EFFECT OF LOW-GRADE ANXIETY ON NEURAL, PSYCHOPHYSICAL AND
PHYSIOLOGICAL ACTIVITY IN AN EXPERIMENTAL PAIN PARADIGM

BY

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<td>Anterior cingulate cortex</td>
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<tr>
<td>AMY</td>
<td>Amygdala</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
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<td>AP</td>
<td>Anxiety-prone group</td>
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<tr>
<td>AR</td>
<td>Autoregressive (method)</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>BOLD</td>
<td>Blood oxygenation-level dependent</td>
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<tr>
<td>bpm</td>
<td>Beats per minute</td>
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<td>BS</td>
<td>Brainstem</td>
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<td>BSI</td>
<td>Brief Symptom Inventory®</td>
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<td>CAU</td>
<td>Caudate</td>
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<td>CER</td>
<td>Cerebellum</td>
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<td>CO</td>
<td>Central opercular cortex</td>
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<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
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<tr>
<td>CUN</td>
<td>Cuneus</td>
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<tr>
<td>DLPFC</td>
<td>Dorsolateral prefrontal cortex</td>
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<tr>
<td>DPFC</td>
<td>Dorsal prefrontal cortex</td>
</tr>
<tr>
<td>Exp</td>
<td>Expectation period</td>
</tr>
<tr>
<td>FFT</td>
<td>Fast Fourier transform (method)</td>
</tr>
<tr>
<td>fMRI</td>
<td>Function magnetic resonance imaging</td>
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<tr>
<td>FO</td>
<td>Frontal operculum</td>
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<td>FP</td>
<td>Frontal pole</td>
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<tr>
<td>FSL</td>
<td>FMRIB (Functional Magnetic Resonance Imaging of the Brain) Software Library</td>
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<tr>
<td>GLM</td>
<td>General linear model</td>
</tr>
<tr>
<td>HF</td>
<td>High frequency (band) (0.15 – 0.40 Hz)</td>
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<tr>
<td>HIP</td>
<td>Hippocampus</td>
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<td>HR</td>
<td>Heart rate</td>
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<tr>
<td>HRV</td>
<td>Heart rate variability</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
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<tr>
<td>IBI</td>
<td>Inter-beat interval</td>
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<tr>
<td>IC</td>
<td>Independent component</td>
</tr>
<tr>
<td>ICA</td>
<td>Independent component analysis</td>
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<tr>
<td>IDL</td>
<td>Interactive Data Language</td>
</tr>
<tr>
<td>IFG</td>
<td>Inferior frontal gyrus</td>
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<tr>
<td>IFG-PO</td>
<td>Inferior frontal gyrus pars opercularis</td>
</tr>
<tr>
<td>I/M-FG</td>
<td>Inferior/middle frontal gyrus</td>
</tr>
<tr>
<td>INS</td>
<td>Insula</td>
</tr>
<tr>
<td>IPL</td>
<td>Inferior parietal lobule</td>
</tr>
<tr>
<td>ITG</td>
<td>Inferior temporal gyrus</td>
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<tr>
<td>LF</td>
<td>Low frequency (band) (0.04 – 0.15 Hz)</td>
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<td>LG</td>
<td>Lingual gyrus</td>
</tr>
<tr>
<td>LOC</td>
<td>Lateral occipital cortex</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>LOC-S</td>
<td>Superior lateral occipital cortex</td>
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<td>LPFC</td>
<td>Lateral prefrontal cortex</td>
</tr>
<tr>
<td>LV</td>
<td>Lateral ventricle</td>
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<tr>
<td>mACC</td>
<td>Mid anterior cingulate cortex</td>
</tr>
<tr>
<td>MeanRR</td>
<td>Mean of the inter-beat intervals</td>
</tr>
<tr>
<td>MELODIC</td>
<td>Multivariate Exploratory Linear Decomposition into Independent Components (FSL software for independent component analysis)</td>
</tr>
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<td>MFG</td>
<td>Middle frontal gyrus</td>
</tr>
<tr>
<td>MRI</td>
<td>Imaging session</td>
</tr>
<tr>
<td>ms</td>
<td>Milliseconds</td>
</tr>
<tr>
<td>MTG</td>
<td>Middle temporal gyrus</td>
</tr>
<tr>
<td>NA</td>
<td>Non-anxious group</td>
</tr>
<tr>
<td>NAc</td>
<td>Nucleus accumbens</td>
</tr>
<tr>
<td>NN50</td>
<td>Number of successive inter-beat interval pairs that differ more than 50 ms</td>
</tr>
<tr>
<td>OcFG</td>
<td>Occipital fusiform gyrus</td>
</tr>
<tr>
<td>OFC</td>
<td>Orbitofrontal cortex</td>
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<td>OL</td>
<td>Occipital lobe</td>
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<td>PC</td>
<td>Precuneus</td>
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<td>PCC</td>
<td>Posterior cingulate cortex</td>
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<td>PCL</td>
<td>Paracentral lobule</td>
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<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>PFC</td>
<td>Prefrontal cortex</td>
</tr>
<tr>
<td>pgACC</td>
<td>Pregenual anterior cingulate cortex</td>
</tr>
<tr>
<td>pg/sg-ACC</td>
<td>Pre-/subgenual anterior cingulate cortex</td>
</tr>
<tr>
<td>PHG</td>
<td>Parahippocampal gyrus</td>
</tr>
<tr>
<td>pNN50</td>
<td>NN50 expressed in percent of the total number of inter-beat intervals</td>
</tr>
<tr>
<td>PoCG</td>
<td>Postcentral gyrus</td>
</tr>
<tr>
<td>Post.</td>
<td>Post-stimulation period</td>
</tr>
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<td>PP</td>
<td>Psychophysical session</td>
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<td>PrCG</td>
<td>Precentral gyrus</td>
</tr>
<tr>
<td>Pre.</td>
<td>Pre-stimulation period</td>
</tr>
<tr>
<td>Pr/Po-CG</td>
<td>Pre-/Postcentral gyri</td>
</tr>
<tr>
<td>PS</td>
<td>Post-stimulus period</td>
</tr>
<tr>
<td>PTSD</td>
<td>Post-traumatic stress disorder</td>
</tr>
<tr>
<td>PUT</td>
<td>Putamen</td>
</tr>
<tr>
<td>RER</td>
<td>Respiratory rate</td>
</tr>
<tr>
<td>RMSSD</td>
<td>Square root of the mean squared differences between successive inter-beat intervals</td>
</tr>
<tr>
<td>ROI</td>
<td>Region-of-interest</td>
</tr>
<tr>
<td>RSA</td>
<td>Respiratory sinus arrhythmia</td>
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<td>RSC</td>
<td>Retrosplenial cortex</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SDNN</td>
<td>Standard deviation of the inter-beat intervals</td>
</tr>
<tr>
<td>sec.</td>
<td>Seconds</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>SFG</td>
<td>Superior frontal gyrus</td>
</tr>
<tr>
<td>sgACC</td>
<td>Subgenual anterior cingulate cortex</td>
</tr>
<tr>
<td>SI</td>
<td>Primary somato-sensory cortex</td>
</tr>
<tr>
<td>SII</td>
<td>Secondary somato-sensory cortex</td>
</tr>
<tr>
<td>SMA</td>
<td>Supplementary motor area</td>
</tr>
<tr>
<td>SMG</td>
<td>Supramarginal gyrus</td>
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<tr>
<td>S/M-TG</td>
<td>Superior/Middle temporal gyri</td>
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<tr>
<td>SPL</td>
<td>Superior parietal lobule</td>
</tr>
<tr>
<td>SPSS®</td>
<td>Statistical Package for the Social Sciences</td>
</tr>
<tr>
<td>STAI</td>
<td>State-Trait Anxiety Inventory</td>
</tr>
<tr>
<td>STG</td>
<td>Superior temporal gyrus</td>
</tr>
<tr>
<td>Stim</td>
<td>Stimulation period</td>
</tr>
<tr>
<td>TE</td>
<td>Echo time</td>
</tr>
<tr>
<td>Temp.</td>
<td>Stimulus temperature</td>
</tr>
<tr>
<td>TFG</td>
<td>Temporal fusiform gyrus</td>
</tr>
<tr>
<td>THAL</td>
<td>Thalamus</td>
</tr>
<tr>
<td>tICA</td>
<td>Tensor independent component analysis</td>
</tr>
<tr>
<td>TOFG</td>
<td>Temporo-occipital fusiform gyrus</td>
</tr>
<tr>
<td>TP</td>
<td>Temporal pole</td>
</tr>
<tr>
<td>TR</td>
<td>Repetition time</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual analog scale</td>
</tr>
<tr>
<td>vMPFC</td>
<td>Ventromedial prefrontal cortex</td>
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ABSTRACT

Morten Sand Hadsel

EFFECT OF LOW-GRADE ANXIETY ON NEURAL, PSYCHOPHYSICAL AND PHYSIOLOGICAL ACTIVITY IN AN EXPERIMENTAL PAIN PARADIGM

Dissertation under the direction of
Robert C. Coghill, Ph.D., Associate Professor of Neurobiology and Anatomy

Anxiety is considered to influence both general health and activities of daily living. Individuals with certain types of clinical anxiety exhibit increased thresholds to painful thermal stimuli, likely to suppress their pain experiences and to facilitate anxiety-specific behavior. It is not known whether pain-related findings from the clinical realm of anxiety also apply to healthy, non-treatment seeking subjects with low-grade anxiety. We therefore employed psychophysical, physiological and functional neuroimaging methods to investigate how inter-individual differences in inherent low-grade anxiety and perceived acute pain are related. Scientific data suggest that low-grade anxiety harbors an adaptive advantage. To explore if this putative advantage reflects a particularly favorable pattern of autonomic and cognitive-emotional processing, we additionally performed analyses of heart rate variability, as their measures are indices of autonomic flexibility and neuro-visceral integration. Results demonstrated that individuals with low-grade anxiety exhibited unique experiential, behavioral, physiological, and brain processing features that putatively set them apart from both non-anxious and clinically anxious subjects.
Low-grade anxiety, presumably as a motivational input, suppressed painful experiences and shaped associated processes. Hence, pain-modulating systems do not respond in a constant fashion to noxious stimuli, but will be engaged according to the overall contextual goal of the organism. Elevated metrics of heart rate variability in the low-grade anxious subjects suggested an ability to maximally harness neurobiological processing systems to ensure optimal behavior and supported claims of an adaptive advantage linked to low-grade anxiety. Consequently, individual mood differences must be taken into account both when designing experiments and when their results are interpreted. More research will be needed to further characterize anxiety-proneness and to allow the involved emotional-motivational and cognitive mechanisms to be fully harnessed for use in clinical situations.
Chapter I

INTRODUCTION

Numerous reports of the effects of anxiety on general mental and physical health have been published (Barlow, 2002). Many of these reports have considered clinical anxiety, which has been invaluable in highlighting the importance of psychosocial elements in the development and maintenance of a variety of disorders, including chronic pain (Turk and Flor, 1984). Results of investigations into the effects of anxiety in the experience of acute pain have also appeared in the last couple of decades. Those results have been ambiguous and have predominantly been based on experimentally induced anxiety (Rhudy and Meagher, 2000; Rhudy et al., 2008). The present dissertation will examine how naturally occurring, non-clinical, low-grade anxiety affects the experience of acute pain. We will consider anxiety-proneness as an “inherent” motivational state and discuss the experimental outcomes in light of competition between different motivations – concepts that have reappeared in recent pain research (Fields, 2004). We will also – in a preliminary fashion – attempt to unravel features of anxiety-proneness itself, an understudied mood/trait category.

Pain

Overview

The International Association for the Study of Pain (IASP) defines pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (Merskey and
Nociception is the process whereby the physical, chemical or thermal energy of a tissue-damaging (nociceptive) stimulus, through activation of specialized nerve endings in the periphery, is transformed into electrical energy, which is propagated as action potentials along sensory, afferent nerves (nociceptors) to multiple levels of the neuraxis (Willis and Coggeshall, 1991). Higher-order interpretation of the nociceptive signals involves cognitive-evaluative and emotional-motivational components, putting the afferent information into a unique intra- and inter-personal context, leading to the subjective experience of pain (Merskey and Bogduk, 1994; Koyama et al., 2005). Pain behavior describes the individual's audible and visible actions that communicate the affected person's suffering to others (Gatchel, 2004). Activity in the effector organs that execute pain behavior is mostly mediated by physiological stress responses characterized by increased activity in the motor-, endocrine-, autonomic- (Bandler and Keay, 1996), immune- (Madden and Felten, 1995; Madden et al., 1995) and opioid-systems (Zubieta et al., 2001). It is important to be aware of the fact that tissue damage is not a necessary element of pain experience (Ramachandran, 1998; Harris, 1999), e.g. as observed in allodynic conditions (pain that occurs without noxious stimulation at the site of pain) (Price, 1999).

**Physiological and pathological aspects of pain**

The experience of pain ensures the integrity of the organism for survival. Pain has a warning function that is able to prevent or limit damage. This is
common-sense knowledge from our daily experiences, but is also scientifically supported by data showing that involved receptors respond to a level of stimulation that is lower than what is necessary to cause actual injury. Also, most types of pain depend on the rate and not on the amount of tissue damage (Hardy et al., 1952; Beecher, 1959). The second physiological function of pain is to support recuperation and healing. The immediate phase following tissue injury will be characterized by fight/flight behavior with the purpose of securing survival of the individual. Depending on the context, this phase likely entails heavy involvement of endogenous analgesic systems, as the experience of pain at this point serves no purpose and would actually impede survival. The motivation for survival is stronger than the motivation to care about injuries. When the individual is out of immediate danger, the motivational focus changes towards the condition of the organism and the extent of the injuries are assessed (Wall, 1979; Bolles and Fanselow, 1980; Melzack et al., 1982). To sustain immobility of the affected body parts, hyperalgesia (increased pain experience to a noxious stimulus) and allodynia are important features of the recuperative phase, and are supported by functional and plastic neuronal changes, including descending facilitation, peripheral and central sensitization and neuronal sprouting (Wall, 1979; Porreca et al., 2002; Jasmin et al., 2003). Central sensitization is a lowering of the firing threshold in neurons at central nociceptive relay stations also seen in pathological/chronic pain conditions (Latremoliere and Woolf, 2009).

Pathological or chronic pain is normally thought of as a condition where there is no physiological purpose in the experience of pain. Some clinicians use
these terms for any pain that has lasted longer than 3 - 6 months. Such a
definition would be most accommodating to idiopathic types of pain, i.e. pain
where there is no identifiable physical cause, or to pain associated with chronic
disease (e.g. diabetic neuropathy). Otherwise, a better definition of chronic pain
might be “pain lasting beyond normal healing time” (Okeson, 1995). In these
cases one could say that pain is going from being the symptom to becoming the
disease. Just as acute pain can be viewed in a phylogenetic perspective, chronic
pain might also serve an evolutionary purpose in the context of partner selection
and mating as signals to the opposite sex of inability to bear offspring or provide
for offspring and mate (Manfredini et al., 2010).

Long-lasting pain/nociception leads to plastic changes in the nervous
system. The neurobiological basis of chronic/neuropathic pain seems to involve
plasticity on several levels within the neuraxis: sensitization of peripheral C-fiber
nerve-endings by release of inflammatory mediators and pro-nociceptive factors
from the injured tissue and from the peripheral nerve ending itself (neurogenic
inflammation) (Gold and Gebhart, 2010); redistribution of the Na\textsubscript{v}1.8 sodium
channel to the axons of peripheral, uninjured C-fibers (positioned adjacent to the
injured primary afferents) (Lai et al., 2002; Lai et al., 2004); spinal sensitization of
second order neurons through increased release of excitatory amino acids from
the primary afferent nerve terminals (Gold and Gebhart, 2010; Quinn et al.,
2010); and activation of descending pain facilitatory pathways originating in the
rostro-ventro-medial medulla (Porreca et al., 2002; Jasmin et al., 2003).
**Pain modulation**

At the spinal level complex modulation of incoming noxious information takes place. Several brainstem systems, in particular the midbrain periaqueductal gray (PAG) and rostral ventromedial medulla (RVM), are involved in nociceptive modulation as well and in coordination of viscero-somatic responses to threatening stimuli (Basbaum and Fields, 1984; Fields et al., 1991; Tracey et al., 2002). Both spinal and bulbar regions are under higher-order control, so-called intra-cortical nociceptive modulation (Rizvi et al., 1991; Floyd et al., 2000; Bingel et al., 2006). Endogenous opioids are among the main modulators of nociceptive signals, although many other directly and indirectly, centrally and peripherally acting modulators exist, including cannabinoids (Vigano et al., 2005; Ibrahim et al., 2006) and cholecystokinin (Faris et al., 1983; Watkins et al., 1984). High levels of opioid receptors and endogenous opioids are found in the PAG and the RVM, as well as in the spinal dorsal horn (Fields et al., 1991). In analgesia, opioids are thought to activate the descending analgesic pathway through disinhibition of tonically active GABAergic interneurons. In the spinal dorsal horn these projections probably activate enkephalinergic interneurons that will inhibit dorsal horn neurons of the ascending nociceptive pathway (Besson and Chaouch, 1987). These endogenous nociceptive/pain modulatory systems also possess the ability to increase nociceptive transmission and induce states of hyperalgesia, depending on the contextual needs (Porreca et al., 2002; Jasmin et al., 2003). Cortico-cortical pain modulation refers to changes in pain experience brought about by processes solely confined to the
cortex itself (Staines et al., 2002; Villemure and Bushnell, 2002; Lorenz et al., 2003; Schaefer et al., 2005). Supporting such ideas would be the observed mismatch between the time courses of the descending opiate system and some of the mood induction and modulation procedures applied in experimental research. The descending opiate system has a slow on-/offset (Price and Barrell, 2000), while many mood induction paradigms show a rapid on- and/or offset of emotional effects (de Wied and Verbaten, 2001). These temporal differences indicate that a pain modulatory circuitry other than the opiate-driven descending system would be operative (Villemure and Bushnell, 2002). Cortico-cortical pain modulatory processes could modify pain perception or the accompanying cognitive appraisals (Wiech and Tracey, 2009).

