontal slices co-planar to the anterior and posterior commissure. The first five volumes of each run were discarded to account for T1-saturation effects. In addition a T1-weighted high-resolution structural head volume with 160 sagittal slices was obtained at Visit 1, using a Magnetization Prepared Rapid Gradient Echo (MP-RAGE) sequence (TR = 2250 ms, TE = 2.6 ms, flip angle = 9 °, 1 x 1 x 1 mm voxel size, FoV = 256 × 256 mm). Presentation 14.9 for Windows (Neurobehavioral Systems Inc., USA) was used to display stimuli, retrieve ratings, and log events. Visual stimuli were presented at a resolution of 1024 x 768 pixels and a frame-rate of 60 Hz on a screen attached to the head-end of the coil. Participant could see the entire display via a mirror attached to the head coil. The thermal stimulation equipment was the same as for the QST.

MR images were pre-processed and analyzed with SPM 8. Volumes were corrected for slice-timing differences using the middle slice as a reference; event onsets were adjusted accordingly. Volume time series were re-aligned and re-sliced to the first volume to account for head motion, using SPM's rigid body transformation with 4th degree B-spline interpolation. Time series were screened for excessive head motion events using ArtRepair (Mazaika, Hoeft, Glover, & Reiss, 2009). Anatomical images were co-registered to the mean realigned functional image, segmented using SPM's MNI (Montreal Neurological Institute) tissue probability maps, and re-sampled at 2 x 2 x 2 mm. Realigned functional images were normalized to MNI space using the parameters retrieved from the segmentation procedure, preserving signal concentrations ("unmodulated").

All co-registration and segmentation results underwent visual quality control and were corrected if necessary. Images were spatially smoothed with a Gaussian full-width at half maximum (FWHM) kernel of 8 mm to improve signal-to-noise ratio.

fMRI—experimental procedure:

The fMRI experiment aimed to test if oxytocin induced changes in BOLD activity in response to tonic heat in the brain. Further, it aimed to obtain VAS ratings of heat intensity and/or unpleasantness. For these purposes, a parametric block design (Büchel, Holmes, Rees, & Friston, 1998) with 24 repetitions and a total duration of 14.6 to 17.2 minutes was employed. A block-outline is provided in Figure 1.1 (lower row). Block length varied between 36.5 and 43 s. Each block started with a visual cue: a red, iconic thermometer was shown on-screen for 1 s to indicate the imminent onset of thermal stimulation, followed by a fixation cross. After 0.5 s the thermode temperature increased rapidly (10 °C / s) from baseline (35 °C) to one of eight target temperatures. These target temperatures ranged from 44.7 °C to 47.5 °C in steps of 0.4 °C and were selected in order to cover the non-painful and painful (but tolerable) spectrum in the majority of male participants. Temperatures were selected by interpolating rating data obtained in a previous study (Zunhammer, Eichhammer, & Busch, 2013). Each target temperature was kept at a constant plateau for 14 s, before it returned to baseline (10 °C / s). The duration of ramp-up and ramp-down varied between 1.3 s for 44.7 °C to 1.5 s for 47.5 °C. Over the course of a run, each target temperature was repeated four times in pseudo-randomized order. Each of the eight temperatures had to be presented once before being repeated, to achieve an even temperature distribution within runs.

Thermal stimulation was followed by 3 to 6 s of rest. Then, a rating phase started: Participants were prompted to use a cursor to rate the preceding stimulus on consecutive displays for perceived "intensity" and "unpleasantness". Intensity and unpleasantness were rated on two VASs occupying 70 % of the width of the display. The VASs were not ticked or numbered. Endpoints were labeled "no stimulus perceived" and "maximally intense" for the intensity rating and "not unpleasant" to "maximally unpleasant" for the unpleasantness rating. Ratings were recorded as integers ranging from 0 to 100, resulting in a 101-point scale. Ratings were entered with a LUMItouch keypad (Photon Control, Canada) held in the right hand. The cursor started at 0 or 100 randomly and could be moved continuously by sustained button press of the index (left) and the middle (right) finger button. Selected ratings could be submitted by pressing the thumb button. Participants were required to submit ratings within 14 s, otherwise the next trial started and the block was discarded. When ratings were submitted in less than 14 s, a fixation cross was displayed for the remaining time. Thus, participants had no influence over the pace of the experiment. The next block followed after another resting interval of 3 to 6 s at fixation.

Before each run, participants were given standardized instructions. Participants were asked to rate "How hot was the stimulus?" on the intensity scale and "How unpleasant was the stimulus?" on the unpleasantness scale. The difference between stimulus intensity and unpleasantness was illustrated by an analogy according to Price et al. (Price, McGrath, Rafii, & Buckingham, 1983). The analogy was adapted by replacing every occurrence of the word "pain" by "heat", to account for the fact that we intended to measure (non-nociceptive) stimulus intensity, not (nociceptive) pain intensity, as in the original protocol. Before the start of the actual fMRI run, two training trials were performed to reduce novelty effects and to reduce sensitization/desensitization effects. Two stimuli with 44.7 and 47.1 °C were presented for this purpose.

fMRI—whole brain analysis

The first part of fMRI analysis aimed at identifying brain regions where oxytocin alters the processing of noxious temperatures. Emphasis was put on separating processes related to stimulation in general and pain-related processes. A standard two-step statistical parametric mapping (SPM) approach was used. In first level analysis a general linear model (GLM) was created for each participant in order to obtain voxel-wise beta estimates of within-subject effects. The thermal stimulation period was modeled as a boxcar regressor with blocks of 14 s duration, corresponding to the plateau phase of thermal stimulation. Stimulus temperature was added onto the stimulation period as a linear parametric modulator, maintaining SPM's default orthogonalization procedure. The stimulation regressor therefore represented activity related to stimulation per-se, whereas the temperature regressor detected activity linearly related to stimulus temperature. Regressors were convolved with SPM's canonical hemodynamic response function. Rating period, the six translational, and the rotational motion parameters were added to the model as nuisance regressors. An autoregressive (AR1) co-variance matrix was used to account for serial correlations. A temporal high-pass filter with a width of 400 s was applied. The eye regions and extra-cerebral tissue were excluded from analysis by using SPM's a-priori brain mask. The beta contrast maps Stimulation > Baseline, Temperature > Baseline, (Oxytocin > Placebo, Stimulation > Baseline), (Oxytocin > Placebo, Temperature > Baseline), as well as the corresponding reverse contrasts, were obtained for each participant.

In order to identify activations at group-level, a second level analysis was performed. Voxel-wise one-sample t-tests were used to test the single-subject contrast maps against the null hypothesis of no effect. The statistical threshold for activation at whole brain level was defined as $p < .05$, applying SPM 8's family-wise error (FWE) correction for multiple comparisons. Regions were labeled with the Harvard-Oxford atlas for cortical (Desikan et al., 2006) and subcortical regions (Frazier et al., 2005), as included in the FSL analysis software (FMRIBs Software Library, www.fmrib.ox.ac.uk/fsl). Common structural names were added where appropriate. Clusters are overlaid for display on the mean structural image. Graphical artwork was prepared using MRIcron 6/2013 for Mac and arranged in panels using gimp.

fMRI—region of interest analysis of the amygdala

The second part of fMRI analysis aimed at quantifying the effects of oxytocin on the processing of noxious temperatures within the amygdala. ROIs for right and left amygdala were defined anatomically by the tissue probability maps from the sub-cortical Harvard-Oxford atlas (Frazier et al., 2005), binarized at a threshold of 50 %.

The SPM-toolbox marsbar (Brett, Anton, Valabregue, & Poline, 2002) was used to obtain percent signal change estimates for each temperature and medication (placebo vs. oxytocin) condition within the ROIs. In order to estimate peri-stimulus activity for each temperature separately, the model for fMRI analysis described above had to be adapted: The eight different temperature stimuli were modeled as eight different boxcar regressors, split into 13 time bins of 2 s duration in a finite impulse response (FIR) model (Ollinger, Shulman, & Corbetta, 2001). Time bins started at the onset of temperature plateau (0 s) and ended 24 s post-stimulus onset, covering stimulus-offset (14 s), plus the subsequent resting period (> 3 s), plus the canonical delay of the HRF (6 s) (Henson & Friston, 2007).

Statistics

Statistics were processed with SPSS 21.0.0.0 for Mac. A repeated-measures MANOVA was used to test effects of oxytocin on QST thresholds. POMS-scores, VAS-ratings, and percent signal changes within amygdala ROIs were analyzed using linear mixed models with maximum likelihood estimation. Sum-of-squares F-tests were performed at a two-tailed $\alpha < 0.05$. For the POMS total score and all sub-scales, the main and interaction effects of the factors oxytocin (oxytocin, placebo) and "within-session time-points" (before session, before testing, after session) were tested. For the VAS results, the effect of oxytocin was tested, while the non-linear relationship between stimulation temperature and ratings of stimulus intensity and unpleasantness was modeled as a second-order polynomial: Mean-centered cofactors Temperature and Temperature^2, as well as the interaction-terms Oxytocin * Temperature and Oxytocin * Temperature^2 were included in the model. For amygdala activity, effect of the factor oxytocin was tested including factor time bin, as well as the linear cofactors temperature, intensity ratings, or unpleasantness ratings (and the respective interaction terms with oxytocin). Within-participant dependencies were modeled by including individual random intercepts and random effects for all factors, cofactors, and interactions, to obtain a maximal random effects structure (Barr, 2013). A "variance components" co-variance matrix was used.

Means are reported ± 1 standard deviation, if not denoted otherwise. Error bars in all figures represent standard errors of the mean (SEM) corrected for repeated measures (Bakeman & Mcarthur, 1996). Graphs were created using GraphPad Prism 5.0 for Windows.

Results

Sample description and medication

Thirty participants successfully completed the study and were eligible for analysis. A detailed participant flow according to the CONSORT criteria is available online, as Appendix 1.1. No adverse event related to oxytocin occurred. Investigators and participants were blinded until all measures were completed, after which the trial was concluded regularly.

Mean age at study inclusion was 24.9 years (range: 19 to 30). Participants were assigned to Group A and B in 50.0 % of cases. After Visit 1, 77.4 % of participants indicated that they believed to have received oxytocin, compared to 53.3 % after Visit 2. Some participants guessed to have received oxytocin twice, despite being reminded that oxytocin was only given at one of the two visits. Nevertheless, participants guessed the right medication in 50.0 % of cases and as such did not differ from chance level. The mean side-effect score was 3.2 ± 2.8 for placebo and 2.9 ± 1.7 for oxytocin and these values did not differ significantly ($p = .82$) according to a paired t-test.

Mood

The POMS total score indicated that participants were generally in good mood before and after the experiment (Nyenhuis, Yamamoto, Luchetta, Terrien, & Parmentier, 1999). Participants showed significant mood changes over the course of the experiment: They described themselves as significantly more fatigued, confused, and less invigorated after the experiment, compared to the two time-points before. Tension, anger, and depression sub-scales did not change significantly. There was a significant interaction-effect of Oxytocin*Time-Points for the POMS total score ($F[2, 60.3] = 4.34$, $p = .017$) and the confusion sub-scale ($F[2, 60.1] = 5.91$, $p = .005$). Post-hoc-test indicated that participants showed a non-significant tendency towards better mood (-4.36 ± 14.96, $t(27) = 1.54$, $p = .135$) and were significantly less confused (-1.75 ± 2.50, $t(27) = 3.67$, $p = .001$) at the post-session time point, after having received oxytocin, as compared to placebo. Tables with full results for the POMS and its sub-scales are provided in Appendix 1.2.

Quantitative sensory testing (QST)

QST testing started 42 ± 2 min after nasal spray administration, at mean. There were no missing values for the QST procedure. No significant differences between oxytocin and placebo conditions could be found for any thermal thresholding procedure according to repeated-measures MANOVA ($F[4, 26] = 0.64$, $p = .64$). Results are detailed in Table 1.

Measure	Placebo	Oxytocin	Statistics df (1, 29)
Cold detection threshold (CDT)	30.98 ± 0.47	30.90 ± 0.41	F = 0.68, p = .414
Warmth detection threshold (WDT)	33.75± 0.79	33.99± 1.01	F = 2.22, p = .147
Cold pain threshold (CPT)	18.37± 7.59	18.79± 7.68	F = 0.24, p = .627
Heat pain threshold (HPT)	44.39± 3.15	44.59± 2.89	F = 0.29, p = .592

Table 1.1: No significant effects of oxytocin on thermal detection and pain thresholds. All means and differences in °C. F-statistics were obtained performing a repeated-measures MANOVA (n = 30, two observations/variable/participant).

fMRI—dataset

On average, fMRI testing started 76 ± 6 min and ended 130 ± 10 min after nasal spray administration. The last three trials of one session were lost due to a technical failure, however

the remaining data from that participant were retained in analysis. At mean, participants completed their ratings within 7.50 ± 1.43 s. Two trials had to be excluded from analysis because ratings were not submitted within 14 s. Overall analysis was based on 1435 trials over 30 participants.

fMRI—ratings

Mean results for intensity and unpleasantness ratings obtained during fMRI scanning are shown in Figure 1.2 a, and b, respectively; dashed lines show the curve of best-fit. Mixed model analysis indicated that the factor oxytocin explained a significant amount of variance in intensity ($F[1, 41.9] = 4.23$, $p = .046$), but not in unpleasantness ratings ($F[1, 41.2] = 1.87$, $p = .18$). As expected, cofactors temperature and temperature^2 were significant predictors of intensity and unpleasantness ratings (all: $p < .001$). None of the interaction terms showed a significant effect ($p > .16$). We therefore conclude that oxytocin decreased intensity ratings after adjusting for repeated measures and the non-linear effects of temperature.

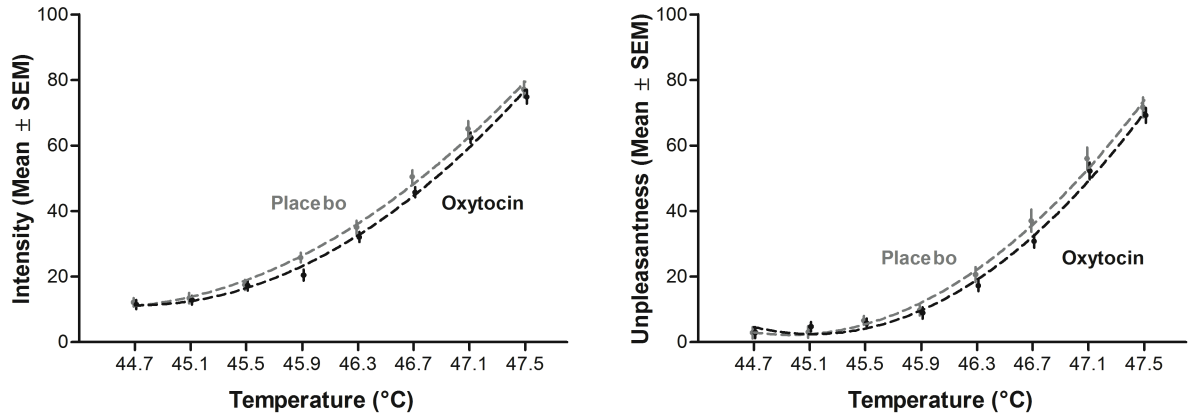


Figure 1.2 a and b: Oxytocin effects on visual analog scale ratings of heat. Parameter estimates indicated that intensity ratings (β = -3.46, 95% CI [-6.86, -0.07], t = -2.06, p = .046) significantly decreased during oxytocin sessions, compared to placebo sessions. A similar, but non-significant trend was apparent for unpleasantness ratings (β = -2.77, 95% CI [-6.97, -1.42], t = -1.37, p = .18). The mean ratings shown were shifted slightly along the x-axis (± 0.02 °C) for the sake of illustration. Error bars represent SEM corrected for repeated measures. n = 30, with 48 observations per variable and participant.

fMRI—whole brain analysis

The contrasts Temperature > Baseline and Stimulation > Baseline were assessed to confirm that our fMRI paradigm was effectively identifying regions of pain processing. Significant activations were found in the bilateral insula, somatosensory cortex II (SII), cingulate cortex, dorsolateral prefrontal cortex (DLPFC), and caudate nucleus. Results of this control analysis are provided in Figure 1.3 and in tabular form in Appendix 1.3.

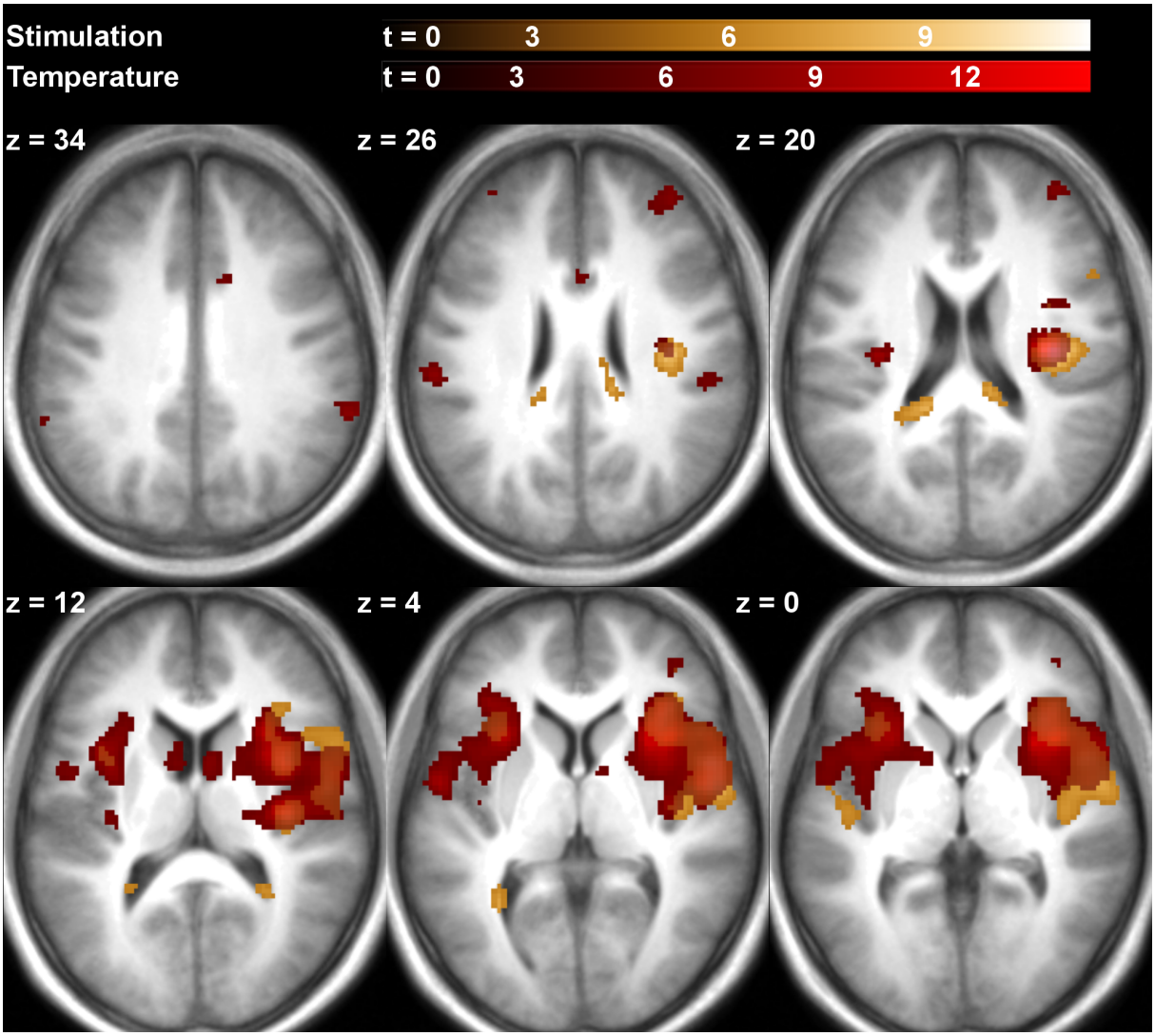


Figure 1.3: Clusters showing increased activity during stimulation period (contrast: Stimulation > Baseline, gold) and brain regions showing a linear activity increase with heat (contrast: Temperature > Baseline, red). The threshold of significance was $p < .05$, corrected for Family-Wise-Error (FWE), with a minimum size of 5 voxels. Left hemisphere is shown left. Regions are labeled according to the Harvard-Oxford Atlas. Abbreviations: DLPFC: Dorsolateral prefrontal cortex, SII: somatosensory cortex II. For coordinates and tabular results see: Appendix 1.3. n = 30 with 48 trials of 14 s duration per variable and participant.

The Oxytocin > Placebo and reverse contrasts were analyzed with respect to the effects of the temperature and the stimulation regressor. No significant effects of oxytocin were found on whole brain level for any of these comparisons at the statistical threshold of $p < .05$ when applying FWE correction. Using a liberal threshold of $p < .001$, uncorrected for multiple comparisons (minimal cluster size 5 voxel), several clusters were identified, which showed decreased activation during the oxytocin sessions. These preliminary results are provided in Figure 1.4 and in tabular form in Appendix 1.4.

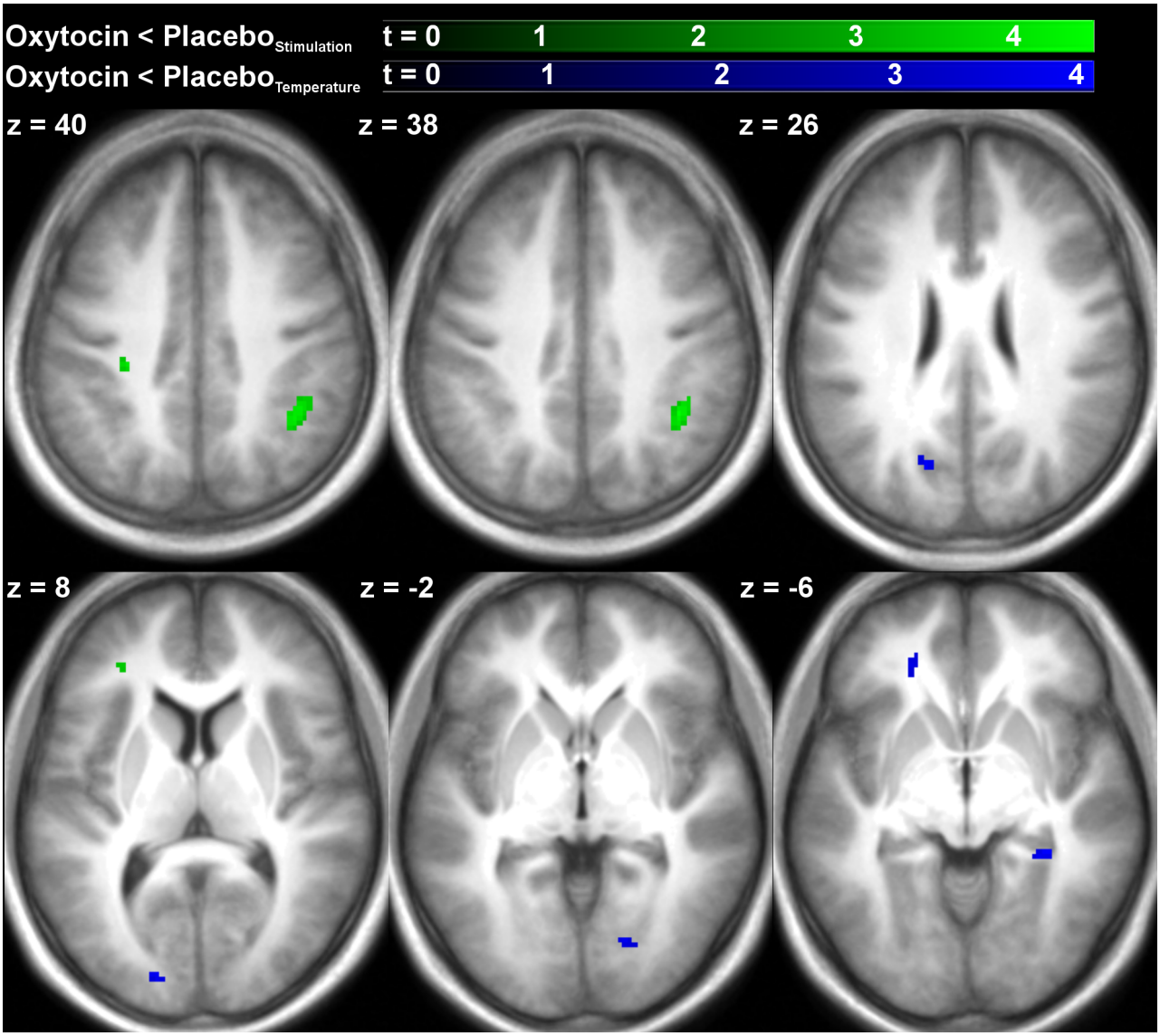


Figure 1.4: Clusters showing decreased stimulus-related activity (green) and decreased temperature related activity (blue) during oxytocin compared to placebo sessions. The statistical threshold was set at $p < .001$, uncorrected for multiple comparisons, with a minimal cluster size of 5 voxel. No significant clusters were found showing increased activation under oxytocin. No significant effects were found when using a threshold of $p < 0.05$ with family-wise-error (FWE) correction for multiple comparisons. Left hemisphere is shown left. Regions are labeled according to the Harvard-Oxford Atlas, with tissue probabilities in %. For coordinates and tabular results see: Appendix 1.4. n = 30 with 48 trials of 14 s duration per variable and participant.

fMRI—region of interest analysis of the amygdala

The percent signal change estimates for right and left amygdala activity are displayed in Figure 1.5. Main effects and interactions between factors oxytocin, time bin, and (mean centered) temperature were tested using mixed model analysis. Oxytocin was found to explain a significant amount of variance in BOLD signal in the left ($F[1, 30.0] = 4.77$, $p = .037$) and right ($F[1, 30.0] = 7.89$, $p = .009$) amygdala. As expected, the time bins explained a significant proportion of variance in both amygdalae (both: $p < .001$). The cofactor temperature did not explain a significant proportion of variance; neither in the left ($F[1, 30.1] = 0.66$, $p = .42$), nor right ($F[1, 32.2] = 1.41$, $p = .24$) amygdala. All interaction terms were non-significant. Intensity-

and unpleasantness-ratings did not explain a significant proportion of variance in amygdala activity, when used as a cofactor in mixed model analysis instead of temperature ($p > .79$ for both variables and hemispheres).

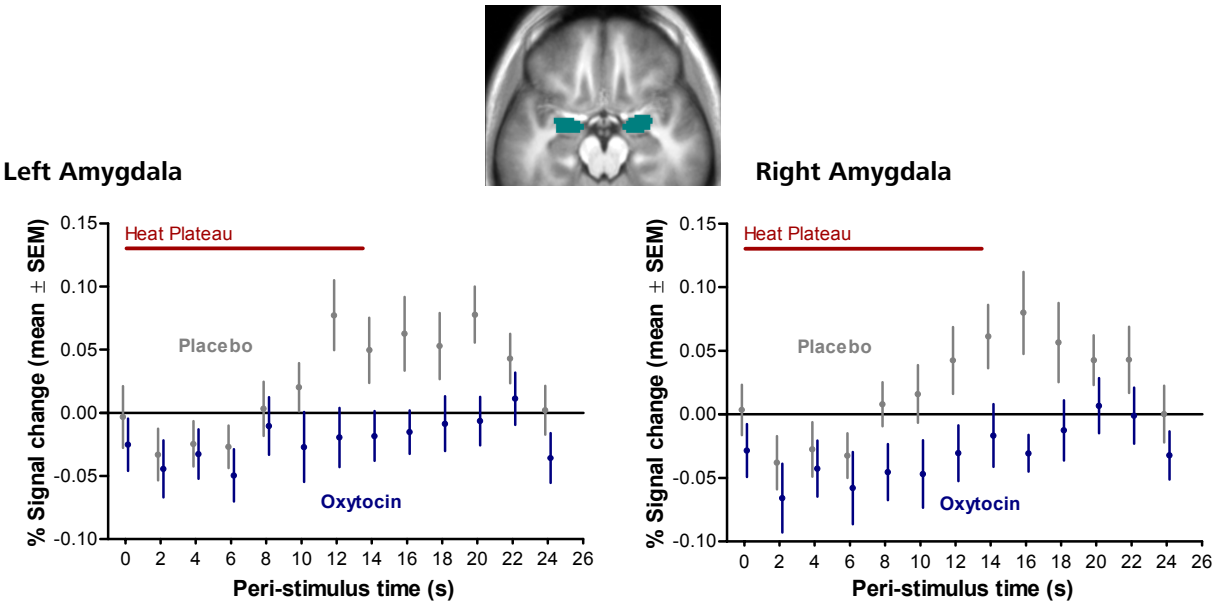


Figure 1.5: Mean time-course of bilateral amygdala activity during stimulation and effects of oxytocin. Parameter estimates indicated that left (β = -0.045, 95% CI [-0.087, -0.003], t = -2.19, p = .037) and right (β = -0.051, 95% CI [-0.088, -0.014], t = -2.82, p = .008) amygdala activity was significantly decreased in oxytocin, compared to placebo sessions. Temperature was not found to significantly predict amygdala activity, the depicted mean % signal changes were therefore pooled across temperatures. Error bars represent SEMs corrected for repeated measures. n = 30 with 48 trials of 14 s duration per variable and participant.

Post-hoc analyses

Oxytocin induced differences in intensity ratings (mean change in ratings) did not correlate with oxytocin induced difference POMS total score (mean change in score, Kendall's tau-b = -.170, p = .21). Oxytocin induced differences in amygdala activity (mean change in signal) did not correlate with oxytocin induced difference in POMS total score (mean change in score, Kendall's tau-b = -.099, p = .62). Similarly, there were no significant correlations between mean changes in intensity- or unpleasantness ratings and mean changes in amygdala activity (all variables and hemispheres: $p > .79$). Mean heat intensity (Kendall's tau-b = .434, p = .001) and mean heat unpleasantness (Kendall's tau-b = .407, p = .001) ratings correlated significantly with HPT (data aggregated across sessions), but not with HDT, CDT, and CPT (all: $p > .11$).

Discussion

Overview

The present neuroimaging study is the first to test the effects of intranasal oxytocin on human heat pain processing with a representative sample size. Oxytocin did not significantly affect thermal thresholds before fMRI (Hypothesis A), but significantly reduced subjects' heat intensity ratings during fMRI (Hypotheses B). A similar, but non-significant trend towards reduced unpleasantness ratings could also be observed. The effects of oxytocin on VAS ratings had a modest (~4 % of the VAS) effect size. No effect of oxytocin on temperature-related cerebral processing was found at whole-brain level using a robust statistical threshold. (Hypotheses C). A ROI analysis showed that oxytocin significantly reduced BOLD responses within the bilateral amygdala (Hypothesis D). Oxytocin induced changes in mood, subjective heat intensity ratings, and amygdala activity did not correlate significantly.

The amygdala, oxytocin, and pain

By showing that oxytocin reduces the hemodynamic response within the amygdala during heat stimulation (see: Figure 1.5), our results point to the established effect of intranasal oxytocin on amygdala activity (for review see: Bethlehem et al., 2013). We could therefore consolidate preliminary evidence by Singer et al. (2008), who found reduced amygdala reactivity in response to painful stimulation in a sub-group of participants, while studying oxytocin effects on empathy.

Contrary to our expectations, whole-brain analysis was unable to identify the amygdala among the regions showing significant stimulus- or temperature-related activity (see: Figure 1.3). Owing to its increased power, a ROI analysis could confirm that both amygdalae were active during stimulation and showed a typical hemodynamic response (see: Figure 1.5). Considering that the canonical delay of the hemodynamic response is ~5 s (Henson, 2006), the observed amygdala activation peak (12-16 s) was related to the second half of the heat stimulus plateau. This time-course suggests that amygdala activity was linked to features of heat stimulus perception, rather than threat evaluation. The latter would be expected to occur earlier, corresponding to the stimulus onset, or to the preceding visual cue, i.e. at a time when the painfulness of the upcoming stimulus is still uncertain. Nevertheless, the amygdala response and the oxytocin effect were not significantly related to temperature, intensity ratings, or unpleasantness ratings. Therefore, general stimulus-related, rather than pain-specific processes might underlie the observed effects. Our results thus provide additional evidence for an effect of oxytocin on stimulus processing in the amygdala, but do not necessarily support the hypothesis of a specific anti-nociceptive effect.

Our fMRI paradigm detected significant, robust, positive linear associations between noxious temperature and BOLD signal in the insula, SII, cingulate cortex, DLPFC and caudate nucleus (see: Figure 1.3). These results are in agreement with previous results for heat pain (Duerden & Albanese, 2013) and verify that our fMRI paradigm was capable of detecting pain-related cerebral processing.

Despite these positive results, no effect of oxytocin on stimulus-, or temperature-related activity could be found with a threshold of $p < 0.05$, after correction for multiple comparisons with the conservative FWE procedure. Clusters of inhibitory oxytocin effects in the posterior hippocampus, the supra-marginal gyrus, as well as several prefrontal, fusiform and occipital regions (see: Figure 1.4) could be found with a more liberal statistical threshold of $p < .001$, voxel size > 5, uncorrected for multiple comparisons. However, we think that strict correction for

multiple comparisons is vital for fMRI research (Vul, Harris, Winkielman, & Pashler, 2009). Therefore, we consider these results preliminary and recommend that they be interpreted with caution.

Rating results

Similar to the amygdala results, our behavioral results do not provide unequivocal support of an analgesic effect of oxytocin. The small oxytocin effect on VAS-ratings was not found to be temperature dependent. In addition intensity, and less so unpleasantness ratings were affected — the opposite would be expected for a clear analgesic effect. We therefore conclude that changes in somatosensory perception and/or changes in other general cognitive states, such as attention, anxiety, or vigilance, may account for the observed oxytocin effects. Our results are in accord with recent findings of Rash and colleagues (2014), who found that intranasal oxytocin reduces pain intensity ratings, pain unpleasantness ratings, and descriptive ratings of cold-pressor pain. Although Kessner et al. (2013) found no effect of oxytocin on heat pain ratings, our results are compatible with the finding that oxytocin enhances the placebo effect. Our participants guessed to have received oxytocin in 77.4 % of cases at Visit 1 and in 53.3 % of cases at Visit 2. This high prevalence of placebo-beliefs in our sample may have driven the effect.

Social interaction was limited in the present experiment, especially within the MR environment and no social stimulus material was involved in the present study. These circumstance may have limited the efficacy of oxytocin in comparison to previous studies, as the effects of oxytocin have repeatedly been shown to depend on social context and associated beliefs (Bartz, Zaki, Bolger, & Ochsner, 2011). The discrepancy between our positive results for the VAS-ratings and our negative results for the thermal thresholding procedure might be explained by a number of points: The rating procedure involved six times more stimulus repetitions than the thresholding procedure, thus entailing more statistical power. Further, the thresholding and the rating procedures differed in timing, thermode location, stimulus dynamics, stimulus intensity, and response type—all of which may have made a difference. Of note, oxytocin may have exerted an effect on pain perception in the anxiogenic environment of the MR-scanner, consistent with the hypothesis of oxytocin as an anxiolytic agent (Bethlehem et al., 2014).

Oxytocin was found to ameliorate negative mood changes occurring over the course of the experimental sessions. Participants described themselves as less confused after the oxytocin session, in comparison to the placebo session. However, these changes in mood were not significantly correlated with changes in intensity ratings or amygdala activity, and therefore may be of limited relevance for the main aim of our study. Of note, the "tension-anxiety" sub-scale of the POMS, a measure related to stress and anxiety, was not significantly affected by oxytocin.

Limitations

The present study was limited to healthy males. The present results might therefore not generalize to the female population, to patient populations, or to clinical forms of pain, We limited our sample to males, because the summary of medical product characteristics for Syntocinon Spray listed uterine contractions as a potential side effect in females (Novartis, 2006), which was deemed a potential source of confound for the testing of pain processing. However, a large review on the safety of intranasal oxytocin indicated that female study participants are not reported to experience more side-effects than their male counterparts (MacDonald et al., 2011). Therefore our concerns might have been unsubstantiated and we encourage future studies on the effects of intranasal oxytocin on heat pain in females.

Thermal stimulation of the skin was the main method of sensory stimulation used in the present study. The present results may therefore not generalize to other forms of (noxious) somatosensory stimulation. In addition, the stimulus protocol used in our fMRI paradigm involved the same heat stimulus intensities for all participants. Individualized stimulus intensities may have increased the power to detect effects of oxytocin by reducing between-subject variability.