A major part of the knowledge about basic nociceptive mechanisms has been derived from animal studies. There are indications of analogous mechanisms in humans that stem from several lines of reasoning, e.g. involved brainstem-spinal cord systems are highly conserved across mammalian species (Price, 1999). Further, opioid drugs able to reduce pain in humans also inhibit nociceptive behavior in animals and decrease neuronal responses in their afferent nociceptive pathways subsequent to noxious stimulation (Fields et al., 1991). In the later years, functional and pharmacological imaging in humans have confirmed and supplemented animal data (Coghill et al., 1994; Treede et al., 2000; Coghill et al., 2001; Zubieta et al., 2001; Liberzon et al., 2002; Coghill et al., 2003; Apkarian et al., 2005; Ribeiro et al., 2005).
Central correlates of pain

Inspired by the Gate Control Theory of Pain (Melzack and Wall, 1965) and by the discovery of endogenous opioid systems (Hughes, 1975), vast efforts were spent at exploring the organization of the dorsal horn of the spinal cord. This is the area where the central terminals of primary afferent axons, including the nociceptors, are located (Light and Perl, 1979; Sugiura et al., 1986) along with excitatory (Todd et al., 2003) and inhibitory (Todd and Sullivan, 1990) interneurons, as well as projection-neurons that convey peripheral information in a rostral direction (Willis and Coggeshall, 1991). Further, the spinal dorsal horn contains axonal terminals of neurons descending from different parts of the brain, including the most studied midbrain periaqueductal grey and the rostral ventromedial medulla, exerting both pro- and anti-nociceptive effects on afferent nociceptive signals (Wall, 1967; Mayer and Price, 1976; Fields et al., 1991; Porreca et al., 2002). It has been established that spinal neuronal circuitry is highly dynamic and subject to both functional (Price et al., 1977; Mendell, 1984; Dubner and Ruda, 1992) and structural plasticity (Bennett et al., 1996), the latter involving non-neuronal components such as recruitment of microglia (DeLeo and Yezierski, 2001).

The majority of studies on spinal and brainstem nociceptive processing have employed behavioral (Helmstetter et al., 1998), neurochemical (Zadina et al., 1997), neuroanatomical (An et al., 1998) and electrophysiological (Fields and Heinricher, 1985) methods, often in combination (Gardell et al., 2002). Due to their invasive nature these investigations have mostly been performed in rodents.
and cats. Obviously, such research could not address cognitive-emotional aspects of the pain experience directly. However, animal research has demonstrated connections between several forebrain regions and bulbo-spinal pain-modulatory circuitry (Rizvi et al., 1991; Cameron et al., 1995; Bandler and Keay, 1996; Rizvi et al., 1996; Hermann et al., 1997; Helmstetter et al., 1998), suggesting both cognitive-emotional influences on, and cortical involvement in, pain processing. Older data also suggested a role of the cortex in pain, and included findings demonstrating nociceptive responsivity in SI in primates (Kenshalo and Isensee, 1983; Kenshalo et al., 1988), the influence of cortical lesions on pain sensitivity (Marshall, 1951) and the observation of painful SI focal seizures (Young and Blume, 1983) in humans. In spite of those earlier data and the “holistic” ideas propagated through the Gate Control Theory and other comprehensive pain models, the general opinion that prevailed more or less until the 1990s asserted that higher brain centers were not involved in pain processing. However, contemporary data and theories underscore that pain is centrally processed, not by static “pain centers”, but through a variety of different and interacting distributed brain networks. Thus, a contextually integrated experience and appropriate autonomic and motor behaviors are created.

Around 1990 functional brain imaging was introduced and made it more feasible to investigate the cortical role in pain. Functional imaging used humans as subjects, which enabled simultaneous employment of psychophysical, psychophysiological and psychometric methods to complement the brain findings with data addressing cognitive-emotional and physiological characteristics of the
participants. During the last two decades the extent of imaging research has increased tremendously, and a considerable amount of data derived from animal studies have gained support through functional imaging in humans (Petrovic et al., 2002; Wager et al., 2007). Pain has been addressed by functional imaging through direct (Coghill et al., 1999) and indirect (a pain model used to address a non-pain question) (Kalisch et al., 2005) types of experiments, as well as some studies of pathological/chronic pain (Geha et al., 2007). In addition to functional imaging, also structural imaging modalities have been employed (Baliki and Apkarian, 2007). In the following, the main findings regarding supra-bulbar pain processing of non-pathological pain will be reviewed, including a discussion on general prefrontal cortex function. See also discussion under the paragraph *Pain and emotions*.

Experimental findings and meta-analyses have revealed a fairly consistent pattern of brain activity associated with pain. This pattern encompasses cortical and sub-cortical regions putatively engaged in several aspects of pain processing. Brain areas that are consistently activated subsequent to noxious stimulation include primary and secondary somatosensory, anterior cingulate, insular and prefrontal cortices, thalamus and the cerebellum (Jones et al., 1991; Talbot et al., 1991; Derbyshire et al., 1997; Coghill et al., 1999; Peyron et al., 1999; Treede et al., 1999; Buchner et al., 2000; Kanda et al., 2000; Peyron et al., 2000; Treede et al., 2000; Coghill et al., 2001; Hofbauer et al., 2001; Chen et al., 2002; Peyron et al., 2002; Coghill et al., 2003; Apkarian et al., 2005; Strigo et al., 2005).
Imaging studies of pain intensity-dependent brain activations have demonstrated that the somatosensory aspects of pain are mainly processed in the brain hemisphere contralateral to stimulation, while putative cognitive-emotional aspects are processed partly bilaterally and partly in the right brain hemisphere (Coghill et al., 2001). Distributed sensory-discriminative processing of pain might help sustain conscious awareness of pain intensity despite extensive cerebral cortical lesions (Coghill et al., 1999). The activated brain areas correspond well with outcomes of anatomical research. Afferent information from the somatosensory thalamus, including nociceptive signals, terminate in SI and SII (Friedman and Murray, 1986; Rausell and Jones, 1991; Shi and Apkarian, 1995), areas shown to encode spatial, temporal and intensity features of noxious and innocuous stimuli (Kenshalo and Isensee, 1983; Kenshalo et al., 1988; Chudler et al., 1990). In SI, a somatotopic organization of nociceptive responses, analogous to that of tactile responses, has been found (Andersson et al., 1997; Vogel et al., 2003). As such an organization is lacking in other cortical areas (Tarkka and Treede, 1993; Xu et al., 1997), it can be presumed that SI is the main structure involved in spatial localization of pain.

The middle and posterior thalamus relay noxious and non-noxious information to the cingulate cortex (Craig and Dostrovsky, 2001), where nociceptive neurons have been found in the anterior part (Koyama et al., 1998; Hutchison et al., 1999). Hypnotically induced alterations in pain unpleasantness were accompanied by corresponding changes in ACC activity (Rainville et al., 1997), suggesting ACC involvement in affective pain processing. This conjecture
has also been supported by patient-reports of reduced pain affect subsequent to cingulotomy (Foltz and White, 1962, 1968).

Direct recordings in humans have revealed nociceptive activity in the insula (Frot and Mauguiere, 2003) and functional imaging has demonstrated a positive correlation between such activity and perceived pain-intensity (Coghill et al., 1999). These findings implicate insula in pain-intensity processing, although a recent lesion study revealed that insula does not seem to be critical for the evaluation and experience of pain (Starr et al., 2009). A large number of studies have linked insula to autonomic regulation (Oppenheimer et al., 1996; Verberne and Owens, 1998) as well as pain affect (Ostrowsky et al., 2002).

The putative involvement of the cingulate and the insular cortices in emotional processing can be traced back to the early postulates of a limbic circuitry (Papez, 1937). Both areas are large and topographically wide-ranging, features that would enable regional specializations (Guldin and Markowitsch, 1983; Augustine, 1996; Vogt, 2005). The cingulate and insular cortices also exhibit widespread connectivity (Mufson and Mesulam, 1982; Vogt and Pandya, 1987; Vogt et al., 1987), implicating these regions in integrative (Augustine, 1996; Kong et al., 2006) as well as interactive (Critchley, 2005) processing. In general, the cingulate cortex monitors environmental events and detects processing conflicts, which are signaled to the prefrontal cortex for it to allocate additional cognitive control. Such control is necessary in resolving conflicts to avoid uncertainty and errors, thereby enabling clear executive commands (Carter et al., 1998; Botvinick et al., 1999; MacDonald et al., 2000). Relatedly, the ACC is
implicated in acquisition and use of social information, as well as cost-of-action considerations related to decision-making (Rushworth et al., 2007b). The insula might be responsible for autonomic-sensory integration, playing an important part in homeostatic regulation (Critchley, 2009).

The prefrontal cortex (PFC) is another area occupying a substantial volume of the brain, located at the rostral end of the neuraxis (Fuster, 2002). Anatomical complexity, results from lesion studies (Petrides, 1982, 1985, 1990) and the observation that a multitude of events correlate with prefrontal cortex activity led to a very compartmentalized view of the region, reminiscent of phrenological principles. Modern theories assume that the different PFC functions are interrelated, mutually supportive and complementary in reaching a unified goal, and that they thereby share common areas and networks (Quintana and Fuster, 1992; Rao et al., 1997; Asaad et al., 1998; Rainer et al., 1998; Prabhakaran et al., 2000; Fuster, 2008). Computational modeling has suggested reverberant reentry of information associated with several putative PFC functions, including working memory and contextual monitoring, consolidated into the concept of the perception-action cycle. This cycle integrates external and internal sensory and motor information at all hierarchical levels between the spinal cord and the cortex (Chavis and Pandya, 1976; Goldman-Rakic and Schwartz, 1982; Barbas and Pandya, 1987; Selemon and Goldman-Rakic, 1988; Seltzer and Pandya, 1989; Barbas and De Olmos, 1990; Petrides and Pandya, 1999), and also sends feedback to prior levels (Pandya and Yeterian, 1990). The PFC is situated at the highest level of this cycle, conveys reentrant feedback to
the posterior association cortex (Petrides and Pandya, 1984; Quintana and Fuster, 1999) and consequently regulates the interaction between the organism and the environment through goal-directed processing (Fuster, 2008).

With progression of phylogenetic development, the PFC grew in size to reach its maximum in humans. The PFC grew more in its lateral than in its ventromedial part (Rakic and Goldman-Rakic, 1982; Fuster, 2002). Lateral PFC (LPFC) is critical for cognitive functioning, especially in the organization of temporal coherence and coordination of actions, while the ventromedial prefrontal cortex (vMPFC) is associated with emotional processing (Fuster, 2001, 2002). This growth is thought to reflect the increasing complexity and individuality of both goals and actions during evolution, in a shift from instinctual to voluntary behavior as well as in the development of reasoning and language (Rakic and Goldman-Rakic, 1982; Fuster, 2002).

The two major executive functions of the PFC are planning and decision-making. Crucial to these functions is executive attention (Fuster, 2002) that encompasses working memory (Fuster and Alexander, 1971; Cohen et al., 1997), preparatory set and inhibitory interference control (Fuster, 2008). All PFC executive control is implemented through coordination of activity in other brain areas involved in executive attention. Accordingly, the neural substrate of working memory comprises networks encompassing areas in the lateral PFC (LPFC) and the posterior parts of the cortex. Working memory is conceptualized as the temporary activation of updated long-term memory networks for organizing actions in the near term, and is often viewed as sustained attention
focused on an internal representation (Miller, 2000; Miller and Cohen, 2001). Preparatory set, defined as the priming of sensory and motor neural structures for the performance of an act contingent on a prior event, also involves activation in parts of the LPFC and premotor regions as well as the basal ganglia (Miller and Cohen, 2001). The functional frame of reference for preparatory set is the near future, while for working memory it is the recent past. However, together the two functions mediate cross-temporal contingency, i.e. they reconcile the past with the future and consequently bridge sequential elements in behavior, thought or speech (Fuster, 1990, 2000a, b). To secure continuity of the sequence currently being enacted, inhibitory control suppresses external and internal influences that might interfere. Inhibition is an important general feature throughout the central nervous system as it enhances and provides contrast to excitatory functions, as seen in attention. In primates, PFC inhibitory control seems mainly associated with ventromedial/medial orbitofrontal brain regions, as well as subcortical areas. Trait anxiety has been associated with reduced recruitment of prefrontal mechanisms to inhibit distracters during processing of an attention-demanding task (Bishop, 2009). PFC receives projections from the mediodorsal thalamic nucleus, but direct thalamocortical nociceptive input is questionable.

Imaging studies of pain have resulted in a variety of activation patterns of the PFC. The activations that have been observed are more non-linearly related to the perception of pain (Coghill et al., 1999). These activations are often found in the dorsal/dorsolateral PFC and are thought to represent cognitive control over
ventromedial prefrontal regions (Lorenz et al., 2002; Lorenz et al., 2003; Kalisch et al., 2006). Actually, dorsal prefrontal modulation of activity in ventral emotional processing regions is often observed (Etkin et al., 2006). In general, pain-related brain deactivations have not been systematically published, but at least one study demonstrated vMPFC deactivation during noxious stimulation (Derbyshire et al., 1997). Such deactivation suggests peri-stimulus PFC activity and conforms to many anecdotal observations as well as the concept of the so-called default-mode brain network (Raichle et al., 2001). A default-mode brain function is thought to represent self-referential processing (Gusnard et al., 2001) and might further reflect coordination of activity between the PFC and other brain areas involved in executive attention.

Consequently, the PFC is probably responsible for cognitive-emotional aspects of pain processing as part of overall goal-directed activity. This has been suggested in a recent fMRI experiment, which demonstrated that assessment of the spatial locations of administered noxious stimuli activated prefrontal, anterior cingulate and posterior parietal cortices, as well as the caudate, in addition to sensory-discriminative brain regions. As the frontoparietal activity resembled spatial discrimination of innocuous stimuli, those brain areas were interpreted to reflect nodes in a cognitive circuitry of evaluation, working memory and decision-making (Oshiro et al., 2007).

Subcortical regions that exhibit relatively consistent pain-related activations in human imaging studies include the thalamus (Derbyshire et al., 1997; Coghill et al., 1999), basal ganglia (Chudler and Dong, 1995; Coghill et al.,...
1999; Becerra et al., 2001; Becerra and Borsook, 2008), cerebellum (Casey et al., 1994; Derbyshire and Jones, 1998; Coghill et al., 1999) and the amygdala (Becerra et al., 2001). As discussed above, thalamus is a relay for afferent information, including nociception, to multiple areas of the cortex (Craig et al., 2002; Craig, 2003b). Nociceptive neurons have been found in the basal ganglia in rodents and (Chudler, 1998), and it is likely that this brain region is part of circuitry involved in detection of novel and salient events and corresponding action selection (Redgrave et al., 2008). Cerebellum has reciprocal spinal connections (Saab and Willis, 2003), modulates nociceptive responses (Saab et al., 2001) and has otherwise been implicated in pain processing (Ekerot et al., 1991b, a; Gao et al., 1996). As nociceptive responses in the cerebellum have been recorded in unconscious individuals, cerebellum is unlikely to be associated with pain perception (Hofbauer et al., 2004). The area might be involved in regulation of nociceptive activity through cortico-cerebellar looping systems analogous to motor control (Ramnani, 2006). The amygdala is a brain region that for years has been implicated in emotional processing (Phelps and LeDoux, 2005). Subsequent to aversive events, like pain, amygdala is involved in attention regulation, conditioning processes and memory-consolidation and retrieval (Zald, 2003). The amygdala activates cholinergic and noradrenergic systems that can have profound effects on attention and memory consolidation (Dalmaz et al., 1993; McGaugh, 2004). Connections between the amygdala and the primary sensory cortices might further enable modulation of attentional and perceptual processes. The latter has been demonstrated by shifts in primary
auditory cortex neuronal tuning to match the frequency of a conditioned stimulus (Weinberger, 2007; Chavez et al., 2009). Amygdala receives nociceptive information through spino-parabrachial-amygdala projections (Bernard et al., 1996).

Interindividual differences in human pain sensitivity are known from anecdotal and experimental reports, and have been attributed to both genetic (Diatchenko et al., 2005) and environmental factors (Leventhal et al., 1998). Functional imaging has demonstrated that subjective pain experiences are in fact associated with objective neurobiological correlates. This was based on findings showing that pain-sensitive subjects exhibited stronger and more frequent pain-induced activity of the primary somatosensory, anterior cingulate, and prefrontal cortices than pain-insensitive individuals (Coghill et al., 2003).

Research has revealed that cognitive factors influence pain processing, e.g., anticipation of pain substantially modulates the subjective pain experience and corresponding brain events. Expectations of pain are associated with activations in brain areas that also are activated during nociceptive stimulation, in particular the anterior insula and anterior cingulate cortex (Hsieh et al., 1999; Ploghaus et al., 1999; Porro et al., 2002; Koyama et al., 2005). Expectations of more intense pain increased brain activity in several regions, including thalamus, insula, PFC, and ACC. When less intense pain was expected than actually administered, a decrease in both pain intensity ratings and brain activations in the primary somatosensory, insular, and anterior cingulate cortices was observed (Koyama et al., 2005). The attentional state of an individual will also influence
the pain experience and associated brain activation patterns, for example it has been demonstrated that when a subject’s attention was diverted away from a painful stimulus, the intensity of the perceived pain decreased along with activation in SI (Bushnell et al., 1999). Similar experiments have also shown attention-related activity in the ACC, thalamus, IC, periaqueductal gray (PAG) and the orbitofrontal cortex (OFC) (Bantick et al., 2002; Tracey et al., 2002). These results from human imaging studies confirm analogous findings from previous electrophysiological experiments in primates that found lower activity in dorsal horn and thalamic neurons when monkeys attended to non-pain than pain-related stimuli (Bushnell et al., 1984; Bushnell and Duncan, 1989).

Price has organized findings from different categories of pain research, including functional imaging, to create a conceptual, integrated framework that links different brain structures with their putative functions. This framework was principally based upon the “separate” sensory and affective dimensions of pain. Sensory-discriminative aspects would mainly be processed by the primary and secondary somatosensory cortices, while nociceptive impulses terminating in the reticular formation, hypothalamus, amygdala and supplementary motor area would have an arousing effect and activate autonomic and somatomotor systems. Depending on contextual and cognitive factors, the ongoing nociception would be perceived as intruding or threatening and lead to an immediate pain unpleasantness. This unpleasantness, often associated with both the intensity and quality of the perceived sensations, will harbor emotions of distress and fear, which putatively are processed in the insular and anterior
cingulate cortices. In particular, when pain persists for a longer period, extended pain affect will gradually develop. Such affect includes depression and anxiety that result from second order appraisals when pain and the accompanying distress are put into a long-term perspective. Second order appraisals are thought to involve the prefrontal cortex. This framework comprises both serial and parallel nociceptive processing and allows for bi-directional flow between most of its components (Price, 1999, 2000).

Attempts to define pain in neuroanatomical terms have also been criticized, e.g., Sullivan stated: “There is no center of the brain where the pain observer sits; there is no point within the nervous system where interpretation of pain experience begins. There is no location within the organism where nociception becomes pain” (Sullivan, 2001). Presently, it seems almost necessary to “compartmentalize” pain to enable the exploration of scientific questions and hypotheses. However, this must be done without losing sight of pain’s unified experiential nature.

**Emotions**

**Overview**

Across disciplines most researchers tend to view human emotion as an adaptive and protective response, which prepares humans for action (McKay et al., 2009). But, affect (dictionary definition: an observed emotional state, see discussion below) motivates us to play, vocalize and interact with other individuals. Without affect, there would be neither pleasure nor pain, and we
would accordingly not feel “alive” (Panksepp, 2008).

Descartes first used the word emotion in 1649, describing “uproar” as in *les emotions de l’âme*. In English and French the word “passion” was used, denoting mental events involving passivity, where an individual would feel inclinations/desires for a certain behavior passively overcoming him/her, rather than emerging from his/her own initiative as indicated by “action”. Similarly, the Latin word “affectus” (affect) meant an experience that one is affected by. The recurrent concept of passivity indicates that certain feelings and behavioral inclinations intrude on ongoing thought and behavior, persisting over time and trying to gain control (Frijda, 2008).

In the course of an emotional event, appraisal, action readiness, and control precedence are normally linked and involve other components as well, including autonomic arousal and cognitive orientation. “Readiness” refers to the preparedness for action if and when appropriate conditions arise, and if relevant action programs are available in the person’s repertoire. “Action readiness” describes the preparedness to maintain or change one’s relationship with the world or oneself. “Control precedence” indicates that emotions take precedence over ongoing behavior or interference from other sources (Frijda, 2008). Additionally, emotions are reward-insensitive, meaning one performs certain actions, e.g. in hate, knowing that the consequences will be negative. On the other hand, emotions are stimulus-governed, as we feel overwhelmingly attracted to specific stimuli in relation to their perceptual salience, e.g. attraction to unattainable sexual targets (Frijda, 2008). The information that emotional
processes work with can come from the environment, individual bodily processes, representational facts, cognitive schemas and from behavioral skills (Frijda, 2008). Emotional processes can be described on different levels. An intentional/phenomenological level would describe events in terms of feelings, aims, desires and expectations, a psychological/functional level would be concerned with habits, programs, information-processing procedures and memory, and finally, a hardware level would concentrate on neural and neurohumoral mechanisms (Dennett, 1987).

**Historical perspectives – Psychology versus Philosophy**

The concept of emotion has changed through times and, depending on the interpreter’s subject area and level of analysis, still is a matter of hot debate. Laymen often view emotions solely as “feelings” attached to significant states or salient events like “being in love” or anger as in road rage. Such views also regard emotions as having no intentionality (intentionality: to be “about” something, have an object), i.e. as being epiphenomenal. These perspectives go back to “pre-Socratic” time when philosophy started to deal with the nature of emotions and emotions were seen as a threat to “reason” and potentially to philosophy. Based on philosophy’s emphasis on reason in general, reason was viewed as the “master” with emotion as the “slave” being suppressed, channeled or in harmony with reason (Solomon, 2008).

Emotions have been central to philosophical and religious debates of ethics. The Stoics interpreted emotions as misguided judgments about life, making
people miserable. In the middle ages, emotions were linked to desires and self-absorbed desires. The Christian preoccupation with sin focused on exploration of greed, gluttony, lust, anger, envy, pride and sloth as emotions, while love, hope and faith were not classified as emotions, but viewed as the highest virtues and equated with reason. Descartes defined “passions” as perceptions of the soul related to movement in the animal spirits (when the mind and the body meet in the pineal gland). David Hume was ahead of his time when he questioned the dominating role of reason and claimed that our passions motivate us for the right behavior. He defined emotion as a specific sensation (“impression”), which was caused by “ideas” and physically stimulated by the “animal spirits” in the blood. These concepts translate well to contemporary views: “ideas” described the cognitive dimension, the “animal spirits” the physiological and the “impressions” the sensational aspects of emotion. Nietzsche concentrated on the instinctual, less rational motives of the human mind and claimed that the passions had more reason than “reason” itself. Following World War I and the upswing of National Socialism in Germany, reason had its comeback in philosophy, demoting the importance of emotions again (Solomon, 2008).

In North America, William James theorized in 1884 that an emotion is a sensation or set of sensations that is caused by a physiological perturbation, which itself is prompted by some kind of perception. He claimed that emotional behavior, just like all other behaviors emanated in the cerebral cortex. Cannon's work on subcortical mechanisms proved James wrong and modified the views on emotional theory. Around this time philosophy and psychology split as academic
disciplines, and the study of emotions were mainly continued within the domain of psychology. In contrast, in Europe emotions took a more central place, involving Heidegger’s ideas of “moods” as our way of “being tuned” to the world and Sartre’s view of emotions as strategies for coping with a difficult world. Today, most philosophers agree that emotions are ways of coping, whether inherited through natural selection or developed through social interactions, and seek an inter-disciplinary approach to its study (Solomon, 2008).

**Components of emotion**

An emotion generally is thought to reflect a domain of phenomena, encompassing feelings (experiences), behaviors and and bodily reactions (autonomics) (Frijda, 2008). These phenomena have sometimes been described to additionally involve appraisals of events, action tendencies and emotion regulation (Fontaine et al., 2007). Also, affective feelings are normally viewed along three dimensions: valence, which denotes their degree of positivity or negativity; degree of the accompanying arousal; and power, indicating their ability to permeate all mental experience (Panksepp, 1998b).

An emotion is considered a collection of interacting and interchanging processes and not a single entity (Lambie and Marcel, 2002). Emotion processes are therefore not linearly organized, and further depend on secondary processes, as personality and state of the organism (Lewis, 2005). How the different components of emotions are related and organized has been expressed in several hypotheses. The basic-emotion or “categorical” hypothesis would
claim that the various components form fixed, coherent packages. Each package is based on common neuronal and neuro-humoral inclinations that jointly activate the different emotional components (Ekman, 1992; Buck, 1999). The multi-componential hypothesis interprets emotions as unordered collections of components that are activated by individual component propensities and how the emotional event is appraised. Consequently, emotions could occupy almost any position in a multidimensional space with as many dimensions as there are components (Grandjean et al., 2008). A third hierarchical hypothesis views the emotional components to differ in their organizational power (Bradley et al., 2001), supporting the interpretation of different emotions as reflecting specific adaptive resources (Frijda, 2008).

**Unconscious emotions**

The question of unconscious emotions has spiked philosophical debate. It seems that there is some consensus that an unfelt feeling does not make sense, but the fact that we are not always aware that we have a certain feeling or sensation, potentially through automatic “appraisals” or subliminal stimulation, is one possible explanation (Solomon, 1993, 2007, 2008). For example, backward masking experiments have shown that valenced reactions to unconsciously perceived emotional stimuli occur in humans (Zajonc, 1980). Further, while most emotions possess intentionality, it is important to be aware of special cases where the object of an emotion is not its cause. Such causes might include physiological factors like an ingested drug or a surgical stimulation of an area of
Sociology of emotions

Examination of emotions from a sociological perspective emphasizes the constraints put on human behavior and interactions by the individuals’ place in social structures as guided by culture. This means that cognitive appraisals, as well as emotional arousal are influenced by culture and social structure in interaction. The key elements of culture in this context involve appropriate feelings and emotional responses in specific situations; emotional experiences and knowledge that build up over time to be utilized in interaction; emotion vocabularies; and feeling and display rules (Stets and Turner, 2008).

The ability to display accepted and expected behavior to emotional stress in social situations is heavily linked to the individual’s capacity to regulate emotions. Gross’ process model of emotion regulation separates antecedent-focused from response-focused processes. Antecedent-focused emotion regulation is thought to take place either on a subconscious level or before the fully fledged emotion has established itself, and is able to change experience, autonomic reactivity and behavior of an event. However, a response-focused process, which denotes conscious re-appraisal of a situation after full experiential and autonomic manifestations, is only able to modify the related behavior (Gross, 1998).
Affect versus emotion

The words affect and emotion are often used interchangeably, but even when used to describe different entities the interpretation varies between authors. However, most of the time definitions of these terms are omitted. One approach views affective responses as evolutionary primitive signals able to activate the autonomic nervous system without major cognitive activity preceding the arousal. This would be the case when speed, intensity and/or duration of a stimulus occur at a high level and encompass distress, startle and surprise responses. In contrast, emotional responses would require higher-order cognitive-evaluative processes and would encode a plan of action. Appraisals and planning are thought to occur after as well as during the experience of an emotion (Stein et al., 2008).

Another approach would view affect as an overarching concept, encompassing raw/reflexive affective reactions, emotions, moods and affective traits/personality. Raw affects are evolutionary ancient reaction patterns to valenced biological information (Panksepp, 1998b). Emotions describe the contextual state involving the composite of experiential, behavioral and physiological components following some form of cognitive process added to the raw affect condition. Emotions last from seconds to minutes, may be hours. Mood represents a “protracted” emotional state lasting for months, may be years, as seen in grief/mourning. Affective traits are basic characteristics of an individual’s personality. They have a genetic foundation, but will be modified by experience over the individual’s lifetime (Panksepp, 2008).
**Conceptual differences between affect and cognition**

Panksepp suggests that affect might be more closely linked to the contextual background activities of the brain than cognitive activities that generate the perceptual foreground contents of consciousness (Panksepp, 2003). His list of major differences between affective and cognitive consciousness includes: Sensory-perceptual processes are based on propagation of signals along discrete afferent channels, while affective tendencies are more holistic in nature and associated with state-dependent functions; activity in sensory-perceptual systems is governed by fast acting neurotransmitters, while state-dependent emotional-motivational networks are to a stronger degree under the influence of neuromodulators like neuropeptides; cognitive brain areas supporting sensory-perceptive functions are located in sensory thalamus, posterior parts of the brain and in dorsolateral prefrontal regions, while affective circuitry is associated within midline mesencephalic and diencephalic territories, as well as the cingulate, and ventromedial prefrontal areas (Panksepp, 2003) (Damasio et al., 2000; Goel and Dolan, 2003; Holstege et al., 2003); important characteristics of emotional states are related to inherited genetic processes as a fundamental form of intentionality or intrinsic-action readiness in the form of an emotional motor-action apparatus (“intentions-in-action”), while perceptually-cognitively based “intentions-to-act”, reflect gradually emerging learned forms of intentionality (Panksepp, 1998b); emotional affect may be a fundamental property of broad-scale analog networks in the generation of emotionally based instinctual action-tendencies, while discrete cognitive
activity is more based on digital neuronal processing.

In summary, cognition involves neocortical processing of information derived mainly from inputs through exteroceptive senses. In contrast, affects are not encoded as information, but reflect diffuse global states generated by deep subcortical brain structures and interacting with primitive, poorly mapped viscerosomatic body representations (Panksepp, 1998b). Consequently, emotional state processes are less amenable to computational simulations than cognitive functions.

**Philosophical aspects – consciousness**

From the junction of philosophy of mind and the relatively new research area of affective neuroscience, hypotheses have suggested that organisms’ survival concerns were the foundations of consciousness. These ideas have sprung from the emphasis on neuronal systems that putatively enable phenomenal experience in an unconditional fashion. Such systems are genetically grounded, but refined through environmental influences, and are found in medial and ventral areas of the brain. From a neuro-evolutionary perspective, primary-process affective experience emerged considerably earlier than cognitive processes enabling secondary (generation of thought) and tertiary (ability to think about thoughts and feelings) forms of consciousness. Consequently, cognitive consciousness was built upon complex instinctual emotional action tendencies. The corresponding affective feeling states might have equated to the first forms of consciousness in brain evolution, as they were
the first principle guides to survival of the organism (Panksepp, 2007).

Affective consciousness can be classified into three general types. First, sensory-affects that are exteroceptively driven and reflect positive and negative aspects of objects and events in the environment. Secondly, homeostatic-affects that are interoceptively driven and reflect peripheral body states such as hunger and thirst. And finally, emotional-affects that embody the arousal of instinctual brain action systems in response to major life challenges, including life-threatening situations that might engender fear, but also encompassing strive for life-supporting stimuli and interactions, as seen in explorative, socio-sexual and maternal behavior (Panksepp, 2008).

**Fear and Anxiety**

Fear and anxiety are aversive, activated states focused on threat and involve intense negative feelings and bodily manifestations. Fear characterizes dread of impending danger with a “fight/flight” response. Anxiety is an apprehensive anticipation of future danger characterized by dysphoric feelings, somatic tension and hypervigilance. Thus, fear has a real eliciting stimulus that anxiety does not have. Consequently, fear is considered as a post-stimulus and anxiety as a pre-stimulus condition. Both fear and anxiety have a common origin in unconscious mobilization towards an as yet poorly defined threat. When the situation is appraised, the emotion can be resolved into fear when reflexive or coping options are available, or into anxiety when such options are not available. Fear may be focused on external sources, as in phobias, while anxiety may be
unfocused, as in generalized anxiety (panic or generalized anxiety disorder). Fear and anxiety can be expressed as short lasting emotional states and also as personality traits. Compared to “normal” fear/anxiety, clinical fear/anxiety is a condition more persistent, with higher intensity in relation to the threat that tends to make the affected person helpless and unable to cope, resulting in impeded daily functioning (Barlow, 2000; Öhman, 2008).

From a factor-analysis of self-reported fear, “fears about interpersonal events or situations”, “fears related to death, injuries, illness, blood and surgical procedures”, “fears of animals”, and “agoraphobic fears” emerged as the 4 most prominent factors (Arrindell et al., 1991). All these factors are related to survival considerations, either in a contextual sense or in an evolutionary perspective (Arrindell et al., 1991; Öhman, 2008). Models of the development of anxiety disorders have been created utilizing traditional diathesis-stress concepts. The most elaborate models were generated based on emotion theory and neuroscientific data (Barlow, 2002). A generalized biological vulnerability encompasses underlying genetic make-up and is based on data showing substantial genetic contribution to traits like anxiety and negative affect (Clark et al., 1994). A generalized psychological diathesis to experience anxiety and related negative affect in general is thought to result from early learning experiences that create a sense of relative uncontrollability in challenging and stressful situations (Nowicki and Strickland, 1973). The co-occurrence of generalized biological and psychological vulnerabilities could under certain stressful conditions produce anxiety disorders, in particular generalized anxiety
disorder. However for the more specific types of anxiety disorders like panic disorder or social phobia, the additional presence of a third and specific psychological vulnerability seems necessary. This diathesis is based on early learning experiences that focus anxiety on certain life circumstances like interoceptive, social or thought processes (Barlow, 2000).

Mostly, fear and anxiety are highly adaptive processes with an obvious evolutionary value. To determine what constitutes pathologic anxiety requires an understanding of behaviors and emotions within the normal range. Some states of anxiety coincide with developmental processes associated with growth, and is not considered pathologic. Consequently, the notion of what deviates from the norm changes over the lifespan of individuals, and the study and classification of anxiety disorders require a developmental perspective on psychopathology (Whiteside and Ollendick, 2009).

Standard diagnostic systems discriminate between different diagnoses for anxiety disorders (American Psychiatric Association, 2000). This classification seems helpful to, at least broadly, collect conditions with similar symptoms and to some degree similar treatment approaches within a uniform category. However, based on pronounced comorbidity between these conditions, some psychologists render the distinctions between them not very important (Bentall, 2007). Also, in recommendation for the upcoming fifth version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V), published by the American Psychiatric Association, a change of the present taxonomy of anxiety disorders has been suggested. General anxiety disorder (GAD) and posttraumatic stress disorder
(PTSD) would potentially be grouped with depression under the label “distress disorders”. Obsessive-compulsive disorders (OCD) would constitute a separate group of obsessive-compulsive spectrum disorders. The rest of the “traditional” anxiety disorders would be grouped together as “fear disorders”. These suggestions are based on several recent findings, especially from factor analytic studies (Slade and Watson, 2006; Gamez et al., 2007; Antony et al., 2009).

Current classification systems of psychopathology are mainly based on descriptive features like signs and symptoms. As in many other fields of medicine, this has led to a call for a mechanism-based classification system to promote insight into the underlying etiological and/or pathophysiological factors. Undoubtedly, such a classification system would improve prevention, diagnosis and treatment of the different conditions, but due to lack of comprehensive disease models, presently this may be an extremely difficult task (Taylor et al., 2009).

It has been suggested that trait anxiety be conceptually distinguished from state anxiety. Trait anxiety would reflect an individual’s predisposition to respond, while state anxiety would be viewed as a transitory, contextual emotion (Spielberger, 1983). Analogous to a transition from a categorical to a dimensional view of emotions in general, the multidimensional interaction model of stress, anxiety and coping recommends a more complex perspective on anxiety than a categorical concept will allow (Endler, 1997). The model involves person variables like traits (including trait anxiety) and physiological and biological components. It also encompasses situation variables, including
stressful events and physical environments. Person variables can interact with one another, as can the situation variables. Further, person variables interact with situation variables leading to a perception of threat that changes state anxiety. The change in state anxiety will generate behavioral and biological reactions, including coping responses. There will also be a feedback from the reactions to both the person and situational variables (Endler, 1997; Endler and Kocovski, 2001).

Low-grade anxiety has been associated with an adaptive advantage. This has been seen in performance psychology where the most favorable level of fear/anxiety/arousal is not confined to extremes, but is of moderate magnitude as demonstrated in animal (Yerkes and Dodson, 1908), and humans (Tecce, 1965; Oxendine, 1970) studies. Recently, data have also suggested that moderate anxiety is linked to lower mortality (Mykletun et al., 2009). Almost no data exist related to low-grade anxiety, and a need for further investigation of this phenotype is desirable. The present project will explore perceptual, physiological and functional brain imaging parameters of healthy, non-treatment-seeking individuals with elevated measures of anxiety and matched non-anxious controls in an experimental pain paradigm.

Motivation

Both emotional feelings and behavior do compellingly indicate their important association with desires and urges. This motivational aspect of emotions has been lost from focus in recent emotional theories (Frijda, 2008).
Regulatory approaches to motivation focus on internal states and biological motives as hunger or fear. On the other hand, purposive approaches concentrate on goals. Organisms work to reduce biological motives, but pursue goals. The two major motivational variable-classes are desire and aversion. Desire is a preference for a behavior whose outcome is preferred to a neutral outcome. Aversion is a preference for a behavior whose outcome is less preferred than a neutral outcome. The hedonic principle states that organisms work to minimize aversive and maximize desirable outcomes (Beck, 2004). In the field of operant conditioning a desirable outcome is a reinforcer, while an aversive outcome is a punisher. Accordingly, any stimulus following a behavior that increases the probability that the behavior will occur again is defined as a reinforcer, while if the probability of re-occurrence decreases is defined as a punisher. Rewards are considered reinforcers (Beck, 2004). Therefore, reward values related not only to pleasure (Cabanac, 1979), but also to fear- (Miller, 1948) and pain- (Leknes et al., 2006) reduction, are considered reinforcers able to motivate behavior to ensure survival and establish homeostasis (Leknes and Tracey, 2008). Both the opioid and the dopamine system have been implicated in these processes (Fanselow, 1986; Fields, 2007). In general, motivational states are triggered by events as appraised and conceptualized as urges that lead to impulsive actions, i.e., they are not the results of planning or foresight and also differ from automatic/habitual behavior (Frijda, 2008). From the neuroscience perspective, motivational processes can be viewed as gain-modulators of decision-making processes (Kouneiher et al., 2009) and are based
on the reward values of different action sets and not cognitive demands (Rushworth et al., 2004). The “optimal level of arousal hypothesis” has influenced motivation theories and states that optimal performance is achieved through the most favorable level of arousal and is illustrated as the peak of the Yerkes-Dodson curve – an inverted U-curve (Yerkes and Dodson, 1908). Early work has shown that this favorable level of arousal, anxiety or drive is normally not confined to either extremes, but is of moderate magnitude and has been demonstrated in animal studies (Yerkes and Dodson, 1908), and for humans, in the areas of problem solving (Tecce, 1965) and motor performance (Oxendine, 1970).