The pathway of intranasal oxytocin into the brain its still unknown. Its peak and retention times in the brain, as well as its bioavailability and metabolism are incompletely understood. Born et al. (Born et al., 2002) found that 40 IU of intranasal vasopressin (a structural sibling of oxytocin) increased concentrations in human cerebrospinal fluid 40 and 80 min after application; no measurements were obtained beyond 80 min, prohibiting the estimation of retention times. A small study assessing oxytocin levels in the human cerebrospinal fluid (Striepens et al., 2013) found elevated oxytocin levels 75 min after intranasal application; again, no measurements were obtained beyond this time point. Oxytocin concentrations in human saliva have been reported to remain elevated for 7 hours after nasal spray application (Ijzendoorn, Bhandari, Veen, & Grewen, 2012). Oxytocin levels were found to peak between 30 and 60 min and return to baseline between 90 and 120 min after nasal application in in-vivo micro-dialysates of rat and mouse brain tissues (Neumann et al., 2013). According to these studies our thresholding procedure (40-60 min post application) might have started too early (Striepens et al., 2013), or too late (Neumann et al., 2013), and our fMRI experiments might have taken too long (130 minutes). The present results may therefore be limited to specific time frames, i.e. 40-60 min for the QST:, and 75-130 min for the rating and fMRI results. Further, no optimal dose of intranasal oxytocin for eliciting behavioral effects has been determined, yet. Since the present study did not include different dosing conditions, it cannot provide information on a probable dose-response relationship. The present findings are therefore limited to the specific oxytocin dose of 32 IU, which may be too low, or even too high (Cardoso, Ellenbogen, Orlando, Bacon, & Joober, 2013; Ijzendoorn et al., 2012). These issues may have obscured further oxytocin effects from detection and may have resulted in an underestimation of actual effect sizes.

Finally, we want to raise awareness for a limitation underappreciated in human intranasal oxytocin research: Oxytocin has repeatedly shown to affect social behavior (Guastella & MacLeod, 2012) and there is no reason why this should not apply to experimental settings, where participants usually interact with experimenters. "Good-participant" behavior might systematically increase under oxytocin, although there is no positive evidence for an oxytocin effect on demand characteristics (Weber & Cook, 1972), yet. Such an oxytocin-induced response bias might confound any behavioral measurement in oxytocin studies — even when a double-blind placebo-control is employed, as in the present study.

Conclusion

In conclusion, our results provide further evidence for a significant, but subtle effect of oxytocin on heat stimulus perception and/or appraisal. They further provide evidence for a significant effect of oxytocin on heat stimulus processing in the amygdala. Preliminary evidence for activity decreases in the hippocampus, prefrontal cortex, parietal cortex, and occipital cortex could be obtained. These regions pose potential targets for further investigations on pain-related oxytocin effects. However, the present study could not support the hypothesis of an anti-nociceptive effect of oxytocin, since the oxytocin induced changes in VAS-ratings and amygdala activity were not found to be temperature-dependent and oxytocin affected intensity, rather than

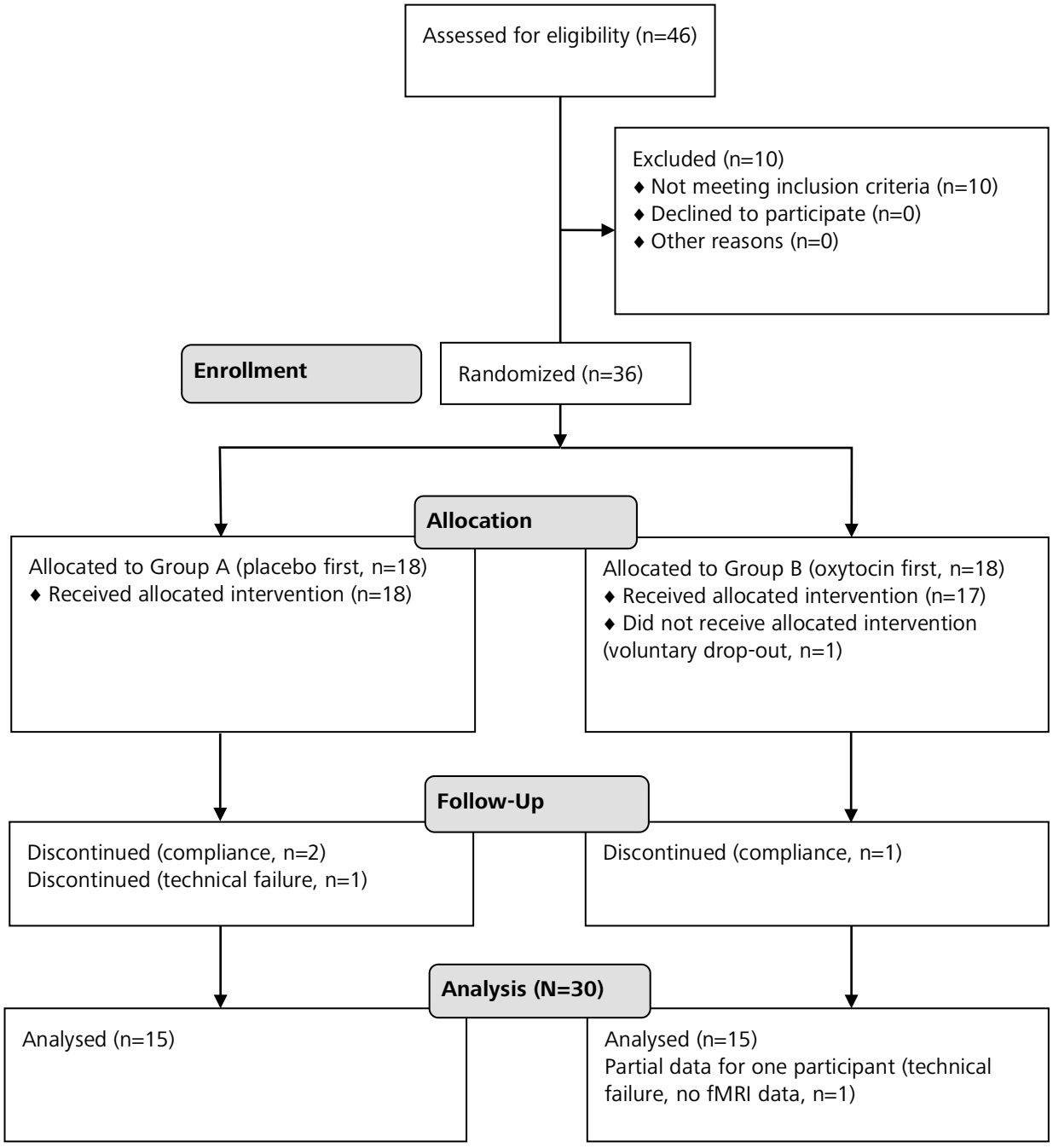
unpleasantness ratings. Future studies are needed to unravel the effects of intranasal oxytocin on pain-related processes.

Acknowledgements:

This is an investigator initiated clinical trial. The study medication was provided at no cost by sigma-tau s.p.a. (Italy). Author M.Z. is supported by a scholarship of the "German National Merit Foundation". This research received no other specific grant from any funding agency in the public, commercial, or not-for-profit sectors. None of the authors has any conflict of interest to disclose.

Appendix 1.1

CONSORT Flowchart



Appendix 1.2

Time-Point	Before Session		Before Testing		After Session	
Medication	Placebo	Oxytocin	Placebo	Oxytocin	Placebo	Oxytocin
n	30	30	30	29	29	29
POMS-Subscale						
Tension	3.3 ± 3.3	3.7 ± 3.3	3.1 ± 3.8	3.6 ± 2.8	4.4 ± 4.5	3.4 ± 2.8
Depression	1.7 ± 2.7	1.5 ± 2.8	1.6 ± 2.6	1.1 ± 2	1.9 ± 3.8	1.0 ± 1.9
Anger	1.6 ± 1.9	2.1 ± 2.2	1.7 ± 1.9	2.2 ± 2.4	1.9 ± 2.8	1.6 ± 2
Vigor	19.6 ± 6.1	18.3 ± 4.8	17.6 ± 5.8	17.7 ± 4.9	15.5 ± 6.3	16.0 ± 5.8
Fatigue	2.5 ± 2.8	3.3 ± 3.1	2.7 ± 2.9	3.3 ± 2.9	7.0 ± 4.7	6.7 ± 5
Confusion	3.5 ± 2.3	3.3 ± 1.7	3.7 ± 2.7	3.5 ± 1.9	5.1 ± 3.3	3.9 ± 2.1
Total	-7.0 ± 13.5	-4.5 ± 11.6	-4.8 ± 15.2	-3.9 ± 11	4.2 ± 18.8	0.1 ± 14.2

Appendix 1.2a: Results for the Profile of Mood States (POMS) and its sub-scales (n = 30), with three data-points missing. Mean scores ± SD. Three data-points missing. Lower POMS total scores indicate better mood.

Factor	Time-Point			Medication			Time-Point* Medication		
	$F(2)$	df_2	p	$F(1)$	df_2	p	$F(2)$	df_2	p
Tension	0.91	60.0	.407	0.02	30.1	.883	2.12	59.2	.129
Depression	0.47	60.4	.627	1.32	30.2	.260	0.75	60.7	.478
Anger	0.12	59.6	.884	0.72	27.8	.404	1.03	58.5	.365
Vigor	15.44	60.5	< .001	0.09	29.7	.763	1.22	60.3	.303
Fatigue	36.03	60.7	< .001	0.32	30.2	.575	1.76	58.8	.182
Confusion	8.16	60.9	.001	3.23	30.1	.082	5.91	60.1	.005
Total	15.46	61.0	< .001	0.06	30.1	.809	4.34	60.3	.017

Appendix Table 1.2b: Mixed model results for the Profile of Mood States (POMS) and its sub-scales. n = 30, with three data-points missing. All mixed models included a random intercept term, as well as random slope terms for time-point and medication.

Appendix 1.3

Region	Hemis-phere	Size (voxel)	Peak coordinates			Peak voxel statistic:	
			x	y	z	Z	p_{FWE}
Stimulation > Baseline							
SII, central (51%)/parietal (12%) operculum	R	2695	38	-16	20	6.92	< .001
White matter (76%)/lat. ventricle (23%)/	R	81	12	-32	22	5.77	< .001
White matter (79%)/lat. ventricle (20%)/	L	210	-14	-40	20	5.65	< .001
Central (43%) operculum	L	30	-36	6	14	5.02	.006
Insula (58%)	L	129	-34	18	0	5.01	.006
White matter (47%)/lat. ventricle (47%)/	R	19	28	-48	12	4.91	.009
Heschl's gyrus (32%), planum polare (26%)	L	46	-46	-16	0	4.88	.010
DLPFC, Frontal pole (71%)	R	9	40	42	6	4.67	.024
Temperature > Baseline							
Insula (33%)	R	4341	34	-16	18	7.76	< .001
Insula (36%)	L	2294	-30	16	4	6.45	< .001
Insula (30%), central operculum (12%)	L	96	-34	-18	20	5.91	< .001
Post. supramarginal (61%), angular gyrus (15%)	R	74	64	-42	34	5.58	.001
Ant. supramarginal (43%), postcentral gyrus (20%)	L	53	-58	-26	26	5.58	.001
DLPFC, Frontal pole (80%)	R	102	38	48	22	5.44	.002
Supplementary motor cortex (46%)	R	82	6	8	64	5.19	.006
Lat. Ventricle (67%), caudate (30%)	L	51	-8	12	14	5.11	.008
SII, Parietal (41%), ant. supramarginal Gyrus (19%)	R	39	54	-28	24	4.99	.014
Ant. cingulate gyrus (26%)	R	8	12	12	34	4.96	.016
Ant. cingulate gyrus (86%)	L/R	18	2	14	26	4.94	.018
DLPFC, Frontal pole (89%)	L	9	-34	48	24	4.90	.020
DLPFC, Frontal pole (67%)	R	27	40	46	4	4.89	.021
Ant. (55%) and post. (18%) supramarginal gyrus	L	8	-60	-44	32	4.84	.026

Appendix 1.3: Whole brain-activity explained by Stimulation > Baseline and a Temperature > Baseline contrasts. Names with tissue probabilities in % are provided according to the Harvard-Oxford Atlas. All p-values were family-wise-error corrected. Only clusters with a minimum size of 5 voxels are reported. Abbreviations: Ant.: anterior, DLPFC: Dorsolateral prefrontal cortex, lat.: lateral L: left, post.: posterior, R: right, SII: somatosensory cortex II.

Appendix 1.4

Region	Hemis-phere	Size (voxel)	Peak Coordinates			Peak voxel statistic:	
			x	y	z	Z	p_{FWE}
Stimulation Regressor: Decreases under oxytocin							
Angular (22%)/supramarginal (14%) gyrus	R	86	40	-48	38	3.88	.410
Frontal Pole (2%)	L	10	-30	38	6	3.62	.684
White matter (80%) near postcentral gyrus (0%)	L	14	-28	-28	40	3.61	.696
Temperature Regressor: Decreases under oxytocin							
Hippocampus (20%)	R	15	34	-38	-6	3.58	.892
Frontal pole (46%)/orbitofrontal (13%) cortex	L	13	-22	38	-8	3.55	.913
Precuneus (37%)/cuneal (22%) cortex	L	15	-16	-70	26	3.49	.945
Calcarine cortex (11%)	L	10	-16	-88	8	3.45	.961
Lingual gyrus (23%)	R	16	18	-74	-2	3.38	.980

Appendix 1.4: Exploratory whole brain-effects of oxytocin on processing of stimulation in general (green) and a linear temperature predictor (blue). Names with tissue probabilities in % are provided according to the Harvard-Oxford Atlas. The statistical threshold was set to $p < .001$, uncorrected for multiple comparisons and a minimal cluster size of 5 voxel. No regions were found which showed increased activation under oxytocin. No significant effects were found when using FWE correction for multiple comparisons. Left hemisphere is shown left in the picture.

STUDY 2

Somatic symptoms evoked by exam stress in university students: the role of alexithymia, neuroticism, anxiety, and depression

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This is a pre-copy-editing, author-produced version of an article published 2013 in PLOS ONE (8:e84911) following peer review. The definitive publisher-authenticated version is available at:
http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0084911

Abstract

BACKGROUND: The etiology of somatization is incompletely understood, but could be elucidated by models of psychosocial stress. Academic exam stress has effectively been applied as a naturalistic stress model, however its effect on somatization symptoms according to ICD-10 and DSM-IV criteria has not been reported so far. Baseline associations between somatization and personality traits, such as alexithymia, have been studied exhaustively. Nevertheless, it is largely unknown if personality traits have an explanatory value for stress induced somatization.

METHODS: This longitudinal, quasi-experimental study assessed the effects of university exams on somatization—and the reversal of effects after an exam-free period. Repeated-observations were obtained within 150 students, measuring symptom intensity before, during, and after an exam period, according to the Screening for Somatoform Symptoms 7-day (SOMS-7d). Additionally, self-reports on health status were used to differentiate between medically explained and medically unexplained symptoms. Alexithymia, neuroticism, trait-anxiety, and baseline depression were surveyed using the Toronto-Alexithymia Scale (TAS-20), the Big-Five Personality Interview (NEO-FFI), the State Trait Anxiety Inventory (STAI), and Beck's Depression Inventory (BDI-II). These traits were competitively tested for their ability to explain somatization increases under exam stress.

RESULTS: Somatization significantly increased across a wide range of symptoms under exam stress, while health reports pointed towards a reduction in acute infections and injuries. Neuroticism, alexithymia, trait anxiety, and depression explained variance in somatization at baseline, but only neuroticism was associated with symptom increases under exam stress.

CONCLUSION: Exam stress is an effective psychosocial stress model inducing somatization. A comprehensive quantitative description of bodily symptoms under exam stress is supplied. The results do not support the stress-alexithymia hypothesis, but favor neuroticism as a personality trait of importance for somatization.

Introduction

Somatization has been defined as the "tendency to experience and communicate somatic distress in response to psychosocial stress and to seek medical help for it" (Lipowski, 1988). Although the re-definition of somatization as a clinical concept and its classification under the psychiatric category "somatic symptom disorders" is a matter of ongoing debate (Dimsdale & Creed, 2009) there is consensus that medically unexplained symptoms (MUS) and a stress-related etiology belong to its core features.

Somatization and personality traits—is alexithymia the key concept?

The causes of somatization have been hypothesized to be multifactorial, involving several mechanisms (for review see: Kellner, 1990; Lipowski, 1988; Rief & Broadbent, 2007). Evidence suggests co-occurrence and shared mechanisms with negative affect, anxiety (De Gucht, Fischler, & Heiser, 2004), neuroticism (Costa & McCrae, 1980; Wise & Mann, 1994), and alexithymia (Mattila et al., 2008). Especially alexithymia, the inability to identify, describe and differentiate emotions, has attracted considerable attention as a potential predisposing factor for somatization (for review see: De Gucht & Heiser, 2003). However, the belief that alexithymia causes or contributes to somatization is mainly based on cross-sectional studies, which do not allow causal inferences (De Gucht & Heiser, 2003; Mattila et al., 2008). Several authors therefore underlined the need for more longitudinal studies (Bailey & Henry, 2007; De Gucht & Heiser, 2003; Mattila et al., 2008). Mechanistically, alexithymia has been hypothesized to affect somatization by modulating physiological responses to stress (Martin & Pihl, 1985). Although this "stress-alexithymia hypothesis" has been experimentally tested on measures of autonomic reactivity (Connelly & Denney, 2007; de Timary, Roy, Luminet, Fillée, & Mikolajczak, 2008), its relevance for somatization induced by a naturalistic psychosocial stressor has, to our knowledge, not been tested to date.

The effects of exam stress on somatization are unknown

The effectiveness of exam stress as a model of psychosocial stress has repeatedly been shown on immunological (Borella et al., 1999; Johannsen, Bjurshammar, & Gustafsson, 2010; Weik & Deinzer, 2010), neuroendocrine (Sherman, Bunyan, Creswell, & Jaremka, 2009; Weik & Deinzer, 2010), physiological, and psychological (Koh, Choe, Song, & Lee, 2006; Loft et al., 2007; Šimić & Manenica, 2012; Spangler, 1997) parameters. Despite these associations, exam stress has not been used to investigate predisposing factors of somatization so far. To our knowledge only Koh and colleagues (2006) determined the effects of exam stress on somatization, showing a significant positive relationship in 38 participants. Still, no quantitative description of somatization symptoms under exam stress is available, although the somatic symptoms of acute exam anxiety have been assessed systematically (Pitts, Winokur, & Stewart, 1961; Ree, French, MacLeod, & Locke, 2008).

The present study investigated somatization by exploring increases in MUS as a reaction to naturalistic psychosocial stress and by competitively testing the explanatory value of several personality traits including alexithymia.

Our first aim was to provide a quantitative description of somatic symptom increases under exam stress including all 53 physical symptoms from the somatization symptom lists of ICD-10 and DSM-IV. It was hypothesized that an exam period affects total symptom scores, as well as

distinct symptoms. Both were expected to increase under exam stress and return to baseline after a period without exams.

Our second aim was to test the predictive value of alexithymia and such related concepts as neuroticism, trait anxiety, and depression for increases in somatization under exam stress. It was hypothesized that alexithymia correlates positively with somatic symptom increases during exam stress, according to the stress-alexithymia hypothesis, and show a stronger association with these increases than neuroticism, state anxiety, or depression.

Methods

The study was approved by the ethics committee of the University of Regensburg and conforms to the Declaration of Helsinki. Informed consent was obtained. Data were collected and analyzed pseudonymously. Written informed consents were obtained.

Study design:

We conducted a longitudinal, quasi-experimental study with a natural event, a reversal period and several control variables. The natural, predictive event was defined as a major university exam. Since exam types vary between academic disciplines, this was specified as an exam being prerequisite for graduation, or contributing to the university degree. Because exams are often clustered the most fearsome and/or distressing exam according to participants' choice was selected. The intervention reversal period was defined as a subsequent exam-free period of 30 days. Repeated observations were obtained at three times within participants: before (Pre-Baseline) and immediately after the predictive exam (Exam Period), as well as after reversal period (Post-Baseline). To guarantee that exam stress did not affect baselines, data was only included when participants reported no exam within the last *and* next 30 days. Moreover, to assure that the effect of the exam was maximal, data for Exam Period was excluded when participants failed to submit their survey within 3 days. Figure 2.1 provides an overview of the study's time course.

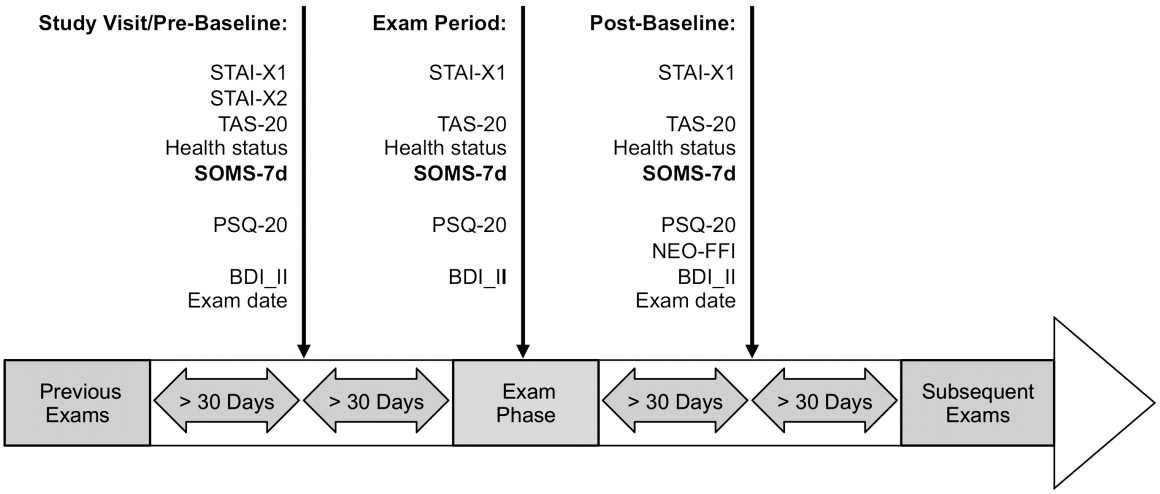

Figure 2.1: Timeline

To increase internal validity, additional control measures were recorded: acute infections, injuries, or exacerbations of pre-existing conditions, i.e. medically explained symptoms (MES), pose an obvious alternative explanation for increases in symptom reports. Therefore, descriptive participant reports of current health status were surveyed, categorized, and subsequently tested for effects of Exam Period. Additional measures of stress, state-anxiety, and negative mood were taken to reassure that Exam Period was an effective psychosocial stressor.

Participants:

For the present study 150 students of the University of Regensburg and the Regensburg University of Applied Sciences were recruited systematically across all faculties by advertisement via bulletins, flyers, and personal appeal at academic lectures. Investigators' relatives, friends, and colleagues were excluded from participation. Past or present internal, neurological, hormonal, or psychiatric disorders were evaluated in a structured interview at study inclusion. Participants with acute conditions and in medical treatment were excluded from participation. Individuals with past or chronic disorders in stable remission were included, but their status was addressed as a potential confound in analysis. Participants received a compensation of 8 Euros per hour.

Procedure:

The only study visit was scheduled at least 30 days before the first major exam. Written informed consent, medical history, and exam dates were obtained. If necessary, exam dates were followed up by telephone interview and participants were advised to report any exams that had to be re-scheduled. In addition, exam dates were retrieved at the end of the Post-Baseline session. All questionnaires were obtained using online forms. Each participant received an e-mail containing a web-link to an online platform and instructions on the day of study visit one (Pre-Baseline), the day of the selected exam (Exam Period) and one month after the last exam according to the participant's specifications (Post-Baseline, see Figure 2.1). All online-questionnaires were identical to the paper versions, with the exception that missing items were prohibited by forced-choice settings.

First aim—state questionnaires:

Symptom intensities of 53 physical symptoms from the somatization symptom lists of ICD-10 and DSM-IV were measured according to the Screening for Somatoform Symptoms 7-day version (SOMS-7d, Hiller, Rief, & Brähler, 2006; Rief & Hiller, 2003), with the difference that instructions asked participants to report perceived impairment for all symptoms, *without* requesting participants to differentiate between MUS and MES. The SOMS-7d was designed to measures impairment by 53 typical somatization symptoms, such as "headache", "bloating", or "back ache" on a 5-point Likert-scale within the last seven days: Scores are 0 (symptom absent or not impairing), 1 (mild), 2 (medium), 3 (severe), and 4 (very severe). SOMS-7d "Symptom Index" was the main outcome measure, calculated by summation of all items (Hiller et al., 2006; Rief & Hiller, 2003, 2008).

Four custom items surveying current health status within the last seven days were used to differentiate between MES and MUS: Item one asked if the participant was feeling healthy today. Item two asked if medical treatment or counsel had recently been taken. Item three asked for the occurrence of any disease or injury and item four for exacerbations of pre-existing conditions. If any item was answered with "yes", participants were required to enter a detailed description of their condition and symptoms into an open form field. Based on these items and responses, three of the authors (M.Z., H.E., V.B.) independently categorized sessions as "evidence for MES" (yesMES), or "no evidence for MES" (noMES). Where raters' categorization did not match, sessions were classified as yesMES. This approach to differentiate between MES and MUS was chosen to preclude that the causal attribution of participants could bias somatization scores.

The Perceived Stress Questionnaire (PSQ-20, Fliege et al., 2005) was used to confirm that the Exam Period was perceived as stressful. The PSQ-20 is a shortened, German adaptation of the original PSQ-30 (Levenstein et al., 1993), with good reliability (Cronbach's $\alpha > .80$). Perceived

stress is measured using 20 negatively and positively worded items, such as "Your problems seem to be piling up", "You have trouble relaxing", or „You have enough time for yourself". Items are rated on a 4-point Likert scale (1 "almost never", 2 "somtimes", 3 "often", 4 "usually").
The German versions of the State-Trait Anxiety Inventory–State Form (STAI-G-X1, Laux, Glanzmann, Schaffner, & Spielberger, 1981) and Beck's Depression Inventory II (BDI-II, Beck, Steer, Ball, & Ranieri, 1996), were used to survey state anxiety and depression levels at all time-points as an intervention check. For consistency, the BDI-II was adapted to a time-span of seven days.

Second aim—trait questionnaires:
Alexithymia and three competing explanatory personality traits were examined for their explanatory value for SOMS-7d scores: The 20-item version of the Toronto-Alexithymia Scale (TAS-20, Bagby, Parker, & Taylor, 1994; Bagby, Taylor, & Parker, 1994; Franz et al., 2008) was the primary measure of alexithymia with respect to our second aim. The TAS-20 surveys alexithymia on the three dimensions "Difficulty identifying feelings", "Difficulty describing feelings", and "Externally oriented thinking style". It uses negatively and positively worded items such as: "I'm often confused about what emotion I am feeling", "I am able to describe my feelings easily", and "Being in touch with emotions is essential". Items are rated on a five-point Likert scale ranging from 1 (strongly disagree) to 5 (strongly agree). From the perspective of test- and measurement-theory the 20-item version of the TAS-20 is the most robust and time-economic instrument to measure Alexithymia currently available. However it has been criticized for its lack of discriminative validity and the fact that it requires a self-evaluation—the very feature alexithymia individuals are impaired in by definition (Kooiman, Spinhoven, & Trijsburg, 2002; Rief, Heuser, & Fichter, 1996; Suslow, Donges, Kersting, & Arolt, 2000). Although defined as a trait measure, we collected the TAS-20 at all three time-points, to follow up reports on its doubtful temporal stability as a side-aim (De Gucht, Fontaine, & Fischler, 2004). For all other analyses the Post-Baseline score of the TAS-20 was used.
Trait-anxiety was surveyed using the State-Trait Anxiety Inventory – Trait Form (STAI-G-X2, Laux et al., 1981). Trait neuroticism was measured by using the Big Five Personality Inventory NEO-FFI (Körner et al., 2008). Trait depression was defined as the mean BDI-II score of both baselines. Although the BDI-II is mostly used as a measure of state depression it has been reported to accurately assess trait-like characteristics (Spielberger, Brunner, Ritterband, & Reheiser, 2003).

Statistics
Statistics were processed with SPSS 21.0.0.0 for Mac OS (Statistical Product and Service Solutions Inc., Chicago, IL, USA), graphs were created using GraphPad Prism 5.0 (GraphPad Software, Inc, La Jolla, CA, USA). Statistical tests were performed at a two-tailed $\alpha < .05$. Means are given ± standard deviation if not denoted otherwise.
For aim one, a mixed linear model was used to estimate the effect of fixed factor time with the levels Pre-Baseline, Exam Period, and Post-Baseline on SOMS-7d Symptom Index, using SPSS's genlinmixed function. To control for potential confounds, fixed factors MES with the levels yesMES and noMES, as well as factor disorder with levels yesDIS (i.e. stable past or chronic disorders) and noDIS (i.e. no disorders reported) were included into the model. To account for individual differences in SOMS-7d intensity score, a random intercept was added for each participant. An autoregressive covariance matrix (AR1) was used to model repeated covariance

within sessions. Robust estimation options and Satterthwaite-approximation were used to account for potential violations of model assumptions and correction of the estimated degrees of freedom for unequal sample sizes. The mixed model allows analyzing repeated-measures data without list-wise exclusion of missing values. Therefore all sessions meeting the deadlines in respect to past/upcoming exams were included in the analysis, even when a participant missed one or two sessions or deadlines. All other control variables besides MES were tested for effects of time analog to the linear mixed model for somatization scores.

For MES, a chi-square test was used to determine if the proportion of reported infections/injuries during Exam Period differed from baselines. Further the mixed model described above was applied to address the question if the TAS-20 is a stable trait measure over time. In addition inter- (Pearson's correlation coefficient) and intra-class correlations (ICC) for the TAS-20 were computed. Friedman's tests were used for an item-by-item analysis of the SOMS-7d in order to identify symptoms increasing during Exam Period. For this item analysis an adapted α-level of $< .001$ was used to control for multiple-comparisons, and post-hoc Wilcoxon's paired rank tests were performed to confirm increases between Exam Period and at least one baseline.

Aim two was to identify the best explanatory trait variable for somatic symptoms at baseline and symptom increase under exam stress. Traits were alexithymia (TAS-20), neuroticism (NEO-FFI), trait anxiety (STAI-X-2), and trait depression (BDI-II). First, a correlation table using Kendall's τ-b was created to explore monotonous relationships. Kendall's τ-b is the non-parametric correlation coefficient of choice for symptom rating scales, especially when comparisons are made between (sub-) samples of different size (Arndt, Turvey, & Andreasen, 1999). For correlational analysis, Symptom Index at baseline was defined as the mean of both baseline sessions and somatization increase was defined as Symptom Index during the Exam Period minus Baseline. Correlation analysis was followed by a modeling approach to determine the best trait predictor of somatization symptoms. Eight variants of the basic model described above (Time, MES, and Disorder [df_1=4]) were compared: Four models were created by adding one of the trait variates: Alexithymia, Neuroticism, Anxiety, or Depression (df_1=5). Four further models included the respective first-degree trait-by-time interaction (df_1=7) in addition. An individual random slope parameter for each trait variate was added to each model to keep the random effects structure of the model "maximal" (Barr, Levy, Scheepers, & Tily, 2013). Finally, Akaike's Information Criterion for finite sample sizes (AICc) and Akaike Weights (Wagenmakers & Farrell, 2004) were used to determine the best-fitting model.

Results

Sample description

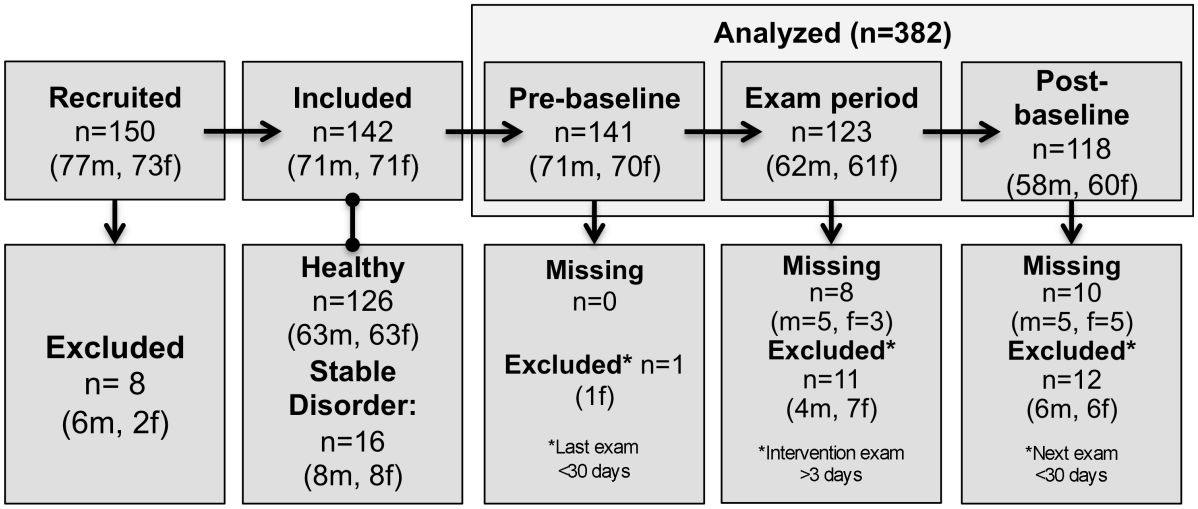

Figure 2.2: Participant flow

Figure 2.2 gives an overview of participant flow. Analysis was based on 142 participants (71 female), of which 107 contributed three, 26 two, and 9 one valid session(s). Mean age at study inclusion was 22.2 ± 2.5 years (range: 18-33). Participants with past or chronic internal, neurological, and psychiatric disorders in stable remission constituted 11 % (8 male, 8 female) of the sample. The mean number of exams reported was 4.9 ± 2.1, ranging from 1 to 12. At mean, the Exam Period survey was submitted 1.0 ± 1.6 days after the exam defined as the intervention. For additional sample information see Appendix 2.1.

Effects of exam stress on somatization

	Pre-Baseline n = 141	**Exam Period** n = 123	**Post-Baseline** n = 118
Primary variable:			
SOMS-7d	11.1 ± 8.9	18.2 ± 14.5	9.7 ± 9.0
Control variables:			
PSQ-20	33.6 ± 17.5	54.0 ± 19.3	29.8 ± 18.3
BDI-II	6.6 ± 6.4	11.5 ± 7.0	5.2 ± 5.4
STAI-G-X1 state	36.9 ± 8.1	42.74 ± 12.3	37.1 ± 10.3
Side aim: temporal stability of the TAS-20			
TAS-20	45.3 ± 9.7	45.9 ± 10.1	44.6 ± 10.2

Table 2.1: The effects of Exam Period on measures of somatic symptoms and stress: Descriptive results. All values represent mean ± SD. **Abbreviations**: BDI-II: Beck's Depression Inventory; PSQ-20: Perceived Stress Questionnaire; SOMS-7d: Screening for Somatoform Symptoms 7-day version; STAI-G-X1 state: State-Trait Anxiety Inventory – State Form; TAS-20: Toronto Alexithymia Scale.

In summary, the Symptom Index and all intervention check variables assessing stress, depression, and state anxiety were found to significantly increase during the Exam Period compared to both Pre and Post Baseline. Inclusion of the factors MES and disorder significantly improved model fit for the prediction of Symptom Index ($\Delta df1$ = +2, $\Delta AICc$ = -20.29) and most control variables. Descriptive results are shown in Table 2.1, the corresponding mixed model results are shown in Table 2.2.