**Neural correlates of emotion**

Traditionally, animal and lesion studies have been instrumental in the research on neurobiological aspects of emotion, but recently neuroimaging investigations in humans have provided additional data. The following is a brief overview of findings derived from different types of studies, including a large and recent meta-analysis of the neuroimaging of emotion (Wager et al., 2008b). Also, other paragraphs in this introduction contain complementary information.

Brainstem periaqueductal gray (PAG) coordinate physiological and behavioral responses to threat consistent with defensive-aggressive emotional responses (Bandler and Shipley, 1994). Because it receives projections from cortical regions like ACC, insula and the PFC, PAG is thought of as an integrative emotional center (Panksepp, 1998a). PAG is consistently activated in
neuroimaging studies of emotion, in particular related to negative emotions (Wager et al., 2008b).

The hypothalamus is involved in the regulation of motivated behavior and homeostasis via autonomic and endocrine functions (Sewards and Sewards, 2003). The medial and intralaminar thalamus has been correlated with emotional and affective processes. Neuroimaging has demonstrated hypothalamic activations, mainly associated with positive emotional experiences and thalamic activity that partly correlated with affect (Wager et al., 2008b).

The amygdala is important in the evaluation of the relevance of sensory cues and directs the organism toward further cue exploration (Davis and Whalen, 2001). This corresponds to human neuroimaging findings, associating amygdala activation with the salience/information value of visual stimuli (Liberzon et al., 2003). The hippocampus’ role in emotional behavior is thought to be memory-related and therefore important in contextual fear conditioning in rodents (Maren et al., 1997). In humans, emotion-related neuroimaging activations of the hippocampus seem to be associated with perceptual rather than experiential events (Wager et al., 2008b).

The basal ganglia are important for planning and initiation of motivationally relevant behaviors. The ventral part, in particular nucleus accumbens, is involved in motivation, reward and learning (Berridge, 2004). Dopamine signaling in the related network is thought to reflect motivated behavior rather than hedonic/reward responses (Berridge and Robinson, 1998). The ventral striatum is among the most frequently activated brain areas in human emotion
Orbitofrontal cortex activity is related to the generation and regulation of affect (Beer et al., 2003) and physiological output (Roberts et al., 2004). Subgenual anterior cingulate and the ventromedial prefrontal cortex are associated with visceromotor control (Vogt et al., 1992) and generation and regulation of stress responses (Sullivan and Gratton, 2002). Prefrontal regions are able to regulate emotions in a goal and strategy-dependent manner (Ochsner et al., 2004; Quirk and Beer, 2006), and self-focused regulatory strategies and inhibition of negative emotions are correlated with activity in the prefrontal cortex (Levesque et al., 2003; Ochsner et al., 2004; Phan et al., 2005; Ohira et al., 2006). Dorsal anterior cingulate cortex has also been linked to affect-related functions (Devinsky et al., 1995). In neuroimaging of emotion, parts of all these areas are activated. Medial OFC and vMPFC seem to be biased towards positive emotion (Wager et al., 2008b).

The anterior insula has been associated with visceromotor control (Yasui et al., 1991) and interoception (Craig, 2002). Its ventral portion is involved in sensory-affective processing and in neuroimaging studies implicated in positive emotion-related activity (Wager et al., 2008b). However, the dorsal portion of the anterior insula is more related to cognitive types of processing (Wager et al., 2004) and associated with negative emotions in neuroimaging investigations (Wager et al., 2008b).

A recent meta-analysis of functional neuroimaging findings in patients with PTSD, social anxiety disorder and specific phobia demonstrated that individuals
suffering from any of these disorders consistently show greater activity than matched control subjects in the amygdala and insula, brain areas that have been associated with negative emotional responses (Etkin and Wager, 2007). A similar pattern was also observed during fear conditioning in healthy subjects. Increased activity in the amygdala and insula were more frequently observed in social anxiety disorder and specific phobia than in PTSD. However, only PTSD sufferers showed hypo-activation in the dorsal/rostral anterior cingulate and the ventromedial prefrontal cortices, brain regions that have been linked to experience and regulation of emotions (Etkin and Wager, 2007).

Models of disease

Overview

Biological predispositions have been considered part of the etiology of disease as far back as Hippocrates (Monroe and Simons, 1991). Plato also emphasized psychological factors as being involved in etiology, e.g., he believed that pain was a product not only of peripheral stimulation but that emotions in the soul and the heart also played a significant role (Bonica, 1990). During the Renaissance, the holistic ideas of medicine formulated by the ancient Greeks changed. Driven by new findings in human anatomy and physiology, and later (19th century) in microbiology, the notion that the mind was able to influence the body was viewed as unscientific. The mind was deemed unnecessary to explain physical processes, and its study was entrusted to philosophers and theologians. The French philosopher René Descartes was a famous advocate of this mind-
body dualism, a dualism that became the foundation of the biomedical reductionist disease model (Gatchel, 1999). This “mechanistic” approach to illness dominated western medicine until the late 19th century when Sigmund Freud’s work brought renewed attention to the role of psychological states. In the 20th century, integration of mind and body started slowly to emerge again (Gatchel, 1999).

By the mid 20th century, studies of the predisposition for or the tendency to suffer from a specific medical condition (diathesis) concentrated on genetic vulnerabilities (Falconer, 1965). Later, genetic markers both for organic (Newman and Liu, 1998) and psychiatric (Silverman et al., 1993) diseases were detected. The impact of environmental stimuli on the development of disease started to be explored around the same time (Selye, 1956). Such environmental influences were conceptualized as stress. Stress is defined as “the physiological, psychological, or mental influences that exert pressure on the organism, taxing or exceeding its capacity to respond” (Lazarus and Folkman, 1984). Research in psychophysiology (see later) and psychoneuroimmunology both deal with how stress makes the organism more susceptible to illness (Ader et al., 1995; Glaser et al., 1999). Originally, diathesis and stress were viewed as separate processes in the etiology of disease, with either one responsible for the development of a given condition. In the 1960s, the two entities were unified into the so-called diathesis-stress model of disease, that state that the onset of an illness results from an interaction between biological diatheses and environmental stress (Spaulding, 1997). This was the original model for
schizophrenia (Bleuler, 1963) but has since been applied to medical conditions like heart disease (Harshfield and Grim, 1997) and diabetes (Lehman et al., 1991).

Originally, diatheses were thought of as physical and genetic predispositions. That changed in the 1970s to also include psychological and social elements when the biopsychosocial disease model was introduced (Engel, 1977). This model is powerful in that it regards an illness as a complex interaction between biological, psychological and social elements. The model also put an end to previous unfortunate conventions of medical practice. Adhering to the reductionist view, medical practice would generally only ascribe a causal role to psychological factors when physical findings were absent or no other reasonable explanation for symptoms could be found. In such cases, the patient would typically be labeled as a psychiatric case (Gatchel, 1999).

Models of pain

Late 19th century models of pain were strictly biomedical approaches that, although partly supported, could not adequately explain pain, as they did not account for any psychosocial input. Von Frey's specificity theory of pain proposed specific peripheral and central mechanisms analogous to other bodily senses. Goldschneider’s pattern theory of pain hypothesized different sensational qualities based on differences in patterning and quantity of nerve impulse discharges (Gatchel, 1999).
In the 20th century, it was observed that only 25% of soldiers in combat hospitals reported pain and requested analgesic medication, while the corresponding proportion of civilian patients with comparable wounds was more than 80%. This was seen as an indication that psychological factors could markedly affect pain (Beecher, 1956). Inspired by those findings, Ronald Melzack and Patrick Wall proposed the gate control theory of pain (Melzack and Wall, 1965). The theory proposed a model for modulating afferent pain signals at the dorsal horn level, either through other sensory nerve impulses or through descending activity from higher centers. Furthermore, pain was not conceptualized as a simple sensory event but as a perceptual process resulting from a complex interaction between emotional-motivational, cognitive-evaluative and sensory-discriminative components. The gate control theory reflected the ideas of the period and the emerging biopsychosocial disease model and led to a paradigm shift in the discipline, influencing clinical diagnostic and treatment procedures as well as pain research. The theory was modified and extended into the neuromatrix theory of pain in the subsequent decades (Melzack, 1990, 1993, 1999). This neuromatrix theory applied findings from neuroscientific research to the area of pain. The pain neuromatrix was postulated to be a neuronal network consisting of somatosensory, thalamocortical and limbic structures that produced “neurosignature” output patterns that would activate perceptual, homeostatic and behavioral programs. A “neurosignature” output pattern was thought to be the individualized and contextual neuronal activation of these effector systems, based on genetic neuronal control and primary and secondary affective, cognitive
and sensory inputs. Because of Melzack’s and Wall’s work, pain was viewed as a multidimensional construct and came to be defined as such by the IASP (Merskey and Bogduk, 1994).

To account for the discrepancy in perceived pain between soldiers and civilians with comparable wounds (Beecher, 1956, 1959), as well as the function of the newly discovered endogenous opioid peptides (Hughes, 1975; Hughes et al., 1975a; Hughes et al., 1975b), the perceptual-defensive-recuperative model of fear and pain was created (Bolles and Fanselow, 1980). This model explained the time course of perceived pain following injury as the product of differential interaction/competition between mutually inhibitory fear- and pain-motivational systems. It was hypothesized that the fear-motivation system would induce defensive behaviors and facilitate the perception of environmental events, including threat and safety. On the other hand, the pain-motivation system would activate recuperative behaviors to promote healing and recovery. It was proposed that fear led to endorphin release, which inhibited pain and recuperative behaviors that otherwise would compete with optimal defensive and attentional performance.

Building on the ideas of the gate control theory, but also on research in cognitive psychology that studied how subjects in pain interact with environmental influences, a comprehensive diathesis-stress model of chronic pain called a psychobiological model was introduced (Flor and Turk, 1984; Turk and Flor, 1984). This model states that predisposing factors like genetic variables, previous trauma or social learning result in a stereotypical pattern of
psychophysiological responses within the affected body system. Genetically based diatheses include congenital pain-insensitivity (Indo et al., 1996; Nagasako et al., 2003) and interindividual differences in pain sensitivity linked to variability in catechol-o-methyltransferase enzyme expression (Diatchenko et al., 2005; Nackley et al., 2007) and, pain inhibitory systems (Mogil et al., 1996; Uhl et al., 1999). Learned predispositions encompass coping mechanisms based in childhood observation of how parents and others interpret and react to various physical symptoms (Leventhal et al., 1998). Pain-related or negative psychosocial stressors would act as eliciting stimuli, activating the autonomic nervous system with resulting nociception-inducing processes and subjective and objective pain responses. These stressors also can serve as unconditioned or conditioned stimuli, with their impact exacerbated by maladaptive and/or deficient behavioral, cognitive or physiological responses. Such responses were coined eliciting responses, as they were also considered capable of inducing and maintaining pain. Among eliciting responses, we find inadequate coping skills (Jensen and Karoly, 1991; Martin et al., 1996; Snow-Turek et al., 1996; Severeijns et al., 2001), misinterpretation of bodily symptoms (Serlie et al., 1995; Serlie et al., 1996; Whitehead and Palsson, 1998), pain anticipation (Price et al., 1999; Koyama et al., 2005), pain memory (Eich et al., 1985), and lack of self-efficacy (insufficient belief that one can successfully execute a course of action to produce a desired outcome in a given situation) (Bandura, 1977; Jensen et al., 1991). Maladaptive pain behaviors can be maintained by anticipation or fear of pain or injury like in respondent conditioning (Schneider et al., 2004) and/or by
receipt of positive and avoidance of negative reinforcers as in operant conditioning (Romano et al., 1992; Turk et al., 1992). Respondent learning is mainly associated with pain-eliciting stimuli, while operant mechanisms are predominantly related to pain-related responses. Frequent problems associated with long-standing pain, of which many are related to different types of learning scenarios and serve to exacerbate and/or maintain the original condition, include: sleep disruption, increased use of nicotine and caffeine, legal and illegal drug abuse, de-conditioning due to inactivity, poor diet, and loss of employment and income. Consequences are often secondary musculoskeletal problems and a deteriorating immune system with development of comorbidities. Unfortunately, pain patients’ histories often also include failed medical and surgical treatments with resulting anger, mistrust and isolation. The psychobiological model allows for complex and non-linear dynamic interactions between physiological, psychological and environmental components necessary to create a comprehensive understanding of pain. This model was originally created to explain chronic musculoskeletal pain but has since been adapted to other kinds of pain, including acute pain.

It is important to emphasize the potential bi-directional impact that exists between comorbid affective and physical disorders (Cohen and Rodriguez, 1995). Research has suggested that persistent pain may negatively influence cognitive (Grigsby et al., 1995) and emotional (Baliki and Apkarian, 2007) processing and presumably result in associated structural (Baliki and Apkarian, 2007) and functional (Baliki et al., 2008) brain changes.
The comprehensive bio-psychosocial and -behavioral concepts place the patient in the center within a holistic framework. These concepts acknowledge that a unidimensional biomedical approach is not only insufficient to explain pain, but also insufficient for assessing and treating pain (Turk and Rudy, 1986). Consequently, a multidimensional approach to pain can more easily account for interindividual differences in pain report and treatment responses and substantiates the need for a more personalized medicine. Accordingly, a multidimensional approach to pain research is inevitable too. Its goal would be to improve diagnostic routines and facilitate the development of new pharmacological and non-pharmacological pain treatment strategies, as well as refining existing human pain models.

**Pain and personality**

In the field of pain, the influence of behavioral medicine and psychology followed largely that of medicine in general. Mid 20th century, G. L. Engel observed patients with atypical facial pain and concluded that no pain exists without involvement of central nervous structures and, in spite of having a physiological basis, that pain was primarily a psychological phenomenon (Engel, 1951). Those observations led to the creation of several pure psychogenic models of pain (Engel, 1959; Blumer and Heilbronn, 1982; Beutler et al., 1986). The models were conceptually similar, assuming that a combination of certain negative psychological characteristics (including depression and denial of emotional problems combined with a family history of depression and chronic
pain), would render the affected individuals especially prone to pain disorders. Thus pain could develop solely by psychological mechanisms, devoid of any physiological input. Some proponents took these psychogenic models to actually describe a pain-prone personality, i.e., a collection of specific traits making a person susceptible to pain disorders (Gatchel and Weisberg, 2000). These views and hypotheses have later been scrutinized and found flawed and in lack of empirical evidence (Turk and Salovey, 1984). Personality profile differences between chronic pain patients and controls may be linked to the chronic illness itself and its consequences rather than to any primary psychopathology (Love and Peck, 1987; Vendrig, 2000). Accordingly, to account for relationships between personality and pain, a *diathesis-stress model of personality disorders in chronic pain* was developed. This model postulated a latent disposition for a personality disorder that under the stress of daily life is kept dormant. However, subsequent to physiological, psychological and social stress related to a painful incident, the organism’s coping ability would be overburdened, resulting in the precipitation of a personality disorder. Such a disorder would then be able to aggravate the chronic pain condition, potentially creating a “vicious circle” (Weisberg and Keefe, 1999; Gatchel and Weisberg, 2000). Consequently, although no obvious causal relationship between personality and pain has been found, this does not mean that personality traits are unimportant for a person’s experience of pain, how a pain disorder progresses or how it needs to be treated (Gatchel and Weisberg, 2000).
Pain treatments that target psychosocial factors

Existing treatments of pain that target behavioral and psychosocial components are, at least to some extent, thought to work by reducing pain-associated unpleasantness (Dellemijn and Fields, 1994; Molton et al., 2007). Such treatments mainly encompass cognitive-behavioral and pharmacological therapies. Cognitive treatment aims at empowering the patient with tools to control his/her cognitive, affective, behavioral and physiological responses through distraction (McCaul and Malott, 1984) and cognitive restructuring (Gil et al., 1990). Such control has been linked to reduced perception of pain and decreased functional disability (Jensen and Karoly, 1991; Tota-Faucette et al., 1993). The objectives of behavioral treatment approaches are to decrease maladaptive and encourage adaptive behaviors (Keefe et al., 1986), thereby increasing the sense of personal competence. This can be achieved by alternating physical activity with rest (Gil et al., 1989) and by implementing appropriate social reinforcements (Keefe et al., 1996). Non-pharmacological methods to modify physiological dysfunction and response systems include relaxation techniques (Carroll and Seers, 1998), biofeedback (Crider and Glaros, 1999) and hypnosis (Jensen and Patterson, 2006). Pharmacological options of non-analgesics include benzodiazepines, as primary anti-anxiety drugs, which have been shown to provide pain relief (Dellemijn and Fields, 1994; Huffman and Stern, 2003).
Pain and emotions

Emotional aspects of the pain experience reach from its intrinsic discomfort to its immediate and/or protracted secondary pain affects, including experiential, behavioral and autonomic consequences (Doan and Wadden, 1989; Philips and Grant, 1991; Price, 2000). Potential antecedent negative affective states and/or genetic susceptibilities are generally thought to predispose, precipitate or exacerbate pain or pain reaction patterns (Beck and Siegel, 1980; Flor et al., 1985; Magni et al., 1994; Carroll et al., 2004; Larson et al., 2004; Roy-Byrne et al., 2008) or contribute to pathology that in itself could be painful (Szekely et al., 2007; Thayer et al., 2009). Anticipation of pain may also lead to emotional distress (Sullivan et al., 2001a; Sullivan et al., 2001b). In reality, it may be impossible to unravel the interconnectedness of pain and associated affective conditions, and, in most clinical instances, it would be unimportant (Wiech and Tracey, 2009). The complex interplay between pain and cognitive-emotional factors is highly context dependent and dynamic, as the differential impact of those factors change over time. Both pain and emotion can be conceptualized as multidimensional constructs that have reciprocal influences on each other and that sometimes overlap (Craig, 2006). The affective dimension of pain is difficult to communicate and more complicated and laborious to assess than the sensory dimension. The latter is, of course, especially limiting in non-human studies. Consequently, in both clinical and research situations, more data have been gathered about pain’s sensory than its emotional aspects. This is true regarding pain-psychology, -physiology and -neurobiology (Craig, 2006).
The different contemporary pain models that have been described were all important because they integrated sensory with affective and cognitive elements. Nevertheless, a sensory-affective dichotomy has been prominent in frameworks exploring physiologic and neurobiologic aspects of pain, where theories have focused on afferent nociceptive pathways and their brain targets. The phylogenetically recent neospinothalamic tract to the ventrobasal thalamus and the somatosensory cortex has been thought to represent the neural basis for the sensory-discriminative dimension of pain. The evolutionary ancient paleospinothalamic or “paramedian” pathway to the medial and intra-laminar thalamus, the limbic system and the frontal lobe, together with spinoreticular, reticulothalamic and spinohypothalamic projections, was thought to represent the neural substrate for the affective component of pain, including pain-related motivational drive and behavioral arousal (Melzack, 1999). It has been found that affective states can influence the unpleasant aspect of pain with little or no change in pain intensity (Villemure and Bushnell, 2002; Villemure et al., 2003; Villemure and Bushnell, 2009). Neuroimaging data also support a dichotomy between affective and sensory dimensions of pain (Rainville et al., 1999). When the sensory dimension of pain was manipulated through hypnotic suggestions, a change in SI activity was observed (Hofbauer et al., 2001). However, hypnotically induced changes in pain’s affective dimension correlated with ACC activity (Rainville et al., 1997).

The effect of emotional factors on pain has been investigated mainly through experiments where specific moods have been induced in healthy
participants or through the study of pain patients exhibiting mood disorders. Mood induction paradigms have become very prevalent, not only in pain research, but also in the realm of psychology research in general. Such paradigms claim to evoke mood states through picture viewing (de Wied and Verbaten, 2001; Meagher et al., 2001), olfactory stimulation (Villemure et al., 2003), music (Tang et al., 2008), film clips (Zillmann et al., 1996; Weisenberg et al., 1998) or by reading or listening to written statements (Zelman et al., 1991). A reduction in pain perception has been observed when positive mood was induced (Zelman et al., 1991; Zillmann et al., 1996; Weisenberg et al., 1998; Meagher et al., 2001). The opposite was true when negative mood was elicited in the participants (Weisenberg et al., 1984; Cornwall and Donderi, 1988; Rhudy and Meagher, 2000; Wunsch et al., 2003; Kenntner-Mabiala and Pauli, 2005). These findings have been explained by the motivational priming theory, proposing that emotional experiences are governed by appetitive and defensive systems that become activated by rewarding and adverse stimuli, respectively. The activated appetitive system would generate positive emotions while the defensive system evokes negative emotions, what would further facilitate future similarly valenced responses from those systems (Lang, 1995).

Although most studies show that human experimental pain is perceived as more intense when concomitant negative moods are being induced, decreased pain perception under the same circumstances has also been observed (al Absi and Rokke, 1991; Rhudy and Meagher, 2000, 2003). These findings are often labeled paradoxical, either because they are difficult to explain, or because they
challenge general opinions and complicates reductionistic thinking. Reduced pain perception when nociceptive stimuli are delivered to an individual exhibiting fear/anxiety has generally been explained by an interaction between emotions and the degree of associated arousal (Rhudy and Williams, 2005). Hence, when especially strong negative affect is evoked by severe threat, it is generally accompanied by a high level of arousal and lowered pain sensitivity. This phenomenon has been labeled stress-induced analgesia (Flor and Grusser, 1999). A valence-by-arousal interaction in emotional control of nociceptive reactions has been confirmed (Rhudy et al., 2008), but the concept of stress-induced analgesia appears fairly vague. As conditions of stress-induced hyperalgesia also exist (Martenson et al., 2009), it seems obvious that the interaction term needs to be expanded by at least one additional factor to explain how fear/anxiety modulates pain perception. One such factor might be the contextual relevance that fear/anxiety has to pain. If anxiety can be attributed to pain, a synergistic effect between them is likely, with hyperalgesic responses sustaining attention to the pain state as the contextually predominant emotional-motivational condition. If fear/anxiety is directed at non-pain-related objects with a stronger motivation than the competing pain-related motivation, suppression of the pain experience will result, facilitating anxiety-related behavior (Weisenberg et al., 1984; al Absi and Rokke, 1991). The latter concept was proposed as the, previously described, perceptual-defensive-recuperative model of fear and pain (Bolles and Fanselow, 1980). The concept is consistent not only with clinical (Beecher, 1959; Melzack et al., 1982) and pharmacological (Fields, 2004; Fields,
2007) observations but also with human experimental findings (al Absi and Rokke, 1991). This hypothesis also conforms to experimental and clinical data showing decreased pain perception when attention is diverted away from pain and increased pain perception when pain is the focus of attention (Villemure and Bushnell, 2002).

Few neuroimaging investigations of experimental emotion and pain interactions have been conducted. Cingulate and orbitofrontal cortex activity is correlated positively with trait-like fear of pain (Fear of Pain Questionnaire utilized), suggesting that putative biological adjustments in brain areas associated with cognitive-emotional pain processing are dependent on affective dispositions (Ochsner et al., 2006). Furthermore, anticipatory anxiety for pain exacerbated pain intensity ratings, and it was correlated to entorhinal cortical activity. These areas have been linked to amplification of aversive events in the priming of behavioral responses that are adaptive to the worst possible outcome. Entorhinal activity further predicted perigenual anterior cingulate cortex (ACC) and mid-insula activity, regions implicated in affective and sensory-autonomic processing, respectively (Ploghaus et al., 2001). Cortical-subcortical circuitry, involving the prefrontal cortex, nucleus accumbens and the amygdala has been associated with effective emotion regulation (Wager et al., 2008a), and might further represent substrates for affective modulation of the pain experience (Wiech and Tracey, 2009).

A recent meta-analysis has revealed considerable overlap between pain- and emotion-related ACC activations (Beckmann et al., 2009). Another
comprehensive review of ACC functional anatomy found overlap mainly between the correlates of pain and fear processing in the ACC (Vogt, 2005). In pain neuroimaging, ACC activity correlates with affective pain processing (Rainville et al., 1997; Tolle et al., 1999; Phillips et al., 2003). In psychopathology, aberrant ACC activity has been observed in PTSD (Liberzon and Martis, 2006), depression (Drevets et al., 2008) and anxiety disorders (Etkin and Wager, 2007). The ACC has been associated with a myriad of putative functions, including conflict monitoring (Rushworth et al., 2007b), reward processing (Rogers et al., 2004) and evaluation of social relevance (Rushworth et al., 2007a; Rushworth et al., 2007b). Together with ventromedial prefrontal regions, the ACC therefore seems to play a major role in decision-making and related cost-benefit processing as part of goal-directed action selection (Walton et al., 2004; Kennerley et al., 2006; Rushworth et al., 2007a; Walton et al., 2007). These functions are highly relevant to pain-emotion processing, especially in situations where competition between different motivational states is present (Fields, 2004).

Traditionally, the insula has been separated into a posterior, sensory part (Fairhurst et al., 2007) and a limbic-related anterior portion (Craig, 2009). Data suggest that the anterior insula is implicated in cognitive evaluation and subjective feeling states (Kong et al., 2008), magnitude assessment (Baliki et al., 2009) monitoring and signaling of interoceptive sensations (Craig, 2002, 2009). Negative emotions, including anxiety, may induce or enhance interoceptive awareness (Salovey, 1992), as expressed by anxiety sensitivity – the tendency to interpret visceral sensations as threatening (Reiss et al., 1986). Anxious
individuals exhibit increased anterior insula activity during emotion processing (Stein et al., 2007), anticipatory anxiety correlates with right insular activity (Simmons et al., 2004) and anxiolytic medication is able to reduce activity in the anterior insula during emotion processing (Paulus et al., 2005). It has been suggested that the anterior insula is capable of creating predictive signals that represent an expected body state, and that anxious individuals may exhibit an increased signal of the difference between experienced and expected states (Paulus and Stein, 2006). All the discussed putative functions of the anterior insula would also apply to pain processing, as pain encompasses affective elements, is often related to anticipatory scenarios, and as knowledge of pain magnitude is crucial for executive action. Pain will further impact homeostatic processes and has even been conceptualized as a homeostatic emotion (Craig, 2003a). Nevertheless, it seems reasonable to assume that depending on the motivational and attentional power of internal or external events, their related integrative insular processes will differentially contribute to homeostasis and survival.

Epidemiological studies of pain in clinical mood disorder populations have generally shown a fairly high level of comorbidity between pain and post-traumatic stress disorder (PTSD) (Gureje, 2008) or major depressive disorder (Bair et al., 2003). Although these data have been interpreted as increased pain sensitivity in mood disorder patients (Wiech and Tracey, 2009), experimental data within this cohort have demonstrated more variable findings. For example, when PTSD sufferers are compared with non-PTSD controls, they generally
demonstrate increased sensitivity to noxious stimuli (Asmundson et al., 2002). However, when trauma-related stimuli are presented to the PTSD group, these individuals exhibit lower pain sensitivity than the control subjects (Pitman et al., 1990). In major depression, patients demonstrate normal to reduced pain sensitivity to noxious skin stimulation (Dickens et al., 2003; Bar et al., 2006) but hyperalgesic responses to ischemic muscle pain (Bar et al., 2005). To explain the diversity in these findings, it is tempting to apply a similar explanation to the one given previously for the disparate experimental data. Thus, trauma-related stimuli may prompt a vivid recall of life-threatening circumstances in PTSD sufferers that shifts attentional and motivational resources from pain to non-pain events. Consequently, to ensure undivided attention to the traumatic stimuli, which for the PTSD sufferers represent the most significant threat, analgesic systems are activated to suppress the competing pain experience. It is not important that pain is a real-time experience while trauma is re-lived in memory, as intrusive memories are one of the hallmarks of the disease (North et al., 2009). Attentional mechanisms may explain the findings in the group of major depressive patients as well (Dickens et al., 2003). Certain superficial forms of pain may not be able to compete with the depressive state itself and are kept under control by analgesic mechanisms. However, severe or unpleasant deep pains might be more intrusive and able to capture the subject’s attention, driven by hyperalgesia.
Epidemiology of psychopathology in chronic pain

Psychopathology occurs frequently among chronic pain sufferers as between 32% and 82% of pain clinic patients exhibit some kind of mood disorder (Sullivan et al., 1992). For example, a chronic pain population exhibited 30% - 54% prevalence of depression (Banks and Kerns, 1996), which in a comparable group was four times larger than in the general population (Sullivan et al., 1992; Magni et al., 1993). Measures of anxiety have also been found to be markedly elevated in chronic pain patients (Snibbe and Peterson, 1980), with anxiety accounting for 16%-54% of variance in pain report, disability and pain-related behavior (McCracken et al., 1996). Rates of psychopathology exceeding the level in the general population have been demonstrated in individuals exhibiting not only chronic but also acute pain (Gatchel et al., 1996) and underscore the importance of the cognitive-psychological elements in all aspects of pain.

Personality disorders are also very common in the chronic pain population (Reich et al., 1983; Reich and Thompson, 1987; Polatin et al., 1993; Gatchel et al., 1994; Gatchel et al., 1996; Weisberg and Keefe, 1999) often overlapping with mood disorders (Flick et al., 1993). Their prevalence ranges between 31% and 59% (Weisberg and Keefe, 1999), which is well above the general population rate of approximately 6% (Samuels et al., 1994).
Methodology

Functional brain imaging and analyses

Functional magnetic resonance brain imaging (fMRI), which is the functional imaging method utilized in the present work, is a brain mapping technique that has the practical advantage of being non-invasive and non-radioactive and the scientific advantage of being able to map global brain activity. FMRI provides a reasonable balance between spatial and temporal resolution and is therefore applicable to a variety of experimental questions (Bandettini, 2009b). The method’s temporal resolution, which is in the range of seconds, is better than that of positron emission tomography but not as good as that of magnetoencephalography. Electrophysiological methods have superior spatial and temporal resolution but are mainly applicable to animal research (Disbrow et al., 2000; Huettel et al., 2004). Ideally, application of multi-disciplinary methods to the same experimental question is preferable, thereby reducing the effect that constriction of a single methods’ resolution might have on the results. Also, the employment of different types of imaging methods (e.g. additional diffusion tensor imaging) during the same scanning session can highlight diverse aspects of individuals in one experimental context (Bandettini, 2009a). The future might also see multimodal imaging procedures in use on a regular basis. These are procedures that entail the parallel acquisition of data through different recording techniques (Ou et al., 2010; Dale and Halgren, 2001). Each mm$^3$ of human cortex on average contains several ten thousands of neurons (Wager et al., 2008b). As brain areas only millimeters or less apart potentially are associated
with different processes and even individual neurons might be part of different functional circuits (Valenstein et al., 1968; Paton et al., 2006), one of the basic challenges of fMRI lies in interpreting the findings in light of the limitations in its spatial resolution. Furthermore, traditional neuroimaging studies are inherently correlative and can not establish any causal relationships. The outcomes of individual fMRI investigations will in the future increasingly be used to create more specific hypotheses that subsequently can be tested by functional connectivity analyses (Wager et al., 2008b). Such analyses, which emphasize activity in interconnected instead of solitary brain areas, do however pose their own sets of challenges (Roebroeck et al., 2009). Also, the use of transcranial magnetic stimulation (TMS) (Bestmann 2008), which is able to temporarily disrupt localized brain function, will probably increase in the near future.

Presently, the majority of techniques employed to analyze fMRI data use regression approaches like the General Linear Model (GLM) where anticipated signal changes are entered as regressors of interest (Friston et al., 1995; Worsley and Friston, 1995). Most regression procedures and functional connectivity and region of interest analyses are hypothesis-driven, and inevitably biased towards preconceived ideas (Omura et al., 2005). The validity of the results will strongly depend on the validity of the assumptions underlying the hypotheses. Even if data-driven regression procedures are performed, e.g., when entering real-time physiological recordings as regressors, the possible presence of non-modeled signals in the data pose problems for its interpretation. Consequently, the signal derived from fMRI is not only composed of the
“targeted” haemodynamic changes but also head motion, scanner and physiological artifacts. Temporal non-orthogonality between the model and noise will enhance the parameter estimates, while an orthogonal relationship between them will inflate the residual error and reduce statistical significance. That measurements are mixtures of the genuine signal sources complicates the attempt to distinguish pure task- or stimulus-related signals and is a general problem not only restricted to imaging.

One analysis method that is able to decompose data into its underlying source processes, thereby not being constrained by predetermined concepts, is the independent component analysis (ICA) (Comon, 1994). ICA is one way of solving the classical Blind Source Separation problem, i.e., to determine n source signals given n or >n mixtures of these signals (Jutten and Herault, 1991). The first step is a whitening process, which removes any correlations in the data and thereby forces the signals to be uncorrelated. Then the whitened signals are orthogonally transformed in an iterative process until a maximal non-normality between them is reached and the source processes emerge (Hyvarinen, 1999). For complex multi component processes like fMRI, a procedure involving a principle component analysis and Bayesian estimation is initially used to infer the number of involved source processes (Beckmann and Smith, 2004).

ICA has been adapted for functional imaging (McKeown et al., 1998; Beckmann and Smith, 2004) and recently also to include tensorial ICA (tICA) for group level analyses (Beckmann and Smith, 2005). Tensorial ICA is a method that breaks down a complex data set into statistically independent spatio-
temporal processes – independent components (ICs) – that characterize co-activating brain regions as putative functional networks (Tamas Kincses et al., 2008). ICs can further be ranked according to the amount of variability they represent in the data-set (McKeown et al., 1998). Tensorial ICA is implemented in MELODIC (Multivariate Exploratory Linear Decomposition into Independent Components) (Beckmann and Smith, 2005), which is a component of the functional image analysis package FSL (Functional Magnetic Resonance Imaging of the Brain Software Library) (Smith et al., 2004) that was employed in the present work. MELODIC describes signal variation across the temporal (time-courses), the spatial (activation maps) and the session/subject domains.

To ensure that a significant proportion of the subjects contributes to an IC’s estimated time course, MELODIC performs a F-test on that time course and the total model fit. For each IC, those statistical results as well as visual inspection of the activation map and its corresponding time-course constitute the basis for identification/classification and potentially selection of the IC for further exploration. In ICA, the time-courses are variance-normalized, only allowing qualitative and not quantitative aspects to be compared between different time-courses (Dr. Aapo Hyvarinen, personal communication).

Tensorial ICA has found application in the unraveling of resting state brain processes (Beckmann et al., 2005; Damoiseaux et al., 2006), which include or are synonymous with default mode brain processes thought to represent self-referential activities (Raichle et al., 2001; Raichle and Snyder, 2007). The rationale behind the employment of tICA to the study of rest is based on the
obvious difficulty to model this state, both when exploring the resting state as such or as interleaved periods between task or stimulus perturbations, as in traditional fMRI. In the present work, we anticipated extensive peri-stimulus activity in networks associated with affect/emotion, and this was one reason for employing a tICA analysis. Other reasons were to explore functionally integrated brain areas and to estimate the amount of variability that certain “emotional networks” would account for in the different experimental groups’ brain activity.

Through comparison of imaging results obtained by ICA and traditional General Linear Model (GLM) approaches, ICA has been confirmed as a sensitive brain-imaging tool, able to differentiate functionally meaningful patterns of spatio-temporal brain activity (Malinen et al., 2007). ICA is a fairly new method in the field of fMRI and publications reporting the application of ICA to experimental pain data are scarce. Therefore, the present work also utilized GLM analyses and compared their results with the ones derived from the tICA approaches. Consequently, degree of consistency with published imaging data could be established.

**Mood-induction**

Mood induction paradigms presumably offer a very controlled experimental setting. However, and despite use of autonomic and introspective control parameters, the validity of induced moods has been questioned. Differences arise dependent on the level of personal significance of the inducing stimulus. Consequently, it is difficult to know if a participant is transported into a
mood state or just respond to the inducing stimuli as an observer with an evaluative attitude. Moreover, if a mood state is induced, is it the specific and targeted one, and how well does this state correspond to analogous “authentic” mood states? Furthermore, how well do features of induced anxiety that lasts for seconds to minutes correspond to the characteristics of life-long anxiety conditions? Another potential problem with mood induction paradigms in pain research regards their temporal trajectories in relation to those of pain modulating systems (Villemure and Bushnell, 2002). Some techniques result in a rapid induction (de Wied and Verbaten, 2001), while others have a slower on- and/or offset of emotional effects (Weisenberg et al., 1998). As the descending opiate system has a slow on-/offset (Price and Barrell, 2000), interpretational difficulties might arise, especially in neuroimaging studies, dependent on type of mood induction technique utilized. Although they are valuable experimental tools, it is obvious that mood induction paradigms have several shortcomings. The present neuroimaging study therefore used individual differences in naturally occurring anxiety-proneness to explore the effect of anxiety on pain, thereby avoiding any additional manipulations beyond graded nociceptive stimulation.