	Model	Coefficient estimates (β ± SEM)
Primary variable:		
SOMS-7d	F(4, 6) = 18.92, p < .001	β_{Exam} = 8.89 ± 1.06, t = 8.42, p = .009 β_{preBL} = 1.77 ± 0.69, t = 2.553, p = .012 β_{MESyes} = 2.44 ± 1.11, t = 2.20, p = .032 β_{yesDIS} = 7.63 ± 3.15, t = 2.42, p = .076
Control variables:		
PSQ-20	F(4, 168) = 55.68, p < .001	β_{Exam} = 24.22 ± 1.95, t = 12.45, p < .001 β_{preBL} = 3.98 ± 1.65, t = 2.410, p = .017 β_{yesMES} = 0.27 ± 2.12, t = 0.13, p = .899 β_{yesDIS} = 15.20 ± 4.25, t = 3.58 p = .001
BDI-II	F(4, 63) = 37.02, p < .001	β_{Exam} = 6.60 ± 0.61, t = 10.80, p < .001 β_{preBL} = 1.69 ± 0.50, t = 3.17, p = .003 β_{yesMES} = 1.04 ± 0.67, t = 1.55, p = .124 β_{yesDIS} = 4.49 ± 1.54, t = 2.92, p = .005
STAI-G-X1 state	F(4, 68) = 10.34, p < .001	β_{Exam} = 5.86 ± 1.19, t = 4.92, p < .001 β_{preBL} = 0.79 ± 0.90, t = 0.88, p = .930 β_{yesMES} = 0.69 ± 1.20, t = 0.58, p = .566 β_{yesDIS} = 6.32 ± 2.40, t = 2.63, p = .011
Side aim: Temporal stability of the TAS-20		
TAS-20	F(4, 377) = 2.71, p = .030	β_{Exam} = 1.80 ± 0.60, t = 3.00, p < .003 β_{preBL} = 0.79 ± 0.56, t = 1.41, p = .164 β_{yesMES} = 0.46 ± 0.93, t = 0.49, p = .623 β_{yesDIS} = 4.14 ± 3.18, t = 1.30, p = .359

Table 2: The effects of Exam Period on measures of somatic symptoms and stress: mixed model results. All linear mixed models included factors time (Pre-Baseline, Exam Period, Post-Baseline), MES (yesMES, noMES) and disorder (yesDIS, noDIS). Degrees of freedom (df_2) may vary due to inequal sample sizes and the Satterthwaite correction used. All analyses were based on 142 subjects contributing 382 data-points. **Abbreviations:** BDI-II: Beck's Depression Inventory; GLM: Generalized Linear Model; MES: Medically Explained Symptoms; preBL: Pre-Baseline; PSQ-20: Perceived Stress Questionnaire; SOMS-7d: Screening for Somatoform Symptoms 7-day version; STAI-G-X1: State-Trait Anxiety Inventory – State Form; TAS-20: Toronto Alexithymia Scale; yesDIS: with stable past/chronic disorders; yesMES: evidence for MES.

A chi-square test indicated that the proportion of participants reporting acute infections and/or injuries (evidence for MES) during Exam Period (8.1 %) was significantly lower ($df = 2, \chi^2 = 7.45$, $p = .024$) than at Pre- (19.1 %) and Post- (18.6 %) Baseline.

Almost all participants (95.0 %) reported at least one symptom causing mild impairment according to the combined ICD-10 and DSM-IV list at baseline. The cumulative proportions of participants reporting at least one moderate, severe, or very severe symptom were 64.9 %, 28.6 %, and 2.7 % respectively. These proportions increased under exam stress (mild: 97.6 %, medium: 80.5 %, severe: 51.2 %, very severe: 16.3 %). The eleven SOMS-7d items listed in Table 2.3 were found to be significantly elevated under exam stress according to Friedman's-tests and post-hoc tests at $\alpha \leq .001$. A full description of results for all SOMS-7d items is available in Appendix 2.2.

Item	Symptom	Pre-Baseline	Exam Period	Post-Baseline	Relative increase in prevalence		Exam effect
					any	severe	
		n = 141 Mean ± SD	n = 123	n = 117	% of baseline		df = 2 X^2_F, sig
1	Headache	0.82 ± 0.92	1.33 ± 1.11	0.71 ± 0.78	30.7	258.8	26.70, ****
2	Abdominal pain	0.65 ± 0.89	0.93 ± 0.98	0.64 ± 0.79	30.7	123.1	14.91, ***
3	Back pain	0.71 ± 0.86	1.24 ± 1.12	0.75 ± 0.84	29.0	474.3	26.85, ****
4	Joint pain	0.33 ± 0.69	0.59 ± 0.88	0.34 ± 0.64	53.2	76.5	14.17, ***
10	Nausea	0.45 ± 0.71	0.76 ± 0.90	0.41 ± 0.66	53.8	317.8	23.64, ****
12	Discomfort around stomach	0.72 ± 0.94	1.24 ± 1.10	0.54 ± 0.83	79.8	199.5	43.44, ****
17	Loss of appetite	0.30 ± 0.68	0.67 ± 0.97	0.22 ± 0.59	136.2	222.9	26.36, ****
20	Frequent diarrhea	0.22 ± 0.60	0.50 ± 0.91	0.19 ± 0.53	105.3	227.9	14.24, ***
30	Excessive tiredness after mild exertion	0.50 ± 0.85	1.03 ± 1.21	0.37 ± 0.62	66.7	487.5	29.88, ****
32	Sexual indifference	0.35 ± 0.69	0.57 ± 0.83	0.23 ± 0.55	80.9	164.8	16.08, ***

Table 2.3: Results for somatization items significantly increasing under exam stress. Symptoms were surveyed according to the Screening for Somatoform Symptoms 7-day version (SOMS-7d). Effects of Exam Period were tested using Friedman's test (X^2_F). The alpha-Level was set to $p \leq .001$ to correct for multiple comparisons. Significant results are depicted as **** $p \leq .0001$, *** $p \leq .001$. All post-hoc differences between baselines and Exam Period were significant as tested with Wilcoxon's paired rank tests (results not shown). Increases in symptom prevalence are shown in % of valid cases for any severity (score ≥ 1) and severe/very severe only (score ≥ 3) at baseline.

The explanatory value of personality traits on stress induced somatization

Mean TAS-20 baseline score was 44.9 ± 9.5 and therefore roughly 4 units below norm values for the German population (Franz et al., 2008). Accordingly, only 8.9 %, or 19.3 %, of participants reached a TAS-20 score of 61 or 52.5—cut-off values suggested for the diagnosis of "alexithymic" (Franz et al., 2008). The significant differences in mean response found for TAS-20 sum scores over time (see Table 2.2) were followed up by computing Pearson's coefficient and the intra-class correlation coefficient (ICC): Correlations were r = .788 for Survey 1 and 2, r = .804 for Survey 2 and 3, and r = .787 (all $p < .001$) for Survey 1 and 3. Single ICC was .786, mean ICC .917.

NEO-FFI-neuroticism scores (Körner et al., 2008) were close to normal values with a mean of 21.0 ± 7.2 points. Mean BDI-II score was 6.0 ± 5.2 and therefore 4 units below a normal student sample (Storch, Roberti, & Roth, 2004). STAI-G-X2 trait anxiety scores were at 39.2 ± 8.7 points and therefore 4 units above a normal student sample (Laux et al., 1981).

Correlations between somatic symptoms and trait variables according to Kendall's τ-b are shown in Table 2.4. NEO-FFI-neuroticism was the only significant trait that correlated with somatic symptom increase during Exam Period, showing a positive monotonous relationship. All trait variables correlated with Symptom Index at baseline. All trait variables were considerably inter-correlated.

	SOMS-7d baseline	SOMS-7d increase	TAS-20 alexithymia	NEO-FFI neuroticism	STAI-G-X2 trait anxiety
SOMS-7d increase	n = 134 τ = .074 p = .212				
TAS-20 alexithymia	n = 132 τ = .228 p < .001	n = 131 τ = -.050 p = .412			
NEO-FFI neuroticism	n = 132 τ = .359 p < .001	n = 131 τ = .152 p = .012	n = 132 τ = .270 p < .001		
STAI-G-X2 trait - anxiety	n = 142 τ = .295 p < .001	n = 134 τ = .074 p = .212	n = 132 τ = .319 p < .001	n = 132 τ = .514 p < .001	
BDI-II baseline depression	n = 142 τ = .413 p < .001	n = 134 τ = .066 p = .271	n = 132 τ = .327 p < .001	n = 132 τ = .470 p < .001	n = 142 τ = .482 p < .001

Table 2.4: Correlations between somatic symptoms (SOMS-7d Symptom Index) at baseline, somatic symptom increase during an Exam Period and personality traits. All tests were performed using Kendall's τ-b. **Abbreviations:** BDI-II: Beck's Depression Inventory; NEO-FFI: Big Five Personality Inventory**,** Neuroticism Subscale; SOMS-7d: Screening for Somatoform Symptoms 7 day version; STAI-G-X2: State-Trait Anxiety Inventory – Trait Form; TAS-20: Toronto Alexithymia Scale, 20-item version.

An information theory driven model selection approach was applied to determine the best explanatory trait variable for the obtained somatization scores. $AICc$ weights indicated that the basic model plus Neuroticism and Neuroticism x Time ($df1 = 7$) was the best-fitting model, with a probability of .97 relative to all equal or smaller models according to Akaike weights. The second-best model was the basic model plus the main effect of neuroticism only, with a probability of only .03. The competing models with TAS-20 and TAS-20 x Time ($\Delta AICc = +31.59$), STAI-G-X2 and STAI-G-X2 x Time ($\Delta AICc = +105.22$), and BDI-II and BDI-II x Time ($\Delta AICc = +81.93$) had only marginal likelihoods of being the best-fitting model.

The final model showed a significant main effect of Neuroticism ($\beta = 0.44 \pm 0.10$[SEM], $t = 4.34$, $p < .001$) and MES ($\beta = 2.55 \pm 1.20$[SEM], $t = 2.10$, $p = .039$), as well as a positive interaction between Neuroticism and Time $F(2, 10) = 5.54$, , $p = .025$, driven by a significant interaction of coefficients Neuroticism x Exam Period ($\beta = 0.42 \pm 0.13$[SEM], $t = 3.30$, $p = .020$). The factor Disorder fell short of the criterion of significance ($p = .128$), which was also missed by all other coefficients. A graphical display of the relationship between Symptom Index, Neuroticism, and Time is given in Figure 2.3.

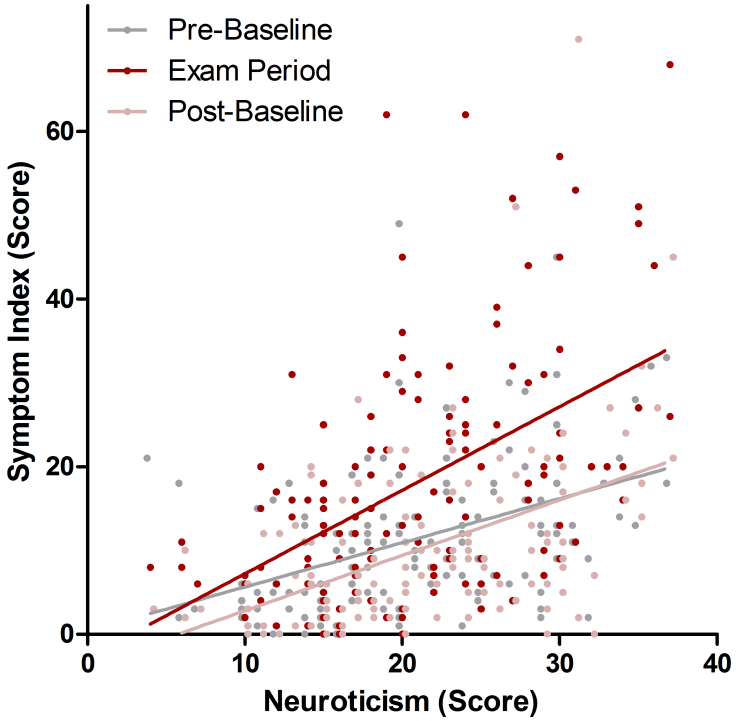

Figure 2.3: The relationship between trait neuroticism, somatic symptoms at baseline, and somatic symptoms under exam stress. There was a significant interaction between neuroticism and exam period (β = 0.42 ± 0.13[SEM], t = 3.30, p = .020), even when accounting for medically explained symptoms and pre-existing disorders. Raw data are shown and simple linear interpolation lines were added for illustrative purposes. To reduce overlap, data points for Pre- and Post-Baseline were shifted by -0.5 and +0.5 points along the x-axis, respectively,

The models including TAS-20 ($F[7, 3] = 16.39, p < .017$), STAI-G-X2 ($F[7, 7] = 15.01, p < .001$), and BDI-II ($F[7, 8] = 16.76, p < .001$) were all explaining a significant amount of variance, however the coefficients TAS-20 x Exam Period ($\beta = 0.101 \pm 0.12$[SEM], $t = 0.86, p = .553$), STAI-G-X2 x Exam Period ($\beta = 0.31 \pm 0.13$[SEM], $t = 2.46, p = .147$) and BDI-II x Exam Period ($\beta = 0.41 \pm 0.23$[SEM], $t = 1.78, p = .183$) failed to do so.

Original data for the present results have been made publicly available as a download. However, the following changes to the raw data file were made: Birthdate, exam date, date of study inclusion, information on illicit drug use, subject of study, original description of medical history and original description of current illness/injuries were deleted and/or replaced by summary variables to ensure participant's privacy.

Discussion

This quasi-experimental study was conducted to provide a comprehensive quantification of somatic symptoms under exam stress in healthy students and to evaluate whether personality traits such as alexithymia can explain their occurrence.

Exam stress increases somatization—typical symptoms

During an exam period, symptom scores showed highly significant increases compared to Pre- and Post-Baselines, even when accounting for participant reports of infections/injuries and pre-existing disorders statistically. This was paralleled by increases in perceived stress, depression, and anxiety. These findings confirm that academic exam periods represent an effective model for psychosocial stress, with a significant impact on somatization. Bodily complaints increased across several domains encompassing pain, gastro-intestinal, and autonomic symptoms. Significant increases during the Exam Period were mainly found for symptoms with a high prevalence at baseline, e.g: headache, back pain, abdominal pain, and nausea. An exception of this trend was bloating, which did not significantly increase although being one of the most common symptoms. The symptom with the highest absolute increase in prevalence across all severities was discomfort/churning around the stomach, which is synonymous to the proverbial "butterflies in the stomach" commonly associated with exams. However, the symptoms with the highest relative increases in prevalence were loss of appetite, frequent diarrhea, and sexual indifference. These can therefore be recommended as the most target specific symptoms of exam stress for future studies. Finally, the symptoms with the strongest relative increases when counting severe/very severe ratings only, were excessive tiredness after mild exertion, back pain, nausea, and headache, indicating that these are the exam stress-related symptoms perceived as the most impairing.

The differentiation of MES and MUS poses a challenging problem in the study of somatization (Klaus et al., 2013). Reports of infections and injuries were obtained in the present study to account for the effects of MES statistically. In addition these reports were found to significantly decline during Exam Period by about half, while predicting increased somatization symptom scores across all sessions. This decrease in MES may be explained by the well-known temporary immune-enhancing effects of acute stressors (Dhabhar, 2009), as well as reductions in social and/or physical activity during the exam preparation period.

Neuroticism is associated with somatization increases under stress

Neuroticism was found to explain a significant amount of variance in somatization under exam stress. Participants with high trait neuroticism scores showed higher symptom scores in general *and* higher symptom increases under exam stress. This finding replicates previous reports of baseline correlations between neuroticism and somatic symptoms (De Gucht, Fischler, et al., 2004; Lane, Carmichael, & Reis, 2011; Noyes et al., 2001; Wise & Mann, 1994) and extends them by showing that neuroticism can explain stress-induced somatization. Neuroticism has been investigated as personality trait moderating stress reactivity since a long time. It has been linked to differences in the appraisal of a stressor, as well as the reactivity to a stressor. Further it has been discussed in conjunction with differences in stress coping behaviors and even differences in exposure to stressors. (Bolger & Zuckerman, 1995; Gunthert, Cohen, & Armeli, 1999). All of these influences might account for the observed relationship.

No support for the stress-alexithymia hypothesis

Although the present study could replicate findings that TAS-alexithymia (for review see: De Gucht & Heiser, 2003), trait anxiety, and baseline depression (De Gucht, Fischler, et al., 2004) are positively associated with somatization at baseline, no such relationship could be found for increases in somatization under exam stress. Compared to neuroticism, TAS-alexithymia, anxiety, and baseline depression had only a marginal likelihood of being the best explanatory variable for the observed variance in Symptom Index. These results do not support our second hypothesis, which claimed that alexithymia was a significant and superior explanatory variable for exam stress induced somatization. This finding is in agreement with experimental studies, which reported that alexithymia was not associated with an increased reactivity to physiological measures of stress (de Timary et al., 2008; Pedrosa Gil et al., 2008). Further our results are in line with a longitudinal study, which could not find a predictive value of alexithymia for the persistence of unexplained physical symptoms in general medical outpatients (C. G. Kooiman, 2004). In accord with earlier conclusions (Lumley, Stettner, & Wehmer, 1996), our results point out that alexithymia and somatization might not be linked by a difference in reactivity to acute stress in the general population. It must be emphasized that exam stress is only one specific form of psychosocial stress. The link between alexithymia and stress reactivity might be different for other forms of psychosocial stress, such as long-term interpersonal stress. Moreover, the role of alexithymia might be different in patient samples, or highly alexithymic sub-populations.

On the temporal stability of the TAS-20

The temporal stability of the TAS-20, especially in clinical samples and under conditions of psychological distress, has been a matter of discussion (De Gucht, Fontaine, et al., 2004). In the present study, significant differences in TAS-20 between Session 2 and 3, but not 2 and 1 could be found. This result somewhat confirms reports of a state and probably stress dependent proportion of TAS-20 scores (De Gucht, Fontaine, et al., 2004; Fukunishi, Kikuchi, Wogan, & Takubo, 1997). However, the small size of session-to-session differences during a period of increased stress leads us to the conclusion that the TAS-20 is a reliable trait measure with only minor state confounds. Pearson's correlation coefficients computed and a ICC computed for all three survey time-points confirm this notion by indicating an acceptable re-tests reliability of $r > .75$.

Limitations

Two baselines, before and after Exam Period, were included in the present study. Since all psychometric scores returned to normal at Post-Baseline, it can be ruled out that the observed increases reflect simple time effects. A number of control variables showed parallel effects for measures of distress (stress, depression, and anxiety) and a reduction in infection and/or injuries, which confirms that the observed effects are due to exam stress. MES were surveyed and categorized to control for participant bias. Nevertheless, several limitations apply to the present study and its interpretation:

The main limitation of the present study is that response bias, driven by social desirability might have confounded all measures. Participants were aware of the study's focus on the effects of exam stress; otherwise exact exam dates could not have been obtained. This might have in- or deflated somatization reports during the Exam Period, especially since it is known that students tend to bias retrospective ratings of pre-exam distress in the direction that maximizes self-esteem (Dewhurst & Marlborough, 2003).

In addition, our sample size decreased with each survey. This might have biased results towards a healthier student sample. Differences between Pre- and Post-Baselines are therefore not discussed, since these might be explained by drifts in sample characteristics and reduced stress levels alike.
Finally, the trait measures NEO-FFI and STAI-G-X2 have been recorded at Pre- or Post-Baseline only, to reduce participant's timely efforts. Despite the high temporal stability of the STAI-G-X2 (Laux et al., 1981), its comparability with the other trait variables might be confounded by subtle state-differences between the baselines and the mentioned change in sample size.

Conclusion

Here we could show that somatization significantly increased under exam stress across a range of symptom dimensions in healthy university students. The present results verify that transient increases in somatization can be evoked by psychosocial stress. The dataset constitutes a valuable basis for future studies on bodily symptoms under psychosocial stress. Further the present findings could not support the stress-alexithymia hypothesis. This highlights that studies in search of personality factors predisposing for somatization should always consider alternative explanatory concepts. Neuroticism was identified as a better correlate of somatization induced by acute exam stress than TAS-alexithymia, trait anxiety, or depression. Perceptions or behaviors related to neuroticism might be of etiological importance for somatization under psychosocial stress and pose interesting targets for future studies.

Acknowledgements:

Funding: Author M.Z. is supported by a scholarship of the "German National Merit Foundation". The funders had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript. This research received no other specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Appendix 2.1

Additional sample information

SAMPLING: The sample size of 150 participants was selected a-priori, based on the resources available for recruiting and testing. Of the 142 participants passed to analysis, 90 participants were recruited during academic winter term 2011/2012 and 52 during summer term 2012. A mean of 126.1 ± 48.3 days passed between study inclusion (Pre-Baseline) and study exclusion (Post-Baseline). Mean exam phase duration (last, minus first exam date) was 19.7 ± 20.0 days, ranging from 0 to 122. Data for all variables was checked for outliers by visual examination of histograms, scatterplots, and by comparison with the available reference sample distributions. No unambiguous outliers were identified.

EXCLUDED FROM STUDY: Eight (6 male, 2 female) participants were excluded from the study due to the following reasons:

Acute disorder or injury (n = 3); Lost to follow up (n = 3); University drop out (n = 1). Voluntary drop out (n = 1). No reason was given for the withdrawal of consent for the voluntary drop out.

PAST/CHRONIC DISORDERS: Sixteen (8 male, 8 female) participants had a history of major internal, or psychiatric disorders, or stable chronic disorders. These were: migraine (n = 5); chronic back pain (n = 3); chronic Joint pain (n = 2); depression (n = 2); tinnitus, urticaria, orofacial pain, social anxiety (each n = 1).

PARTICIPANTS' FIELDS OF STUDY (IN % OF VALID CASES.): Psychology/medicine 26.8 %; Humanities 27.5 %; Natural sciences, engineering, mathematics, or informatics 22.5 %; Economics 5.6 %; others in 17.6 %.

PARTICIPANTS' DRUG HABITS (IN % OF VALID CASES): Regular alcohol consumption: 83.7 %; regular nicotine consumption: 22.7 %; regular caffeine consumption: 66.7 %; regular consumption of cannabis/illicit drugs: 0.7 %

Appendix 2.2

Item No	Symptom	Pre-Baseline n = 141 (f = 70, m = 71)			Exam Period n = 123 (f = 61, m = 62)			Post-Baseline n = 117 (f = 59, m = 58)			Relative Increase		Exam Effect	
		Symptom Index	% Prevalence		Symptom Index	% Prevalence		Symptom Index	% Prevalence		% Baseline Prevalence		X^2_F	Sig.
		Mean±SD	Any/Severe		Mean±SD	Any/Severe		Mean±SD	Any/Severe		Any/Severe			
1	Headache	0.82±0.92	53.2	5.7	1.33±1.11	70.7	16.3	0.71±0.78	55.1	3.4	30.7	258.8	26.70	****
2	Abdominal pain	0.65±0.89	42.6	4.3	0.93±0.98	57.7	5.7	0.64±0.79	45.8	0.8	30.7	123.1	14.91	***
3	Back pain	0.71±0.86	50.4	2.8	1.24±1.12	67.5	15.4	0.75±0.84	54.2	2.5	29.0	474.3	26.85	****
4	Joint pain	0.33±0.69	23.4	2.8	0.59±0.88	37.4	3.3	0.34±0.64	25.4	0.8	53.2	76.5	14.17	***
5	Pain in legs/ arms	0.29±0.54	25.5	0.7	0.51±0.80	36.6	1.6	0.43±0.71	33.1	0.8				
6	Chest pain	0.09±0.35	7.1	0.7	0.21±0.62	13.8	1.6	0.19±0.51	14.4	0.8				
7	Rectal pain	0.11±0.35	9.2	0.0	0.15±0.54	8.9	1.6	0.06±0.33	4.2	0.8				
8	Pain during sexual intercourse	0.06±0.31	4.3	0.7	0.07±0.25	6.5	0.0	0.08±0.32	5.9	0.0				
9	Pain during urination	0.06±0.34	4.3	0.7	0.08±0.35	6.5	0.8	0.03±0.18	3.4	0.0				
10	Nausea	0.45±0.71	33.3	0.7	0.76±0.90	50.4	3.3	0.41±0.66	32.2	0.8	53.8	317.8	23.64	****
11	Bloating	0.89±0.93	57.4	6.4	0.91±0.91	60.2	4.9	0.75±0.97	46.6	8.5				
12	Discomfort/ churning around stomach	0.72±0.94	44.0	6.4	1.24±1.10	71.5	14.6	0.54±0.83	35.6	3.4	79.8	199.5	43.44	****
13	Vomiting (excluding pregnancy)	0.04±0.26	2.8	0.0	0.03±0.28	1.6	0.8	0.05±0.29	3.4	0.0				
14	Regurgitation	0.20±0.50	16.3	0.7	0.36±0.77	22.8	3.3	0.11±0.31	11.0	0.0				
15	Hiccup or heartburn	0.18±0.49	14.9	0.7	0.17±0.47	13.0	0.0	0.17±0.42	15.3	0.0				
16	Food intolerance	0.23±0.58	16.3	1.4	0.33±0.76	19.5	3.3	0.24±0.62	16.1	2.5				
17	Loss of appetite	0.30±0.68	19.9	2.8	0.67±0.97	41.5	7.3	0.22±0.59	15.3	1.7	136.2	222.9	26.36	****
18	Bad taste in mouth or coated tongue	0.45±0.76	31.9	3.5	0.49±0.79	33.3	3.3	0.32±0.64	24.6	1.7				
19	Dry mouth	0.24±0.57	17.7	0.7	0.38±0.75	26.0	4.1	0.27±0.59	20.3	0.8				
20	Frequent diarrhea	0.22±0.60	14.9	2.1	0.50±0.91	30.1	4.9	0.19±0.53	14.4	0.8	105.3	227.9	14.24	***
21	Discharge of fluid from anus	0.05±0.28	3.5	0.0	0.07±0.34	4.9	0.0	0.02±0.13	1.7	0.0				
22	Frequent urination	0.33±0.62	27.0	1.4	0.60±0.94	36.6	6.5	0.24±0.60	16.9	1.7				
23	Frequent defecation	0.25±0.58	18.4	0.7	0.44±0.80	27.6	3.3	0.15±0.48	11.0	0.8				
24	Palpitations	0.13±0.42	10.6	0.0	0.33±0.70	23.6	3.3	0.11±0.41	8.5	0.8				
25	Feelings of pressure around precordium	0.09±0.31	8.5	0.0	0.24±0.61	18.7	1.6	0.13±0.44	9.3	0.8				
26	Sweating	0.23±0.51	19.1	0.7	0.44±0.84	26.8	4.1	0.22±0.53	16.9	0.0				
27	Flushing or blushing	0.23±0.56	17.7	0.7	0.45±0.78	29.3	2.4	0.19±0.44	17.8	0.0				
28	Breathlessness without exertion	0.08±0.36	5.7	0.7	0.15±0.57	9.8	2.4	0.08±0.36	5.9	0.0				

Item No	Symptom	Pre-Baseline n = 141 (f = 70, m = 71)			Exam Period n = 123 (f = 61, m = 62)			Post-Baseline n = 117 (f = 59, m = 58)			Relative Increase		Exam Effect	
		Symptom Index	% Prevalence		Symptom Index	% Prevalence		Symptom Index	% Prevalence		% Baseline Prevalence		X^2_F	Sig.
		Mean±SD	Any/Severe		Mean±SD	Any/Severe		Mean±SD	Any/Severe		Any/Severe			
29	Hyperventilation	0.11±0.43	8.5	1.4	0.21±0.62	13.8	3.3	0.07±0.31	5.1	0.0				
30	Excessive tiredness on mild exertion	0.50±0.85	31.9	5.0	1.03±1.21	52.0	17.1	0.37±0.62	30.5	0.8	66.7	487.5	29.88	****
31	Blotchiness or discoloration of skin	0.10±0.40	6.4	0.0	0.31±0.73	19.5	2.4	0.09±0.39	6.8	0.8				
32	Sexual indifference	0.35±0.69	26.2	2.8	0.57±0.83	39.8	4.9	0.23±0.55	17.8	0.8	80.9	164.8	16.08	***
33	Unpleasant sensations around genitals	0.11±0.39	8.5	0.7	0.14±0.50	9.8	0.8	0.04±0.20	4.2	0.0				
34	Impaired coordination or balance	0.13±0.39	10.6	0.0	0.28±0.63	20.3	1.6	0.08±0.30	6.8	0.0				
35	Paralysis or myasthenia	0.01±0.12	1.4	0.0	0.07±0.34	4.9	0.0	0.03±0.16	2.5	0.0				
36	Difficulty swallowing or lump in throat	0.18±0.55	11.3	1.4	0.16±0.55	10.6	2.4	0.08±0.27	7.6	0.0				
37	Loss of voice	0.11±0.40	9.2	0.7	0.07±0.32	5.7	0.0	0.07±0.31	5.1	0.0				
38	Urinary retention	0.01±0.08	0.7	0.0	0.04±0.24	3.3	0.0	0.03±0.21	1.7	0.0				
39	Hallucinations	0.06±0.23	5.7	0.0	0.10±0.32	8.9	0.0	0.04±0.24	3.4	0.0				
40	Loss of touch or pain sensation	0.01±0.08	0.7	0.0	0.04±0.24	3.3	0.0	0.04±0.20	4.2	0.0				
41	Paresthesias	0.14±0.41	12.1	0.0	0.25±0.64	17.1	2.4	0.11±0.34	10.2	0.0				
42	Double vision	0.05±0.30	3.5	0.7	0.07±0.31	4.9	0.0	0.01±0.09	0.8	0.0				
43	Blindness	0.01±0.08	0.7	0.0	0.01±0.09	0.8	0.0	-	-	0.0				
44	Deafness	0.03±0.17	2.8	0.0	0.01±0.09	0.8	0.0	0.03±0.16	2.5	0.0				
45	Seizures	0.01±0.12	1.4	0.0	0.07±0.34	4.9	0.0	0.04±0.24	3.4	0.0				
46	Amnesia	0.06±0.30	5.0	0.0	0.11±0.40	7.3	0.0	0.05±0.26	4.2	0.0				
47	Fainting	0.01±0.08	0.7	0.0	-	-	0.0	0.01±0.09	0.8	0.0				
48	Painful menstruation	0.51±0.96	27.1	5.7	0.56±0.99	29.5	6.6	0.47±0.91	25.0	6.7				
49	Irregular menstruation	0.41±0.96	20.0	5.7	0.67±1.22	31.1	13.1	0.33±0.84	18.3	5.0				
50	Excessive menstrual bleeding	0.17±0.54	11.4	1.4	0.20±0.65	9.8	3.3	0.15±0.49	10.2	0.0				
51	Vomiting during pregnancy	-	-	-	-	-	-	-	-	-				
52	Unusual/excessive vaginal discharge	0.24±0.52	20.0	0.0	0.48±0.81	31.1	3.3	0.31±0.65	22.0	1.7				
53	Erectile or ejaculatory dysfunction	0.06±0.37	2.8	1.4	0.02±0.13	1.6	0.0	0.05±0.22	5.2	0.0				

Appendix 2.2: SOMS items under exam stress: Full results. Symptoms were surveyed according to the Screening for Somatoform Symptoms 7 day (SOMS-7d)**.** Effects of Exam Period were tested using Friedman's test (X^2_F). The alpha-Level was set to ≤ 0.001 to correct for multiple comparisons. Significant results are depicted as **** $p \leq 0.0001$, *** $p \leq 0.001$. All post-hoc differences between baselines and Exam Period were significant as tested with Wilcoxon's paired rank tests (results not shown). Increases in symptom prevalence are shown in %of valid cases for any severity (score ≥ 1) and severe/very severe only (score ≥ 3) at baseline.

STUDY 3

Do cardiorespiratory variables predict the antinociceptive effects of deep and slow breathing?

Matthias Zunhammer, Peter Eichhammer, Volker Busch

This is a pre-copy-editing, author-produced version of an article published 2013 in Pain Medicine (14:843–854) following peer review. The definitive publisher-authenticated version is available at: http://onlinelibrary.wiley.com/journal/10.1111/%28ISSN%291526-4637

Abstract

Deep and slow breathing (DSB) is a central part of behavioral exercises used for acute and chronic pain management. Its mechanisms of action are incompletely understood.

OBJECTIVES: 1) To test the effects of breathing frequency on experimental pain perception in a dose dependent fashion. 2) To test the effects of breathing frequency on cardiorespiratory variables hypothesized to mediate DSB analgesia. 3) To determine the potential of the cardiorespiratory variables to mediate antinociceptive DSB effects by regression analysis.

DESIGN: Single-blind, randomized, crossover trial.

SUBJECTS: Twenty healthy participants.

INTERVENTIONS: Visually paced breathing at 0.14 Hz, 0.10 Hz, 0.06 Hz, and resting frequency.

OUTCOME MEASURES: Cardiorespiratory variables: RR-interval (= 60 s/heart rate), standard deviation of the RR-interval (SDRR), and respiratory CO_2. Experimental pain measures: Heat pain thresholds, cold pain thresholds, pain intensity ratings, and pain unpleasantness ratings.

RESULTS: 1) There was no significant effect of DSB frequency on experimental pain perception. 2) SDRR and respiratory CO_2 were significantly modulated by DSB frequency, while RR-interval was not. 3) Baseline-to-DSB and session-to-session differences in RR-interval significantly predicted pain perception within participants: Prolonged RR-intervals predicted lower pain ratings and shortened RR-intervals predicted higher pain ratings. SDRR and respiratory CO_2 were not found to predict pain perception.

CONCLUSION: The present study could not confirm hypotheses that the antinociceptive effects of DSB are related to changes in breathing frequency, heart rate variability, or hypoventilation/hyperventilation when applied as a short-term intervention. It could confirm the notion that increased cardiac parasympathetic activity is associated with reduced pain perception.

Introduction

Deep and slow breathing (DSB) techniques are used in the multimodal therapy of chronic pain as a behavioral method of pain management (Morone & Greco, 2007). Most DSB techniques are characterized by a reduction of breathing frequency and a diaphragmal emphasis of breathing with a long exhalation phase. In clinical practice, biofeedback techniques often pace the frequency of DSB, i.e., the patient is instructed to inhale/exhale according to a moving bar shown on a screen (Lehrer, Vaschillo, & Vaschillo, 2000). Despite the widespread popularity and some evidence of its effectiveness in painful conditions (Chambers, Taddio, Uman, & McMurtry, 2009; Friesner, Curry, & Moddeman, 2005; Hassett et al., 2007) and experimental settings (Busch et al., 2012; Cogan & Kluthe, 1981; Zautra, Fasman, Davis, & Craig, 2010), the mechanisms of action of DSB are still not fully understood.

The majority of studies have suggested that the antinociceptive effects of DSB are mediated by psychological mechanisms, such as facilitation of emotional regulation (Zautra et al., 2010), relaxation (Busch et al., 2012; Cogan & Kluthe, 1981) or distraction (Chambers et al., 2009). In addition, expectancy and placebo effects have to be considered (Buhle, Stevens, Friedman, & Wager, 2012; Subotnik & Shapiro, 1984). However, there is a body of evidence suggesting that DSB has elementary physiological consequences that may directly or indirectly moderate antinociceptive effects: Slow breathing frequencies have been reported to prolong RR-intervals (= 60[beats]/heart rate [beats/s] by definition; Pöyhönen, Syväoja, Hartikainen, Ruokonen, & Takala, 2004) and studies using heart rate biofeedback found that prolonged RR-intervals are associated with diminished pain perception (Reeves & Shapiro, 1982; Victor, Mainardi, & Shapiro, 1978). Further, DSB is likely to influence pulmonary gas exchange. Faster breathing frequencies may lead to hypocapnia and very slow breathing frequencies may lead to hypercapnia. The latter has been shown to cause antinociceptive effects (Stokes III., Chapman, & Smith, 1948). Moreover, increases in heart rate variability (HRV) depend on breathing frequency. It is known that HRV reaches its maximum at a breathing frequency of approximately 0.10 Hz (6 breaths/min), the so-called "resonant frequency" (Lehrer et al., 2003; Vaschillo, Vaschillo, & Lehrer, 2006; Vaschillo, Vaschillo, Pandina, & Bates, 2011). Based on this observation, it has been hypothesized in the field of HRV biofeedback that DSB may modulate autonomic nervous system (ANS) activity and pain perception most efficiently at the resonant frequency (Chalaye, Goffaux, Lafrenaye, & Marchand, 2009).

In summary, depending on the breathing frequency, DSB might affect a number of cardiorespiratory variables that might in turn mediate changes in pain perception. To our knowledge, the impact of DSB frequency on pain perception has not been studied so far in a dose-dependent fashion. It is further unclear, which of the mentioned cardiorespiratory variables are influenced by DSB frequency and at the same time able to predict antinociceptive effects.

Therefore, the first objective of the present study was to test experimentally if pain perception was significantly affected by four different paced DSB frequencies. Pain perception was measured using thermal pain thresholds and by retrieving ratings of tonic heat stimuli. The second study objective was to test the effects of four paced breathing frequencies on the cardiorespiratory variables RR-interval, standard deviation of the RR-interval (SDRR), and respiratory CO_2. Finally, the third study objective was to determine if DSB-initiated changes in the measured cardiorespiratory variables could predict changes in nociception. Based on the studies mentioned earlier, it was expected that increases in RR-interval, SDRR, and respiratory CO_2 predict anti-nociception.