**Psychophysiology**

Psychophysiology can be defined as the use of physiological signals to understand psychological processes (Cacioppo et al., 2007). In psychology, interpretation of self-reports has often been a means of understanding emotions (Cacioppo et al., 1999). However, self-reports are only moderately related to
somato-visceral and behavioral features of emotion (Mauss et al., 2005), as they suffer from contextual manipulations (Gagnon and Peretz, 2003) and distortions (Schwarz and Clore, 1983). Additionally, some aspects of emotion can not be reported (Bradley, 2000), and emotional processes may occur without emotional experience (Winkielman et al., 2005). The goals of psychophysiology of emotion are therefore to complement and clarify insights obtained from self-reports, and to break down component processes to gain additional insights into the emotional experience (Larsen et al., 2008).

Psychophysiology research has concentrated on studying physiological signals associated with activity in three different parts of the nervous system. Traditionally, the emphasis was on the peripheral nervous system, including both the autonomic and the somatic portions, while more recently work has focused on the central nervous system. Afferent somato-visceral activity serves as feedback to update central commands, but it can also shape the emotional experience either by emotion-specific autonomic patterns, by cognitive labeling of unexplained physiological arousal or by somato-visceral illusions (Cacioppo et al., 1992). This underscores the bi-directional influence between the brain and autonomic, endocrine, and immune functions, functions that previously were thought to possess mainly automaticity that could not be consciously perturbed. Here, only aspects related to autonomic nervous system processes will be reviewed. Autonomically induced somato-visceral activity was originally thought to precede and generate emotional experiences so that different patterns of somato-visceral activity produced different emotions (James, 1884). An
alternative theory (Cannon, 1927) was proposed claiming that different emotions generate different patterns of somato-visceral activity. This theory originated from animal research showing that autonomic events are too slow, insensitive and undifferentiated to influence emotions (Cannon, 1927). Also arguing against James’ theory was the finding that spinal cord injuries in humans have minor impact on the experience of emotions (Chwalisz et al., 1988). A third model acknowledged that somato-visceral activity could generate emotional experiences but suggested that the same pattern of autonomic activity could give rise to different emotions, based on contextual cognitive evaluation and labeling of the undifferentiated physiological arousal state (Schachter and Singer, 1962). Subsequent research has produced results both supporting and contradicting each theory (Larsen et al., 2008), making it likely that components from each view may differentially be expressed depending on the situation.

Another debate in psychophysiology has dealt with the specificity of the relationship between a discrete emotion and its related autonomic activity. It has been suggested that each discrete emotion is associated with an innate affect program that coordinates changes in the organism’s biological states (Levenson et al., 1990). Meta-analyses have only partly confirmed such claims. For example, results have shown that fear was more associated with heart rate increase than anger was but not associated with increased skin conductance compared to a control group (Cacioppo et al., 2000). One way to explain variability within these results is to distinguish between “strategic” and “tactical” aspects of emotions. Tactics denote context-specific action-patterns drawn upon
to achieve narrow goals. This implies that different behaviors might be necessary to deal with different situations and that different behaviors require different viscero-somatic support due to varying metabolic demands (Zajonc and Daniel, 1992). On the other hand, strategies orchestrate actions to achieve broad goals, like securing food supplies and avoiding predators (Lang et al., 1990). Results from the meta-analyses also asserted that negative emotions in general elicit greater autonomic activity than positive emotions (Taylor, 1991; Cacioppo et al., 2000). To extrapolate the significance of findings from experimental emotion research to real life contexts is always difficult and may be further complicated by the fact that different emotion inductions seem to elicit different autonomic patterns (Zajonc and Daniel, 1992) (Rainville et al., 2006).

**Heart rate variability**

Although no uniting theory regarding specificity of emotional autonomic activity has been developed so far, a recent investigation employing principal component and heart rate variability (HRV) analyses found that measures of parasympathetic and sympathetic activity could differentiate discrete emotions even when end-organ responses could not (Rainville et al., 2006). This shows the utility of HRV analyses in obtaining differential information about the activity in the autonomic nervous system.

Heart rate variability (HRV) describes variation of the intervals between consecutive heartbeats (Malik, 1996) and not variability in heart rate (HR) *per se*. HR is increased by sympathetic and decreased by parasympathetic activity.
(Levy, 1990) and determined by sympathetic and vagal activation or withdrawal or inverse activity among them, although vagal function dominates dynamic HR regulation (Saul, 1990). HRV therefore represents autonomic inputs to the sinoatrial node of the heart and their differential contribution to cardiac chronotropy (Allen et al., 2007). HRV is also affected by other components, including endocrine (Brandenberger et al., 1985) and mechanical-haemodynamic factors (Bernardi et al., 1989) and circadian rhythmicity (Furlan et al., 1990). Such fluctuations in cardiovascular parameters have been observed since the 16th century, but detailed investigation into these phenomena did not start until mid 20th century when more advanced research techniques became available (Cohen and Taylor, 2002). Animal studies at that time concluded that the observed cardiovascular oscillations were autonomically based (Guyton and Harris, 1951). Other studies found congruent temporal patterns in sympathetic preganglionic activity and arterial pressure waves (Mayer waves) (Preiss and Polosa, 1974), concluding that such waves originate from sympathetically mediated vascular resistance fluctuations. Further, respiratory patterns were temporally associated with arterial pressure and sympathetic outflow that were seen as a link between respiratory and cardiovascular centers in the brain (Preiss et al. 1975). In the years that followed, non-invasive cardiovascular recording methods were developed and led to numerous studies in humans. Today, the so-called respiratory sinus arrhythmia (RSA) is viewed as representing both autonomic neural fluctuations (Fouad et al., 1984; Akselrod et al., 1985; Koizumi et al., 1985; Taylor and Eckberg, 1996) and mechanically induced central blood volume
changes (Toska and Eriksen, 1993; Guzzetti et al., 1994; Scheffer et al., 1994; Triedman and Saul, 1994). However, their exact contributions to HR and arterial pressure fluctuations are still equivocal (Cohen and Taylor, 2002).

Improved computing power and methods, especially frequency domain analyses (Penaz et al., 1978; Akselrod et al., 1981), generated new ways to approach and interpret HRV data (Cohen and Taylor, 2002). Because the sino-atrial node of the heart responds very quickly to vagal (circa 150 ms latency), in contrast to sympathetic (circa 1-2 s latency), influences (Spear et al., 1979; Levy, 1997), the differential impact of sympathetic and vagal activity on HR can be probed by power-spectral analyses of heart beat-to-beat recordings (Pagani et al., 1986). High frequency power reflects parasympathetic activity and RSA (Malik, 1996), while the interpretation of low frequency power is more ambiguous, as it reflects both sympathetic and parasympathetic events (Japundzic et al., 1990).

In clinical medicine, measures of HRV are mainly used for their predictive value. Originally, it was discovered that changes in HRV predicted fetal distress prior to changes in HR itself (Hon and Lee, 1963). Today, HRV is accepted as a reliable predictor of diabetic neuropathy (Ewing et al., 1985; Braune and Geisendorfer, 1995; Pagani, 2000) and of survival following acute myocardial infarction (Wolf et al., 1978; Lombardi et al., 2001; Huikuri et al., 2003). In the basic sciences, HRV has been employed as a quantitative marker of autonomic activity and has been central in exploring comprehensive models of emotional dynamics (Benarroch, 1993; Porges, 2007). The F-16 fighter plane was
deliberately designed to be unstable in order to enhance its maneuverability (Hoh and Mitchell, 1982). Analogously, and based on non-linear systems chaos and complexity theories, physiological “instability” as reflected by increased measures of HRV, enhances the organism’s capability to efficiently react and adapt to environmental challenges (Davis, 1958; Goldberger and West, 1987b, a; Friedman, 2007). Such adaptations are thought to occur through dynamic interactions between involved components, and therefore HRV has been used as a proxy for cardiac vagal control to index autonomic flexibility and neuro-visceral integration (Thayer and Lane, 2000; Friedman, 2007). Consequently, physiological variability is a sign of health crucial in sustaining homeostasis, while reduced variability is associated with pathology (Goldberger, 1992, 1996, 1997). These concepts are supported by data linking high HRV with stress-resiliency (Fabes and Eisenberg, 1997) and low HRV with risk for cardiovascular disease and mortality (Thayer and Lane, 2007) or psychopathology (Rottenberg, 2007). Anxiety in its episodic, persistent and pathological forms has been associated with low HRV and reduced vagal responsiveness (see Friedman, 2007 for review). In the present work, we employed both time- and frequency-domain HRV analyses to index neuro-visceral flexibility and integration in the study groups.

The area of psychophysiology still remains somewhat esoteric for many scientists, but it has fascinating prospects as it addresses important aspects of present day life-style diseases. A major part of this research focuses on the role of moods and emotions on general health and well-being and how emotions can
be exploited in the prevention and treatment of physical pathology. Chapter IV contains further information on HRV, including methodological aspects.

**Aims**

From this introductory discussion, it is clear that the relationship between anxiety and pain is complex. We employed psychophysical, physiological and fMRI methods to investigate how inter-individual differences in anxiety-proneness and perceived heat pain are associated. We hypothesized that, compared to non-anxious individuals, anxiety-prone subjects would exhibit decreased pain perception, reflecting suppression of pain to facilitate anxiety-specific behavior. This hypothesis was based on the perceptual-defensive-recuperative model of fear and pain (Bolles and Fanselow, 1980) and recent publications on state-dependent opioid control of pain (Fields, 2004; Fields, 2007). Most data regarding those concepts have been derived from animal experiments or from clinical observations. Consequently, our paradigm also investigated state-dependent hypo-/analgesia in a conflict situation between competing motivations in a human experiment. The innate anxiety-proneness of the participants represented the non-contextual/non-pain motivator. We recorded heart and respiratory rate as measures of arousal.

The ventromedial prefrontal cortex (vMPFC) has been implicated in emotional processing and related to emotional-autonomic integration, motivational states and decision-making. Hypo-activity of the vMPFC has been observed in clinical anxiety conditions and in animal fear conditioning. We
therefore chose to focus our imaging analysis on activity in this brain area and its associated regions. In functional magnetic resonance imaging (fMRI) studies of pain, the vMPFC exhibits stimulus-related deactivation, underscoring peri-stimulus activity of this area. This corresponds to observations of default mode network activity, whereby prefrontal cognitive processing is reduced to reallocate resources to the processing of salient events. As peri-stimulus and brain network activity is difficult to assess with traditional fMRI techniques, we employed a tensor independent component analysis (tICA). TICA is a model-free method that breaks down a data set into statistically independent spatio-temporal components (ICs), enabling unbiased insight into activity patterns of functionally integrated brain areas. ICs can be ranked according to the amount of variability they represent in the data set. We hypothesized that, compared to the non-anxious individuals, the anxiety-prone subjects would exhibit vMPFC hypoactivity, and that the ICs encompassing the vMPFC would account for more variability in their brain activation. To statistically explore the tICA findings, general linear model analyses were used. As to our knowledge, tICA has never been applied to pain imaging data before, we further sought to compare previously well established pain related brain activation patterns derived through GLM with analogous patterns obtained from the tICA. Coherence between the two methods would thus validate the tICA for use with pain data.

Heart rate variability (HRV) power spectral analyses have been used to index autonomic flexibility and neuro-visceral integration, reflecting the organism's ability to efficiently react and adapt to environmental challenges.
Moderate anxiety has been reported to bestow an adaptive advantage onto the affected individuals, but it is not known if these putative advantageous characteristics reflect a pattern of autonomic and cognitive-emotional processing that would be expressed by increased measures of HRV. To explore this question, we performed HRV analyses on anxiety-prone and non-anxious participants. As both groups were healthy, the HRV results cannot be viewed as indicating or predicting degrees of health but more as a proxy for flexibility in central processing networks, where high flexibility presumably reflects an “adaptive advantage”. We tested the hypothesis that anxiety-prone individuals would exhibit greater measures of HRV compared to a non-anxious control group, both during environmental stress in the form of noxious stimulation and during a pre-experimental rest period.

Relatively few investigations have explored the correlates of central heart rate (HR) control under conditions of experimental pain, in particular anxiety-specific HR-related brain activity. We therefore obtained individual real-time HR recordings to delineate areas of HR-related cortical processing through our imaging analyses. Our hypothesis predicted an inverse relationship between HR and vMPFC activity in HR-related brain processing but minor or no insula and dorsal ACC involvement due to putative limited cognitive participation. As the perigenual division of the ACC has been implicated in emotional and autonomic processing, we predicted that this area would exhibit anxiety-specific HR-related activity. We further predicted that the pain-related activations would demonstrate a partial spatial overlap with neural correlates of HR in regions of higher-order
processing in the prefrontal cortex, putatively providing a link between pain and autonomic processing.

In summary, our aims were to study the effect of a motivational bias, represented by anxiety-proneness, on pain perception, associated brain processing, heart and respiratory rate. Furthermore, we aimed to delineate anxiety-specific heart rate variability profiles, as a proxy for autonomic flexibility and neuro-visceral integration. Finally, our objective was to identify anxiety-related neural correlates of heart rate-related brain processing. The findings will additionally be interpreted in the context of inter-individual differences in acute pain.
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Chapter II

EFFECT OF ANXIETY-PRONENESS ON NEURAL, PSYCHOPHYSICAL AND PHYSIOLOGICAL ACTIVITY IN EXPERIMENTAL PAIN

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The following manuscript has been completed and submitted. Stylistic specificities are due to the requirements of the journal it was prepared for. Morten Hadsel and Dr. Robert Coghill designed the paradigm. Morten Hadsel, Dr. Alexandre Quevedo and Dr. Yoshitetsu Oshiro collected the data. Dr. Timothy Houle acted as a statistical consultant. Dr. Robert Kraft was responsible for operating the fMRI scanner. Morten Hadsel performed the analyses and prepared the manuscript. Dr. Robert Coghill acted in an advisory and editorial capacity.
Abstract

Competition between emotional-motivational states shapes adaptive behavior, as when fear/anxiety and pain interact. If pain competes with non-pain related fear/anxiety, the state with the stronger motivational potency is favored, while the competitor will be suppressed. Our objective was to investigate how inter-individual differences in inherent low-grade anxiety (anxiety-proneness) and perceived acute pain were related, and to characterize patterns of associated brain activity. Twelve healthy volunteers experienced and evaluated innocuous and noxious thermal stimuli during functional magnetic resonance imaging, while heart and respiratory rates were recorded. Psychometric assessment was performed two weeks post-imaging and divided the group into six anxiety-prone, and six non-anxious subjects. Compared to the non-anxious individuals, the anxiety-prone subjects suppressed their perception of pain and exhibited lower heart-and respiratory rates. The psychophysical findings were to a large extent reflected in the associated patterns of brain activity. Functional imaging findings also revealed relative prefrontal hypo-activity in the anxiety-prone subjects, potentially indicating biased decision-making processing. Networks encompassing ventromedial prefrontal cortex activity accounted for considerably more variability in brain activation in the anxiety-prone than in the non-anxious group. There was a tendency whereby those networks engaged cortical areas in the anxiety-prone and subcortical regions in the non-anxious subjects, reflecting putative differential anti-nociceptive strategies. Our data suggest that anxiety-proneness is characterized by unique experiential, behavioral, physiological and
brain processing features and represents an emotional state distinct from no anxiety and from clinical anxiety conditions. The data underscore that dynamic interactions between emotional-motivational states direct goal-directed action and hence the selection of pain-modulatory strategies.
Introduction

Emotions are action dispositions that drive appetitive and aversive response systems (Lang et al., 1990), and competition between emotional-motivational states directs context-dependent adaptive behavior (Fields, 2007). This is seen during noxious stimulation where, depending upon motivational state, opioids are involved in inhibition or facilitation of pain (Fields, 2004). Analogously, in anxiety disorders rigid cognitive patterns bias action tendencies to facilitate these disorders’ perseverative behaviors like hypervigilance and threat recall (Friedman et al., 2000). Pain and anxiety interactions have been studied in post-traumatic stress disorder (PTSD). Individuals suffering from PTSD exhibit increased thresholds to painful thermal stimuli (Geuze et al., 2007), likely representing suppression of their pain experiences to facilitate anxiety-specific behavior. Responses to noxious stimulation in healthy subjects undergoing fear- and anxiety-induction include both hyperalgesia (Cornwall and Donderi, 1988; Rhudy and Meagher, 2000) and analgesia (Malow, 1981; Rhudy and Meagher, 2000).

Compared to non-PTSD controls, PTSD sufferers exhibit hypo-activity of prefrontal brain areas in response to aversive stimuli (Shin et al., 2005). Findings from neuroimaging of PTSD and animal studies of fear conditioning (Morgan and LeDoux, 1995) have shaped traditional views that ventro-medial prefrontal cortex (vMPFC) hypo-activity is associated with dis-inhibition of the amygdala, resulting in over-expression of fear, and development of anxiety due to lack of fear extinction (Rauch et al., 2006).
It is not known whether pain-related psychophysical and functional imaging findings from the clinical realm of anxiety also apply to healthy, non-treatment seeking subjects with increased levels of anxiety. Psychophysical, physiological and fMRI methods were employed to investigate how inter-individual differences in anxiety-proneness and perceived heat pain related. We hypothesized that, compared to non-anxious individuals, anxiety-prone subjects would exhibit decreased pain perception, reflecting suppression of pain to facilitate anxiety-specific behavior.

In functional magnetic resonance imaging (fMRI) studies of pain, the vMPFC exhibits stimulus-related deactivation (Derbyshire et al., 1997), underscoring peri-stimulus activity of this area. This corresponds to observations of default mode network activity, where prefrontal cognitive processing is reduced to reallocate resources to the processing of salient events (Gusnard et al., 2001). As peri-stimulus and also brain network activity is difficult to assess with traditional fMRI techniques, we employed a tensor independent component analysis (tICA). TICA is a model-free method that breaks down a data set into statistically independent spatio-temporal components (ICs), enabling unbiased insight into activity patterns of functionally integrated brain areas (Tamas Kincses et al., 2008). ICs can be ranked according to the amount of variability they represent in the data-set (McKeown et al., 1998). We hypothesized that, compared to the non-anxious individuals, the anxiety-prone subjects would exhibit vMPFC hypo-activity, and that the ICs encompassing the vMPFC would
account for more variability in their brain activation. To statistically explore the tICA findings, general linear model (GLM) analyses were used.