Methods

Participants:

Twenty healthy volunteers (50 % male) were recruited by advertisement among university students. Absence of acute infections, obesity, internal, neurological, or psychiatric disorders, acute or chronic pain, as well as absence of analgesic, psychoactive, cardiovascular, or anti-allergic medication was ascertained in a structured clinical inter-view with a certified specialist. Written informed consent was obtained from all participants. The study was approved by the local ethics committee and is conform to the Declaration of Helsinki (59th WMA General Assembly, 2009). Participants were informed that the study aim was to determine the effect of "several breathing exercises" on pain perception, but it was not explicated that the study focused on breathing frequency and that one session involved paced breathing at their own baseline frequency. Participants were not given any suggestion that a certain breathing condition may be more effective than the others.

Procedure:

The present study was designed as a randomized, repeated-measures, single-blind trial with four sessions per participant. The experimental conditions were paced breathing at:

1) 0.14 Hz (8.4 breaths/min),
2) 0.10 Hz (6 breaths/min),
3) 0.06 Hz (3.6 breaths/min), and
4) the individual's resting frequency.

The breathing conditions were arranged in 20 different intervention sequences balanced for first session. Participants were randomly assigned to these intervention sequences using a free online tool (Urbaniak & Plous, 2011). To get accustomed to the laboratory setting, all participants completed an additional training session before the first testing session, where paced breathing was exercised at different breathing frequencies and pain measurement procedures were practiced. Sessions were scheduled at least 7 days apart, to rule out carryover effects between the experimental conditions. Sessions were held always at the same time of day (± 1 hour) to control for circadian effects. All testing was performed in a quiet room, with participants sitting in a comfortable reclining chair in a semi-supine position. Participants were sitting for at least 10 min before any measurement was started. A full timeline of the procedures is shown in Table 3.1.

<table>
<tr><th colspan="2">0 min</th><th>10 min</th><th colspan="2">20 min</th><th>30 min</th><th colspan="2">40 min</th><th>50 min</th><th colspan="2">60 min</th><th>70 min</th><th>80 min</th><th>End</th></tr>
<tr><td colspan="3" rowspan="3">Exclusion criteria
Vital Signs
Electrode Setup</td><td colspan="3">ECG Measures
CO_2 Measures</td><td colspan="3">Thermal Thresholding</td><td colspan="4">Tonic Heat Testing</td><td rowspan="3">Vital Signs</td></tr>
<tr><td colspan="2">7 min</td><td>7 min</td><td colspan="3">30 min</td><td colspan="4">30 min</td></tr>
<tr><td>Rest</td><td colspan="10">Paced Breathing
(at 0.14, 0.10, or 0.06 Hz, or at Resting Frequency)</td></tr>
</table>

Table 3.1: Timeline of experimental procedures, ECG=electrocardiogram.

For baseline resting period, participants were asked to relax, breathe normally, and sit quietly for 7 min. For the rest of the session, breathing was paced visually, with a vertically moving bar presented on-screen using EZ-Air Plus (BFE, retrieved from http://www.bfe.org/breathpacer.htm). The inspiration-to-expiration ratio was set to 1:2 for all frequencies (Eckberg, Kifle, & Roberts, 1980; Strauss-Blasche et al., 2000). In each session, participants were instructed to pace their breathing according to a script adapted from Lehrer et al. (Lehrer et al., 2000) focusing on a "constant diaphragmal breathing rhythm, while using pursed lips to control exhalation volume", emphasizing that they should "breathe in a relaxed way" and "not try too hard." They were further instructed to maintain paced breathing during and between all measurements. For the resting frequency condition, the breath pacer was set to the individual's mean resting frequency of that day's 5 min of rest.

R-R intervals and heart rate variability measures:

The "EXG" module of a "Biofeedback Expert 2000" system (Schuhfried, Mödling, Austria) was used to obtain a standard three lead setup electrocardiograms (ECGs). ECGs were recorded during 7 min of rest and 7 min of paced breathing at a sampling frequency of 1 kHz with instantaneous R-R interval detection. An ECG dataset was defined as the last 5 min of a given measuring period. Recording and data processing was performed following the appropriate guidelines (Malik et al., 1996).

SDRR was defined as the only outcome measure of HRV for the present study. Frequency domain measures of HRV, such as low- (LF) and high-frequency power (HF) were included for descriptive purposes, but excluded from further analysis, as they are only valid indices of ANS function, when breathing frequency is constant between experimental conditions (Ritz & Dahme, 2006). LF and HF represent the spectral power of HRV in frequency bands of 0.04–0.15 Hz and 0.15–40 Hz, respectively. Respiratory sinus arrhythmia (RSA) usually is the main source of HF power under resting conditions with a peak around 0.20 Hz. Paced breathing according to our experimental conditions switches RSA power below 0.15 Hz and therefore from HF to LF. The subsequent increase in LF and decrease in HF would therefore reflect the filter properties inherent to these measures instead of meaningful changes in vagal activity. The time domain measure root mean square successive difference (RMSSD) was dismissed from further analysis due to similar reasons (Berntson, Lozano, & Chen, 2005).

All HRV variables were calculated using the software Kubios-HRV (Niskanen, Tarvainen, Ranta-Aho, & Karjalainen, 2004). All recordings were inspected visually and artifacts corrected with the software's built-in filter function. Recordings with more than three artifacts were to be excluded from analysis.

Respiratory measures and CO_2:

Respiratory amplitude was recorded using the "RESP" module of the biofeedback equipment. The module equals a strain gauge with a resolution of 0.2 mm and a sampling-rate of 200 Hz. Recordings were visually checked and artifacts corrected. Analysis was performed using an adapted version of the open-source algorithm "peakdet" (Billauer, 2007) in combination with a moving-average low-pass filter with a window size of 0.5 s. End-tidal partial pressure of respiratory CO_2 was measured at the end of the resting and breathing intervals using a tight-fitting anatomic mask, in combination with a CO_2 sensor connected to a Sirecust (SC) 9000XL (Siemens, Erlangen, Germany) health monitor. The CO_2 values of five subsequent breaths were recorded and averaged.

Testing of Pain Perception

Temperature stimuli were applied to the volar surface of the left lower arm with a 30 x 30 mm thermode connected to a Thermosensory Analyzer II (Medoc, Ramat Yishai, Israel). The thermode was kept in place by an elastic strap, while the arm comfortably rested on a tablet attached to the chair. The experimenter relocated the thermode once after the thresholding procedure and once after half of the tonic heat rating procedure. The three sites of stimulation were 3 cm, 9 cm, and 15 cm above the wrist, used in balanced sequence.

Heat and cold pain thresholds were determined using the method of limits according to established protocols (Busch et al., 2012). Nociceptive perception was measured using cold pain threshold (CPT) and heat pain threshold (HPT). In short, five stimuli were presented as decreasing (CPT) or increasing (HTP) temperatures starting from a baseline of 32 °C. Participants were instructed to press a stop button at pain threshold defined according to a read-aloud script (Rolke et al., 2006). Stimuli were separated by jittered interstimulus intervals of 11 ± 1 s. The first stimulus was defined as a trial stimulus and discarded from analysis. Thresholds were defined as the mean of the four other stimuli.

As a second measure of pain perception, a rating procedure (Geuze et al., 2007) was employed: 18 heat stimuli with temperature levels of 43, 44, 45, 46, 47, and 48 °C were applied. Each level was presented three times. All stimuli were applied in pseudorandomized sequences, ensuring that no temperature occurred twice in a row. Sequences were randomly assigned across participants and conditions. All heat stimuli were applied for 20 s duration, oscillating ± 1 °C around the temperature levels at 0.5 Hz (speed: 2 °C/s) to reduce adaption/sensitization effects (Schmahl et al., 2006). Thermode temperature ramp-up was set to 2 °C/s, the interstimulus interval was 30 s. After nine stimuli, thermode location was switched. Before the procedure and after changing thermode location, two preconditioning stimuli of 43 and 47 °C were applied to reduce adaption/sensitization effects. Participants could stop a stimulus if deemed unbearable at any time by pressing a button. In this case, the following interstimulus interval was automatically prolonged to keep testing duration constant. During the interstimulus intervals, participants were asked to enter ratings of pain intensity and unpleasantness on two visual analog scales (VASs) shown on-screen using a mouse. The VASs that ranged from "0" to "100", were ticked and labeled in steps of 10, and the cursor always started at "0." Instructions were provided to the participant before the procedure by a read-aloud script. The distinction between pain intensity and pain unpleasantness was illustrated according to established protocols (Price, McGrath, Rafii, & Buckingham, 1983) and resulted in two variables: pain intensity ratings and pain unpleasantness ratings. Both were calculated as the mean individual rating response to temperature steps 45, 46, 47, and 48°C. Ratings for 43 and 44°C were excluded from analysis as preliminary results indicated that ratings did not significantly differ from 0.

Statistics

SPSS 19.0.0.2 for Mac OS X (IBM, Armonk, New York) was used for statistical analysis. All statistical tests were performed using SPSS's GENLINMIXED procedure for generalized linear model (GLM) analysis with robust covariance estimation to correct for potential violations of the model assumptions. Before analysis, all variables were z-transformed to ensure grand mean centering and to standardize results. Further unstructured autoregressive covariance matrixes (no assumptions on repeated covariance structure are made) were used to model repeated covariance within participants across time and sessions for objectives one and three. For objective two, a first-order autoregressive covariance matrix (measures closer in time are expected to show

stronger covariance) was used due to the limited degrees of freedom available. Within each study objective, Bonferroni correction was used to account for multiple comparisons.

For objective one, the effects of the factor "breathing frequency" on the dependent variables CPT, HPT, pain intensity ratings, and pain unpleasantness ratings were tested using four separate one-way mixed analyses of variance (ANOVAs). For objective two, the effects of the fixed factors "time" (two levels: 5 min of baseline vs 5 min of paced breathing), "breathing frequency" (four levels: paced baseline frequency, 0.14 Hz, 0.10 Hz, and 0.06 Hz), and the interaction term "time x breathing frequency" on the dependent variables RR-interval, SDRR, and respiratory CO_2 were tested with three separate 2 x 4 mixed ANOVAs. Significant effects were followed up using planned contrasts (Bonferroni corrected), testing all differences between baseline and paced breathing, as well as all differences between the four paced breathing conditions. For objective three, within-session changes from resting period to paced breathing (paced breathing minus resting period) and resting period measures of RR-interval, SDRR, and CO_2 were defined as predictive scalar factors for the dependent variables CPT, HPT, pain intensity ratings, and pain unpleasantness ratings in 12 separate GLMs. Pearson's standardized residuals were examined for all models to guarantee robustness of the results. As a rule of thumb, 5% of cases can be expected to show an absolute value greater than 2.0 (Field, 2009). So for the present sample size of 74 sessions, 3.7 sessions were expected to show an absolute value above this limit. Therefore, models with four values above 2.0 were to be reexamined, excluding these particular cases.

Results

General:

The mean age of the sample was 24.4 (range: 20.7–28.6) years. Two participants reported side effects: One participant experienced paresthesias in the upper limbs, as well as hiccups during three sessions. He was excluded from analysis as only one valid session remained. Another participant reported dizziness after the resting frequency condition. All data from this session were excluded from analysis except for the baseline measures. For another participant's session respiratory belt, RR-interval, and SDRR data were lost due to equipment failure. Inspection of respiratory belt data indicated that all participants were able to maintain a stable breathing pattern throughout the HRV measurements, except for one participant who showed a respiratory rate of 0.89 Hz during the 0.06 Hz breathing frequency condition. Data from this session were excluded from analysis except for baseline measures. In total, the sample included 74 sessions within 19 participants, with one additional missing session for breathing frequency, RR, and SDRR, and no missing sessions for respiratory CO_2.

Condition	**Breathing Frequency** (breaths/min)		**RR-interval** (ms)		**SDRR** (ms)		**Respiratory CO_2** (% vol)	
	Baseline	Paced	Baseline	Paced	Baseline	Paced	Baseline	Paced
0.06 Hz (3.6 breaths/min)	14.3 ± 4.2	3.7 ± 0.2	813.6 ± 124.4	819.5 ± 107.8	59.3 ± 21.9	98.0 ± 27.2	5.1 ± 0.4	5.4 ± 0.7
0.10 Hz (6.0 breaths/min)	14.2 ± 3.4	6.0 ± 0.2	839.2 ± 122.4	853.2 ± 105.0	70.5 ± 22.7	109.2 ± 33.0	4.9 ± 0.4	4.9 ± 0.6
0.14 Hz (8.4 breaths/min)	14.0 ± 3.5	8.5 ± 0.3	816.3 ± 132.4	829.7 ± 114.2	63.1 ± 21.8	84.8 ± 20.9	4.9 ± 0.5	4.9 ± 0.7
Paced resting frequency	14.0 ± 4.1	14.4 ± 4.0	847.7 ± 131.9	858.1 ± 120.6	62.7 ± 20.1	62.1 ± 20.7	4.9 ± 0.5	4.6 ± 0.7

Table 3.2: Descriptive results for the cardiorespiratory variables: Means ± standard deviation. n=19 (74 sessions). Baseline data was recorded during 5 min at rest. Subsequently, 5 min of paced breathing according to the experimental conditions were recorded. SDRR = standard deviation of the RR-interval

Descriptive results for cardiorespiratory variables are shown in Table 3.2, and additional HRV results including frequency domain measures are shown in Appendix 3.1. Descriptive results for variables of pain perception are shown in Table 3.3. Shapiro–Wilk's test for normality performed on within-subject centered variables indicated a non-normal distribution of within-participant differences for breathing frequency ($p < .001$), HTP ($p = .032$), and RR-interval ($p = .012$). Intra-individual differences in CPT, pain intensity ratings, pain unpleasantness ratings, CO_2, and SDRR were normally distributed.

Condition	Cold Pain Thresholds (°C)	Heat Pain Thresholds (°C)	Heat Intensity Ratings (Points)	Heat Unpleasantness Ratings (Points)
0.06 Hz (3.6 breaths/min)	12.3 ± 9.5	46.8 ± 2.0	42.4 ± 17.4	41.4 ± 18.2
0.10 Hz (6.0 breaths /min)	10.6 ± 9.1	46.5 ± 2.5	43.9 ± 19.5	43.3 ± 19.2
0.14 Hz (8.4 breaths /min)	12.3 ± 8.0	46.6 ± 1.8	42.1 ± 19.6	41.1 ± 19.6
Paced resting frequency	9.2 ± 8.0	46.9 ± 1.8	44.6 ± 19.5	41.5 ± 20.4

Table 3.3: Descriptive results for the somatosensory variables: Means ± standard deviation, n=19 (74 sessions) Somatosensory variables were recorded during constant paced breathing following the cardiorespiratory recordings. Intensity- and Unpleasantness-Ratings are shown as participants' mean ratings for stimuli with 45, 46, 47, and 48 °C of thermode temperature on a 0-100 point visual analog scale.

Objective 1: Effects of different paced breathing frequencies on pain perception

No measure of pain perception was found significantly affected by the experimental condition. There were no significant differences in the effect of different slow-paced breathing frequencies on four variables on pain perception (see Table 3.4).

Variable	*F*(3, 70)	p	p_{Bonf}
Cold Pain Threshold	2.599	.059	.236
Heat Pain Threshold	0.263	.852	1
Pain Intensity Ratings	1.242	.301	1
Pain Unpleasantness Ratings	0.568	.638	1

Table 3.4: General Linear Model results for the effects of breathing frequency on pain perception. Breathing conditions were: paced baseline frequency, 0.14 Hz, 0.10 Hz, and 0.06 Hz. n=19 (74 sessions). p_{Bonf} = Bonferroni corrected p-Value.

Objective 2: Effects of different paced breathing frequencies on physiological variables

RR-interval was not found significantly affected by factors Time or Breathing Frequency, but significant Time x Breathing Frequency interactions were found for SDRR and CO_2 (see Table 3.5).

Variable	df_2	Model Term	F	p	p_{Bonf}
		Time	0.02	.889	1
RR-interval	141	Breathing Frequency	0.63	.595	1
		Time x Breathing Frequency	1.66	.178	.534
		Time	38.82	<.001	**<.001**
SDRR	141	Breathing Frequency	22.22	<.001	**<.001**
		Time x Breathing Frequency	16.69	<.001	**<.001**
		Time	0.78	.379	1
Respiratory CO_2	142	Breathing Frequency	5.28	.002	**.006**
		Time x Breathing Frequency	5.01	.002	**.006**

Table 3.5: General Linear Models testing the effects of slow paced breathing frequency on cardiorespiratory variables. df_1 = 7. Significant effects are marked bold. **Abbreviations:** SDRR, standard deviation of the RR-interval; p_{Bonf}, Bonferroni corrected p-Value.

For SDRR, planned comparisons revealed that all breathing exercises with the exception of paced resting frequency significantly increased mean SDRR compared with baseline. Further, mean SDRR significantly increased with decreasing breathing frequencies until the 0.1 Hz condition. The 0.06 Hz condition did not significantly increase SDRR in comparison to the 0.1 Hz

condition and the 0.14 Hz condition. Significant changes from baseline were only found for paced resting frequency, which decreased CO_2. However, respiratory CO_2 during the 0.06 Hz breathing condition was significantly elevated compared to resting frequency, 0.10 Hz, and 0.14 Hz conditions. Results for planned comparisons are shown in Table 3.6.

			SDRR			Respiratory CO_2		
Planned Contrast			t	p	p_{Bonf}	t	p	p_{Bonf}
Resting compared to Breathing Period (within-session)								
0.06 Hz, rest	vs	0.06 Hz, paced	-8.37	<.001	**<.001**	-2.49	.023	.233
0.10 Hz, rest	vs	0.10 Hz, paced	-6.33	<.001	**<.001**	0.41	.685	1
0.14 Hz, rest	vs	0.14 Hz, paced	0.23	<.001	**<.001**	0.82	.422	1
Resting frequency, rest	vs	Resting frequency, paced	-2.14	.820	1	3.88	.001	**.012**
Between-session comparisons of Breathing Period								
0.06 Hz, paced	vs	0.10 Hz, paced	1.88	.048	.475	3.90	.001	**.012**
0.06 Hz, paced	vs	0.14 Hz, paced	5.04	.077	.773	3.91	.001	**.011**
0.06 Hz, paced	vs	Resting frequency, paced	4.04	<.001	**.001**	4.11	.001	**.008**
0.10 Hz, paced	vs	0.14 Hz, paced	8.47	.001	**.007**	-0.01	.993	1
0.10 Hz, paced	vs	Resting frequency, paced	5.56	<.001	**<.001**	2.02	.060	.595
0.14 Hz, paced	vs	Resting frequency, paced	-8.37	<.001	**<.001**	2.02	.059	.594

Table 3.6: Planned paired contrasts following up significant effects of slow paced breathing frequency on cardiorespiratory variables n=19 (74 sessions), Significant effects are marked bold. Abbreviations: SDRR, standard deviation of the RR-interval; p_{Bonf}, Bonferroni corrected p-Value.

Objective 3: Predicting pain perception by DSB-induced cardiorespiratory changes

Table 3.7 shows the results of twelve regression analyses testing the relationships between the cardiorespiratory and nociceptive variables. Including RR-interval change and RR-interval at baseline improved model fit for pain intensity ratings and pain unpleasantness ratings compared to an intercept-only model. Changes in RR-interval and baseline RR-interval both were highly significant predictors of pain unpleasantness ratings. For pain intensity ratings, change in RR-interval was a predictor of borderline significance and RR-interval at baseline was a highly significant predictor. Beta-coefficients indicated that in sessions where the RR-interval increased by 10ms from baseline, pain unpleasantness ratings decreased by -0.23 ± 0.07 points. Beta-coefficients further indicated that in sessions where RR-interval was elevated by 10 ms at baseline, pain intensity ratings were -0.53 ± 0.11 points lower and pain unpleasantness ratings were -0.56 ± 0.12 points lower[2]. Change in RR-interval and baseline RR-interval could not explain significant proportions of variance for CPT or HPT and could not improve model fit. The same held true for, changes in SDRR and respiratory CO_2. Examination of Pearson's residuals indicated that six cases were $z > 2.0$ for pain unpleasantness ratings, (the largest case being -2.328). Re-analysis excluding the particular cases did not change the results in respect to model fit, direction of effect, and the criterion of significance. Further, a visual examination of the results indicated that one session could represent an outlier with undue influence on the linear results. Excluding this session did not change the results in respect to model fit, direction of effect, or the criterion of significance. All pairs of somatosensory variables were re-examined

[2]An increase by 10 ms of RR-interval corresponds to an increase in heart rate by 0.9 beats/minute at mean breathing frequency. A decrease by 10 ms of RR-interval corresponds to a decrease in heart rate by 0.8 beats/minute at mean breathing frequency.

using one cardiorespiratory variable at a time for descriptive purposes and can be found in Appendix 3.2.

Variable	Cofactor	Model Term	Effect			Coefficient	
			F(4, 70)	p	p_{Bonf}	beta ± (SE)	ΔAICC
Cold Pain Thresholds	RR-interval	Intercept	2.12	.128	1	0.083 ± 0.204	
		RR-interval change	0.02	.894	1	-0.008 ± 0.058	5.710
		RR-interval baseline	3.97	.050	.600	-0.102 ± 0.051	
	SDRR	Intercept	1.35	.266	1	0.097 ± 0.205	
		SDRR change	1.21	.275	1	0.078 ± 0.071	5.343
		SDRR baseline	1.27	.263	1	-0.062 ± 0.055	
	Respiratory CO_2	Intercept	0.27	.762	1	0.105 ± 0.210	
		CO_2 change	0.48	.489	1	0.050 ± 0.073	7.081
		CO_2 baseline	0.10	.757	1	0.022 ± 0.071	
Heat Pain Thresholds	RR-interval	Intercept	3.64	.031	.372	0.292 ± 0.123	
		RR-interval change	0.80	.376	1	0.066 ± 0.074	2.919
		RR-interval baseline	4.74	.033	.396	0.150 ± 0.069	
	SDRR	Intercept	0.35	.709	1	0.309 ± 0.133	
		SDRR change	0.22	.638	1	0.027 ± 0.056	5.522
		SDRR baseline	0.56	.456	1	0.037 ± 0.049	
	Respiratory CO_2	Intercept	0.18	.839	1	0.281 ± 0.135	
		CO_2 change	0.09	.765	1	-0.018 ± 0.061	7.293
		CO_2 baseline	0.31	.578	1	0.036 ± 0.064	
Intensity Ratings	RR-interval	Intercept	11.19	< .001	**< .001**	0.211 ± 0.204	
		RR-interval change	7.61	.007	.084	-0.120 ± 0.044	-4.796
		RR-interval baseline	22.1	< .001	**< .001**	-0.310 ± 0.066	
	SDRR	Intercept	3.22	.046	.552	0.048 ± 0.206	
		SDRR change	4.41	.039	.468	-0.074 ± 0.035	0.598
		SDRR baseline	0.03	.853	1	-0.014–0.075	
	Respiratory CO_2	Intercept	1.04	.359	1	-0.008 ± 0.187	
		CO_2 change	2.02	.160	1	0.075 ± 0.053	0.534
		CO_2 baseline	0.21	.650	1	0.020 ± 0.043	
Unplea-santness Rating	RR-interval	Intercept	12.74	< .001	**< .001**	0.128 ± 0.183	
		RR-interval change	11.46	.001	**.012**	-0.134 ± 0.040	-5.080
		RR-interval baseline	23.51	< .001	**< .001**	-0.324 ± 0.067	
	SDRR	Intercept	0.32	.731	1	-0.019 ± 0.192	
		SDRR change	0.60	.440	1	-0.039 ± 0.050	-1.798
		SDRR baseline	0.12	.736	1	0.026 ± 0.075	
	Respiratory CO_2	Intercept	0.76	.474	1	0.020 ± 0.181	
		CO_2 change	0.01	.919	1	0.006 ± 0.063	-2.629
		CO_2 baseline	1.48	.227	1	0.072 ± 0.059	

Table 3.7: General Linear Models testing if changes in cardiorespiratory variables predict measures of pain perception. Cardiorespiratory variables at rest were included in analysis to account for session-to-session baseline differences. n = 19 (74 sessions). Significant effects are marked bold.
$\Delta AICC = AICC_{Model} - AICC_{Intercept\ only}$ (a lower AICC indicates increased model fit). **Abbreviations:** AICC: Akaike's information criterion corrected for finite sample sizes; SDRR: standard deviation of the RR-interval.

Discussion

Overview

The present study tested effects of slow paced breathing frequencies on experimental pain perception (Objective 1), cardiorespiratory function (Objective 2), and examined the relationship between changes in cardiorespiratory variables and experimental measures of pain perception (Objective 3). Results indicated that breathing frequency had no significant effect on experimental pain perception and RR-interval. Only SDRR and respiratory pCO_2 were significantly affected by different breathing frequencies. Nevertheless, neither SDRR nor respiratory CO_2, could predict intra-individual changes in pain perception. In contrast, baseline-to-breathing as well as session-to-session differences in RR-interval were found to predict changes in pain perception. Prolonged RR-intervals predicted lower pain unpleasantness ratings, while shortened RR-intervals predicted higher ratings.

Breathing frequency was not found to determine antinociceptive effects

The present study could not add evidence to the hypothesis that breathing frequency is the determining factor of DSB-induced hypoalgesia. This stands in contrast to two recent studies that could show an effect of slow-paced breathing on experimental pain perception using paced breathing at normal frequency as a control condition (Martin et al., 2012; Zautra et al., 2010). The best explanation for these conflicting results might be that the present study was the first to test the effects of slow breathing in a dose-dependent fashion, i.e., using several slow breathing frequencies.

On the one hand, this approach might have diminished statistical power. On the other hand, the use of several slow breathing conditions might have reduced expectation effects. Finally, these results could point toward a third variable underlying DSB analgesia. Besides breathing frequency, the present study particularly aimed to follow up two other hypotheses on physiological phenomena accompanying DSB: modulation of cardiac autonomic function and modulation of respiratory gas concentrations.

RR-Interval not found influenced by DSB, yet best predictor of pain ratings

RR-interval was not influenced by DSB, yet the best predictor of pain ratings. ANS activity is closely linked to nociception (Craig, 2003; Schlereth & Birklein, 2008). RR-interval, heart rate, as well as HRV in general have been interpreted as measures of general ANS function in the past, "although inferences are clearly restricted to the level of the heart" (Ritz, 2009). Nevertheless, these measures of cardiac autonomic function have been shown to be interrelated with pain perception in different contexts (Appelhans & Luecken, 2008; Rainville, Bao, & Chrétien, 2005; Tousignant-Laflamme, Rainville, & Marchand, 2005).

Accordingly, we found that pain unpleasantness ratings significantly decreased when RR-interval increased during DSB. Although this effect did not reach significance for CPT, HPT, and pain intensity ratings, the same direction of effect could be observed (see Table 3.7 and Appendix 3.2). Similarly, in sessions with elevated RR-interval at baseline, pain unpleasantness ratings, and pain intensity ratings were decreased significantly. As increases in RR-interval are commonly interpreted as increases in parasympathetic activity or sympathetic withdrawal, our results are in accord with earlier animal (Ren, Zhuo, Randich, & Gebhart, 1993) and human (Pereira et al., 2010) studies linking increases in cardiac parasympathetic tone and sympathetic withdrawal to

antinociceptive effects. However, it cannot be concluded that the DSB exercise was causally related to changes in cardiac ANS function and the associated effects on pain perception as breathing frequency was not found to significantly modulate RR-interval. Small but meaningful increases in RR-interval from baseline to DSB that remained undetected due to a lack of statistical power may have led to this outcome. The finding that paced DSB was insufficient to influence RR-interval stands in line with some earlier finding (Anderson, McNeely, & Windham, 2010; Ritz, 2009; Song & Lehrer, 2003), but in contrast to others (Martin et al., 2012; Pöyhönen et al., 2004) and indicates that DSB alone might not be efficient for manipulating heart rate. Biofeedback procedures designed to increase RR-interval might be more successful in doing so (Reeves & Shapiro, 1982; Victor et al., 1978) and should be considered as an intervention in future studies.

Hear rate variability during slow breathing was not found to predict anti-nociception

SDRR is the most general measure of HRV. It has been used as an indirect measure of cardiac ANS activity and used to infer on general ANS function (Malik et al., 1996; Thayer & Sternberg, 2006; Zhong, Jan, Ju, & Chon, 2006). Increases in SDRR are commonly interpreted to reflect parasympathetic activation and/or sympathetic withdrawal referring to experiments using cholinergic and beta-adrenergic receptor blockade (Berntson, Cacioppo, & Quigley, 1994; Bittiner & Smith, 1986). However, it is long known that slow breathing frequencies and increases in respiratory volume lead to dramatic increases in measures of HRV by mechanisms that obscure the association between HRV measures and the psychophysiologically relevant portion of ANS activity (Billman, 2011; Ritz, 2009). Notwithstanding, HRV increases have been hypothesized to indicate resonance between the RSA mechanism and the baroceptor reflex (Vaschillo et al., 2011), and proposed to be therapeutically beneficial when used to exercise parasympathetic function (Lehrer et al., 2000). Taking this hypotheses one step further, Chalaye et al. 2009 postulated that "slow deep breathing and HR biofeedback would produce the largest cardiac changes and the strongest analgesic responses" on experimental pain in a one-session experiment with healthy participants. Based on their finding of coinciding differences in pain tolerance thresholds and SDRR amplitude between a distraction and a slow breathing condition, they concluded: "modulation of HR and pain share a common neurophysiological pathway".

Our present experiment followed up this hypothesis by testing if DSB induced intra-individual SDRR amplitude changes can predict antinociceptive DSB effects in a dose-dependent fashion. Indeed, we could replicate that DSB at frequencies of around 0.1 Hz ("the resonant frequency") causes large increases in SDRR (Hirsch & Bishop, 1981; Vaschillo et al., 2006) and therefore general HRV. Consequently, SDRR should have been expected to predict a good degree of variation in experimental pain measures if a functional relationship between pain perception and general HRV existed. The fact that large DSB-induced changes in HRV did not predict any difference in pain perception is in accord with recent results by Martin et al. who could not find a relationship between breathing-induced changes in RMSSD and pain ratings (Martin et al., 2012). Together, these findings question if the "resonance" mechanism is relevant for DSB-induced antinociception, at least when applied without long-term training (Lehrer et al., 2000). It further underlines that HRV amplitude changes seen during slow breathing may reflect "mechanical effects of respiration" (Billman, 2011), rather than appropriate correlates of ANS function. The finding that RR-interval could predict differences in pain perception, while SDRR could not, supports the notion that RR-interval (or heart rate) at baseline is a better correlate of the

psychophysiologically relevant ANS portion than HRV (Grossman & Kollai, 1993; Paul Grossman & Taylor, 2007; Kollai & Mizsei, 1990).

Paced breathing at baseline and very slow frequencies induced hyper/hypoventilation

Hypercapnia-facilitated analgesia was the second candidate mechanism to be examined in the present study. Measures of respiratory CO_2 were used to detect potential consequences of DSB on respiratory gas exchange as hypercapnia had been shown to modulate acute pain perception (Stokes III. et al., 1948). Indeed, significant differences in respiratory CO_2 could be found, which indicated that participants tended to develop hypercapnia during the 3.6 breaths/min condition and hypocapnia during the paced resting frequency condition. These findings encourage the monitoring of hyperventilation/hypoventilation in the study of DSB exercises and replicate the finding that paced breathing at resting frequency is not just a simple "distraction" control condition, but has measurable consequences on respiratory gas exchange (Pinna, Maestri, La Rovere, Gobbi, & Fanfulla, 2006). Nevertheless, respiratory CO_2 could not explain relevant differences in any experimental measure of nociception. The magnitude of hyperventilation/hypoventilation might have been too small to lead to significant somatosensory consequences.

Limitations

The present findings have to be considered in the context of the following limitations: DSB effects were studied using experimental pain in healthy participants and may not generalize to clinical forms of pain or patient populations with ANS dysregulation. Caution must be exercised when comparing the results of our DSB protocol with other studies using breathing, relaxation, or distraction exercises: e.g., participants maintained visually paced breathing during sensory measurements at any time and only received a single training session. Therefore, the whole procedure might have been too demanding to have a relaxing effect. The simultaneous execution of paced DSB and thermal thresholding might also have diminished the sensitivity of the CPT and HPT measurements, which are affected by reaction time (Yarnitsky & Ochoa, 1990). We particularly emphasize that prolonged DSB training might yield entirely different results. It might enhance the effect of DSB by several mechanisms, but particularly because association effects were suggested to play a role in pain appraisal (Hallman, Olsson, von Schéele, Melin, & Lyskov, 2011). Lastly, the decision to exclude participants reporting side effects from analysis might have led to an underestimation of hyperventilation/hypoventilation effects.

Conclusions:

The present study supports the hypothesis that pain is as a homeostatic emotion (Craig, 2002; Zautra et al., 2010) depending on ANS function (Appelhans & Luecken, 2008) as inter-individual RR-interval differences at rest and changes in RR-interval were associated with measures of experimental pain perception. However, as the experimental conditions have neither influenced experimental pain perception directly, nor indirectly by altering RR-interval, we cannot conclude that breathing frequency is a determining factor of the proposed antinociceptive effects of DSB. Similarly, highly significant breathing induced changes in HRV and respiratory gas concentration could not predict antinociceptive DSB effects.

The present results do not exclude the possibility that DSB therapies might be beneficial for chronic pain patients. DSB might have a different impact on patients with ANS dysfunction, and other mechanisms of action could account for its therapeutic efficacy. In this context, potential

long-term training effects of DSB on cardiorespiratory function and pain perception might be of particular interest for future studies. As mentioned earlier, psychophysiological mechanisms such as "facilitation of emotional regulation" (Zautra et al., 2010), "relaxation" (Busch et al., 2012; Cogan & Kluthe, 1981), or "distraction" (Chambers et al., 2009) pose relevant alternative mechanisms of action to be considered. Based on our results, we encourage further biofeedback studies to determine the relevant mechanisms of action of slow breathing exercises.

Appendix 3.1

Condition	SDRR (ms)		RMSSD (ms)		LF (ms^2)		HF (ms^2)	
	Baseline	Paced	Baseline	Paced	Baseline	Paced	Baseline	Paced
0.06 Hz (3.6 breaths/min)	59.3 ± 21.9	98.0 ± 27.2	55.7 ± 29.6	49.5 ± 18.2	1438 ± 2150	9812 ± 5423	1735 ± 1753	623 ± 525
0.10 Hz (6.0 breaths/min)	70.5 ± 22.7	109.2 ± 33.0	69.9 ± 32.7	79.8 ± 38.5	1198 ± 1368	9656 ± 5310	2948 ± 2383	1726 ± 1932
0.14 Hz (8.4 breaths/min)	63.1 ± 21.8	84.8 ± 20.9	60.8 ± 31.0	65.5 ± 24.6	1554 ± 1890	5644 ± 2243	2242 ± 1798	885 ± 1168
Paced resting frequency	62.7 ± 20.1	62.1 ± 20.7	60.4 ± 26.9	60.8 ± 23.7	1167 ± 1433	821 ± 747	1919 ± 1473	1916 ± 1263

Appendix 3.1: Descriptive HRV Results—Frequency domain measures: Means ± standard deviation. n = 19 (74 sessions). Baseline data was recorded during 5 min at rest. Subsequently, 5 min of paced breathing according to the experimental conditions were recorded. **Abbreviations:** SDRR, standard deviation of the RR-interval; RMSSD, root mean squared of successive difference; LF, low frequency power; HF, high frequency power.