**Materials and Methods**

**Subjects**

Twelve volunteers with an average age of 25.4 years (SD = 1.7) participated in this study. The group had mixed ethnic composition, consisting of 6 Caucasians, 2 African-Americans, 2 Asians, 1 African-Hispanic and 1 Hispanic. All subjects were right-handed and reported being healthy, non-smokers, drug- and pain free with no history of prolonged pain or drug abuse. The seven female participants were scheduled for experimental testing before day eight or after day twenty of their menstrual cycle. Each subject gave written, informed consent. All procedures were approved by the Institutional Review Board of Wake Forest University School of Medicine.

**Experimental design and stimulus paradigm**

A psychophysical training session familiarized the subjects with the experimental procedures and was followed by an imaging session one week later. Two weeks subsequent to the imaging session, a psychometric assessment was performed.

During training, participants first received three series of eight randomly delivered 5-second thermal stimuli to their left volar forearm, ranging between 35°C and 49°C, and one series between 25°C and 35°C. Pain intensity scores
from the first three series were used to characterize stimulus-response relationships. During the second part of the training and in the imaging session, participants lay supine with stimuli delivered to their left lower leg. The stimulus paradigm is explained in figure 1. Highly noxious (49°C), moderately noxious (47°C) or innocuous (25°C) stimuli were kept at target temperature for 23 seconds, with a rise/fall rate of 4°C per second. Nine experimental series, three per stimulus temperature, were administered in pseudorandom order to each blinded participant per session. Overall series order was further randomized between subjects.

A thermal stimulator (TSA II, Medoc Ltd., Ramat Yishai, Israel) with a 16 x 16 mm contact thermode was used to deliver all stimuli. To avoid long-term suppression or sensitization of nociceptive afferents (Price and Dubner, 1977), the thermode was moved to “naïve” skin sites before each stimulus series. The thermode was placed on pre-marked skin sites in a counter-balanced fashion across subjects to avoid order effects.

**Visual analog scale (VAS) use**

VAS ratings were performed with sliding scales. These scales have been well validated for sensory ratings of pain and exhibit ratio scale properties (Price et al., 1983; Price et al., 1994). Following each stimulus series, perceived pain intensity and –unpleasantness were rated. Pain unpleasantness was not evaluated in a traditional manner, but was incorporated in an unpleasantness/pleasantness rating. Such a rating was preferred, as the present experiment
also acquired data intended for analysis of relief perceptions at pain offset, results that will be published separately. Real-time ratings, including cold sensation, were additionally performed during training. The pain intensity and cold sensation scales both had a 0 – 10 range, while the unpleasantness/pleasantness scale spanned from -10 to +10. “No pain sensation” and “Most intense pain imaginable” anchored the pain intensity scale and “No cold sensation” and “Painfully cold” the cold sensation scale. The unpleasantness/pleasantness scale had a neutral position in the middle and was anchored to the left by “Most unpleasant imaginable” and to the right by “Most pleasant imaginable”.

**Acquisition of continuous psychophysical and physiological data**

Real-time VAS ratings, heart rate (HR) and respiratory rate (RER) were sampled with a digital chart recorder (PowerLab/4sp, AD Instruments, Inc, Colorado Springs, CO). Due to technical difficulties, two 25°C and three 47°C physiological recording series were missing (4.6% of all series), and all data pertaining to these series were excluded from the HR and RER analyses. HR and RER were used as indicators of autonomic arousal, as implemented in other studies (Pitman et al., 1987; Kalisch et al., 2005), and will be examined in more detail in separate manuscripts.
**Psychometric and coping assessment**

Anxiety was measured by the anxiety scale (0 – 4 range) of the Brief Symptom Inventory® (BSI®) (Derogatis and Melisaratos, 1983; Derogatis, 1993), and this scale’s raw scores were used in all anxiety-related analyses. To avoid confounds related to experimental fear/anxiety, psychometric assessment followed 14 days after the imaging session. Consequently, the researchers were blinded to the participants’ fear/anxiety levels, and the participants were not aware of the study’s focus on anxiety during the experiment. Concurrent with the BSI®, the COPE questionnaire (Carver et al., 1989), worded for dispositional coping responses, was also administered. COPE is a multi-dimensional coping inventory, which we employed to probe for possible differences in stress responding between the anxiety-prone and the non-anxious individuals.

**Image acquisition and image processing**

Functional MRI data were acquired on a 1.5T General Electric Twin-Speed LX scanner with a birdcage quadrature head coil (General Electric Medical Systems, Milwaukee, WI). For functional imaging, two-dimensional blood oxygenation level-dependent (BOLD) images of the whole brain were acquired continuously by an echo-planar technique [40 ms echo time (TE); 2s repetition time (TR); 28 x 5-mm-thick slices; 3.72 x 3.75 mm in-plane resolution; 90° flip angle; no slice gaps] (Ogawa et al., 1990). Each functional acquisition series lasted 350 seconds (175 volumes), during which the subjects were requested to keep their eyes closed. High-resolution structural images were acquired using a
three-dimensional spoiled gradient-echo sequence (600 ms inversion time; 9.1 ms TR; 20° flip angle; 1.98 ms TE; 256 x 196 matrix; 1.5 mm section thickness with no gap between sections; 0.9375 x 0.9375 in-plane resolution; 24 cm field of view).

The functional image analysis package FSL version 4.0 [Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (Center for FMRIB, University of Oxford, Oxford, UK)] (Smith et al., 2004) was used for image processing and statistical analysis. The following pre-processing was applied to the data: movement correction; masking of non-brain voxels (Smith, 2002); spatial smoothing by 5 mm with a 3D isotropic Gaussian kernel; nonlinear high-pass temporal filtering with a cutoff period identical to 1.5 times the block length; scaling of each functional image by its mean global intensity when applying a general linear model (GLM); and variance-normalization of the time-courses for the tICA. Each subject’s functional images were registered to their structural data and converted into standard stereotaxic space (Talairach and Tournoux, 1988; Jenkinson and Smith, 2001; Jenkinson et al., 2002). Magnetic field inhomogeneities can be caused by the proximity of air-filled spaces (nasal cavity and sinuses) to the ventral prefrontal brain, resulting in fMRI artifacts and signal loss (Devlin et al., 2000; Gorno-Tempini et al., 2002). Pre-registration thresholding was employed to remove areas of signal loss from the activation maps.
Statistical analyses of the imaging data

Tensorial ICA (Beckmann and Smith, 2005) as implemented in MELODIC (Multivariate Exploratory Linear Decomposition into Independent Components) Version 3.05 was carried out on the whole group of 12 individuals and on a dichotomized group, created through a median split of the BSI anxiety raw scores. The dichotomization led to an anxiety-prone group with BSI anxiety raw scores > 0 (n = 6) and to a non-anxious group with BSI anxiety raw scores = 0 (n = 6). MELODIC describes signal variation across the temporal (time-courses), the spatial (activation maps), and the session/subject domains. To ensure that a significant proportion of the subjects contributes to an IC’s estimated time course, MELODIC performs a F-test on that time course and the total model fit. Only significant ICs that had median response amplitudes significantly different than zero and, according to visual inspection, did not represent artifacts or physiological activity were extracted for further exploration. On the resulting ICs, a comparison between the anxiety-prone and the non-anxious groups was performed for networks following stimulus- and peri-stimulus-related time-courses. Due to the central role of the vMPFC in fear/emotional and decision-making processing, selection of networks following peri-stimulus-related time courses was based on the incorporation of vMPFC activity. The neural correlates of HR-related brain processing will be dealt with in a separate paper.

Through comparison of imaging results obtained by ICA and traditional GLM approaches ICA has been confirmed as a sensitive brain-imaging tool, able to differentiate functionally meaningful patterns of spatio-temporal brain activity
As ICA is a fairly new method in the field of fMRI and publications reporting the application of ICA to experimental pain data are scarce, we sought to validate our tICA findings through additional GLM analyses both for the stimulus- and peri-stimulus related investigations. The GLM analyses would also statistically explore group differences that emerged from the tICA. For the GLM, clusters of voxels with a Z-score > 2.3 and a p-value < 0.05 were considered statistically significant (Worsley et al., 1992). To examine group differences in stimulus-related activity, a boxcar regressor (stimulus-periods = +1; rest-periods = -1), convolved with a gamma-variate model of the hemodynamic response function (mean lag: 6 sec.; SD: 3 sec.) was utilized. To pursue group differences in peri-stimulus-related vMPFC-encompassing network activity, individual series-specific regressors based on relative vMPFC activity were utilized to identify brain areas that co-activated with the vMPFC. These individual regressors originated from a region-of-interest (ROI) analysis. This analysis employed a mask derived from the vMPFC activation in the peri-stimulus related spatial map with the highest median response amplitude of the whole group during the 49°C experimental series. The ROI analysis was additionally performed to address anxiety-differential vMPFC activity by experimental period. While we limited the tICA and the GLM analyses to the 49°C experimental series, the ROI analysis examined BOLD activity across all three temperature paradigms.
Processing and statistical analyses of psychophysical, physiological and ROI data

SPSS® version 15.0 (SPSS Inc., Chicago, IL) was employed for mixed model analyses of variance (ANOVA) on all non-imaging data. A two-tailed probability value of 0.05 was used as significance criterion in all analyses. Exact p-values were also reported. Where appropriate, Bonferroni-corrected post-hoc tests were used to examine significant effects. Anxiety score, although representing a continuous variable, was entered as a factor in the analyses due to the fixed number of anxiety score levels. For visualization-purposes, the participants were dichotomized into anxiety-prone and non-anxious subjects, as described for the tICA.

For the retrospective VAS ratings, a linear mixed model with random effects, including session (training versus the imaging session), stimulus (temperature), series, and anxiety score, was employed. The real-time VAS data sets collected during the training session were explored by analogous a priori models. A Spearman correlation analysis of the subjects’ average retrospective VAS scores during the imaging session and their BSI anxiety scores was also performed for each stimulus temperature.

The stimulus-response data were evaluated with a random effects model in which anxiety-specific differences would be demonstrated by a significant interaction between the anxiety score and the natural logarithm of the effective stimulus temperature [35°C (skin temperature) subtracted from the actual stimulus temperature] in predicting the VAS rating (the dependent variable).
For the physiological and the ROI data analyses, stimulus, block, period and anxiety scores were examined with parsimonious models, collapsing across series. The rational for collapsing across series was based on uniform retrospective VAS ratings across analogous stimulus series. Each ROI time-series was indexed to the average of its initial ten values.

For BOLD signals and physiological recordings, outliers were defined as observations beyond three inter-quartile ranges above or below the upper or lower quartile, respectively. One outlier-series (0.93% of all series) was detected and excluded in toto from all analyses.

Results

Psychometric and coping scores

Average BSI anxiety raw score was 0.35, which placed the participants at the 50th percentile of the normative sample of adult non-patients. The anxiety-prone subjects’ mean BSI anxiety raw score was 0.70 while being 0.00 for the non-anxious individuals, corresponding to the 78th (range: 35th%-97th%) and the 22nd percentile of the reference population, respectively. No statistically significant differences between the anxiety-prone and the non-anxious groups were found for any of the coping scales of the COPE inventory (all p-values > 0.05).
Psychophysical responses

The average retrospective VAS pain unpleasantness/pleasantness ratings were fully confined to the range of unpleasantness for the noxious stimuli and in every respect mirrored the pain intensity ratings. Hence, for clarity, only the pain intensity ratings will be reported here. The anxiety-prone subjects perceived identical thermal stimuli as less intense than the non-anxious individuals, demonstrated by the participants’ VAS ratings in response to brief stimuli to the arm [Anxiety x ln(Effective Stimulus Temperature): (F(5, 68.16) = 7.426, p < 0.001)] (Fig. 2B) and by real-time [Stimulus x Period x Anxiety: F(45, 193.32) = 4.606, p < 0.001] (Fig. 2A) and retrospective [Stimulus x Anxiety: F(10, 44.66) = 6.202, p < 0.001] (Fig. 2C) VAS ratings of prolonged stimuli to the leg. A substantial similarity between real-time peak ratings and retrospective ratings of pain intensity was found (Fig. 2A). Further, a strong negative correlation existed between average retrospective VAS scores (49°C stimuli) and the BSI Anxiety scores (r_s = -0.73, p = 0.008) (Fig. 3). All subjects perceived the 25°C stimulus as cooler than the baseline temperature of 35°C [Main effect Period: F(3, 100.45) = 36.130, p < 0.001].

Functional imaging

In the non-anxious, the anxiety-prone and across all subjects, the tICA identified 17, 21 and 12 ICs, of which 8, 14 and 9 ICs, respectively, had median response amplitudes significantly different than zero. After excluding ICs reflecting artifacts and peri-stimulus related activity not encompassing the
vMPFC, 3 ICs in the non-anxious and 6 in the anxiety-prone group remained and were further explored.

**Stimulus-related findings**

We examined the IC that, according to its spatial map and corresponding stimulus-related time-course during the 49°C experimental series, reflected typical pain-related brain activation. This IC accounted for 15.7% and 12.9% of explained variability in brain activation in the non-anxious and the anxiety-prone group, respectively. Further analyses of this IC revealed that all subjects and the anxiety-specific groups engaged highly similar brain networks (Fig. 4A) with almost identical time-courses (Fig. 4B). As these time-courses were variance-normalized, their quantitative aspects could not be compared.

However, some areas within these qualitatively similar networks displayed anxiety-specific volumetric and Z-score differences, potentially harboring differential quantitative effects. Statistical GLM exploration confirmed these differences (Suppl. Fig. 1) and revealed that, corresponding to their higher pain ratings, the non-anxious group exhibited significantly stronger activation of thalamus, the primary somatosensory (SI) and the anterior cingulate (ACC) cortices than the anxiety-prone group. Conversely, right anterior insula activity, which usually is positively correlated with perceived pain (Coghill et al., 1999), was greater in the anxiety-prone than in the non-anxious individuals. Further, the dorsal prefrontal cortex (DPFC) activated in the anxiety-prone and not in the non-anxious group. These GLM results supported the tICA findings both on a global
and regional level, and overall demonstrated conventional pain-related brain activations that were highly similar among the different groups.

The stimulus-related IC in the anxiety-prone group also included vMPFC activity that followed the inverse time course of the other brain areas found in this network. Two additional stimulus-related ICs encompassing vMPFC deactivations during the 49°C experimental series emerged in the anxiety-prone group. Please see Supplemental Figure 2 for description and discussion.

Peri-stimulus related findings

The IC that accounted for most of the explained variance in brain activation (13.1%) in the anxiety-prone group during the 49°C experimental series exhibited peri-stimulus activations involving the vMPFC area (Brodmann’s area 10, pre- and subgenual ACC and orbitofrontal cortex), temporal poles, hippocampus and amygdala (Fig. 5A). An IC that encompassed a similar activation pattern was also found in the non-anxious group, but accounted for only 6.9% of explained variability in brain activity. As this IC did not reach significance, it was omitted from detailed exploration (although depicted in Fig. 5 for comparison). All groups revealed pre-stimulus activity that, with a slight delay, deactivated abruptly following noxious stimulation and gradually recovered during the post-stimulus periods (Fig. 5B).

The non-anxious and anxiety-prone groups each revealed two additional ICs that encompassed peri-stimulus activations of the vMPFC during the 49°C experimental condition (Suppl. Fig. 3). These data indicated a propensity of the
non-anxious group to activate sub-cortical areas such as the amygdala and the brainstem. On the other hand, the anxiety-prone group tended to engage cortical areas, including somato-sensory processing regions. These group-specific activity patterns were also confirmed by the GLM exploration of brain regions co-activating with the vMPFC (Suppl. Fig. 4).

Consistently, the vMPFC exhibited peri-stimulus related activity. Collectively, the ICs that encompassed vMPFC activity accounted for 60.1% of explained and 19.9% of total variability in the anxiety-prone group’s brain activation. The corresponding percentages for the non-anxious group were 14.4% and 6.4%.

ROI analysis of the vMPFC

The anxiety-prone subjects exhibited a significantly lower level of BOLD activity throughout the 49°C experimental series than the non-anxious participants. For the 47°C series, this pattern was reversed, but with a smaller group-difference than during the 49°C series. No difference in BOLD level between the two groups could be detected in the 25°C experimental condition [Stimulus x Anxiety: F(18, 637.00) = 2.181, p = 0.003] (Fig. 6A). The vMPFC deactivated during thermal stimulation and in the post-stimulus periods. The deactivations were temperature-dependent, as more deactivation was observed with increasing stimulus-temperature [Stimulus x Period: F(6, 1213.72) = 8.088, p < 0.001] (Fig. 6B). The largest average vMPFC deactivation took place in block 1, a time point where uncertainty about the nature of the stimulus was first
resolved. Further, the average vMPFC rest-period BOLD activity was higher during block 2 and 3 than during block 1 and 4. This implies that pre-stimulus vMPFC activity increased after the quality of the stimulus had been revealed and did not decline until the last stimulus of the series was due [Block x Period: F(9, 1321.89) = 2.840, p = 0.003] (Fig. 6C).

**Physiological responses**

*Heart rate*

Average HR decreased with increasing anxiety-scores [Main effect Anxiety: F(9, 55.78) = 8.833, p < 0.001]. In the imaging session, the non-anxious individuals’ average HR was elevated by 6.3%, 7.2% and 15.8% for the 25°C, 47°C and 49°C experimental series, respectively, compared to the anxiety-prone subjects’ HR [Session x Stimulus x Anxiety: F(10, 2222.60) = 7.619, p < 0.001]. In the psychophysical session a similar anxiety-specific heart rate pattern emerged, but was not statistically significant (Suppl. Fig. 5).

*Respiratory rate*

During the imaging session, the non-anxious subjects exhibited a 15.7% higher RER than the anxiety-prone subjects [Main effect Anxiety: F(9, 115.54) = 4.505, p < 0.001] (Suppl. Fig. 6).
Discussion

Consistently, throughout three different VAS rating procedures, with stimulation at two different body sites, and from recordings during two different sessions, anxiety-prone subjects perceived noxious thermal stimuli as less painful than the non-anxious individuals. The anxiety-prone subjects exhibited an overall lower heart- and respiratory rate compared to the non-anxious individuals. During the 49°C experimental series, vMPFC hypo-activity was observed in the anxiety-prone relative to the non-anxious group. ICs encompassing peri-stimulus related vMPFC activity accounted for considerably more variability in brain activation in the anxiety-prone than in the non-anxious group. Among qualitative group differences in those ICs was a tendency towards cortical processing in the anxiety-prone and sub-cortical processing in the non-anxious individuals. Stimulus-related right insular and dorsomedial prefrontal activity emerged in the anxiety-prone, relative to the non-anxious subjects.