Appendix 3.2

	RR-interval		SDRR		Respiratory CO_2	
	Baseline	Paced	Baseline	Paced	Baseline	Paced
Cold pain thresholds	-0.10 ± 0.05 p = .066	-0.10 ± 0.05 p = .038	-0.07 ± 0.06 p = .271	0.07 ± 0.07 p = .362	0.05 ± 0.07 p = .533	0.06 ± 0.08 p = .489
Heat pain thresholds	0.11 ± 0.034 p = .005	0.13 ± 0.05 p = .016	0.05 ± 0.05 p = .340	0.03 ± 0.05 p = .588	0.03 ± 0.06 p = .652	0.00 ± 0.06 p = .968
Intensity Ratings	-0.21 ± 0.06 p = .001	-0.28 ± 0.05 p < .001	-0.02 ± 0.08 p = .792	-0.04 ± 0.04 p = .331	0.03 ± 0.05 p = .580	0.09 ± 0.06 p = .181
Unpleasantness Ratings	-0.24 ± 0.07 p = .002	-0.28 ± 0.05 p < .001	-0.03 ± 0.08 p = .734	-0.01 ± 0.05 p = .804	0.07 ± 0.06 p = .228	0.07 ± 0.07 p = .282

Appendix 3.2: Simple regressions between cardiorespiratory and somatosensory variables. Results of Robust General Linear Models depicting Beta ± (SE). Repeated-covariance from 74 sessions within 19 participants was modeled using an unstructured covariance matrix. Significant effects are marked bold. "Baseline" data was recorded during a 5 min interval at rest. Paced data were recorded in 5 min interval while the participant was performing paced breathing according to the experimental condition. **Abbreviations:** SDRR, standard deviation of the RR-interval.

STUDY 4

rTMS over the cerebellum modulates temperature detection and pain thresholds through peripheral mechanisms.

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This is a pre-copy-editing, author-produced version of an article published 2011 in Brain Stimulation (4:210-7.e1) following peer review. The definitive publisher-authenticated version is available at: http://www.brainstimjrnl.com/article/S1935-861X%2810%2900166-X

Abstract

BACKGROUND: Repetitive transcranial magnetic stimulation (rTMS) of motor and prefrontal cortex has been shown to modulate pain perception. Even though evidence suggests an involvement of cerebellar structures in pain processing, the effect of rTMS over the cerebellum on pain perception has not yet been investigated.
OBJECTIVE/HYPOTHESIS: This study aimed to test the effects of rTMS over the cerebellum on sensory perception, particularly controlling for peripheral stimulation effects. Methods Sensory perception was determined as temperature detection and temperature pain thresholds. Experiment one explored the effects of four different rTMS protocols (flat figure-of-eight coil; 120 % motor resting threshold; 1000 stimuli; 1 Hz and 10 Hz; medial and right lateral cerebellum) on sensory thresholds in 10 healthy volunteers using pairwise comparisons. The most efficient protocol of experiment one was compared in a second experiment with two control conditions (rTMS with a sham coil over the cerebellum [sham] and repetitive magnetic stimulation [rMS] of the neck) by using robust statistics (MANOVA).
RESULTS: The first experiment demonstrated pronounced effects on sensory perception for 1Hz rTMS over the lateral cerebellum. The second experiment confirmed this result in comparison to sham. However, rMS over the neck had a similar effect like rTMS over the cerebellum.
CONCLUSIONS: Our findings suggest that changes in sensory perception after rTMS over the cerebellum are largely due to stimulation effects on peripheral structures and support recent reports of analgesic effects of neck rMS. They advocate the critical review of the proposed analgesic effects of rTMS and encourage the future use of proper control conditions in rTMS research.

Introduction

Repetitive transcranial magnetic stimulation (rTMS) is a noninvasive brain stimulation technique that is currently examined as a treatment option for a large variety of neuropsychiatric disorders, including chronic pain (Leung et al., 2009). In rTMS neuronal discharges are repeatedly induced in superficial brain areas by magnetic pulses, delivered through the scalp by a magnetic coil (Hallett, 2000). rTMS of the primary motor cortex has been demonstrated to exert analgesic effects lasting from minutes to days in various chronic pain disorders (André-Obadia, Mertens, Gueguen, Peyron, & Garcia-Larrea, 2008; Khedr et al., 2005; Lefaucheur, Drouot, Ménard-Lefaucheur, Keravel, & Nguyen, 2006; Lefaucheur, Drouot, & Nguyen, 2001; Passard et al., 2007; Pleger et al., 2004). Analgesic effects of rTMS of the motor cortex (Johnson, Summers, & Pridmore, 2006; Lefaucheur et al., 2010; Summers, Johnson, Pridmore, & Oberoi, 2004; Tamura et al., 2004) and prefrontal areas (Borckardt et al., 2007) could further be evidenced for experimentally induced pain. Modulation of neuronal excitability in cortical and subcortical regions like the thalamus has been proposed as a putative mechanism of rTMS induced analgesic effects (Lefaucheur et al., 2001; Leo & Latif, 2007). This view is supported by evidence for altered cortico-spinal excitability in several forms of chronic pain (Mhalla, de Andrade, Baudic, Perrot, & Bouhassira, 2010; Strutton, Theodorou, Catley, McGregor, & Davey, 2005; Turgut & Altun, 2009) and studies demonstrating that the analgesic effects of rTMS correlate with normalization of cortical excitability in chronic pain patients (Lefaucheur et al., 2006; Schlaier et al., 2007).

Apart from motor and prefrontal cortex no other brain regions have been investigated as targets for pain modulation. Among the regions ignored so far is the cerebellum, although several lines of evidence highlight it as a promising target. Both animal and human studies have demonstrated the involvement of the cerebellum in several aspects of pain processing (Moulton, Schmahmann, & Borsook, 2010). Moreover the cerebellum has strong connections with the motor cortex via the cerebello-thalamo-cortical pathway, which can be modulated by rTMS (Daskalakis et al., 2004; Fierro et al., 2007; Langguth et al., 2008). Accordingly, rTMS over the cerebellum has been shown to have an impact on a variety of neurobiologic functions (Gironell et al., 2002; Oliveri et al., 2009). An important confounder in the investigation of cerebellar rTMS is the concomitant stimulation of peripheral structures such as neck muscles (Gerschlager, Christensen, Bestmann, & Rothwell, 2002; Ugawa et al., 1991). This is of special relevance in the investigation of pain thresholds, because magnetic stimulation of neck muscles is known to have analgesic effects (Smania et al., 2005).

In this study, we performed two experiments to explore the effects of rTMS over the cerebellum on pain and temperature perception in healthy humans. In the first experiment, we screened several stimulation parameters for their effect on sensory thresholds. In the second experiment, the most promising rTMS protocol was retested and compared with two control conditions. Repetitive magnetic stimulation (rMS) of the neck was performed to control for effects of muscle stimulation. Sham rTMS over the cerebellum was applied as a further control condition, because measures of pain perception are known to be particularly susceptible to placebo effects (Krummenacher, Candia, Folkers, Schedlowski, & Schönbächler, 2010). Nociceptive and non-nociceptive sensation was assessed by quantitative sensory testing of temperature and pain detection thresholds.

Methods

General information

Study participants were recruited among students and hospital personnel through advertisement. They were free of analgesic or centrally active medication and free of any acute or chronic medical diagnosis. The study was approved by the local ethics committee and written informed consent was obtained from all participants. The study conformed to the most recent version of the Helsinki Declaration (59th WMA General Assembly, 2009).

For rTMS, participants were seated in a comfortable chair. TMS was delivered by a Magstim super rapid stimulator (Magstim Co, Dyfed, UK) connected to a figure-of-eight coil (double-circular 70 mm coil). Resting motor threshold (RMT) of the motor cortex was determined according to standard procedures before the first experimental session (Rossini et al., 1994). rTMS was performed at 120 % RMT or at 60 % maximum stimulator output (MSO) when RMT exceeded 50 % MSO. The limit of 60 % MSO was set due to safety considerations and as higher stimulation strengths were found to be hardly tolerable by the participants in a previous study (Langguth et al., 2008). Low frequency stimulation was applied at 1 Hz rTMS (1 train, 1000 stimuli), high-frequency stimulation at 10 Hz (20 trains, 50 stimuli/train, inter-train interval of 20 s). The handle of the coil was pointed upward and held in place by a mechanical arm. The current in the coil was thus directed downward during the reversal phase of the biphasic stimulus, inducing an upward current in the region of interest (Ugawa, Uesaka, Terao, Hanajima, & Kanazawa, 1995). All used coils were precooled with ice packs to prevent overheating. Net duration of stimulation was about 17 min for the 1 Hz protocol and about 8 min for the 10 Hz protocol.

Before and after rTMS treatment, sensory measurements were performed while participants were sitting in a comfortable chair. Changes in nociceptive and non-nociceptive sensation were determined as threshold temperatures, using an adapted version of the method of limits (Fruhstorfer, Lindblom, & Schmidt, 1976; Summers et al., 2004). Temperature stimuli were applied to the thenar of the right hand, using a 30 x 30 x 30 mm thermode connected to a sensory Analyzer II (Medoc, Ramat Yishai, Israel). Measurements were only retrieved from the right hand ipsilateral to the stimulation site, according to the ipsilateral motor representation of the cerebellum. Temperature stimuli were presented as ramping thermode temperatures starting from a baseline of 32 °C. For the temperature detection thresholds "cold detection threshold" (CT) and "warmth detection threshold" (WT) participants were requested to press a response button as soon as they perceived "the slightest change in temperature". Temperatures increased or decreased with a rate of 0.5 °C for detection thresholds. For pain thresholds "cold pain threshold" (CPT) and "heat pain threshold" (HPT), participants were requested to press a response button as soon as the perceived heat or cold started "to be accompanied by the slightest feeling of pain." Temperatures increased or decreased with a rate of 1 °C for pain thresholds. On pressing the response button, the reached temperature was recorded and temperature automatically returned to baseline with 10 °C/s. Automatic safety temperature limits were set, so that whenever a temperature of 0 °C or 50.5 °C was reached, the thermode temperature rapidly returned to baseline. Participants could not see the operator screen and no visual or auditory cues were present to signal stimulus onset. Ramping stimuli were separated by automatically timed, jittered intervals of 10 ± 1 s at baseline temperature.

Statistics were processed with SPSS 17.0 (SPSS Inc, Chicago, IL). Graphs were processed with GraphPad Prism 5.0 (GraphPad Software, Inc, San Diego, CA). Significance levels for all statistical tests were $\alpha < .05$ Shapiro-Wilk test for normality was used to test if the data was sampled from a Gaussian distribution. Means are displayed ± standard deviation if not denoted otherwise.

Experiment 1

Baseline measurements (15 min)	**Repetitive transcranial magnetic stimulation** (20 min):	**Posttreatment measurements** (15 min)
Cold detection threshold (4x) and Warm detection threshold (4x) and Cold pain threshold (4x) and Heat pain threshold (4x).	1 Hz; lateral cerebellum or 10 Hz; lateral cerebellum or 1 Hz; medial cerebellum or 10 Hz; medial cerebellum	Cold detection threshold (4x) and Warm detection threshold (4x) and Cold pain threshold (4x) and Heat pain threshold (4x).

Experiment 2

Baseline measurements (25 min)	**Repetitive (transcranial) magnetic stimulation** (25min):	**Posttreatment measurements** (25 min)
Cold detection threshold (8x) and Warm detection threshold (8x) and Cold pain threshold (6x) and Heat pain threshold (6x).	1 Hz; lateral cerebellum or 1 Hz; lateral neck or 1 Hz; lateral cerebellum (sham)	Cold detection threshold (8x) and Warm detection threshold (8x) and Cold pain threshold (6x) and Heat pain threshold (6x).

Table 4.1 Experimental protocol of Experiments 1 and 2

Experiment 1

The aim of the first experiment was to explore different rTMS protocols (medial and lateral cerebellum, low and high frequency) for their potential to alter sensory detection and pain thresholds. Ten volunteers (4 male, 6 female, mean age 24.4 years, range 22.2-25.8) were tested in four sessions, each time applying one of four rTMS stimulation protocols. Two stimulation frequencies, 1 Hz and 10 Hz, were tested at each of the two stimulation sites, which were located over the medial and the lateral cerebellum (Table 4.1). The order of treatment was randomly assigned. The stimulation targets were localized using a neuronavigation system (Brainvision, Brainlab, Munich, Germany) in combination with individual anatomical MRI scans, retrieved from each participant. Stimulation areas were chosen over lobule VII of the cerebellar vermis (medial) and over Crus II of the right lateral cerebellar hemisphere (lateral) as defined in previous experiments (Langguth et al., 2008). The neuronavigation system ensured correct localization of the coil over the target during the application of rTMS. Once the target area was found, the coil was held in place by a mechanical arm and the experimenter constantly monitored its position over the target region. Ramping temperature stimuli for CT, WT, CPT, and HPT were presented in blocks and in this given order (Summers et al., 2004; Verdugo & Ochoa, 1992). Temperature detection thresholds were assessed first, as they are known to be influenced by immediately antecedent temperature pain stimuli (Heldestad, Linder, Sellersjö, & Nordh, 2010; Quiton & Greenspan, 2008). A block consisted of four subsequent temperature stimuli for CT and WT and of three for CPT and HPT. The means of all thresholds in a given block were defined as CT, WT, CPT, and HPT, respectively.

In case of missing data at least two valid measurements per block had to be obtained to include the value. The sensory testing procedure took approximately 15 min of time (Table 4.1). CT, WT, CPT, and HPT were compared for pre- and post-rTMS differences by two-tailed repeated measures *t*-tests for each rTMS condition. No correction for multiple comparisons was applied in this experiment, because the purpose was to screen for potential effects of the different stimulation protocols.

Experiment 2

Based on the results of the first experiment, low-frequency rTMS stimulation over the lateral cerebellum was chosen as a treatment condition for the second experiment. The second experiment aimed to answer the question, whether 1 Hz rTMS over the lateral cerebellum significantly modulates warm and cold detection and pain thresholds in comparison to (1) sham TMS and (2) active rMS over neck muscles. rMS of the lateral neck was applied as control condition in addition to standard sham rTMS, to account for rTMS effects on cervical somatosensory afferents as a potential confounding factor.

No participant of the first experiment was tested in the second experiment. Each of the twelve volunteers of the second experiment (5 males, 7 females, mean age 31.8 years, range 20.6-55.0) was tested three times under one of the following conditions: low-frequency active stimulation over the lateral cerebellum, low-frequency active stimulation of the lateral neck, and low-frequency sham stimulation over the lateral cerebellum using a sham coil (Table 4.1). The order of the stimulation conditions was random. Neuro-navigated coil localization in the first experiment revealed only minimal inter-individual differences with respect to the stimulation site and indicated sufficient accuracy for coil positioning according to anatomic landmarks, as shown in previous studies (Herwig, Satrapi, & Schönfeldt-Lecuona, 2003; Langguth et al., 2006). Thus, in the second experiment, coil positioning was performed according to established standard procedures based on anatomic landmarks. For stimulation over the right lateral cerebellum the center of the coil was positioned 20 mm below the inion and 20 mm lateral from the mid-sagittal plane (Fierro et al., 2007). Stimulation of the neck was performed by placing the center of the coil 100 mm below the inion and 20 mm lateral to the mid-sagittal plane resulting approximately at the level of the fifth cervical vertebra and thus in sufficient distance from cerebellum and brainstem. Once the target area was found, the coil was held in place by a mechanical arm and its position over the target region was constantly monitored by the experimenter. Sham rTMS was performed with a sham coil (Magstim Co) at 1 Hz over the lateral cerebellum. The sham coil does not emit magnetic pulses, but mimics the characteristic "clicking" sounds of rTMS. The temperature detection and pain measurement procedures were slightly modified for the second experiment with the aim to reduce data variability. Conformity of instructions was now ensured by reading the instructions to the participant from a script. Moreover, the number of temperature measurements underlying the threshold values was increased by performing the whole thresholding procedure twice, with a 3 min break in between. This was done to reduce the influence of missing data and to reduce intra-individual variability. Apart from these modifications, temperature and pain thresholds were assessed identically as in experiment 1. The sensory testing procedure took approximately 25 min of time (Table 4.1). Within-subject effects of the independent factors Time (pre-rTMS versus post-rTMS) and Treatment (cerebellum versus neck versus sham) were tested using a repeated-measures, multivariate analysis of variance (MANOVA) that included all thresholding variables (CT, WT, CPT, HPT). Repeated-measures *t*-tests were used as planned comparisons to determine the directions of effect. To assess, if the

effect of verum rTMS over the cerebellum was significantly larger than the effect of neck or sham rTMS, the planned comparisons cerebellum versus neck (pre-rTMS versus post-rTMS), cerebellum versus sham (pre-rTMS versus post-rTMS), and neck versus sham (pre-rTMS versus post-rTMS) were performed for all variables.

Results

Mild and transient muscle pain in the neck was experienced by most participants after rTMS over neck and cerebellum. Mild headache (after rMS of the neck) was reported by one and transient concentration problems (after active rTMS over the cerebellum) by another participant. Fortunately, no nausea, as previously reported for low frequency stimulation of the right lateral cerebellum was observed in any of the 22 participants as a side effect of rTMS (Satow et al., 2002).

Experiment 1

Shapiro-Wilk tests indicated that all but three measures were potentially sampled from a Gaussian distribution and thus the assumption of normality was maintained. Results indicated significant differences between before and after low-frequency stimulation over the lateral cerebellum for CT (n = 10, $t = 2.34$, $p = .044$), WT (n = 10, $t = 2.91$, $p = .017$), and HPT (n = 10, $t = 3.27$, $p = .010$). Significant differences were further found for the lateral (n = 10, $t = 2.67$, $p = .026$) and medial (n = 10, $t = 2.59$, $p = .029$) 10 Hz rTMS conditions for CT. Descriptive results are given in Table 4.2. Several participants reached machine safety limits during CTP testing pre-rTMS. These measures were excluded from analysis. Based on these results with significant differences in three of four sensory parameters, low-frequency rTMS stimulation over the lateral cerebellum was chosen as the most promising stimulation protocol for the second experiment.

rTMS	Lateral, 10 Hz		Medial, 10 Hz		Lateral, 1Hz		Medial, 1Hz	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Cold detection thresholds	30.93 ± 0.11	30.25 ± 0.25	30.99 ± 0.13	30.36 ± 0.33	31.01 ± 0.10	30.57 ± 0.20	31.02 ± 0.14	30.87 ± 0.19
Warmth detection thresholds	32.91 ± 0.09	33.15 ± 0.09	33.05 ± 0.12	33.18 ± 0.18	32.80 ± 0.06	33.15 ± 0.15	32.92 ± 0.06	32.94 ± 0.10
Cold pain thresholds	12.97 ± 2.70	10.18 ± 2.00	14.70 ± 2.64	12.75 ± 2.38	13.79 ± 2.84	14.52 ± 2.79	14.38 ± 2.96	15.89 ± 2.77
Heat pain thresholds	46.62 ± 0.79	47.61 ± 1.07	46.60 ± 0.76	46.58 ± 1.05	44.93 ± 0.89	46.08 ± 0.96	43.85 ± 1.20	44.12 ± 0.99

Table 4.2: Descriptive results of the first experiment. Means ± SD. All values in °C. Abbreviations: rTMS: repetitive transcranial magnetic stimulation.

Experiment 2

From 12 participants, 10 completed all study procedures. Two participants had to be excluded from the study, as they constantly reached the temperature safety limit of 50 °C pre-rTMS during HPT-testing. One participant's HPT difference for the sham condition was identified as an outlier (net HTP change 15.17 C, $\Delta Z = 2.58$) by using box plots and excluded from analysis. This reduced the total sample size for the repeated measures MANOVA to n = 9, because of case-wise exclusion. Shapiro-Wilk tests indicated that all measures were potentially sampled from a Gaussian distribution and thus the assumption of normality was maintained. The repeated measures 2 x 3 MANOVA with the variables HPT, CPT, WT, CT, and within-subject factors Time (pre-rTMS versus post-rTMS) and Treatment (cerebellum versus neck versus sham) indicated a significant interaction between Treatment and Time (Wilk's lambda = 0.265, $F(8) = 3.059$, $p = .014$). No significant main effects of Treatment or Time were found. This

suggested that temperature thresholds differed significantly in at least one mean vector pairing in one of the four measures. Mauchly's test indicated that the assumption of sphericity had not been violated for any variable, still all results are reported applying Greenhouse-Geisser correction. Significant Time versus Treatment interactions were found for CT and HPT (Figures 3.1a and 3.1d), but not for WT or CPT (Figures 3.1b and 3.1c). There was a significant main effect of Time for WT (Figure 3.1b), and a near significant main effect of Time for CT and HPT. This suggests a general reduction in thermal sensitivity across all Treatment conditions, which is most likely a consequence of the repeated heat and cold pain measurements (Heldestad et al., 2010; Quiton & Greenspan, 2008). Descriptive results are given in Table 4.3. Planned comparisons for the Time x Treatment interactions between verum rTMS over the cerebellum and sham rTMS indicated no significant differences for WT or CPT and near significant differences for CT (n = 9, $t = 1.93$, $p = .090$). The increases in HPT (n = 9, $t = 4.00$, $p = .004$) were highly significant. Planned comparisons for the Time x Treatment interactions indicated no significant differences between rTMS over the cerebellum and rMS of the neck for CT, WT, CPT, or HPT, although the latter was nearly significant (n = 9, $t = 2.84$, $p = .071$). Planned comparisons for the Time x Treatment interactions between rMS of the neck and sham rTMS indicated no significant differences for WT and CPT, but for CT (n = 9, $t = 2.82$, $p = .022$) and HPT (n = 9, $t = 3.53$, $p = .007$).

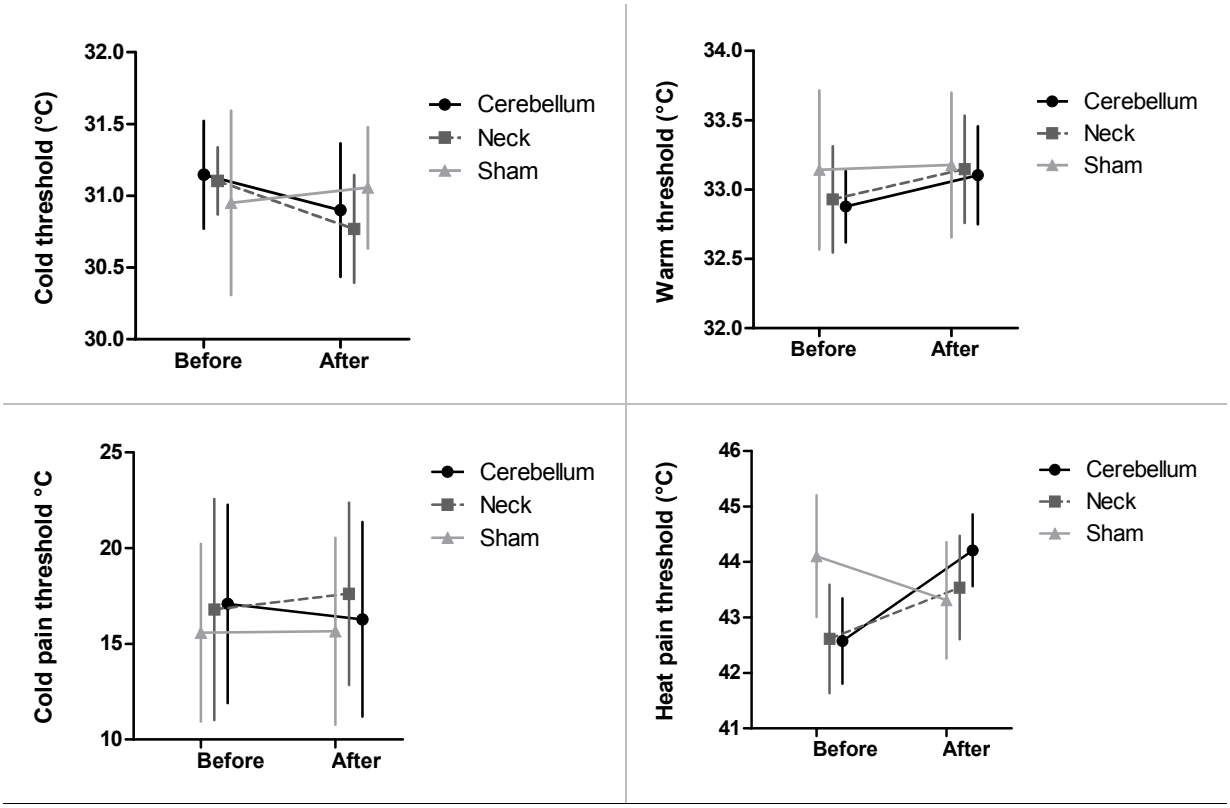

Figure 3.1a: Cold detection threshold (CT) before and after rTMS. There was a near significant main effect of Time F(1) = 4.70, partial $\eta^2 = 0.370$, p = .062. The interaction between factors Time and Treatment was found significant: F(2; 1.62) = 4.65, partial $\eta^2 = 0.368$, **p = .036**.

Figure 3.1b: Warm detection threshold (WT) before and after rTMS. There was a significant main effect of Time F(1) = 5.40, partial η2 = 0.403, **p = .049**.

Figure 3.1c: Cold pain threshold (CPT) before and after rTMS. There were no significant or near significant main effects or interactions.

Figure 3.1d: Heat pain threshold (HPT) before and after rTMS. There was a near significant main effect of Time F(1) = 3.73, partial η2 = 0.318, p = 0.089. The interaction between factors Time and Treatment was found significant F(2; 1.448) = 13.036, partial η2 = 0.620, **p = .002**.

All means are displayed ± standard deviation.

rTMS	**Cerebellum**		**Neck**		**Sham**	
	Pre	Post	Pre	Post	Pre	Post
Cold detection thresholds	31.15 ± 0.38	30.90 ± 0.47	31.10 ± 0.23	30.77 ± 0.38	30.95 ± 0.64	31.06 ± 0.42
Warmth detection thresholds	32.88 ± 0.26	33.10 ± 0.35	32.93 ± 0.38	33.15 ± 0.39	33.14 ± 0.57	33.18 ± 0.52
Cold pain thresholds	17.09 ± 5.18	16.28 ± 5.09	16.79 ± 5.78	17.62 ± 4.76	15.58 ± 4.64	15.66 ± 4.88
Heat pain thresholds	42.58 ± 2.31	44.21 ± 1.94	42.61 ± 2.93	43.54 ± 2.80	44.11 ± 3.30	43.31 ± 3.15

Table 4.3: Descriptive results of the second experiment. Means ± SD, all values in °C. **Abbreviations:** rTMS: repetitive transcranial magnetic stimulation.

Discussion

This is the first study to examine the potential of rTMS over the cerebellum to modulate temperature and pain thresholds in humans. In two separate experiments, 1 Hz rTMS over the lateral cerebellum was consistently found to significantly increase HPT and to decrease CT by trend. However, main result of the study is that similar changes of CT and HPT occurred after the control condition, in which rMS was performed over the neck. Therefore, the observed effects of rTMS over the lateral cerebellum on thermal perception are most likely mediated via stimulation of peripheral structures and not via modulation of cerebellar activity. Though we cannot exclude a potential surplus effect of cerebellar rTMS over rMS of the neck on thermal sensation with the current sample size, our results suggest that such an effect might be smaller than the effect of magnetic stimulation of peripheral structures of the neck.

The results of the current study indicate that the observed effects of rTMS over the cerebellum on thermal perception are rather mediated via stimulation of cervical muscles or afferents than via modulation of cerebellar activity. This is in accordance with studies on the effects of rMS of the neck, where 10 sessions of rMS of myofascial trigger points at the trapezius muscle resulted in pain relief outlasting the stimulation by several weeks (Smania et al., 2005). Also single sessions of rMS were shown to relief pain caused by localized musculoskeletal processes (Pujol et al., 1998). The more pronounced analgesic effect of rMS as compared with transcutaneous electrical nerve stimulation (TENS) has been explained by the fact that magnetic stimulation achieves higher stimulation intensities than electrical stimulation without local patient discomfort (Smania et al., 2005). The higher stimulation intensities achieved with rMS result in stimulation induced rhythmic muscle contractions, which may trigger mechanisms similar to those underlying analgesia after isometric muscle contractions (Hoeger Bement, Dicapo, Rasiarmos, & Hunter, 2008; Staud, Robinson, & Price, 2005). However, the exact peripheral mechanisms by which rMS of the neck and rTMS over the posterior skull modulate pain remain incompletely understood. Potential mechanisms of action include modulation of afferent fiber excitability, gate control mechanisms in dorsal horn and brainstem, counter irritation mechanisms similar to the "diffuse noxious inhibition controls" (DNIC), activation of the sympathetic nervous system, and central release of endogenous analgesic substances such as opioids, dopamine, or serotonin (Bragin, 1986; Heldmann, Kerkhoff, Struppler, Havel, & Jahn, 2000; Melzack, 1999; Schlaier et al., 2007; Sluka & Walsh, 2003; Smania et al., 2005). Clarification of the underlying mechanisms will require further research.

Nevertheless, the current pilot study provides additional evidence for a pain relieving effect of repetitive magnetic muscle stimulation, though replication in a larger sample is necessary before definite conclusions can be drawn. Several previous studies have found effects of TMS over the cerebellum using different stimulation protocols (Fierro et al., 2007; Fisher, Lai, Baker, & Baker, 2009; Gironell et al., 2002; Koch et al., 2009; Langguth et al., 2008; Oliveri et al., 2009; Satow et al., 2002; Ugawa, Uesaka, Terao, Hanajima, & Kanazawa, 1994; Ugawa et al., 1995). Flat figure-of-eight coils, as well as angled double-coned coils have been used in various orientations, with different stimulation intensities and at different locations. Just recently, (Ugawa, 2009) rightfully advocated a systematic reassessment of cerebellar stimulation thresholds to settle the inconsistent use of cerebellar stimulation protocols.

The stimulation of superficial structures is well known as an important confounding factor in the investigation of rTMS (Mennemeier et al., 2009; Rossi et al., 2007). For rTMS over the

cerebellum, it has been shown that effects on cortico-spinal excitability are largely mediation via stimulation of peripheral structures such as muscles or afferent fibers (Gerschlager et al., 2002). These potent peripheral effects of rTMS at the posterior skull are further substantiated in the current study.

A recent study also evidenced the risk of concomitant stimulation of deep brainstem structures with TMS over the cerebellum when using a double coned-coil (Fisher et al., 2009). However, the risk of concomitant stimulation of the brainstem or the dorsal columns seems negligible in the current study, as the magnetic pulse of a flat figure-of-eight Magstim coil as used in our study decays rapidly with increasing distance to the coil (Thielscher & Kammer, 2004).

Our data further highlight, that results of rTMS studies in general may depend to a large extent on the adequate choice of the control condition. Being aware that all available control conditions have shortcomings, the best choice among the available options depends on the specific experimental context (Langguth et al., 2008). Along these lines, our results stress the importance to account for peripheral effects of rTMS, especially when the dependent variable is known to be influenced by somatosensory modulation.

In conclusion, rTMS effects over the cerebellum did not differ significantly from those obtained by rMS over the lateral neck in the current study. A pain relieving effect via cerebellar modulation could not be demonstrated, yet these results are showing for the first time that rTMS over the neck can induce significant reductions in heat pain perception and non-noxious cold perception by recruiting peripheral mechanisms. With the given sample size, the current study can only serve as a pathfinder in the search for effects of cerebellar rTMS. Further studies will be needed to investigate to which extent the analgesic effect of muscle stimulation depends on stimulation parameters such as frequency, intensity, and localization. Relying on our results, we lastly argue for a critical examination of the proposed analgesic effects, which were reported for rTMS of various other cranial stimulation sites. Peripheral stimulation effects of rTMS might be underestimated in their impact on experimental, as well as clinical pain.

Acknowledgments

We thank Sandra Pfluegl and Helene Niebling for technical assistance.

CONCLUDING DISCUSSION

On measuring pain

The methods used to obtain information on pain perception for this dissertation stem from the domain of classic psychophysics. In the following section, an overview on available methods of pain measurement is provided, the challenges in studying pain are discussed, and potential future directions are presented. Finally, the pro and contra of the methods used for this dissertation are examined and the obtained results are summarized.

Methods of measuring pain

A multitude of attempts have been made to measure human pain. An overview of methods used in the past is provided in Table D.1 without claims of completeness.

Domain	Method	Example reference
Classic Psychophysics	Thresholding: Method of constant stimuli	(Strigo, Carli, & Bushnell, 2000)
	Thresholding: Method of limits	(Rolke, Magerl, et al., 2006)
	Thresholding: Staircase method	(Rolke, Magerl, et al., 2006)
	Thresholding: Method of adjustment	(Horn, Blischke, Kunz, & Lautenbacher, 2012)
	Descriptive scales (e.g. verbal, numeric or item based scales)	(Melzack, 1975)
	Analog scales/cross-modality matching/magnitude estimation (e.g. visual analog scales, handgrip, finger-span)	(Breivik, Björnsson, & Skovlund, 2000; Gracely, McGrath, & Dubner 1978)
Modern Psychophysics	Signal-detection theory designs (based on two-alternative or two interval forced choice tasks)	(Lloyd & Appel, 1976)
Behavioral	Facial expressions	(Prkachin, 2009)
	Vocalization (infants only)	(Mijović et al., 2010)
	Observation of general behavior (e.g. activity, sleep, medication intake)	(Keefe, Bradley, & Crisson, 1990)
Physiological	Microneurography (i.e. in-vivo afferent nerve recordings of peripheral nerves)	(Mano, Iwase, & Toma, 2006)
	Reflex myography (e.g. Nociceptive flexion reflex, Eyeblink reflex)	(Skljarevski & Ramadan, 2002; Holle et al., 2011)
	Electroencephalography (e.g. laser evoked potentials, contact heat evoked potentials, gamma band oscillations)	(Handwerker & Kobal, 1993; Greffrath, Baumgärtner, & Treede, 2007)
	Pupillary dilation	(Ellermeier & Westphal, 1995)
	Heart rate variability (as a measure of autonomic nervous system activity)	(Tousignant-Laflamme, Rainville, & Marchand, 2005; Loggia, Juneau, & Bushnell, 2011)
	Galvanic skin response (as a measure of autonomic nervous system activity)	(Loggia et al., 2011)
Neuroimaging	Functional magnetic resonance imaging (fMRI), positron emission tomography (PET), single photon emission computed tomography (SPECT)	(Apkarian, Bushnell, Treede, & Zubieta, 2005)

Table D.1: Measures in human pain research.

Most of the methods listed in Table D.1 can only be used in combination with experimental pain stimulation; an overview on experimental methods of pain induction used in the past is given in Table D.2, again, without claims of completeness.

Stimulus type	Stimulus	Example reference
Heat	Hot plate	(Rolke, Magerl, et al., 2006)
	Hot water bath	(Verne, Himes, et al., 2003)
	Hot light bulb ("Hardy-Wolff-Goodell Dolorimeter")	(Tousignant, 2011)
	Laser	(Bornhövd et al., 2002)
Cold	Cold plate	(Rolke, Magerl, et al., 2006)
	Water bath ("Cold pressor")	(Lovallo, 1975)
	Thermal-grill illusion	(Boettger, Schwier, & Bär, 2011)
Mechanical	Sharp force	(Rolke, Magerl, et al., 2006)
	Blunt force (e.g. on the nail bed)	(Göbel, Heller, Nowak, & Westphal, 1988; Woollard & Carmichael, 1933)
	Incision pain	(Reitz et al., 2012)
	Distension devices in gastrointestinal tract (esophageal/colonic/rectal distension)	(Paine, Kishor, Worthen, Gregory, & Aziz, 2009; Verne, Robinson, Vase, & Price, 2003)
Other	Ischemia (cuff around arm)	(Fillingim et al., 1997)
	Capsaicin (paste, injection)	(Tamura et al., 2004)
	Ascorbic acid	(Favilla et al., 2014)
	Muscle pain (exercise induced, hypertonic saline injections)	(Dannecker, Price, O'Connor, & Robinson, 2008; Owen, Clarke, Ganapathy, Prato, & St Lawrence, 2010)
	Electric shock	(Rhudy, Williams, McCabe, Rambo, & Russell, 2006)

Table D.2: Methods of experimental pain stimulation in human research.

Difficulties in measuring pain

How to measure pain? Despite all past efforts, this still is a central question of pain research and the field of psychophysics. All measures listed in Table D.1 can yield valuable information on pain-related processes. However, no single method has been established as a generally accepted, unbiased measure of perceived pain so far. All methods in Table D.1 are limited in the scope of application, reliability and validity. The shortcomings of the available pain measurements are based on a number of basic problems complicating the study of pain:

- **Problem 1.)** Pain is still defined as a psychological, subjective phenomenon (International Association for the Study of Pain, 2002, p. 210). There currently is no objective criterion to determine if a given stimulus is perceived as painful with certainty. A participant's rating of a stimulus as "painful" or "not painful" cannot be verified as "correct" and "incorrect". Participants' responses have to be relied on.

  Consequently, pain is mostly studied in so called "Type 2-experiments" and "appearance" rather than "performance" tasks, according to the classification of psychophysiological experiments by Kingdom & Prins (2010), pp. 18–24.[3]

[3] Signal detection theory designs have been suggested to circumvent Problem 1 in the past (Coppola & Gracely, 1983; Handwerker & Kobal, 1993), however this view has been challenged (see: Rollman, 1977).

- **Problem 2.)** Stimulus-related pain exists in a continuum with other somatosensory domains. For example low-dose heat, electric shocks, or capsaicin are perceived as warm, tingling, or itching before becoming painful with higher doses. To my knowledge there is no experimental method of pain induction where sub-threshold stimulation is not accompanied by any sensation (see: Table D.2). Therefore, non-nociceptive somatosensory perception has to be considered as a confounding factor.
- **Problem 3.)** Response bias (see: Furnham, 1986) may affect any self-report measures of pain: Expectations, motivations, and the social context associated with an experiment (e.g. see: Aslaksen, Myrbakk, Høifødt, & Flaten, 2007) may all falsify participants' responses in pain measurements. Nevertheless, these factors might also have a genuine influence on the perception of pain (for review see: Wiech, & Tracey, 2013). A notable example is the placebo effect; an expectation, which might both bias pain measurements (i.e. alter the nature of the measurement, causing a "deviation from the truth", Hróbjartsson, Kaptchuk, & Miller, 2011) *and* influence the experience of pain (Wiech, & Tracey, 2013).
- **Problem 4.)** Both habituation and sensitization may occur with prolonged or repeated pain stimulation; these effects can be peripherally and/or centrally mediated and add systematic variance to pain measurements (Breimhorst, Hondrich, Rebhorn, May, & Birklein, 2012; Vierck, Cannon, Fry, Maixner, & Whitsel, 1997; Werner, Lassen, Pedersen, & Kehlet, 2002).
- **Problem 5.)** Ethical conduct in pain research is of primary importance. Restrictions apply (International Association for the Study of Pain, 2014) in addition to the standards of ethical conduct in human research (59th WMA General Assembly, 2009): For example the quality and duration of pain endured in research settings must be limited.

Fortunately, an appropriate study design can compensate for the inevitable shortcomings of pain measures and problems listed above. Proper study design is the key feature of scientific experiments and the prerequisite for a meaningful interpretation of results. Three basic features (e.g. see: Ruxton & Colegrave, 2011) of experiments that help to make valid inferences are:

- **Blinding and control conditions**
- **Random allocation** of participants to the studied conditions
- **Replications** of measurements within a representative and sufficient sample size

Discussion of methods and study designs used

Study design: blinding and control conditions

In **Study 1** a double-blind, placebo controlled crossover design was used; participants received oxytocin at one study visit and a placebo at another. Neither the participants nor the experimenters knew the order in which the medication was given during testing. The double-blind placebo design is the gold standard of experimental control; it effectively minimizes both experimenter bias and participant response bias (Ruxton & Colegrave, 2011). Crossover designs are superior in statistical power compared to parallel-group designs since every participant acts as his or her own control, greatly reducing random between-participant variability (Ruxton & Colegrave, 2011). The absence of carryover effects was assumed for **Study 1,** based on the known pharmacokinetics of oxytocin and the choice of an appropriate wash-out phase of ≥ 7 days between study visits. Known sensitization and desensitization effects of repeated thermal stimulation were reduced by employing a training session and pre-conditioning stimuli (Quiton & Greenspan, 2008).

Study 2 was not an experimental study, but a questionnaire survey. A "quasi-experimental" longitudinal design (for review see: Fife-Schaw, 2012) was employed to examine the effects of an exam period in university students. Again, participants acted as their own control. Two baseline measures were obtained as a control condition, one before and one after the exam period measurement. The choice of two baselines excluded that simple time effects explained the results. Further, control variables, such as perceived stress and current infections/injuries, were assessed to confirm that exam period was an effective psychosocial stressor and to rule out alternative explanations. Quasi-experimental study designs are often applied when "natural" treatments, interventions, or conditions cannot be controlled experimentally for practical or ethical purposes (Fife-Schaw, 2012). Effects of conditions can be observed under more "realistic" circumstances and thus entail higher external validity than in strictly controlled experimental settings (Fife-Schaw, 2012). However, quasi-experimental approaches are less capable in controlling for the confounding effects of third variables (Fife-Schaw, 2012), which is a considerable threat to the internal validity of the results. A specific threat to the internal validity of the effects observed in **Study 2** is that no blinding could be applied due to the longitudinal design. Therefore experimenter bias and participant response bias cannot be excluded, which considerably limits the interpretation of results.

The choice of a valid placebo (also: sham) condition for behavioral (**Study 3**) and electrophysiological (**Study 4**) interventions is challenging. The nature of the verum- and placebo-conditions is difficult to conceal from participants and even more difficult to conceal from experimenters. **Studies 3 and 4** both had single-blind crossover design, since it was not feasible to achieve double-blind conditions, e.g. by using different experimenters for treatment application and outcome measurement.

Single-blind designs largely exclude participant response bias, but are susceptible to experimenter bias (Ruxton & Colegrave, 2011), which therefore cannot be excluded for **Studies 3 and 4**. To achieve blinding of participants for **Study 3**, no information was given about the explicit hypotheses before debriefing. A design with four different paced breathing conditions was chosen, in which the hypothesized effect was expected to show a non-linearly relationship with heart-rate variability. Since breathing frequency was the only discernible difference between sessions, and the range of breathing frequencies were not known a-priori, the explicit hypotheses

were hard to guess. To achieve blinding of participants in **Study 4** a sham stimulation condition and an ectopic verum stimulation condition were used. Again, participants were not informed about the hypothesized effects of the different conditions before debriefing.
Again, **Studies 3 and 4** had designs with repeated visits, treatments, and measurements within participants. In both studies a wash-out phase of ≥ 7 days was observed to minimize carry-over effects of breathing exercises or rTMS from one study visit to the other. However, no training visit was used in the two studies. Therefore sensitization and desensitization effects (Quiton & Greenspan, 2008) may have added to the random variability within the data.

Study design: randomization

For **Studies 1, 3, and 4** participants were randomly allocated to the treatment sequence. Treatment order was strictly balanced across participants to exclude a systematic impact of habituation, sensitization, or other time effects on the results. For **Study 2,** a random assignment to academic exams was not possible, of course—thus the quasi-experimental design.

Study design: choice of sample

Regarding the nature of replications, all four studies investigated pain in student samples: In **Studies 1, 3, and 4** healthy students were tested. In **Study 2** a naturalistic student sample was obtained. Therefore, all four studies suffer from a shortcoming in external validity that is common to the field of pain research: The results obtained might not generalize to the general population or sufferers of chronic pain. However, the use of "unnatural" model situations in pain research has two good reasons: First, studies on pain models are more resource efficient than trials in patient populations. Second, a basic understanding of the mechanisms in health is necessary to allow an understanding of the highly diverse mechanisms of pathological pain. The dilemma of bridging-the-gap between experimental pain research and clinical application can only be solved by developing refined methods, by translational research approaches, and by replication of findings. Additionally, the sample of **Study 1** was restricted to male participants, since the effects of oxytocin are likely to be sex-specific (Theodoridou, Penton-Voak, & Rowe, 2013). Any conclusions drawn from **Study 1** are therefore limited to the male population.

Study design: replications

A sufficient sample size allows generalizing findings to the population and to detect an existing effect with an acceptable probability. But how many replications have to be chosen for a sample size to be sufficient? The answer depends on several factors: E.g. the actual size of the effect in question, potential interaction effects, the reliability of the measurement, the natural variability within the sample, as well as the information available a-priori.
The sample size of **Study 1** was chosen based on a power-analysis for heat an cold pain thresholds: The smallest important absolute effect size of oxytocin compared to placebo was defined as +0.5 °C for heat and -1.5 °C for cold pain thresholds, as this approximates 50 % of the effect of a low dose (10 mg) of intravenous morphine (Pavlakovic, Tigges, & Crozier, 2009). Standard deviations and test-retest correlations for heat pain thresholds (SD = 1.42 °C, r = 0.88) and cold pain thresholds (SD = 7.44°C, r = 0.91) were estimated based on the results of 18 male participants from **Studies 3 and 4**. For a paired two-tailed *t*-test[4] with a Bonferroni-corrected α-error probability $\alpha_{Bonf} < .05$ and a desired power (β-error probability) $\beta > .80$, a minimal sample

[4] The statistical models were simplified for the purposes of the power calculation. The general linear (mixed) model used for analysis was assumed to entail more power to detect an effect.

size of 21 (heat pain threshold) and 44 (cold pain threshold) participants was estimated. A sample size of 36 was chosen as a compromise between the two values. In regard to the fMRI measures and pain rating procedures such power calculations were not conducted due to the lack of a-priori data.

For **Study 2** the sample size of 150 participants was based on the following calculation: For the SOMS-7d a re-test reliability of $r = .76$ and a population mean of 6.25 ± 8.65 points of the Symptom Index has been reported (Hiller, Rief, & Brähler, 2006; Rief & Hiller, 2008). A paired two-tailed *t*-test[1] at an α-error probability $\alpha < .05$ would require a sample size of 128, to detect a change of 1.5 points in the Symptom Index with a power of $\beta > .80$. This minimum sample size was rounded to 150 participants to account for the expected dropout rate.

No a-priori sample size calculations were performed for **Study 3**, which certainly is a limitation. The aim was to achieve a statistical power comparable to the study generating the study hypothesis (Chalaye, Goffaux, Lafrenaye, & Marchand, 2009).

For **Study 4**, no estimations of power were made due to the nature of the study: Two separate experiments were performed; the first identified potential hypotheses, while the second aimed to test and verify the hypotheses. Retrospectively, Type-II errors are a minor concern in **Study 4,** since effects of stimulation were found for most measures.

Methods of psychophysics: thresholding

Thresholding procedures according to the "method of limits" were used in **Studies 1, 3, and 4** to infer on pain perception: Slowly decreasing and increasing temperatures were applied to the skin by using an electrically heated/cooled plate ("thermode"). Participants were instructed to press a button when the temperature reached the heat or cold pain threshold.

These thresholding procedures are fast, efficient, reliable, and established in the field (Rolke, Baron, et al., 2006). The automated, computerized execution of the protocol, the standardized, scripted definition of thresholds, and other precautions make the technique an acceptably objective measurement. Nevertheless, the procedure has shortcomings: First, thresholding cannot discern between changes in pain perception, pain appraisal, response criterion (i.e. the individual, internal definition of the pain threshold), and response speed. These confounding factors can only be compensated by appropriate experimental design, e.g. by blinding participants to the studied conditions (see above). Second, thresholding measures can be confounded by changes in non-nociceptive somatosensation, since the stimulus shifts from the non-nociceptive to the nociceptive range (see: Problem 2). Therefore, non-nociceptive warmth and cold detection thresholds always were assessed in parallel, as a control. Third, pain thresholding according to the method of limits involve transient and low pain intensities only, which reduces the face-validity of the measure. Moreover the low intensities might reduce the reliability of the measurement, since Quiton & Greenspan (2008) showed that higher pain intensities are judged more reliably within- and between-individuals.

Methods of psychophysics: visual analog scale ratings

To account for the last-mentioned shortcoming of the thresholding procedure, **Studies 1 and 3** additionally included a pain-rating procedure: The VAS is an established and time-efficient method for obtaining ratings of perceived experimental and clinical pain (Breivik, Björnsson, & Skovlund, 2000; Quiton & Greenspan, 2008; Rosier, Iadarola, & Coghill, 2002). In **Studies 1 and 3** participants were asked to rate tonic heat stimuli of 14 s duration on such a VAS. Although the VAS rating procedure shares almost all advantages and disadvantages of the thresholding procedure, considerably higher stimulation intensities could be used, which might have reduced variability and increased validity of the measurement (Quiton & Greenspan, 2008).

Methods: self-assessment questionnaires

Study 2 is the only study of this dissertation that investigated pain in its naturalistic form. A questionnaire was used to obtain self-report measures of pain and other bodily symptoms. The Screening for Somatoform Symptoms 7-day version (SOMS 7d) is not a classic pain scale, but was developed as a measure of somatization (Rief & Hiller, 2008). Nevertheless, the SOMS 7d includes the most common idiopathic forms of pain (headache, backache, intestinal pain, joint pain) and surveys a number of typical bodily symptoms occurring under stress. Furthermore, it is well established according to the quality criteria of test theory (Rief & Hiller, 2008). Questionnaires are a fast and efficient way to obtain detailed population estimates of disease prevalence and to identify potential causes. However, like all self-descriptions, questionnaires are highly susceptible to response bias (see: Furnham, 1986).

Methods: functional magnetic resonance imaging (fMRI)

Positron emission tomography (PET, e.g. Talbot et al., 1991), single photon emission computed tomography (SPECT, e.g. Krabbe, Henriksen, & Olesen, 1984), and functional magnetic resonance imaging (fMRI, e.g. Davis, Wood, Crawley, & Mikulis, 1995) have been adopted for the study of pain early after the advent of modern neuroimaging. Hundreds of neuroimaging experiments have since then been conducted to study various aspects of human pain (for review see: Duerden & Albanese, 2013). Like **Study 1**, many studies used fMRI, in combination with the analysis approach "statistical parametric mapping" (SPM, Friston et al., 1995). With fMRI and SPM, the cerebral correlates of human pain could successfully be identified (for review see: Apkarian, Bushnell, Treede, & Zubieta, 2005; Duerden & Albanese, 2013). Especially the insula, SI, SII, and the anterior cingulate cortex, have repeatedly been confirmed as correlates of human pain processing (Apkarian, Bushnell, Treede, & Zubieta, 2005; Duerden & Albanese, 2013). However, a number of limitations apply to these techniques:

- **Limitation a)** The blood oxygenation level dependent (BOLD) signal measured by fMRI is a unitless metabolic correlate of neuronal mass activity (Logothetis, 2008). Its ability to qualify and quantify cerebral processes is constrained:

  > The fMRI signal cannot easily differentiate between function-specific processing and neuromodulation, between bottom-up and top-down signals, and between excitation and inhibition. Moreover the magnitude of the fMRI signal is no accurate estimate of activity differences between brain regions, or even between different tasks within the same region. (Logothetis, 2008)

- **Limitation b)** The low signal-to-noise-ratio of fMRI leads to a low reliability and a high statistical uncertainty (Smith et al., 2005). This problem is aggravated by the fact that fMRI studies are costly and therefore their sample size is usually limited. Consequently, the regions found to be associated with pain vary considerably between fMRI studies of pain, despite similar experimental designs (Apkarian et al., 2005; Duerden & Albanese, 2013)
- **Limitation c)** SPM involves the calculation of more than 100,000 regressions for a whole brain analysis. However, there is no generally accepted standard procedure to correct for multiple comparisons in SPM (compare: Vul, Harris, Winkielman, & Pashler, 2009 and Lieberman & Cunningham, 2009).
- **Limitation d)** "The brain is never inactive" (Balduzzi, Riedner, & Tononi, 2008), therefore there is no „zero" baseline condition in fMRI. Simple comparisons with an alleged "baseline"-activity, or deficient control condition, can easily lead to confounded results. As an example, many studies in pain research used subtractive designs with categorical predictors in the past, i.e. they simply compared painful stimulus conditions and with no stimulation (Duerden & Albanese, 2013). Non-nociceptive somatosensory processing almost certainly confounds such results (Tseng et al., 2009).

The problem of selecting an adequate baseline conditions is part of a general problem in fMRI analysis: Inferences regarding the effects of a stimulation condition on brain activity are based on the assumption of "pure insertion", i.e. that the stimulation condition of interest "inserts" only a single additional process compared to the baseline condition and that this additional process does not interact with other processes (Friston et al., 1996). However, this assumption has been identified as flawed from early on (Friston et al., 1996). One method to relax the assumption of pure insertion is the use of a parametric fMRI design (Büchel, Holmes, Rees, & Friston, 1998): In parametric fMRI designs, scalar, instead of categorial predictors are used to model the cerebral process in question. E.g. temperature intensities or rated pain intensities are employed as linear (or higher order) predictors of BOLD signal, instead of simply using a categorical predictor. The potential for contamination by nuisance processes is therefore reduced. Consequently, parametric designs have successfully been applied to discriminate between perceived pain processing and accompanying non-nociceptive processes (Büchel et al., 2002).

Aim of the fMRI experiment in **Study 1** was to test effects of intranasal oxytocin on cerebral heat pain processing. A parametric fMRI design was employed to address the issues mentioned above. Stimulus temperature was used as a linear predictor for brain activity related to noxious temperature processing. As a result, our approach could identify most canonical candidate regions of the pain matrix—despite using the conservative family-wise-error (FWE) correction for multiple comparisons. The VAS-intensity and -unpleasantness ratings acquired during fMRI indicated that the applied stimulus conditions had the desired effect. Further, by using the parametric design, the effects of oxytocin on temperature-related activity could be tested separately from oxytocin effects on stimulation-related activity. Nevertheless, no effect of oxytocin could be found in whole-brain analysis at a FWE corrected statistical threshold, although behavioral effects of oxytocin were found for the VAS-intensity ratings.

A region of interest (ROI) analysis was the second type of fMRI analysis used in **Study 1** (Poldrack, 2007): In ROI analysis certain brain regions are pre-selected and treated as functional units. The BOLD-signal is estimated for the ROI as a whole, instead of making inferences on every single voxel separately. The ROI approach therefore reduces the multiple comparisons problem and greatly improves statistical power (Poldrack, 2007). Further, by applying a finite

impulse response (FIR) model (Ollinger, Shulman, & Corbetta, 2001), a ROI analysis allows to evaluate the time-course of the BOLD signal after stimulus presentation. Note that the selection of ROIs has to be performed independently from any paralleling whole-brain analysis; otherwise the rate of false-positive results is dramatically inflated (Vul, Harris, Winkielman, & Pashler, 2009). The ROI analysis in **Study 1** was used to test a-priori hypotheses regarding the effect of oxytocin on amygdala activity. Anatomic tissue probability maps were used to define the ROIs, independently from whole-brain analysis. With this approach, main effects of oxytocin could be found within the amygdala.

Methods: outlook

While the studies of this dissertation were being conducted, new methods were developed, which might solve some limitations of pain measurements listed above:

The combined use of skin conductance and pupil dilation measures has recently been proposed as a reliable measure of pain (Geuter, Gamer, Onat, & Büchel, 2014). Although these findings have to be replicated independently, such a procedure may pose a resource-efficient alternative to neuroimaging experiments.

As mentioned, the BOLD signal is no absolute measurement of cerebral blood flow. The obtained signal depends on sources of inter- and intra-individual physiological variance, such as cerebral blood flow, cerebral blood volume, tissue specific features, cardiac noise, and acquisition variance, such as scanner noise and field inhomogeneity (Bulte et al., 2012; Logothetis, 2008; Smith et al., 2005). Therefore, cerebral blood oxygenation in traditional fMRI can only be measured reliably in terms of relative, within-subject contrasts for stimulation conditions that vary within a session (Tracey & Johns, 2010). Conditions that cannot easily be manipulated within a session, such as chronic and recurrent pain, cannot be effectively studied with traditional fMRI. Two new techniques have recently been proposed to overcome this limitation: Arterial spin labeling (ASL) is a direct and quantitative measure of blood flow (Tracey & Johns, 2010) and might be useful to study chronic recurrent pain. Another new technique presented recently is respiratory calibrated fMRI (Bulte et al., 2012). Here, breathing gas concentrations are manipulated in a calibration scan to allow an absolute estimate of blood oxygenation levels.

Further, new development in neuroimaging analysis might solve shortcomings of the SPM approach and advance neuroimaging towards becoming an objective measure of pain. Four variants of machine learning have been proposed to allow for a reliably prediction and/or quantification of perceived pain: "Gaussian process" modeling (Marquand et al., 2010), "support vector machine" modeling (Brodersen et al., 2012; Brown, Chatterjee, Younger, & Mackey, 2011), "ν-method" modeling (Prato, Favilla, Zanni, Porro, & Baraldi, 2011), and "least absolute shrinkage and selection operator-regularized principal components regression" (LASSO-PCR, Wager et al., 2013). All of these techniques first estimate the spatio-temporal pattern of activity exhibited in fMRI volumes in response to an experimental condition (e.g. pain vs. no-pain) or a regressor (e.g. pain ratings) from a "training" dataset. The trained patterns are then used to classify (and/or quantify) activity within a second set of neuroimaging data as (more or less) painful. In contrast to the classical SPM techniques, which compute the relationship between conditions/regressors and fMRI signal for each brain-voxel separately, the patterns of machine learning are multi-dimensional, combining spatial and temporal patterns. The future will show the impact of these methods on pain research and clinical practice. It is worth noting that the data obtained in **Study 1** might be suitable for an analysis with one of these strategies.

Discussion of results and outlook

Study 1: The effects of intranasal oxytocin on pain perception and processing in healthy male—a randomized trial

For **Study 1**, an effective sample size of 30 was obtained; the study was therefore considerably larger than almost all previous studies investigating experimental heat pain with fMRI (see: Duerden & Albanese, 2013). **Study 1** could provide first evidence that intranasal oxytocin modulates ratings of heat intensity and amygdala activity in response to heat stimuli. However, the effects were independent of temperature (i.e. pain stimulus intensity) and therefore could not support the hypotheses that intranasal oxytocin has a specific effect on pain processing (Rash, Aguirre-Camacho, & Campbell, 2013). The observed effects might reflect changes in anxiety and/or reward sensitivity as suggested by Bethlehem, Baron-Cohen, van Honk, Auyeung, & Bos (2014), or an enhanced placebo responses as shown by Kessner, Sprenger, Wrobel, Wiech, & Bingel (2013). The mechanisms by which oxytocin modulates thermal stimulus processing, as well as the relevance of this modulatory effect for clinical applications, must be assessed in future studies. In particular, a repetition of the present study with a female sample is desirable, since the effects of oxytocin might be sex-specific (Theodoridou, Penton-Voak, & Rowe, 2013).

There are several future directions for **Study 1:** A second fMRI experiment testing the effects of oxytocin on emotional modulation of pain (Rhudy, Williams, McCabe, Nguyen, & Rambo, 2005) was conducted in parallel to **Study 1** and its results are currently under review. As mentioned above, another future direction of **Study 1** is the re-analysis of the dataset with machine learning techniques (Marquand et al., 2010; Wager et al., 2013). These analyses might have an improved statistical power to detect effects of oxytocin. In addition, a side-project of **Study 1** is waiting for analysis: Within a month of the last experimental session, participants were asked to recognize the pictures shown during the emotional modulation experiment amongst other distracting images. These data could shed light on the effects of oxytocin on emotional memory processes.

Study 2: Somatic symptoms evoked by exam stress in university students: the role of alexithymia, neuroticism, anxiety, and depression.

In **Study 2**, 150 university students provided a detailed, quantitative symptom-by-symptom description of their bodily reactions during a period of academic exam stress. Symptoms of pain were amongst the items showing the strongest increases. All control variables indicated that the observed symptoms were due to exam stress, rather than time effects, acute infections, or injuries. Moreover neuroticism could be identified as a significant explanatory personality trait for symptom increases under exam stress. No such relationship could be found for trait anxiety, trait depression, or alexithymia. The descriptive and quantitative results of **Study 2** may help to establish academic exam stress as a model of psychosocial stress in the field of pain research; complementary to models of experimental stress induction (Kirschbaum, Pirke, & Hellhammer, 1993) or diary studies (Bolger & Zuckerman, 1995).

In parallel to **Study 2**, data were retrieved to describe the effects of exam stress on sleep reports and drug-related behavior. This parallel study has been analyzed and is currently being revised after a first submission (Zunhammer, Eichhammer, & Busch, submitted for publication). Further, a follow-up to **Study 2** is currently being conducted, which aims to disentangle the relative contributions of reporting behavior and actual symptom occurrence under exam stress using different participant instructions.

Study 3: Do cardiorespiratory variables predict the antinociceptive effects of deep and slow breathing?

Study 3 could not confirm the main hypotheses tested: Breathing exercises had no effect on pain perception, neither via breathing frequency, nor via cardiac parasympathetic activity, nor via hyperventilation. Nevertheless, baseline and within-session associations of pain perception and heart rate could be found. **Study 3** therefore successfully replicated the finding that experimental pain perception is linked to ANS activity (Appelhans & Luecken, 2008), but could not elucidate the modulatory effects or mechanisms of breathing exercises.

The results of **Study 3** highlight an underrated limitation of heart rate variability (HRV) measurements, when used as a tool to infer on ANS activity: Breathing frequency is one of the strongest determinants of heart rate variability. Although this fact has been acknowledged since long (Hirsch & Bishop, 1981; Pinna, Maestri, La Rovere, Gobbi, & Fanfulla, 2006), the relevance of breathing induced changes in HRV for parasympathetic tone in general, and the psychophysiological consequences in particular, are a matter of debate (Grossman & Kollai, 1993; Hirsch & Bishop, 1981; Kollai & Mizsei, 1990). Breathing induced changes in HRV might reflect peripheral, rather than central modulations of ANS activity and be irrelevant for the proposed antinociceptive effects of breathing exercises (Martin et al., 2012). In the light of the present results, the study by Chalaye et al. (2009) that contributed the main hypotheses might have been confounded by the lack of adequate control conditions and an incomplete randomization of treatment sequences. **Study 3** demonstrates that the choice of proper control conditions in behavioral and alternative pain treatments is difficult, yet of central importance. No follow-up experiments or analyses on **Study 3** are currently being planned.

Study 4: rTMS over the cerebellum modulates temperature detection and pain thresholds through peripheral mechanisms

Similar conclusions can be drawn from **Study 4**, which investigated the effects of cerebellar rTMS on pain perception: A first exploratory experiment identified 1Hz rTMS over the lateral cerebellum as an effective method of pain modulation. However, a second experiment including additional control conditions identified an important alternative explanation for the observed effects: Repetitive magnetic stimulation (rMS) of peripheral neck had similar effects as cerebellar rTMS. Two interpretations can be drawn from this result: a) the sham rTMS simulation currently used in TMS research is insufficient, especially since it does not simulate the obvious, unpleasant side effects of verum TMS, such as muscle twitches and pinching sensations. b) rMS stimulation of peripheral tissues might affect pain perception via peripheral pathways.

In the meantime Albu et al., 2013 followed up **Study 4** by testing local and distal effects of 1 Hz rMS over the neck. They replicated the effects of rMS on non-nociceptive thresholds, while effects on nociceptive thresholds were only apparent by trend. Furthermore our study has been cited in two reviews articles (Mylius, Borckardt, & Lefaucheur, 2012; Tomlinson, Davis, & Bracewell, 2013). No follow-up experiments or analyses on **Study 4** are currently being planned.

Bibliography

59th WMA General Assembly. (2009). Declaration of Helsinki. Ethical principles for medical research involving human subjects. *Journal of the Indian Medical Association*, *107*(6), 403–5.

Albu, S., Gómez-Soriano, J., Bravo-Esteban, E., Palazon, R., Kumru, H., Avila-Martin, G., … Taylor, J. (2013). Modulation of thermal somatosensory thresholds within local and remote spinal dermatomes following cervical repetitive magnetic stimulation. *Neuroscience Letters*, *555*, 237–42. doi:10.1016/j.neulet.2013.06.067

American Psychological Association. (2010). *Publication Manual of the American Psychological Association* (6th ed., p. 288). London: American Psychological Association.

Anderson, D. E., McNeely, J. D., & Windham, B. G. (2010). Regular slow-breathing exercise effects on blood pressure and breathing patterns at rest. *Journal of Human Hypertension*, 24(12), 807–13. doi:10.1038/jhh.2010.18

André-Obadia, N., Mertens, P., Gueguen, a, Peyron, R., & Garcia-Larrea, L. (2008). Pain relief by rTMS: differential effect of current flow but no specific action on pain subtypes. *Neurology*, *71*(11), 833–40. doi:10.1212/01.wnl.0000325481.61471.f0

Apkarian, A. V., Bushnell, M. C., Treede, R.-D., & Zubieta, J.-K. (2005). Human brain mechanisms of pain perception and regulation in health and disease. *European Journal of Pain (London, England)*, *9*(4), 463–84. doi:10.1016/j.ejpain.2004.11.001

Appelhans, B. M., & Luecken, L. J. (2008). Heart rate variability and pain: associations of two interrelated homeostatic processes. *Biological Psychology*, *77*(2), 174–82. doi:10.1016/j.biopsycho.2007.10.004

Arndt, S., Turvey, C., & Andreasen, N. C. (1999). Correlating and predicting psychiatric symptom ratings: Spearman's r versus Kendall's tau correlation. *Journal of Psychiatric Research*, *33*(2), 97–104.

Aslaksen, P. M., Myrbakk, I. N., Høifødt, R. S., & Flaten, M. a. (2007). The effect of experimenter gender on autonomic and subjective responses to pain stimuli. *Pain*, *129*(3), 260–8. doi:10.1016/j.pain.2006.10.011

Bagby, R. M., Taylor, G. J., & Parker, J. D. (1994). The Twenty-item Toronto Alexithymia Scale--II. Convergent, discriminant, and concurrent validity. *Journal of Psychosomatic Research*, *38*(1), 33–40.

Bailey, P. E., & Henry, J. D. (2007). Alexithymia, somatization and negative affect in a community sample. *Psychiatry Research*, *150*(1), 13–20. doi:10.1016/j.psychres.2006.05.024

Bakeman, R., & Mcarthur, D. (1996). Picturing repeated measures: Comments on Loftus, Morrison, and others. *Behavior Research Methods, Instruments, & Computers*, *28*(4), 584–589. doi:10.3758/BF03200546

Balduzzi, D., Riedner, B. A., & Tononi, G. (2008). A BOLD window into brain waves. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(41), 15641–2. doi:10.1073/pnas.0808310105

Baliki, M. N., Geha, P. Y., & Apkarian, A. V. (2009). Parsing pain perception between nociceptive representation and magnitude estimation. *Journal of Neurophysiology*, *101*(2), 875–87. doi:10.1152/jn.91100.2008

Barr, D. J., Levy, R., Scheepers, C., & Tily, H. J. (2013). Random effects structure for confirmatory hypothesis testing: Keep it maximal. *Journal of Memory and Language*, *68*(3), 255–278. doi:10.1016/j.jml.2012.11.001

Bartz, J. A, Zaki, J., Bolger, N., & Ochsner, K. N. (2011). Social effects of oxytocin in humans: context and person matter. *Trends in Cognitive Sciences*, 15(7), 301–9. doi:10.1016/j.tics.2011.05.002

Beck, A. T., Steer, R. A., Ball, R., & Ranieri, W. (1996). Comparison of Beck Depression Inventories -IA and -II in psychiatric outpatients. *Journal of Personality Assessment*, *67*(3), 588–97. doi:10.1207/s15327752jpa6703_13

Berntson, G. G., Lozano, D. L., & Chen, Y.-J. (2005). Filter properties of root mean square successive difference (RMSSD) for heart rate. *Psychophysiology*, 42(2), 246–52. doi:10.1111/j.1469-8986.2005.00277.x

Bethlehem, R. A. I., Baron-Cohen, S., van Honk, J., Auyeung, B., & Bos, P. a. (2014). The oxytocin paradox. *Frontiers in Behavioral Neuroscience*, *8*(February), 1–5. doi:10.3389/fnbeh.2014.00048

Bethlehem, R. A. I., van Honk, J., Auyeung, B., & Baron-Cohen, S. (2013). Oxytocin, brain physiology, and functional connectivity: a review of intranasal oxytocin fMRI studies. *Psychoneuroendocrinology*, *38*(7), 962–74. doi:10.1016/j.psyneuen.2012.10.011

Billauer, E. (2007). peakdet: Peak detection using MATLAB. Retrieved from http://www.billauer.co.il/peakdet.html

Billman, G. E. (2011). Heart rate variability - a historical perspective. *Frontiers in Physiology*, 2(November), 86. doi:10.3389/fphys.2011.00086

Bittiner, S. B., & Smith, S. E. (1986). Beta-adrenoceptor antagonists increase sinus arrhythmia, a vagotonic effect. *British Journal of Clinical Pharmacology*, 22(6), 691–5.

Boettger, M. K., Schwier, C., & Bär, K.-J. (2011). Sad mood increases pain sensitivity upon thermal grill illusion stimulation: implications for central pain processing. *Pain*, *152*(1), 123–30. doi:10.1016/j.pain.2010.10.003

Bolger, N., & Zuckerman, A. (1995). A framework for studying personality in the stress process. *Journal of Personality and Social Psychology*, *69*(5), 890–902.

Borckardt, J. J., Smith, A. R., Reeves, S. T., Weinstein, M., Kozel, F. A., Nahas, Z., … George, M. S. (2007). Fifteen minutes of left prefrontal repetitive transcranial magnetic stimulation acutely increases thermal pain thresholds in healthy adults. *Pain Research & Management: The Journal of the Canadian Pain Society = Journal de La Société Canadienne Pour Le Traitement de La Douleur*, *12*(4), 287–90.

Borella, P., Bargellini, A., Rovesti, S., Pinelli, M., Vivoli, R., Solfrini, V., & Vivoli, G. (1999). Emotional stability, anxiety, and natural killer activity under examination stress. *Psychoneuroendocrinology*, *24*(6), 613–27.

Born, J., Lange, T., Kern, W., McGregor, G. P., Bickel, U., & Fehm, H. L. (2002). Sniffing neuropeptides: a transnasal approach to the human brain. *Nature Neuroscience*, *5*(6), 514–6. doi:10.1038/nn849

Bornhövd, K., Quante, M., Glauche, V., Bromm, B., Weiller, C., & Büchel, C. (2002). Painful stimuli evoke different stimulus-response functions in the amygdala, prefrontal, insula and somatosensory cortex: a single-trial fMRI study. *Brain : A Journal of Neurology*, *125*(Pt 6), 1326–36. doi:10.1093/brain/awf137

Bragin, E. O. (1986). Opioid and catecholaminergic mechanisms of different types of analgesia. *Annals of the New York Academy of Sciences*, *467*, 331–44.

Breimhorst M, Hondrich M, Rebhorn C, May A, Birklein F. (2012). Sensory and sympathetic correlates of heat pain sensitization and habituation in men and women. *European Journal of Pain*.;16(9):1281–92. doi:10.1002/j.1532-2149.2012.00133.x.

Breivik, E. K., Björnsson, G. A., & Skovlund, E. (2000). A comparison of pain rating scales by sampling from clinical trial data. *The Clinical Journal of Pain*, *16*(1), 22–8.

Brett, M., Anton, J.-L., Valabregue, R., & Poline, J.-B. (2002). Region of interest analysis using an SPM toolbox. In *Presented at the 8th International Conference on Functional Mapping of the Human Brain*. Sendai.

Brodersen, K. H., Wiech, K., Lomakina, E. I., Lin, C.-S., Buhmann, J. M., Bingel, U., … Tracey, I. (2012). Decoding the perception of pain from fMRI using multivariate pattern analysis. *NeuroImage*, *63*(3), 1162–70. doi:10.1016/j.neuroimage.2012.08.035

Brown, J. E., Chatterjee, N., Younger, J., & Mackey, S. (2011). Towards a physiology-based measure of pain: patterns of human brain activity distinguish painful from non-painful thermal stimulation. *PloS One*, *6*(9), e24124. doi:10.1371/journal.pone.0024124

Büchel, C., Bornhovd, K., Quante, M., Glauche, V., Bromm, B., & Weiller, C. (2002). Dissociable neural responses related to pain intensity, stimulus intensity, and stimulus awareness within the anterior cingulate cortex: a parametric single-trial laser functional magnetic resonance imaging study. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *22*(3), 970–6.

Büchel, C., Holmes, A. P., Rees, G., & Friston, K. J. (1998). Characterizing stimulus-response functions using nonlinear regressors in parametric fMRI experiments. *NeuroImage*, *8*(2), 140–8. doi:10.1006/nimg.1998.0351

Buhle, J. T., Stevens, B. L., Friedman, J. J., & Wager, T. D. (2012). Distraction and placebo: two separate routes to pain control. *Psychological Science*, 23(3), 246–53. doi:10.1177/0956797611427919

Bulte, D. P., Kelly, M., Germuska, M., Xie, J., Chappell, M. A., Okell, T. W., … Jezzard, P. (2012). Quantitative measurement of cerebral physiology using respiratory-calibrated MRI. *NeuroImage*, *60*(1), 582–91. doi:10.1016/j.neuroimage.2011.12.017

Busch, V., Magerl, W., Kern, U., Haas, J., Hajak, G., & Eichhammer, P. (2012). The effect of deep and slow breathing on pain perception, autonomic activity, and mood processing--an experimental study. *Pain Medicine (Malden, Mass.)*, 13(2), 215–28. doi:10.1111/j.1526-4637.2011.01243.x

Campbell, J. N., & LaMotte, R. H. (1983). Latency to detection of first pain. *Brain Research*, *266*(2), 203–8.

Cauda, F., Torta, D. M.-E., Sacco, K., Geda, E., D'Agata, F., Costa, T., … Amanzio, M. (2012). Shared "Core" Areas between the Pain and Other Task-Related Networks. *PLoS ONE*, *7*(8), e41929. doi:10.1371/journal.pone.0041929

Cardoso, C., Ellenbogen, M. A, Orlando, M. A., Bacon, S. L., & Joober, R. (2013). Intranasal oxytocin attenuates the cortisol response to physical stress: a dose-response study. *Psychoneuroendocrinology*, 38(3), 399–407. doi:10.1016/j.psyneuen.2012.07.013

Chalaye, P., Goffaux, P., Lafrenaye, S., & Marchand, S. (2009). Respiratory effects on experimental heat pain and cardiac activity. *Pain Medicine (Malden, Mass.)*, *10*(8), 1334–40. doi:10.1111/j.1526-4637.2009.00681.x

Chambers, C. T., Taddio, A., Uman, L. S., & McMurtry, C. M. (2009). Psychological interventions for reducing pain and distress during routine childhood immunizations: a systematic review. *Clinical Therapeutics*, 31 Suppl 2, S77–S103. doi:10.1016/j.clinthera.2009.07.023

Cogan, R., & Kluthe, K. B. (1981). The role of learning in pain reduction associated wih relaxation and patterned breathing. *Journal of Psychosomatic Research*, 25(6), 535–9.

Connelly, M., & Denney, D. R. (2007). Regulation of emotions during experimental stress in alexithymia. *Journal of Psychosomatic Research*, *62*(6), 649–56. doi:10.1016/j.jpsychores.2006.12.008

Coppola, R., & Gracely, R. H. (1983). Where is the noise in SDT pain assessment? *Pain*, *17*(3), 257–66.

Costa, P. T., & McCrae, R. R. (1980). Somatic complaints in males as a function of age and neuroticism: a longitudinal analysis. *Journal of Behavioral Medicine*, *3*(3), 245–57.

Cox, J. J., Reimann, F., Nicholas, A. K., Thornton, G., Roberts, E., Springell, K., … Woods, C. G. (2006). An SCN9A channelopathy causes congenital inability to experience pain. *Nature*, *444*(7121), 894–8. doi:10.1038/nature05413

Craig, A. D. (2003). A new view of pain as a homeostatic emotion. *Trends in Neurosciences*, 26(6), 303–7. doi:10.1016/S0166-2236(03)00123-1

Craig, A. D. B. (2002). How do you feel? Interoception: the sense of the physiological condition of the body. *Nature Reviews. Neuroscience*, 3(8), 655–66. doi:10.1038/nrn894

Crettaz, B., Marziniak, M., Willeke, P., Young, P., Hellhammer, D., Stumpf, A., & Burgmer, M. (2013). Stress-induced allodynia--evidence of increased pain sensitivity in healthy humans and patients with chronic pain after experimentally induced psychosocial stress. *PloS One*, *8*(8), e69460. doi:10.1371/journal.pone.0069460

Dannecker, E. A., Price, D. D., O'Connor, P. D., & Robinson, M. E. (2008). Appraisals of pain from controlled stimuli: relevance to quantitative sensory testing. *British Journal of Health Psychology*, *13*(Pt 3), 537–50. doi:10.1348/135910707X230985

Darwin, E. (1796). Section XIV, 8. In G. Alethoup, R. Shimmin, & K. Edkins (Eds.), *The Project Gutenberg EBook of Zoonomia, Vol. I Or, the Laws of Organic Life* (2005th ed.). The Project Gutenberg.

Daskalakis, Z. J., Paradiso, G. O., Christensen, B. K., Fitzgerald, P. B., Gunraj, C., & Chen, R. (2004). Exploring the connectivity between the cerebellum and motor cortex in humans. *The Journal of Physiology*, *557*(Pt 2), 689–700. doi:10.1113/jphysiol.2003.059808

Davis, K. D., Wood, M. L., Crawley, A. P., & Mikulis, D. J. (1995). fMRI of human somatosensory and cingulate cortex during painful electrical nerve stimulation. *Neuroreport*, *7*(1), 321–5.

De Gucht, V., & Heiser, W. (2003). Alexithymia and somatisation: quantitative review of the literature. *Journal of Psychosomatic Research*, *54*(5), 425–34. doi:10.1016/S0022-3999(02)00467-1

De Gucht, V., Fischler, B., & Heiser, W. (2004). Personality and affect as determinants of medically unexplained symptoms in primary care; A follow-up study. *Journal of Psychosomatic Research*, *56*(3), 279–85. doi:10.1016/S0022-3999(03)00127-2

De Gucht, V., Fontaine, J., & Fischler, B. (2004). Temporal stability and differential relationships with neuroticism and extraversion of the three subscales of the 20-item Toronto Alexithymia Scale in clinical and nonclinical samples. *Journal of Psychosomatic Research*, *57*(1), 25–33. doi:10.1016/S0022-3999(03)00577-4

De Timary, P., Roy, E., Luminet, O., Fillée, C., & Mikolajczak, M. (2008). Relationship between alexithymia, alexithymia factors and salivary cortisol in men exposed to a social stress test. *Psychoneuroendocrinology*, *33*(8), 1160–4. doi:10.1016/j.psyneuen.2008.06.005

Desikan, R. S., Ségonne, F., Fischl, B., Quinn, B. T., Dickerson, B. C., Blacker, D., … Killiany, R. J. (2006). An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *NeuroImage*, *31*(3), 968–80. doi:10.1016/j.neuroimage.2006.01.021

Dewhurst, S. A., & Marlborough, M. A. (2003). Memory bias in the recall of pre-exam anxiety: the influence of self-enhancement. *Applied Cognitive Psychology*, *17*(6), 695–702. doi:10.1002/acp.903

Dhabhar, F. S. (2009). Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection and immunopathology. *Neuroimmunomodulation*, *16*(5), 300–17. doi:10.1159/000216188

Di Simplicio, M., Massey-Chase, R., Cowen, P. J., & Harmer, C. J. (2009). Oxytocin enhances processing of positive versus negative emotional information in healthy male volunteers. *Journal of Psychopharmacology (Oxford, England)*, *23*(3), 241–8. doi:10.1177/0269881108095705

Dimsdale, J., & Creed, F. (2009). The proposed diagnosis of somatic symptom disorders in DSM-V to replace somatoform disorders in DSM-IV--a preliminary report. *Journal of Psychosomatic Research*, *66*(6), 473–6. doi:10.1016/j.jpsychores.2009.03.005

Domes, G., Lischke, A., Berger, C., Grossmann, A., Hauenstein, K., Heinrichs, M., & Herpertz, S. C. (2009). Effects of intranasal oxytocin on emotional face processing in women. *Psychoneuroendocrinology*. doi:10.1016/j.psyneuen.2009.06.016

Dubin, A. E., & Patapoutian, A. (2010). Nociceptors: the sensors of the pain pathway. *The Journal of Clinical Investigation*, *120*(11), 3760–72. doi:10.1172/JCI42843

Duerden, E. G., & Albanese, M.-C. (2013). Localization of pain-related brain activation: a meta-analysis of neuroimaging data. *Human Brain Mapping*, *34*(1), 109–49. doi:10.1002/hbm.21416

Eckberg, D. L., Kifle, Y. T., & Roberts, V. L. (1980). Phase relationship between normal human respiration and baroreflex responsiveness. *The Journal of Physiology*, 304, 489–502.

Ellermeier, W., & Westphal, W. (1995). Gender differences in pain ratings and pupil reactions to painful pressure stimuli. *Pain*, *61*(3), 435–9.

Favilla, S., Huber, A., Pagnoni, G., Lui, F., Facchin, P., Cocchi, M., … Porro, C. A. (2014). Ranking brain areas encoding the perceived level of pain from fMRI data. *NeuroImage*, *90*, 153–162. doi:10.1016/j.neuroimage.2014.01.001

Field, A. P. (2009). *Discovering Statistics Using SPSS* (3rd ed.). Sage Publications.

Fierro, B., Giglia, G., Palermo, A., Pecoraro, C., Scalia, S., & Brighina, F. (2007). Modulatory effects of 1 Hz rTMS over the cerebellum on motor cortex excitability. *Experimental Brain Research. Experimentelle Hirnforschung. Expérimentation Cérébrale*, *176*(3), 440–7. doi:10.1007/s00221-006-0628-y

Fillingim, R. B., Maixner, W., Girdler, S. S., Light, K. C., Harris, M. B., Sheps, D. S., & Mason, G. A. (1997). Ischemic but not thermal pain sensitivity varies across the menstrual cycle. *Psychosomatic Medicine*, *59*(5), 512–20.

Fisher, K. M., Lai, H. M., Baker, M. R., & Baker, S. N. (2009). Corticospinal activation confounds cerebellar effects of posterior fossa stimuli. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *120*(12), 2109–13. doi:10.1016/j.clinph.2009.08.021

Fife-Schaw, C. (2012). Quasi-experimental Designs. In G. M. Breakwell, J. A. Smith, & D. B. Wright (Eds.), *Research Methods in Psychology* (4th ed., p. 616). SAGE Publications Ltd.

Fliege, H., Rose, M., Arck, P., Walter, O. B., Kocalevent, R.-D., Weber, C., & Klapp, B. F. (2005). The Perceived Stress Questionnaire (PSQ) reconsidered: validation and reference values from different clinical and healthy adult samples. *Psychosomatic Medicine*, *67*(1), 78–88. doi:10.1097/01.psy.0000151491.80178.78

Franz, M., Popp, K., Schaefer, R., Sitte, W., Schneider, C., Hardt, J., … Braehler, E. (2008). Alexithymia in the German general population. *Social Psychiatry and Psychiatric Epidemiology*, *43*(1), 54–62. doi:10.1007/s00127-007-0265-1

Frazier, J. A., Chiu, S., Breeze, J. L., Makris, N., Lange, N., Kennedy, D. N., … Biederman, J. (2005). Structural brain magnetic resonance imaging of limbic and thalamic volumes in pediatric bipolar disorder. *The American Journal of Psychiatry*, *162*(7), 1256–65. doi:10.1176/appi.ajp.162.7.1256

Friesner, S. A., Curry, D. M., & Moddeman, G. R. (2005). Comparison of two pain-management strategies during chest tube removal: relaxation exercise with opioids and opioids alone. *Heart & Lung: The Journal of Critical Care*, 35(4), 269–76. doi:10.1016/j.hrtlng.2005.10.005

Friston, K. J., Holmes, A. P., Poline, J. B., Grasby, P. J., Williams, S. C., Frackowiak, R. S., & Turner, R. (1995). Analysis of fMRI time-series revisited. *NeuroImage*, *2*(1), 45–53. doi:10.1006/nimg.1995.1007

Friston, K. J., Price, C. J., Fletcher, P., Moore, C., Frackowiak, R. S., & Dolan, R. J. (1996). The trouble with cognitive subtraction. *NeuroImage*, *4*(2), 97–104. doi:10.1006/nimg.1996.0033

Fruhstorfer, H., Lindblom, U., & Schmidt, W. C. (1976). Method for quantitative estimation of thermal thresholds in patients. *Journal of Neurology, Neurosurgery, and Psychiatry*, *39*(11), 1071–5.

Fukunishi, I., Kikuchi, M., Wogan, J., & Takubo, M. (1997). Secondary alexithymia as a state reaction in panic disorder and social phobia. *Comprehensive Psychiatry*, *38*(3), 166–70.

Furnham, A. (1986). Response bias, social desirability and dissimulation. *Personality and Individual Differences*, *7*(3), 385–400. doi:10.1016/0191-8869(86)90014-0

Gatchel, R. J., Polatin, P. B., & Kinney, R. K. (1995). Predicting outcome of chronic back pain using clinical predictors of psychopathology: A prospective analysis. *Health Psychology*, *14*(5), 415–420. doi:10.1037//0278-6133.14.5.415

Gauriau, C., & Bernard, J.-F. (2004). A comparative reappraisal of projections from the superficial laminae of the dorsal horn in the rat: the forebrain. *The Journal of Comparative Neurology*, *468*(1), 24–56. doi:10.1002/cne.10873

Gerschlager, W., Christensen, L. O. D., Bestmann, S., & Rothwell, J. C. (2002). rTMS over the cerebellum can increase corticospinal excitability through a spinal mechanism involving activation of peripheral nerve fibres. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *113*(9), 1435–40.

Geuter, S., Gamer, M., Onat, S., & Büchel, C. (2014). Parametric trial-by-trial prediction of pain by easily available physiological measures. *Pain*, (February). doi:10.1016/j.pain.2014.02.005

Geuze, E., Westenberg, H. G. M., Jochims, A., de Kloet, C. S., Bohus, M., Vermetten, E., & Schmahl, C. (2007). Altered pain processing in veterans with posttraumatic stress disorder. *Archives of General Psychiatry*, 64(1), 76–85. doi:10.1001/archpsyc.64.1.76

Gironell, A., Kulisevsky, J., Lorenzo, J., Barbanoj, M., Pascual-Sedano, B., & Otermin, P. (2002). Transcranial magnetic stimulation of the cerebellum in essential tremor: a controlled study. *Archives of Neurology*, *59*(3), 413–7.

Göbel, H., Heller, O., Nowak, T., & Westphal, W. (1988). [The correspondence between pain stimulus and pain sensation.]. *Schmerz (Berlin, Germany)*, *2*(4), 205–11. doi:10.1007/BF02527918

Gracely, R. H., McGrath, F., & Dubner, R. (1978). Ratio scales of sensory and affective verbal pain descriptors. *Pain*, *5*(1), 5–18.

Greffrath, W., Baumgärtner, U., & Treede, R.-D. (2007). Peripheral and central components of habituation of heat pain perception and evoked potentials in humans. *Pain*, *132*(3), 301–11. doi:10.1016/j.pain.2007.04.026

Grossman, P., & Kollai, M. (1993). Respiratory sinus arrhythmia, cardiac vagal tone, and respiration: within- and between-individual relations. *Psychophysiology*, *30*(5), 486–95.

Grossman, P., & Taylor, E. W. (2007). Toward understanding respiratory sinus arrhythmia: relations to cardiac vagal tone, evolution and biobehavioral functions. *Biological Psychology*, 74(2), 263–85. doi:10.1016/j.biopsycho.2005.11.014

Guastella, A. J., & MacLeod, C. (2012). A critical review of the influence of oxytocin nasal spray on social cognition in humans: Evidence and future directions. *Hormones and Behavior*, 1–9. doi:10.1016/j.yhbeh.2012.01.002

Guastella, A. J., Hickie, I. B., McGuinness, M. M., Otis, M., Woods, E. A., Disinger, H. M., … Banati, R. B. (2012). Recommendations for the standardisation of oxytocin nasal administration and guidelines for its reporting in human research. *Psychoneuroendocrinology*. doi:10.1016/j.psyneuen.2012.11.019

Gunthert, K. C., Cohen, L. H., & Armeli, S. (1999). The role of neuroticism in daily stress and coping. *Journal of Personality and Social Psychology*, *77*(5), 1087–100.

Hallett, M. (2000). Transcranial magnetic stimulation and the human brain. *Nature*, *406*(July), 147–150.

Hallman, D. M., Olsson, E. M. G., von Schéele, B., Melin, L., & Lyskov, E. (2011). Effects of heart rate variability biofeedback in subjects with stress-related chronic neck pain: a pilot study. *Applied Psychophysiology and Biofeedback*, 36(2), 71–80. doi:10.1007/s10484-011-9147-0

Handwerker, H. O., & Kobal, G. (1993). Psychophysiology of experimentally induced pain. *Physiological Reviews*, *73*(3), 639–71.

Hashmi, J. A., & Davis, K. D. (2008). Effect of static and dynamic heat pain stimulus profiles on the temporal dynamics and interdependence of pain qualities, intensity, and affect. *Journal of Neurophysiology*, *100*(4), 1706–15. doi:10.1152/jn.90500.2008

Hassett, A. L., Radvanski, D. C., Vaschillo, E. G., Vaschillo, B., Sigal, L. H., Karavidas, M. K., … Lehrer, P. M. (2007). A pilot study of the efficacy of heart rate variability (HRV) biofeedback in patients with fibromyalgia. *Applied Psychophysiology and Biofeedback*, 32(1), 1–10. doi:10.1007/s10484-006-9028-0

Hawrylycz, M. J., Lein, E. S., Guillozet-Bongaarts, A. L., Shen, E. H., Ng, L., Miller, J. A., … Jones, A. R. (2012). An anatomically comprehensive atlas of the adult human brain transcriptome. *Nature*, *489*(7416), 391–9. doi:10.1038/nature11405

Heckel, A., Rothmayr, C., Rosengarth, K., Hajak, G., Greenlee, M. W., & Eichhammer, P. (2011). Aversive faces activate pain responsive regions in the brain. *Neuroreport*, *22*(11), 548–53. doi:10.1097/WNR.0b013e328348b54a

Heldestad, V., Linder, J., Sellersjö, L., & Nordh, E. (2010). Reproducibility and influence of test modality order on thermal perception and thermal pain thresholds in quantitative sensory testing. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *121*(11), 1878–1885. doi:10.1016/j.clinph.2010.03.055

Heldmann, B., Kerkhoff, G., Struppler, A., Havel, P., & Jahn, T. (2000). Repetitive peripheral magnetic stimulation alleviates tactile extinction. *Neuroreport*, *11*(14), 3193–8.

Helmchen, C., Mohr, C., Erdmann, C., Binkofski, F., & Büchel, C. (2006). Neural activity related to self- versus externally generated painful stimuli reveals distinct differences in the lateral pain system in a parametric fMRI study. *Human Brain Mapping*, *27*(9), 755–65. doi:10.1002/hbm.20217

Henson, R. (2006). Efficient Experimental Design for fMRI. In K. Friston, J. Ashburner, S. Kiebel, T. Nichols, & W. Penny (Eds.), *Statistical Parametric Mapping: The Analysis of Functional Brain Images* (1st ed., pp. 193–210). New York: Academic Press.

Henson, R., & Friston, K. (2007). Convolution Models for fMRI. In W. D. Penny, K. J. Friston, J. T. Ashburner, S. J. Kiebel, & T. E. Nichols (Eds.), *Statistical Parametric Mapping: The Analysis of Functional Brain Images* (1st ed., pp. 178–192). Academic Press.

Herwig, U., Satrapi, P., & Schönfeldt-Lecuona, C. (2003). Using the international 10-20 EEG system for positioning of transcranial magnetic stimulation. *Brain Topography*, *16*(2), 95–9.

Hiller, W., Rief, W., & Brähler, E. (2006). Somatization in the population: from mild bodily misperceptions to disabling symptoms. *Social Psychiatry and Psychiatric Epidemiology*, *41*(9), 704–12. doi:10.1007/s00127-006-0082-y

Hirsch, J., & Bishop, B. (1981). Respiratory sinus arrhythmia in humans: how breathing pattern modulates heart rate. *The American Journal of Physiology*, *241*(4), H620–9.

Hoeger Bement, M. K., Dicapo, J., Rasiarmos, R., & Hunter, S. K. (2008). Dose response of isometric contractions on pain perception in healthy adults. *Medicine and Science in Sports and Exercise*, *40*(11), 1880–9. doi:10.1249/MSS.0b013e31817eeecc

Holle, D., Gaul, C., Krebs, S., Naegel, S., Diener, H.-C., Kaube, H., … Obermann, M. (2011). Nociceptive blink reflex and pain-related evoked potentials in hypnic headache. *Cephalalgia : An International Journal of Headache*, *31*(11), 1181–8. doi:10.1177/0333102411412629

Horn, C., Blischke, Y., Kunz, M., & Lautenbacher, S. (2012). Does pain necessarily have an affective component? Negative evidence from blink reflex experiments. *Pain Research & Management : The Journal of the Canadian Pain Society = Journal de La Société Canadienne Pour Le Traitement de La Douleur*, *17*(1), 15–24.

Hróbjartsson, A., Kaptchuk, T. J., & Miller, F. G. (2011). Placebo effect studies are susceptible to response bias and to other types of biases. *Journal of Clinical Epidemiology*, *64*(11), 1223–9. doi:10.1016/j.jclinepi.2011.01.008

Iannetti, G. D., & Mouraux, A. (2010). From the neuromatrix to the pain matrix (and back). *Experimental Brain Research. Experimentelle Hirnforschung. Experimentation Cerebrale*, 1–12. doi:10.1007/s00221-010-2340-1

van Ijzendoorn MH, Bhandari R, van der Veen R, Grewen KM, Bakermans-Kranenburg MJ. (2012) Elevated Salivary Levels of Oxytocin Persist More than 7 h after Intranasal Administration. *Front Neurosci.*;6(December):174. doi:10.3389/fnins.2012.00174.

International Association for the Study of Pain. (2002). *Classification of chronic pain : descriptions of chronic pain syndromes and definitions of pain terms*. (H. Merskey & N. Bogduk, Eds.) (2nd ed., p. 210). Seattle: IASP Press.

International Association for the Study of Pain. (2014). Ethical Guidelines for Pain Research in Humans.

Jackson, T., Iezzi, T., Chen, H., Ebnet, S., & Eglitis, K. (2005). Gender, interpersonal transactions, and the perception of pain: an experimental analysis. *The Journal of Pain : Official Journal of the American Pain Society*, *6*(4), 228–36. doi:10.1016/j.jpain.2004.12.004

Johannsen, A., Bjurshammar, N., & Gustafsson, A. (2010). The influence of academic stress on gingival inflammation. *International Journal of Dental Hygiene*, *8*(1), 22–7. doi:10.1111/j.1601-5037.2009.00397.x

Johnson, S., Summers, J., & Pridmore, S. (2006). Changes to somatosensory detection and pain thresholds following high frequency repetitive TMS of the motor cortex in individuals suffering from chronic pain. *Pain*, *123*(1-2), 187–92. doi:10.1016/j.pain.2006.02.030

Julius, D. (2013). *TRP channels and pain. Annual review of cell and developmental biology* (Vol. 29, pp. 355–84). doi:10.1146/annurev-cellbio-101011-155833

Kandel, E., Schwartz, J., Jessell, T., Siegelbaum, S., & Hudspeth, A. J. (2013). *Principles of Neural Science* (5th ed., p. 1709). McGraw Hill Professional.

Keefe, F. J., Bradley, L. A., & Crisson, J. E. (1990). Behavioral assessment of low back pain: identification of pain behavior subgroups. *Pain*, *40*(2), 153–60.

Kellner, R. (1990). Somatization. Theories and research. *The Journal of Nervous and Mental Disease*, *178*(3), 150–60.

Kessner, S., Sprenger, C., Wrobel, N., Wiech, K., & Bingel, U. (2013). Effect of oxytocin on placebo analgesia: a randomized study. *JAMA : The Journal of the American Medical Association*, *310*(16), 1733–5. doi:10.1001/jama.2013.277446

Khedr, E. M., Kotb, H., Kamel, N. F., Ahmed, M. A., Sadek, R., & Rothwell, J. C. (2005). Longlasting antalgic effects of daily sessions of repetitive transcranial magnetic stimulation in central and peripheral neuropathic pain. *Journal of Neurology, Neurosurgery, and Psychiatry*, *76*(6), 833–8. doi:10.1136/jnnp.2004.055806

Kingdom, F., & Prins, N. (2010). *Psychophysics* (1st ed., p. 296). London: Academic Press.

Kirsch, P., Esslinger, C., Chen, Q., Mier, D., Lis, S., Siddhanti, S., … Meyer-Lindenberg, A. (2005). Oxytocin modulates neural circuitry for social cognition and fear in humans. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *25*(49), 11489–93. doi:10.1523/JNEUROSCI.3984-05.2005

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*(1-2), 76–81.

Klaus, K., Rief, W., Brähler, E., Martin, A., Glaesmer, H., & Mewes, R. (2013). The distinction between "medically unexplained" and "medically explained" in the context of somatoform disorders. *International Journal of Behavioral Medicine*, *20*(2), 161–71. doi:10.1007/s12529-012-9245-2

Koch, G., Brusa, L., Carrillo, F., Lo Gerfo, E., Torriero, S., Oliveri, M., … Stanzione, P. (2009). Cerebellar magnetic stimulation decreases levodopa-induced dyskinesias in Parkinson disease. *Neurology*, *73*(2), 113–9. doi:10.1212/WNL.0b013e3181ad5387

Koh, K. B., Choe, E., Song, J. E., & Lee, E. H. (2006). Effect of coping on endocrinoimmune functions in different stress situations. *Psychiatry Research*, *143*(2-3), 223–34. doi:10.1016/j.psychres.2005.04.008

Kollai, M., & Mizsei, G. (1990). Respiratory sinus arrhythmia is a limited measure of cardiac parasympathetic control in man. *The Journal of Physiology*, *424*, 329–42.

Kooiman, C. G. (2004). Alexithymia Does Not Predict the Persistence of Medically Unexplained Physical Symptoms. *Psychosomatic Medicine*, *66*(2), 224–232. doi:10.1097/01.psy.0000116714.38868.06

Kooiman, C. G., Spinhoven, P., & Trijsburg, R. W. (2002). The assessment of alexithymia: a critical review of the literature and a psychometric study of the Toronto Alexithymia Scale-20. *Journal of Psychosomatic Research*, *53*(6), 1083–90.

Körner, A., Drapeau, M., Albani, C., Geyer, M., Schmutzer, G., & Brähler, E. (2008). Deutsche Normierung des NEO-Fünf-Faktoren-Inventars [German Norms for the NEO-Five Factor Inventory]. *Zeitschrift Für Medizinische Psychologie*, *17*(2-3), 133–144.

Krabbe, A., Henriksen, L., & Olesen, J. (1984). Tomographic determination of cerebral blood flow during attacks of cluster headache. *Cephalalgia : An International Journal of Headache*, *4*(1), 17–23.

Kramer, J. L. K., Haefeli, J., & Jutzeler, C. R. (2012). An objective measure of stimulus-evoked pain. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *32*(38), 12981–2. doi:10.1523/JNEUROSCI.3175-12.2012

Krummenacher, P., Candia, V., Folkers, G., Schedlowski, M., & Schönbächler, G. (2010). Prefrontal cortex modulates placebo analgesia. *Pain*, *148*(3), 368–74. doi:10.1016/j.pain.2009.09.033

Kubzansky, L. D., Mendes, W. B., Appleton, A. A., Block, J., & Adler, G. K. (2012). A heartfelt response: Oxytocin effects on response to social stress in men and women. *Biological Psychology*, 1–9. doi:10.1016/j.biopsycho.2012.02.010

Lane, R. D., Carmichael, C., & Reis, H. T. (2011). Differentiation in the momentary rating of somatic symptoms covaries with trait emotional awareness in patients at risk for sudden cardiac death. *Psychosomatic Medicine*, *73*(2), 185–92. doi:10.1097/PSY.0b013e318203b86a

Langguth, B., de Ridder, D., Dornhoffer, J. L., Eichhammer, P., Folmer, R. L., Frank, E., … Hajak, G. (2008). Controversy: Does repetitive transcranial magnetic stimulation/ transcranial direct current stimulation show efficacy in treating tinnitus patients? *Brain Stimulation*, *1*(3), 192–205. doi:10.1016/j.brs.2008.06.003

Langguth, B., Eichhammer, P., Zowe, M., Landgrebe, M., Binder, H., Sand, P., & Hajak, G. (2008). Modulating cerebello-thalamocortical pathways by neuronavigated cerebellar repetitive transcranial stimulation (rTMS). *Neurophysiologie Clinique = Clinical Neurophysiology*, *38*(5), 289–95. doi:10.1016/j.neucli.2008.08.003

Langguth, B., Zowe, M., Landgrebe, M., Sand, P., Kleinjung, T., Binder, H., … Eichhammer, P. (2006). Transcranial magnetic stimulation for the treatment of tinnitus: a new coil positioning method and first results. *Brain Topography*, *18*(4), 241–7. doi:10.1007/s10548-006-0002-1

Laux, L., Glanzmann, P., Schaffner, P., & Spielberger, C. D. (1981). Das State-Trait-Angstinventar. Theoretische Grundlagen und Handanweisung. Weinheim: Beltz Test GmbH.

Lee, H.-J., Macbeth, A. H., Pagani, J. H., & Young, W. S. (2009). Oxytocin: the great facilitator of life. *Progress in Neurobiology*, *88*(2), 127–51. doi:10.1016/j.pneurobio.2009.04.001

Lefaucheur, J. P., Drouot, X., & Nguyen, J. P. (2001). Interventional neurophysiology for pain control: duration of pain relief following repetitive transcranial magnetic stimulation of the motor cortex. *Neurophysiologie Clinique = Clinical Neurophysiology*, *31*(4), 247–52.

Lefaucheur, J. P., Drouot, X., Ménard-Lefaucheur, I., Keravel, Y., & Nguyen, J. P. (2006). Motor cortex rTMS restores defective intracortical inhibition in chronic neuropathic pain. *Neurology*, *67*(9), 1568–74. doi:10.1212/01.wnl.0000242731.10074.3c

Lefaucheur, J.-P., Jarry, G., Drouot, X., Ménard-Lefaucheur, I., Keravel, Y., & Nguyen, J.-P. (2010). Motor cortex rTMS reduces acute pain provoked by laser stimulation in patients with chronic neuropathic pain. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *121*(6), 895–901. doi:10.1016/j.clinph.2009.12.028

Lehrer, P. M., Vaschillo, E., & Vaschillo, B. (2000). Resonant frequency biofeedback training to increase cardiac variability: rationale and manual for training. *Applied Psychophysiology and Biofeedback*, 25(3), 177–91.

Lehrer, P. M., Vaschillo, E., Vaschillo, B., Lu, S.-E., Eckberg, D. L., Edelberg, R., … Hamer, R. M. (2003). Heart rate variability biofeedback increases baroreflex gain and peak expiratory flow. *Psychosomatic Medicine*, 65(5), 796–805. doi:10.1097/01.PSY.0000089200.81962.19

Leo, R. J., & Latif, T. (2007). Repetitive transcranial magnetic stimulation (rTMS) in experimentally induced and chronic neuropathic pain: a review. *The Journal of Pain: Official Journal of the American Pain Society*, *8*(6), 453–9. doi:10.1016/j.jpain.2007.01.009

Leung, A., Donohue, M., Xu, R., Lee, R., Lefaucheur, J.-P., Khedr, E. M., … Chen, R. (2009). rTMS for suppressing neuropathic pain: a meta-analysis. *The Journal of Pain: Official Journal of the American Pain Society*, *10*(12), 1205–16. doi:10.1016/j.jpain.2009.03.010

Levenstein, S., Prantera, C., Varvo, V., Scribano, M. L., Berto, E., Luzi, C., & Andreoli, a. (1993). Development of the Perceived Stress Questionnaire: a new tool for psychosomatic research. *Journal of Psychosomatic Research*, *37*(1), 19–32.

Lieberman, M. D., & Cunningham, W. a. (2009). Type I and Type II error concerns in fMRI research: re-balancing the scale. *Social Cognitive and Affective Neuroscience*, *4*(4), 423–8. doi:10.1093/scan/nsp052

Liem, E. B., Joiner, T. V, Tsueda, K., & Sessler, D. I. (2005). Increased sensitivity to thermal pain and reduced subcutaneous lidocaine efficacy in redheads. *Anesthesiology*, *102*(3), 509–14.

Lipowski, Z. J. (1988). Somatization: the concept and its clinical application. *The American Journal of Psychiatry*, *145*(11), 1358–68.

Lloyd, M. A., & Appel, J. B. (1976). Signal detection theory and the psychophysics of pain: an introduction and review. *Psychosomatic Medicine*, *38*(2), 79–94.

Loft, P., Thomas, M. G., Petrie, K. J., Booth, R. J., Miles, J., & Vedhara, K. (2007). Examination stress results in altered cardiovascular responses to acute challenge and lower cortisol. *Psychoneuroendocrinology*, *32*(4), 367–75. doi:10.1016/j.psyneuen.2007.02.004

Loggia, M. L., Juneau, M., & Bushnell, M. C. (2011). Autonomic responses to heat pain: Heart rate, skin conductance, and their relation to verbal ratings and stimulus intensity. *Pain*, *152*(3), 592–8. doi:10.1016/j.pain.2010.11.032

Loggia, M. L., Mogil, J. S., & Bushnell, M. C. (2008). Experimentally induced mood changes preferentially affect pain unpleasantness. *The Journal of Pain : Official Journal of the American Pain Society*, *9*(9), 784–91. doi:10.1016/j.jpain.2008.03.014

Logothetis, N. K. (2008). What we can do and what we cannot do with fmri. *Nature*, *453*(7197), 869–878. doi:10.1038/nature06976

Lovallo, W. (1975). The cold pressor test and autonomic function: a review and integration. *Psychophysiology*, *12*(3), 268–82.

Lumley, M. A., Stettner, L., & Wehmer, F. (1996). How are alexithymia and physical illness linked? A review and critique of pathways. *Journal of Psychosomatic Research*, *41*(6), 505–18. doi:10.1016/S0022-3999(96)00222-X

MacDonald, E., Dadds, M. R., Brennan, J. L., Williams, K., Levy, F., & Cauchi, A. J. (2011). A review of safety, side-effects and subjective reactions to intranasal oxytocin in human research. *Psychoneuroendocrinology*, 36(8), 1114–26. doi:10.1016/j.psyneuen.2011.02.015

Magerl, W., Krumova, E. K., Baron, R., Tölle, T., Treede, R.-D., & Maier, C. (2010). Reference data for quantitative sensory testing (QST): refined stratification for age and a novel method for statistical comparison of group data. *Pain*, *151*(3), 598–605. doi:10.1016/j.pain.2010.07.026

Malik, M., Bigger, T. J., Camm, J. A., Kleiger, R. E., Malliani, A., Moss, A. J., & Schwartz, P. J. (1996). Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *European Heart Journal*, 17(3), 354–81.

Mameli, S., Pisanu, G. M., Sardo, S., Marchi, A., Pili, A., Carboni, M., … Agabio, R. (2014). Oxytocin nasal spray in fibromyalgic patients. *Rheumatology International*, (c). doi:10.1007/s00296-014-2953-y

Mano, T., Iwase, S., & Toma, S. (2006). Microneurography as a tool in clinical neurophysiology to investigate peripheral neural traffic in humans. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *117*(11), 2357–84. doi:10.1016/j.clinph.2006.06.002

Marquand, A., Howard, M., Brammer, M., Chu, C., Coen, S., & Mourão-Miranda, J. (2010). Quantitative prediction of subjective pain intensity from whole-brain fMRI data using Gaussian processes. *NeuroImage*, *49*(3), 2178–89. doi:10.1016/j.neuroimage.2009.10.072

Martin, J. B., & Pihl, R. O. (1985). The stress-alexithymia hypothesis: theorectical and empirical considerations. *Psychotherapy and Psychosomatics*, *43*(4), 169–76.

Martin, S. L., Kerr, K. L., Bartley, E. J., Kuhn, B. L., Palit, S., Terry, E. L., … Rhudy, J. L. (2012). Respiration-induced hypoalgesia: exploration of potential mechanisms. *The Journal of Pain: Official Journal of the American Pain Society*, 13(8), 755–63. doi:10.1016/j.jpain.2012.05.001

Mattila, A. K., Kronholm, E., Jula, A., Salminen, J. K., Koivisto, A.-M., Mielonen, R.-L., & Joukamaa, M. (2008). Alexithymia and somatization in general population. *Psychosomatic Medicine*, *70*(6), 716–22. doi:10.1097/PSY.0b013e31816ffc39

May, A. (2007). Neuroimaging: visualising the brain in pain. *Neurological Sciences : Official Journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology*, *28 Suppl 2*, S101–7. doi:10.1007/s10072-007-0760-x

Mayer, J. D., Allen, J. P., & Beauregard, K. (1995). Mood inductions for four specific moods: A procedure employing guided imagery vignettes with music. *Journal of Mental Imagery*, *19*(1-2), 133–150.

Mazaika, P. K., Hoeft, F., Glover, G. H., & Reiss, A. L. (2009). *Methods and Software for fMRI Analysis of Clinical Subjects*. *NeuroImage* (Vol. 47, p. S58). doi:10.1016/S1053-8119(09)70238-1

Mazzola, L., Isnard, J., & Mauguière, F. (2006). Somatosensory and pain responses to stimulation of the second somatosensory area (SII) in humans. A comparison with SI and insular responses. *Cerebral Cortex (New York, N.Y. : 1991)*, *16*(7), 960–8. doi:10.1093/cercor/bhj038

McMahon, S., & Wall, P. D. (2013). *Wall and Melzack's Textbook of Pain* (6th ed., p. 1153). Philadelphia, Pa: Saunders.

Meagher, M. W., Arnau, R. C., & Rhudy, J. L. (2001). Pain and emotion: effects of affective picture modulation. *Psychosomatic Medicine*, *63*(1), 79–90.

Melzack, R. (1975). The McGill Pain Questionnaire: major properties and scoring methods. *Pain*, *1*(3), 277–99.

Melzack, R. (1999). From the gate to the neuromatrix. *Pain*, *Suppl 6*(1), S121–6.

Mendell, L. M. (2013). Constructing and deconstructing the gate theory of pain. *Pain*, *155*(2), 210–216. doi:10.1016/j.pain.2013.12.010

Mennemeier, M., Triggs, W., Chelette, K., Woods, A., Kimbrell, T., & Dornhoffer, J. (2009). Sham Transcranial Magnetic Stimulation Using Electrical Stimulation of the Scalp. *Brain Stimulation*, *2*(3), 168–173. doi:10.1016/j.brs.2009.02.002

Mense, S. (2009). Algesic agents exciting muscle nociceptors. *Experimental Brain Research*, *196*(1), 89–100. doi:10.1007/s00221-008-1674-4

Mhalla, A., de Andrade, D. C., Baudic, S., Perrot, S., & Bouhassira, D. (2010). Alteration of cortical excitability in patients with fibromyalgia. *Pain*, *149*(3), 495–500. doi:10.1016/j.pain.2010.03.009

Mijović, B., Silva, M., Van den Bergh, B. R. H., Allegaert, K., Aerts, J.-M., Berckmans, D., & Van Huffel, S. (2010). Assessment of pain expression in infant cry signals using empirical mode decomposition. *Methods of Information in Medicine*, *49*(5), 448–52. doi:10.3414/ME09-02-0033

Millan, M. J. (2002). Descending control of pain. *Progress in Neurobiology*, *66*(6), 355–474.

Moore, R. A., Derry, S., & Wiffen, P. J. (2013). Challenges in design and interpretation of chronic pain trials. *British Journal of Anaesthesia*, *111*(1), 38–45. doi:10.1093/bja/aet126

Morone, N. E., & Greco, C. M. (2007). Mind-body interventions for chronic pain in older adults: a structured review. *Pain Medicine (Malden, Mass.)*, 8(4), 359–75. doi:10.1111/j.1526-4637.2007.00312.x

Moulton, E. a, Schmahmann, J. D., Becerra, L., & Borsook, D. (2010). The cerebellum and pain: passive integrator or active participator? *Brain Research Reviews*, *65*(1), 14–27. doi:10.1016/j.brainresrev.2010.05.005

Mylius, V., Borckardt, J. J., & Lefaucheur, J.-P. (2012). Noninvasive cortical modulation of experimental pain. *Pain*, *153*(7), 1350–63. doi:10.1016/j.pain.2012.04.009

Neumann, I. D., Maloumby, R., Beiderbeck, D. I., Lukas, M., & Landgraf, R. (2013). Increased brain and plasma oxytocin after nasal and peripheral administration in rats and mice. *Psychoneuroendocrinology*, *38*(10), 1985–93. doi:10.1016/j.psyneuen.2013.03.003

Niskanen, J.-P., Tarvainen, M. P., Ranta-Aho, P. O., & Karjalainen, P. A. (2004). Software for advanced HRV analysis. *Computer Methods and Programs in Biomedicine*, 76(1), 73–81. doi:10.1016/j.cmpb.2004.03.004

Novartis, P. G. (2006). *Gebrauchsinformation: Informationen für den Anwender Syntocinon-Spray*. Novartis Pharma GmbH.

Nyenhuis, D. L., Yamamoto, C., Luchetta, T., Terrien, a, & Parmentier, a. (1999). Adult and geriatric normative data and validation of the profile of mood states. *Journal of Clinical Psychology*, *55*(1), 79–86.

Noyes, R., Langbehn, D. R., Happel, R. L., Stout, L. R., Muller, B. A., & Longley, S. L. (2001). Personality dysfunction among somatizing patients. *Psychosomatics*, *42*(4), 320–9.

Olausson, H., Wessberg, J., Morrison, I., McGlone, F., & Vallbo, A. (2010). The neurophysiology of unmyelinated tactile afferents. *Neuroscience and Biobehavioral Reviews*, *34*(2), 185–91. doi:10.1016/j.neubiorev.2008.09.011

Oliveri, M., Bonnì, S., Turriziani, P., Koch, G., Lo Gerfo, E., Torriero, S., … Caltagirone, C. (2009). Motor and linguistic linking of space and time in the cerebellum. *PloS One*, *4*(11), e7933. doi:10.1371/journal.pone.0007933

Ollinger, J. M., Shulman, G. L., & Corbetta, M. (2001). Separating processes within a trial in event-related functional MRI. *NeuroImage*, *13*(1), 210–7. doi:10.1006/nimg.2000.0710

Owen, D. G., Clarke, C. F., Ganapathy, S., Prato, F. S., & St Lawrence, K. S. (2010). Using perfusion MRI to measure the dynamic changes in neural activation associated with tonic muscular pain. *Pain*, *148*(3), 375–86. doi:10.1016/j.pain.2009.10.003

Paine, P., Kishor, J., Worthen, S. F., Gregory, L. J., & Aziz, Q. (2009). Exploring relationships for visceral and somatic pain with autonomic control and personality. *Pain*, *144*(3), 236–44. doi:10.1016/j.pain.2009.02.022

Passard, A., Attal, N., Benadhira, R., Brasseur, L., Saba, G., Sichere, P., … Bouhassira, D. (2007). Effects of unilateral repetitive transcranial magnetic stimulation of the motor cortex on chronic widespread pain in fibromyalgia. *Brain: A Journal of Neurology*, *130*(Pt 10), 2661–70. doi:10.1093/brain/awm189

Pavlaković, G., Tigges, J., & Crozier, T. A. (2009). Effect of buspirone on thermal sensory and pain thresholds in human volunteers. *BMC Clinical Pharmacology*, *9*, 12. doi:10.1186/1472-6904-9-12

Pedrosa Gil, F., Bidlingmaier, M., Ridout, N., Scheidt, C. E., Caton, S., Schoechlin, C., & Nickel, M. (2008). The relationship between alexithymia and salivary cortisol levels in somatoform disorders. *Nordic Journal of Psychiatry*, *62*(5), 366–73. doi:10.1080/08039480801983554

Pereira, E. a C., Lu, G., Wang, S., Schweder, P. M., Hyam, J. A, Stein, J. F., … Green, A. L. (2010). Ventral periaqueductal grey stimulation alters heart rate variability in humans with chronic pain. *Experimental Neurology*, 223(2), 574–81. doi:10.1016/j.expneurol.2010.02.004

Perkins, F. M., & Kehlet, H. (2000). Chronic pain as an outcome of surgery. A review of predictive factors. *Anesthesiology*, *93*(4), 1123–33.

Petrovic, P., Kalisch, R., Singer, T., & Dolan, R. J. (2008). Oxytocin attenuates affective evaluations of conditioned faces and amygdala activity. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *28*(26), 6607–15. doi:10.1523/JNEUROSCI.4572-07.2008

Pinna, G. D., Maestri, R., La Rovere, M. T., Gobbi, E., & Fanfulla, F. (2006). Effect of paced breathing on ventilatory and cardiovascular variability parameters during short-term investigations of autonomic function. *American Journal of Physiology. Heart and Circulatory Physiology*, 290(1), H424–33. doi:10.1152/ajpheart.00438.2005

Pitts, F. N., Winokur, G., & Stewart, M. A. (1961). Psychiatric syndromes, anxiety symptoms and responses to stress in medical students. *The American Journal of Psychiatry*, *118*, 333–40.

Pleger, B., Janssen, F., Schwenkreis, P., Völker, B., Maier, C., & Tegenthoff, M. (2004). Repetitive transcranial magnetic stimulation of the motor cortex attenuates pain perception in complex regional pain syndrome type I. *Neuroscience Letters*, *356*(2), 87–90. doi:10.1016/j.neulet.2003.11.037

Poldrack, R. A. (2007). Region of interest analysis for fMRI. *Social Cognitive and Affective Neuroscience*, *2*(1), 67–70. doi:10.1093/scan/nsm006

Pollock, V., Cho, D. W., Reker, D., & Volavka, J. (1979). Profile of Mood States: the factors and their physiological correlates. *The Journal of Nervous and Mental Disease*, *167*(10), 612–4. doi:10.1097/00005053-197910000-00004

Pöyhönen, M., Syväoja, S., Hartikainen, J., Ruokonen, E., & Takala, J. (2004). The effect of carbon dioxide, respiratory rate and tidal volume on human heart rate variability. *Acta Anaesthesiologica Scandinavica*, 48(1), 93–101. doi:10.1080/13548500903322791

Prato, M., Favilla, S., Zanni, L., Porro, C. A, & Baraldi, P. (2011). A regularization algorithm for decoding perceptual temporal profiles from fMRI data. *NeuroImage*, *56*(1), 258–67. doi:10.1016/j.neuroimage.2011.01.074

Prescott, S. A., & Ratté, S. (2012). Pain processing by spinal microcircuits: afferent combinatorics. *Current Opinion in Neurobiology*, *22*(4), 631–9. doi:10.1016/j.conb.2012.02.010

Price, D. D., McGrath, P. A., Rafii, A., & Buckingham, B. (1983). The validation of visual analogue scales as ratio scale measures for chronic and experimental pain. *Pain*, *17*(1), 45–56.

Prkachin, K. M. (2009). Assessing pain by facial expression: facial expression as nexus. *Pain Research & Management : The Journal of the Canadian Pain Society = Journal de La Société Canadienne Pour Le Traitement de La Douleur*, *14*(1), 53–8.

Pud, D., Eisenberg, E., Sprecher, E., Rogowski, Z., & Yarnitsky, D. (2004). The tridimensional personality theory and pain: harm avoidance and reward dependence traits correlate with pain perception in healthy volunteers. *European Journal of Pain (London, England)*, *8*(1), 31–8. doi:10.1016/S1090-3801(03)00065-X

Pujol, J., Pascual-Leone, A., Dolz, C., Delgado, E., Dolz, J. L., & Aldomà, J. (1998). The effect of repetitive magnetic stimulation on localized musculoskeletal pain. *Neuroreport*, *9*(8), 1745–8.

Quiton, R. L., & Greenspan, J. D. (2008). Across- and within-session variability of ratings of painful contact heat stimuli. *Pain*, *137*(2), 245–56. doi:10.1016/j.pain.2007.08.034

Rainville, P., Bao, Q. V. H., & Chrétien, P. (2005). Pain-related emotions modulate experimental pain perception and autonomic responses. *Pain*, 118(3), 306–18. doi:10.1016/j.pain.2005.08.022

Rainville, P., Carrier, B., Hofbauer, R. K., Bushnell, M. C., & Duncan, G. H. (1999). Dissociation of sensory and affective dimensions of pain using hypnotic modulation. *Pain*, *82*(2), 159–71.

Rash, J. A., Aguirre-Camacho, A., & Campbell, T. S. (2013). Oxytocin and Pain: A Systematic Review and Synthesis of Findings. *The Clinical Journal of Pain*, *00*(00), 1–10. doi:10.1097/AJP.0b013e31829f57df

Rash, J. A., & Campbell, T. S. (2014). The effect of intranasal oxytocin administration on acute cold pressor pain: a placebo-controlled, double-blind, within-participants crossover investigation. *Psychosomatic Medicine*, 76(6), 422–9. doi:10.1097/PSY.0000000000000068

Read, D., & Loewenstein, G. (1999). Enduring Pain for Money : Decisions Based on the Perception and Memory of Pain. *Journal of Behavioral Decision Making*, *17*(April 1998), 1–17.

Ree, M. J., French, D., MacLeod, C., & Locke, V. (2008). Distinguishing Cognitive and Somatic Dimensions of State and Trait Anxiety: Development and Validation of the State-Trait Inventory for Cognitive and Somatic Anxiety (STICSA). *Behavioural and Cognitive Psychotherapy*, *36*(03), 313–332. doi:10.1017/S1352465808004232

Reeves, J. L., & Shapiro, D. (1982). Heart rate biofeedback and cold pressor pain. *Psychophysiology*, 19(4), 393–403.

Reitz, S., Krause-Utz, A., Pogatzki-Zahn, E. M., Ebner-Priemer, U., Bohus, M., & Schmahl, C. (2012). Stress regulation and incision in borderline personality disorder--a pilot study

modeling cutting behavior. *Journal of Personality Disorders*, *26*(4), 605–15. doi:10.1521/pedi.2012.26.4.605

Ren, K., Zhuo, M., Randich, A., & Gebhart, G. F. (1993). Vagal afferent stimulation-produced effects on nociception in capsaicin-treated rats. *Journal of Neurophysiology*, 69(5), 1530–40.

Rhudy, J. L., Williams, A. E., McCabe, K. M., Nguyen, M. A. T. V, & Rambo, P. (2005). Affective modulation of nociception at spinal and supraspinal levels. *Psychophysiology*, *42*(5), 579–87. doi:10.1111/j.1469-8986.2005.00313.x

Rhudy, J. L., Williams, A. E., McCabe, K. M., Rambo, P. L., & Russell, J. L. (2006). Emotional modulation of spinal nociception and pain: the impact of predictable noxious stimulation. *Pain*, *126*(1-3), 221–33. doi:10.1016/j.pain.2006.06.027

Rief, W., & Broadbent, E. (2007). Explaining medically unexplained symptoms-models and mechanisms. *Clinical Psychology Review*, *27*(7), 821–41. doi:10.1016/j.cpr.2007.07.005

Rief, W., & Hiller, W. (2003). A new approach to the assessment of the treatment effects of somatoform disorders. *Psychosomatics*, *44*(6), 492–8. doi:10.1176/appi.psy.44.6.492

Rief, W., & Hiller, W. (2008). *SOMS – Screening für Somatoforme Störungen Manual* (2nd ed.). Bern: Verlag Hans Huber, Hogrefe AG.

Rief, W., Heuser, J., & Fichter, M. M. (1996). What does the Toronto Alexithymia Scale TAS-R measure? *Journal of Clinical Psychology*, *52*(4), 423–429. doi:10.1002/(SICI)1097-4679(199607)52:4<423::AID-JCLP6>3.3.CO;2-C

Ringkamp, M., Peng, Y. B., Wu, G., Hartke, T. V, Campbell, J. N., & Meyer, R. a. (2001). Capsaicin responses in heat-sensitive and heat-insensitive A-fiber nociceptors. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *21*(12), 4460–8.

Ritz, T. (2009). Studying noninvasive indices of vagal control: the need for respiratory control and the problem of target specificity. *Biological Psychology*, 80(2), 158–68. doi:10.1016/j.biopsycho.2008.08.003

Ritz, T., & Dahme, B. (2006). Implementation and interpretation of respiratory sinus arrhythmia measures in psychosomatic medicine: practice against better evidence? *Psychosomatic Medicine*, 68(4), 617–27. doi:10.1097/01.psy.0000228010.96408.ed

Rolke, R., Baron, R., Maier, C., Tolle, T. R., Treede, R., Beyer, A., … Wasserka, B. (2006). Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): standardized protocol and reference values. *Pain* (Vol. 123, pp. 231–43). doi:10.1016/j.pain.2006.01.041

Rolke, R., Magerl, W., Campbell, K. A., Schalber, C., Caspari, S., Birklein, F., & Treede, R.-D. (2006). Quantitative sensory testing: a comprehensive protocol for clinical trials. *European Journal of Pain (London, England)*, *10*(1), 77–88. doi:10.1016/j.ejpain.2005.02.003

Rollman, G. B. (1977). Signal detection theory measurement of pain: a review and critique. *Pain*, *3*(3), 187–211.

Rosier, E. M., Iadarola, M. J., & Coghill, R. C. (2002). Reproducibility of pain measurement and pain perception. *Pain*, *98*(1-2), 205–16.

Rossi, S., Ferro, M., Cincotta, M., Ulivelli, M., Bartalini, S., Miniussi, C., … Passero, S. (2007). A real electro-magnetic placebo (REMP) device for sham transcranial magnetic stimulation (TMS). *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *118*(3), 709–16. doi:10.1016/j.clinph.2006.11.005

Rossini, P. M., Barker, A. T., Berardelli, A., Caramia, M. D., Caruso, G., Cracco, R. Q., … Lücking, C. H. (1994). Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroencephalography and Clinical Neurophysiology*, *91*(2), 79–92.

Roy, M., Peretz, I., & Rainville, P. (2008). Emotional valence contributes to music-induced analgesia. *Pain*, *134*(1-2), 140–7. doi:10.1016/j.pain.2007.04.003

Ruxton, G. D., & Colegrave, N. (2011). *Experimental design for the life sciences.* (G. D. Ruxton & N. Colegrave, Eds.) (3rd ed., p. 208). Oxford: Oxford Univ. Press.

Salomons, T. V, Johnstone, T., Backonja, M.-M., Shackman, A. J., & Davidson, R. J. (2007). Individual differences in the effects of perceived controllability on pain perception: critical role of the prefrontal cortex. *Journal of Cognitive Neuroscience*, *19*(6), 993–1003. doi:10.1162/jocn.2007.19.6.993

Satow, T., Mima, T., Hara, H., Oga, T., Ikeda, A., Hashimoto, N., & Shibasaki, H. (2002). Nausea as a complication of low-frequency repetitive transcranial magnetic stimulation of the posterior fossa. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *113*(9), 1441–3.

Schepers, R. J., & Ringkamp, M. (2010). Thermoreceptors and thermosensitive afferents. *Neuroscience and Biobehavioral Reviews*, *34*(2), 177–84. doi:10.1016/j.neubiorev.2009.10.003

Schlaier, R., Eichhammer, P., Langguth, B., Doenitz, C., Binder, H., & Brawanski, A. (2007). Effects of spinal cord stimulation on cortical excitability in patients with chronic neuropathic pain: A pilot study. *European Journal of Pain*, *11*, 863–868. doi:10.1016/j.ejpain.2007.01.004

Schlereth, T., & Birklein, F. (2008). The sympathetic nervous system and pain. *Neuromolecular Medicine*, 10(3), 141–7. doi:10.1007/s12017-007-8018-6

Schmahl, C., Bohus, M., Esposito, F., Treede, R.-D., Di Salle, F., Greffrath, W., … Seifritz, E. (2006). Neural correlates of antinociception in borderline personality disorder. *Archives of General Psychiatry*, 63(6), 659–67. doi:10.1001/archpsyc.63.6.659

Sherman, D. K., Bunyan, D. P., Creswell, J. D., & Jaremka, L. M. (2009). Psychological vulnerability and stress: the effects of self-affirmation on sympathetic nervous system responses to naturalistic stressors. *Health Psychology: Official Journal of the Division of Health Psychology, American Psychological Association*, *28*(5), 554–62. doi:10.1037/a0014663

Šimić, N., & Manenica, I. (2012). Exam experience and some reactions to exam stress. *Human Physiology*, *38*(1), 67–72. doi:10.1134/S0362119712010161

Simons, L. E., Moulton, E. A., Linnman, C., Carpino, E., Becerra, L., & Borsook, D. (2014). The human amygdala and pain: evidence from neuroimaging. *Human Brain Mapping*, *35*(2), 527–38. doi:10.1002/hbm.22199

Singer, T., Snozzi, R., Bird, G., Petrovic, P., Silani, G., Heinrichs, M., & Dolan, R. J. (2008). Effects of oxytocin and prosocial behavior on brain responses to direct and vicariously experienced pain. *Emotion (Washington, D.C.)*, *8*(6), 781–91. doi:10.1037/a0014195

Skljarevski, V., & Ramadan, N. M. (2002). The nociceptive flexion reflex in humans – review article, *96*, 3–8.

Sluka, K. A., & Walsh, D. (2003). Transcutaneous electrical nerve stimulation: basic science mechanisms and clinical effectiveness. *The Journal of Pain: Official Journal of the American Pain Society*, *4*(3), 109–21. doi:10.1054/jpai.2003.434

Smania, N., Corato, E., Fiaschi, A., Pietropoli, P., Aglioti, S. M., & Tinazzi, M. (2005). Repetitive magnetic stimulation: a novel therapeutic approach for myofascial pain syndrome. *Journal of Neurology*, *252*(3), 307–14. doi:10.1007/s00415-005-0642-1

Smith, S. M., Beckmann, C. F., Ramnani, N., Woolrich, M. W., Bannister, P. R., Jenkinson, M., … McGonigle, D. J. (2005). Variability in fMRI: a re-examination of inter-session differences. *Human Brain Mapping*, *24*(3), 248–57. doi:10.1002/hbm.20080

Song, H.-S., & Lehrer, P. M. (2003). The effects of specific respiratory rates on heart rate and heart rate variability. *Applied Psychophysiology and Biofeedback*, 28(1), 13–23.

Spangler, G. (1997). Psychological and physiological responses during an exam and their relation to personality characteristics. *Psychoneuroendocrinology*, *22*(6), 423–41.

Spielberger, C. D., Brunner, T. M., Ritterband, L. M., & Reheiser, E. C. (2003). The nature and measurement of depression. *Revista Internacional de Psicología Clínica Y de La Salud= International Journal of Clinical and Health Psychology*, *3*(2), 209–234.

Staud, R., Robinson, M. E., & Price, D. D. (2005). Isometric exercise has opposite effects on central pain mechanisms in fibromyalgia patients compared to normal controls. *Pain*, *118*(1-2), 176–84. doi:10.1016/j.pain.2005.08.007

Sterzer, P., & Kleinschmidt, A. (2010). Anterior insula activations in perceptual paradigms: often observed but barely understood. *Brain Structure & Function*, *214*(5-6), 611–22. doi:10.1007/s00429-010-0252-2

Stokes III., J. I., Chapman, W. P., & Smith, L. H. (1948). Effects of hypoxia and hypercapnia on perception of thermal cutaneous pain. *The Journal of Clinical Investigation*, 27(3 Pt 1), 299–304. doi:10.1172/JCI101958

Storch, E. A., Roberti, J. W., & Roth, D. A. (2004). Factor structure, concurrent validity, and internal consistency of the Beck Depression Inventory-Second Edition in a sample of college students. *Depression and Anxiety*, *19*(3), 187–9. doi:10.1002/da.20002

Strauss-Blasche, G., Moser, M., Voica, M., McLeod, D. R., Klammer, N., & Marktl, W. (2000). Relative timing of inspiration and expiration affects respiratory sinus arrhythmia. *Clinical and Experimental Pharmacology & Physiology*, 27(8), 601–6.

Striepens, N., Kendrick, K. M., Hanking, V., Landgraf, R., Wüllner, U., Maier, W., & Hurlemann, R. (2013). Elevated cerebrospinal fluid and blood concentrations of oxytocin following its intranasal administration in humans. *Scientific Reports*, *3*, 3440. doi:10.1038/srep03440

Strigo, I. A., Carli, F., & Bushnell, M. C. (2000). Effect of ambient temperature on human pain and temperature perception. *Anesthesiology*, *92*(3), 699–707.

Strutton, P. H., Theodorou, S., Catley, M., McGregor, A. H., & Davey, N. J. (2005). Corticospinal excitability in patients with chronic low back pain. *Journal of Spinal Disorders & Techniques*, *18*(5), 420–4.

Subotnik, K. L., & Shapiro, D. (1984). Heart rate biofeedback and cold pressor pain: effects of expectancy. *Biofeedback and Self-Regulation*, 9(1), 55–75.

Summers, J., Johnson, S., Pridmore, S., & Oberoi, G. (2004). Changes to cold detection and pain thresholds following low and high frequency transcranial magnetic stimulation of the motor cortex. *Neuroscience Letters*, *368*(2), 197–200. doi:10.1016/j.neulet.2004.07.008

Suslow, T., Donges, U. S., Kersting, A., & Arolt, V. (2000). 20-Item Toronto Alexithymia Scale: do difficulties describing feelings assess proneness to shame instead of difficulties symbolizing emotions? *Scandinavian Journal of Psychology*, *41*(4), 329–34.

Talbot, J. D., Marrett, S., Evans, A. C., Meyer, E., Bushnell, M. C., & Duncan, G. H. (1991). Multiple representations of pain in human cerebral cortex. *Science (New York, N.Y.)*, *251*(4999), 1355–8.

Tamura, Y., Okabe, S., Ohnishi, T., N Saito, D., Arai, N., Mochio, S., … Ugawa, Y. (2004). Effects of 1-Hz repetitive transcranial magnetic stimulation on acute pain induced by capsaicin. *Pain*, *107*(1-2), 107–15. doi:10.1016/j.pain.2003.10.011

Thayer, J. F., & Sternberg, E. (2006). Beyond heart rate variability: vagal regulation of allostatic systems. *Annals of the New York Academy of Sciences*, 1088, 361–72. doi:10.1196/annals.1366.014

Theodoridou, A., Penton-Voak, I. S., & Rowe, A. C. (2013). A direct examination of the effect of intranasal administration of oxytocin on approach-avoidance motor responses to emotional stimuli. *PloS One*, *8*(2), e58113. doi:10.1371/journal.pone.0058113

Thielscher, A., & Kammer, T. (2004). Electric field properties of two commercial figure-8 coils in TMS: calculation of focality and efficiency. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *115*(7), 1697–708. doi:10.1016/j.clinph.2004.02.019

Todd, A. J. (2010). Neuronal circuitry for pain processing in the dorsal horn. *Nature Reviews. Neuroscience*, *11*(12), 823–36. doi:10.1038/nrn2947

Tomlinson, S. P., Davis, N. J., & Bracewell, R. M. (2013). Brain stimulation studies of non-motor cerebellar function: A systematic review. *Neuroscience and Biobehavioral Reviews*. doi:10.1016/j.neubiorev.2013.03.001

Tousignant-Laflamme, Y., Rainville, P., & Marchand, S. (2005). Establishing a link between heart rate and pain in healthy subjects: a gender effect. *The Journal of Pain: Official Journal of the American Pain Society*, 6(6), 341–7. doi:10.1016/j.jpain.2005.01.351

Tousignant, N. (2011). The rise and fall of the dolorimeter: pain, analgesics, and the management of subjectivity in mid-twentieth-century United States. *Journal of the History of Medicine and Allied Sciences*, *66*(2), 145–79. doi:10.1093/jhmas/jrq024

Tracey, I. (2008). Imaging pain. *British Journal of Anaesthesia*, 101(1), 32–9. doi:10.1093/bja/aen102

Tracey, I., & Johns, E. (2010). The pain matrix: reloaded or reborn as we image tonic pain using arterial spin labelling. *Pain*, *148*(3), 359–60. doi:10.1016/j.pain.2009.11.009

Tracey, I., & Mantyh, P. W. (2007). The cerebral signature for pain perception and its modulation. *Neuron*, *55*(3), 377–91. doi:10.1016/j.neuron.2007.07.012

Treede, R. D., Meyer, R. A., Raja, S. N., & Campbell, J. N. (1995). Evidence for two different heat transduction mechanisms in nociceptive primary afferents innervating monkey skin. *The Journal of Physiology*, *483 (Pt 3*, 747–58.

Tseng, M.-T., Tseng, W.-Y. I., Chao, C.-C., Lin, H.-E., & Hsieh, S.-T. (2009). Distinct and shared cerebral activations in processing innocuous versus noxious contact heat revealed by functional magnetic resonance imaging. *Human Brain Mapping*, *000*. doi:10.1002/hbm.20902

Turgut, N., & Altun, B. U. (2009). Cortical disinhibition in diabetic patients with neuropathic pain. *Acta Neurologica Scandinavica*, *120*(6), 383–8. doi:10.1111/j.1600-0404.2009.01235.x

Ugawa, Y. (2009). Can we see the cerebellar activation effect by TMS over the back of the head? *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *120*(12), 2006–7. doi:10.1016/j.clinph.2009.09.003

Ugawa, Y., Day, B. L., Rothwell, J. C., Thompson, P. D., Merton, P. A., & Marsden, C. D. (1991). Modulation of motor cortical excitability by electrical stimulation over the cerebellum in man. *The Journal of Physiology*, *441*(1), 57–72. doi:10.1016/j.clinph.2006.09.006

Ugawa, Y., Uesaka, Y., Terao, Y., Hanajima, R., & Kanazawa, I. (1994). Magnetic stimulation of corticospinal pathways at the foramen magnum level in humans. *Annals of Neurology*, *36*(4), 618–24. doi:10.1002/ana.410360410

Ugawa, Y., Uesaka, Y., Terao, Y., Hanajima, R., & Kanazawa, I. (1995). Magnetic stimulation over the cerebellum in humans. *Annals of Neurology*, *37*(6), 703–13. doi:10.1002/ana.410370603

Urbaniak, G. C., & Plous, S. (2011). Research Randomizer 3.0. [Computer software]. Retrieved March 24, 2011, from http://www.randomizer.org/

Van Damme, S., Legrain, V., Vogt, J., & Crombez, G. (2010). Keeping pain in mind: a motivational account of attention to pain. *Neuroscience and Biobehavioral Reviews*, *34*(2), 204–13. doi:10.1016/j.neubiorev.2009.01.005

Van Houdenhove, B. (2000). Psychosocial stress and chronic pain. *European Journal of Pain (London, England)*, *4*(3), 225–8. doi:10.1053/eujp.2000.0189

Vaschillo, E. G., Vaschillo, B., & Lehrer, P. M. (2006). Characteristics of resonance in heart rate variability stimulated by biofeedback. *Applied Psychophysiology and Biofeedback*, 31(2), 129–42. doi:10.1007/s10484-006-9009-3

Vaschillo, E. G., Vaschillo, B., Pandina, R. J., & Bates, M. E. (2011). Resonances in the cardiovascular system caused by rhythmical muscle tension. *Psychophysiology*, 48(7), 927–36. doi:10.1111/j.1469-8986.2010.01156.x

Verdugo, R., & Ochoa, J. L. (1992). Quantitative somatosensory thermotest. A key method for functional evaluation of small calibre afferent channels. *Brain: A Journal of Neurology*, *115 (Pt 3)*, 893–913.

Verne, G. N., Himes, N. C., Robinson, M. E., Gopinath, K. S., Briggs, R. W., Crosson, B., & Price, D. D. (2003). Central representation of visceral and cutaneous hypersensitivity in the irritable bowel syndrome. *Pain*, *103*(1-2), 99–110. doi:10.1016/S0304-3959(02)00416-5

Verne, G. N., Robinson, M. E., Vase, L., & Price, D. D. (2003). Reversal of visceral and cutaneous hyperalgesia by local rectal anesthesia in irritable bowel syndrome (IBS) patients. *Pain*, *105*(1-2), 223–30. doi:10.1016/S0304-3959(03)00210-0

Victor, R., Mainardi, J. A., & Shapiro, D. (1978). Effects of biofeedback and voluntary control procedures on heart rate and perception of pain during the cold pressor test. *Psychosomatic Medicine*, 40(3), 216–26.

Vierck, C. J., Cannon, R. L., Fry, G., Maixner, W., & Whitsel, B. L. (1997). Characteristics of temporal summation of second pain sensations elicited by brief contact of glabrous skin by a preheated thermode. *Journal of Neurophysiology*, *78*(2), 992–1002.

Villemure, C., & Bushnell, M. C. (2009). Mood influences supraspinal pain processing separately from attention. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *29*(3), 705–15. doi:10.1523/JNEUROSCI.3822-08.2009

Vul, E., Harris, C., Winkielman, P., & Pashler, H. (2009). Puzzlingly High Correlations in fMRI Studies of Emotion, Personality, and Social Cognition. *Perspectives on Psychological Science*, *4*(3), 274–290. doi:10.1111/j.1745-6924.2009.01125.x

Wagenmakers, E.-J., & Farrell, S. (2004). AIC model selection using Akaike weights. *Psychonomic Bulletin & Review*, *11*(1), 192–6.

Wager, T. D., Atlas, L. Y., Lindquist, M. A., Roy, M., Woo, C.-W., & Kross, E. (2013). An fMRI-based neurologic signature of physical pain. *The New England Journal of Medicine*, *368*(15), 1388–97. doi:10.1056/NEJMoa1204471

Weber, S. J., & Cook, T. D. (1972). Subject effects in laboratory research: An examination of subject roles, demand characteristics, and valid inference. *Psychological Bulletin*, *77*(4), 273–295. doi:10.1037/h0032351

Weik, U., & Deinzer, R. (2010). Alterations of postawakening cortisol parameters during a prolonged stress period: Results of a prospective controlled study. *Hormones and Behavior*, *58*(3), 405–9. doi:10.1016/j.yhbeh.2010.06.001

Werner, M. U., Lassen, B., Pedersen, J. L., & Kehlet, H. (2002). Local cooling does not prevent hyperalgesia following burn injury in humans. *Pain*, *98*(3), 297–303.

Whalen, P. J. (2007). The uncertainty of it all. *Trends in Cognitive Sciences*, *11*(12), 499–500. doi:10.1016/j.tics.2007.08.016

Wiech, K., & Tracey, I. (2009). The influence of negative emotions on pain: behavioral effects and neural mechanisms. *NeuroImage*, *47*(3), 987–94. doi:10.1016/j.neuroimage.2009.05.059

Wiech, K., & Tracey, I. (2013). Pain, decisions, and actions: a motivational perspective. *Frontiers in Neuroscience*, *7*(April), 46. doi:10.3389/fnins.2013.00046

Wise, T. N., & Mann, L. S. (1994). The relationship between somatosensory amplification, alexithymia, and neuroticism. *Journal of Psychosomatic Research*, *38*(6), 515–21.

Woolf, C. J., & Ma, Q. (2007). Nociceptors--noxious stimulus detectors. *Neuron*, *55*(3), 353–64. doi:10.1016/j.neuron.2007.07.016

Woollard, H. H., & Carmichael, E. A. (1933). The testis and referred pain. *Brain*, *56*(3), 293–303. doi:10.1093/brain/56.3.293

World Health Organisation. (2011). WHO-DALY-Report. Retrieved from http://www.who.int/healthinfo/global_burden_disease/estimates_regional/en/

Yarnitsky, D., & Ochoa, J. L. (1990). Studies of heat pain sensation in man: perception thresholds, rate of stimulus rise and reaction time. *Pain*, 40(1), 85–91.

Zautra, A. J., Fasman, R., Davis, M. C., & Craig, A. D. B. (2010). The effects of slow breathing on affective responses to pain stimuli: an experimental study. *Pain*, 149(1), 12–8. doi:10.1016/j.pain.2009.10.001

Zhong, Y., Jan, K.-M., Ju, K. H., & Chon, K. H. (2006). Quantifying cardiac sympathetic and parasympathetic nervous activities using principal dynamic modes analysis of heart rate variability. *American Journal of Physiology. Heart and Circulatory Physiology*, 291(3), H1475–83. doi:10.1152/ajpheart.00005.2006

Zubieta, J.-K., Heitzeg, M. M., Smith, Y. R., Bueller, J. A., Xu, K., Xu, Y., … Goldman, D. (2003). COMT val158met genotype affects mu-opioid neurotransmitter responses to a pain stressor. *Science (New York, N.Y.)*, *299*(5610), 1240–3. doi:10.1126/science.1078546

Zunhammer, M., Eichhammer, P., & Busch, V. (2013). Do cardiorespiratory variables predict the antinociceptive effects of deep and slow breathing? *Pain Medicine (Malden, Mass.)*, *14*(6), 843–54. doi:10.1111/pme.12085

Zunhammer, M., Eichhammer, P., & Busch, V. (Submitted). Effects of exam stress on sleep quality: the role of alcohol, caffeine and nicotine.

Zunhammer, M., Eichhammer, P., Franz, J., Hajak, G., & Busch, V. (2012). Effects of acupuncture needle penetration on motor system excitability. *Neurophysiologie Clinique = Clinical Neurophysiology*, *42*(4), 225–30. doi:10.1016/j.neucli.2012.02.134