Pain perception is highly context-dependent. E.g. immediately following injuries, pain is generally not reported as the organism follows needs for survival and security through escape or rescue. However, as soon as those primary needs have been met, pain emerges to support recuperative behavior (Beecher, 1959; Melzack et al., 1982). The perceptual-defensive-recuperative model of fear and pain explains the time course of pain subsequent to injury as a result of competition and mutual inhibition between fear- and pain-motivational systems (Bolles and Fanselow, 1980). Motivational processes can be viewed as gain-modulators of decision-making processes (Kouneiher et al., 2009) and are based
on the reward values of different action sets and not on cognitive demands (Rushworth et al., 2004). Reward values related not only to pleasure (Cabanac, 1979), but also to fear- (Miller, 1948) and pain- (Leknes et al., 2006) reduction, are considered reinforcers able to motivate behavior to ensure survival and establish homeostasis (Leknes and Tracey, 2008). Both the opioid and the dopamine system have been implicated in these processes (Fanselow, 1986; Fields, 2007). The perceptual-defensive-recuperative model of fear and pain has been supported by animal work addressing both innate and learned fear stimuli (Fanselow, 1986). Human studies have shown that if fear/anxiety can be attributed to pain, a synergistic effect is likely with hyperalgesic responses sustaining attention to the pain state as the contextually predominant emotional-motivational condition. However, if fear/anxiety is associated with non-pain-related entities that possess a stronger motivational power than that of the competing pain, suppression of the pain experience will result, facilitating anxiety-related behavior (Weisenberg et al., 1984; Al Absi and Rokke, 1991). The latter scenario is consistent with our results. As our participants were graduate students, it seems likely that the anxiety-prone subjects' anxiety was related to factors such as scholastic performance, balancing private life with academic requirements, financial and/or romantic problems (Dahlin et al., 2005; Brimstone et al., 2007; Smith et al., 2007). The emotional-motivational state associated with these worries may have outcompeted the pain-related motivations and led to the perception of less pain than the non-anxious individuals.
In our data, activity in the networks encompassing the spatially most extensive vMPFC activation was carried by regions implicated in emotional/fear processing (Phan et al., 2002; Etkin and Wager, 2007), in particular affect-guided decision-making (Davidson, 2003). These networks are thought to encode the values associated with sensory stimuli (Lee et al., 2007; Ostlund and Balleine, 2007) and to be involved in stimulus selection (Rushworth et al., 2005; Rudebeck et al., 2008). The ROI analysis demonstrated stimulus-related vMPFC deactivation (Fig. 6B). This deactivation might imply that cognitive prefrontal processes were shut down to favor pre-attentive systems to guide behavior (Arnsten and Goldman-Rakic, 1998), as seen in self-referential processing (Gusnard et al., 2001). The deactivation was temperature-dependent, likely reflecting that the more intrusive the stimulus was, the more resources were allocated to stimulus processing.

The ROI analysis also demonstrated vMPFC hypo-activity throughout the entire 49°C experimental series in the anxiety-prone relative to the non-anxious subjects (Fig. 6A). Inter-individual variation in pain experience correlates with differential patterns of brain activity (Coghill et al., 2003), and dispositional mood states influence cognitive-emotional brain processing (Hamann and Canli, 2004). Hence, mood may be crucial to creating personalized pain experiences and brain activation patterns (Koyama et al., 2005). In our data, the anxiety-prone subjects’ vMPFC hypo-activity could reflect that the associated decision-making process was biased to facilitate anxiety-related behavior. When the painful stimulus was powerful enough to pose a challenge to the anxiety-related action
tendencies, as during the 49°C experimental series, the stimulus was interpreted as behaviorally less relevant than the anxiety-related worries, thereby downplaying the decision-making process. Accordingly, no vMPFC hypo-activity was observed during the 47°C condition. Presumably, the anxiety-prone individuals exhibited a constitutive inclination towards anxiety-related behavior that, through mechanisms other than modulation of decision-making processes, likely accounted for the experiential group difference also during the 47°C experimental series.

A learning effect from the preceding training session and knowledge about uniformity of stimuli across individual experimental series probably influenced brain activity in both groups. The participants probably set up their action tendencies partly in a preemptive fashion, and partly when they became aware of the quality of the stimulus during block 1 in each experimental series. The latter scenario is depicted in figure 6C, where rest-period vMPFC activity is lower during uncertainty about the quality of upcoming stimuli at the beginning and end of an experimental series.

The vMPFC hypo-activity that was observed in the anxiety-prone group likely reflected a different phenomenon from what has been described in clinical states of anxiety. In such states vMPFC hypo-activity has been associated with amygdala dis-inhibition, over-expression of fear and development of anxiety (Rauch et al., 2006). If vMPFC hypo-activity were an intrinsic feature of the anxiety-prone subjects conforming to this theory, we would expect to observe it during all the stimulus conditions and not, as in our data, only during the 49°C
experimental state. Also, PTSD sufferers exhibit relative hypo-activity of prefrontal brain areas in response to aversive (Shin et al., 2005), and stress-induced hypo-/analgesia (SIA) subsequent to noxious stimulation (Pitman et al., 1990). If vMPFC hypo-activity during 49°C stimulation represented contextual prefrontal dis-inhibition of the amygdala’s fear processing with resulting SIA, we would expect the participants to exhibit signs of physiological arousal (Pitman et al., 1990). However, our data demonstrated less physiological arousal in the anxiety-prone than in the non-anxious individuals. Consequently, neither of these alternative hypotheses can explain the anxiety-prone subjects’ behavioral and brain imaging findings.

The tICA revealed that the networks encompassing the vMPFC during 49°C stimulation accounted for substantially more variability in brain activation in the anxiety-prone than in the non-anxious subjects. It could therefore be speculated that the anxiety-prone individuals exhibited a greater extent of dynamic interactions within cognitive-emotional networks, as opposed to the non-anxious subjects. Such interactions could underlie an advantageous capacity of the anxiety-prone individuals to optimize goal-directed behavior. Low-grade anxiety has been described as positive and adaptive (Endler and Kocovski, 2001) and these tICA findings might reflect this idea. Future studies will determine if low-grade anxiety represents a specific subclinical trait or is a condition on a developmental trajectory towards clinical anxiety.

The tICA and GLM analyses of the 49°C experimental series demonstrated that networks encompassing the vMPFC exhibited group-
differential spatial patterns of activity (Suppl. Figs. 3 and 4). The non-anxious subjects activated brainstem and amygdala circuitry, implicated in top-down regulation of nociceptive activity at the bulbo-spinal level (Bingel et al., 2006). The anxiety-prone group engaged cortical networks, including higher-order executive and somato-sensory domains. These networks could reflect cortico-cortical pain modulation (Lorenz et al., 2003) and represent substrates for emotional regulation of pain (Villemure and Bushnell, 2002; Rhudy et al., 2004).

The GLM imaging analyses results supported both the stimulus and peri-stimulus related tICA findings. We interpreted this consistency between the two methods as validation of tICA for use in pain- and affective-related fMRI studies. The dorsomedial prefrontal cortex exhibited greater stimulus-related activity in the anxiety-prone than in the non-anxious group during the 49°C experimental condition (Suppl. Fig. 1), potentially reflecting autonomic modulatory processes (Napadow et al., 2008). The anxiety-prone group additionally demonstrated peri-stimulus related dorsolateral prefrontal cortex activity (Suppl. Fig. 3), possibly representing goal-dependent emotion regulation (Kalisch et al., 2005). Right insula/frontal operculum exhibited greater stimulus-related activity in the anxiety-prone than in the non-anxious group during the 49°C condition (Suppl. Figs. 1 and 3). Right insula might bridge sensory and cognitive aspects of pain (Kong et al., 2006; Starr et al., 2009), tune arousal levels (Critchley et al., 2000), and also signal safety (Christianson et al., 2008). Such a safety signal might reflect the anxiety-prone group’s tendency to suppress novel stimuli.
Our results underscore that pain-modulating systems do not respond in a linear fashion to noxious stimuli, but will be engaged according to the overall contextual goal of the organism and guided by competing motivational inputs. Inherent low-grade anxiety is one such input that, if potent enough, might suppress painful experiences and shape associated processes. Consequently, our data suggest that anxiety-prone individuals exhibit unique experiential, behavioral, physiological and brain processing features that set them apart from both non-anxious and clinically anxious subjects. More research will be needed to further characterize anxiety-proneness and to allow the involved emotional-motivational and cognitive mechanisms to be fully harnessed for use in clinical situations.
Fig. 1. Stimulus paradigm. A: One experimental block consisted of a stimulus period and a 35°C baseline. Each stimulus period involved either a highly noxious (49°C), moderately noxious (47°C) or innocuous (25°C) stimulus that was kept at target temperature for 23 seconds, with a rise/fall rate of 4°C per second. For analytical purposes, the baseline was divided into pre-stimulus rest and expectation periods and a post-stimulus period. B: One experimental series was made up of four contiguous, repeated blocks. Nine series, three per stimulus temperature, were administered in pseudorandom order to each blinded participant per session.
A

“Rest period”, 35°C, 20 s duration
“Expectation period”, 35°C, 10 s duration, auditory cue at start
“Stimulus period”, 49°C, 47°C or 25°C
“Post-stimulus period”, 35°C, 20 s duration

B

Block 1  Block 2  Block 3  Block 4
Fig. 2. Average visual analog scale (VAS) pain intensity ratings for the non-anxious (n = 6) and the anxiety-prone (n = 6) groups. A: Real-time ratings during the 49°C experimental series from the psychophysical session. Gray bars represent the stimulus periods. Average retrospective ratings from the same series were over-plotted and show a considerable coherence with the real-time peak ratings. B: Ln-fitted thermal stimulus-response curves. C: Retrospective ratings of all stimulus temperatures plotted separately for the psychophysical (PP) and the imaging (MRI) session. Error bars: SEM. Across all experiments and rating procedures, the anxiety-prone subjects perceived noxious thermal stimuli as less painful than the non-anxious individuals did.
**ANOVA (non-dichotomized)**
Stimulus X Period X Anxiety: $F(45, 193.32) = 4.606, p < 0.001$

**ANOVA (non-dichotomized)**
Anxiety X Ln(Eff.stim.temp.): $F(5, 68.16) = 7.426, p < 0.001$

**ANOVA (non-dichotomized)**
Main effect Session: $F(1, 88.01) = 3.673, p = 0.059$
Main effect Stimulus: $F(2, 44.68) = 69.520, p < 0.001$
Stimulus X Anxiety: $F(10, 44.66) = 6.202, p < 0.001$
Stimulus X Anxiety X Session: $F(10, 44.90) = 0.431, p = 0.924$
Fig. 3. Average retrospective visual analog scale (VAS) pain intensity scores from the imaging session plotted as function of Brief Symptom Inventory® (BSI®) anxiety scores. N = 12. Negative correlation between pain intensity and anxiety scores confirmed an anxiety-specific perceptual response pattern to noxious stimuli.
Fig. 4. A: Tensor independent component analysis spatial maps reflecting pain-related activation during the 49°C experimental series. These spatial maps were identified in independent components that accounted for 15.7% and 12.9% of explained variability in brain activation in the non-anxious (n = 6) and the anxiety-prone group (n = 6), respectively. Left side of images corresponds to the right brain. B: Average stimulus-related time-courses corresponding to the spatial maps presented in A. Note that the time-courses were variance-normalized, not allowing quantitative comparisons between the groups. Gray bars represent stimulus periods. The spatial maps reproduced repeatedly published brain activation patterns related to pain processing. Together with their corresponding time courses, they demonstrated considerable inter-group similarities, although areas like thalamus and insulae exhibited anxiety-specific volumetric and z-score differences. ACC, Anterior cingulate cortex; BOLD, Blood oxygenation-level dependent; DLPFC, Dorsolateral prefrontal cortex; SI, Primary somato-sensory cortex; SII, Secondary somato-sensory cortex; SMA, Supplementary motor area; vMPFC, Ventromedial prefrontal cortex.
Fig. 5.  A: Tensor independent component analysis spatial maps reflecting networks involving the most prominent vMPFC activation in each group during the 49°C experimental series. These spatial maps were identified in independent components that accounted for 6.9% and 13.1% of explained variability in brain activation in the non-anxious (n = 6) and the anxiety-prone group (n = 6), respectively. The non-anxious group’s component did not reach significance, but is shown here to enable comparison with the other groups. Left side of images corresponds to the right brain.  B: Average peri-stimulus related time-courses corresponding to the spatial maps presented in A. Note that the time-courses were variance-normalized, not allowing quantitative comparisons between the groups. Gray bars represent stimulus periods. Substantial inter-group similarities included pre-stimulus vMPFC activity that deactivated abruptly following noxious stimulation and gradually recovered during the post-stimulus periods. ACC, Anterior cingulate cortex; BOLD, Blood oxygenation-level dependent; IC, Independent component; OFC, Orbitofrontal cortex; TFG, Temporal fusiform gyrus; TP, Temporal pole; vMPFC, Ventromedial prefrontal cortex.
Fig. 6. Region-of-interest analysis of the vMPFC. A: During the 49°C experimental series the anxiety-prone subjects exhibited vMPFC hypo-activity when compared to the non-anxious individuals. B: The vMPFC deactivated in a temperature-dependent fashion during the stimulus/post-stimulus periods. C: The largest stimulus-related vMPFC deactivation took place in block 1. The average vMPFC rest-period BOLD activity was higher during block 2 and 3 than during block 1 and 4. BOLD, Blood oxygenation-level dependent; Exp, Expectation; Stim, Stimulation; PS, Post-stimulation. Error bars: SEM.
ANOVA (non-dichotomized)
Stimulus X Anxiety: $F(18, 637.00) = 2.181$, $p = 0.003$
Stimulus X Period: $F(6, 1213.72) = 8.088$, $p < 0.001$
Block X Period: $F(9, 1321.89) = 2.840$, $p = 0.003$
Suppl. Fig. 1. Average brain activation maps identified with a general linear model analysis of the 49°C experimental series, using a stimulus-related regressor. All subjects (n = 12) as well as the non-anxious (n = 6) and the anxiety-prone (n = 6) individuals were analyzed, and the anxiety-specific groups were contrasted using a fixed effects analysis. Consistent with their higher pain intensity ratings, the non-anxious subjects exhibited significant greater activation in thalamus, ACC, SI and SII than the anxiety-prone individuals. However, the right insula/frontal operculum, DPFC and cerebellum demonstrated a higher level of activity in the anxiety-prone individuals when compared with the non-anxious subjects. These findings supported the results of the tensor independent component analysis (Fig. 4), both on a global and on an anxiety-specific, regional level. ACC, Anterior cingulate cortex; CAU, Caudate; CER, Cerebellum; CUN, Cuneus; DPFC, Dorsal prefrontal cortex; FO, Frontal operculum; FP, Frontal pole; IFG, Inferior frontal gyrus; INS, Insula; LOC, Lateral occipital cortex; LPFC, Lateral prefrontal cortex; MFG, Middle frontal gyrus; MTG, Middle temporal gyrus; OFC, Orbitofrontal cortex; PCC, Posterior cingulate cortex; PCL, Paracentral lobule; PrCG, Precentral gyrus; PUT, Putamen; SI, Primary somato-sensory cortex; SII, Secondary somato-sensory cortex; SMA, Supplementary motor area; THAL, Thalamus; vMPFC, Ventromedial prefrontal cortex.
Putative brain networks encompassing stimulus-related brain activity with deactivation of the vMPFC. These independent components (ICs) emerged from the tensor independent component analysis of the 49°C experimental series in the anxiety-prone group. IC3 and IC5 encompassed a main stimulus-related activation of the SMA and the mid-ACC. SMA activity has been linked to control and generation of endogenously triggered action {Deiber, 1999 #997} and cognitive time-management {Rubia, 2004 #1026; Macar, 2004 #1024}, mostly related to motor activity. Here, SMA activity could reflect prospective motor action associated with VAS ratings, tracking of temporal progression of the stimulus series and inhibition of escape/withdrawal programs. The SMA might also govern top-down and/or cortico-cortical pain-modulatory processes. Somatotopic sensory organization of the human SMA {Arienzo, 2006 #1081} and report of pain relief following repetitive transcranial magnetic stimulation of the motor cortex in neuropathic pain patients {Lefaucheur, 2006 #1107} support this hypothesis. The ACC activation might, together with prefrontal areas, reflect intentional monitoring and action selection {Carter, 2007 #1444; Van Veen, 2002 #1441}. ACC, Anterior cingulate cortex; BOLD, Blood oxygenation-level dependent; BS, Brainstem; CAU, Caudate; CER, Cerebellum; FO, Frontal operculum; FP, Frontal pole; IC, Independent component; IFG, Inferior frontal gyrus; IFG-PO, Inferior frontal gyrus pars opercularis; INS, Insula; ITG, Inferior temporal gyrus; LOC-S, Superior lateral occipital cortex; MFG, Middle frontal gyrus; MTG, Middle temporal gyrus; OFC, Orbitofrontal cortex; PC, Precuneus; PCL, Paracentral lobule; PoCG, Postcentral gyrus; PrCG, Precentral
gyrus; SFG, Superior frontal gyrus; SMA, Supplementary motor area; STG, Superior temporal gyrus; TP, Temporal pole; THAL, Thalamus; vMPFC, Ventromedial prefrontal cortex.
Suppl. Fig. 3. Putative brain networks encompassing peri-stimulus-related activations of the vMPFC. These independent components (ICs) emerged from the tensor independent component analysis of the 49°C experimental series in the non-anxious and the anxiety-prone groups.

IC3 in the non-anxious and IC4 in the anxiety-prone group revealed peri-stimulus-related activity in widespread somato-sensory cortical areas together with prefrontal activations. These putative networks might reflect short-term, dynamic adaptation of representational maps in SI {Iguchi, 2001 #1066; Schaefer, 2005 #1060}, including tuning of sensory receptive fields {Quevedo, 2007 #7}, to optimize stimulus processing. A prefrontal-cortical gating system has been suggested to underlie these SI modulatory processes {Schaefer, 2005 #897; Staines, 2002 #898}. In IC4 in the anxiety-prone group, the DLPFC might represent the prefrontal component, linking sensations and memory with decisions and actions in an adaptive fashion {Seo, 2007 #1116}. DLPFC activity could also reflect increased attention {Iguchi, 2001 #1066; Iguchi, 2002 #1059} and be a source of emotion regulation {Kalisch, 2005 #1153; Ochsner, 2002 #881; Ochsner, 2004 #574}. The vMPFC/OFC, which activated in both groups, supply data on affective state and derive decisions from reward estimates {Krawczyk, 2002 #1114}.

Peri-stimulus-related activations of the lateral occipital cortices were also found in IC3 of the non-anxious and in IC4 of the anxiety-prone groups. These activations might reflect mental reasoning processes {Goel, 2001 #1051; Johnson-Laird, 2001 #1052; Knauff, 2003 #1050; Ruff, 2003 #1018}, using
temporal tracking information to arrive at probability estimates concerning the remainder of the experiment. Such estimates could be used to select anti-nociceptive and attentional strategies. Parietal working memory sites involve maintenance of problem information {Ruff, 2003 #1018}.

IC11 in the non-anxious and IC8 in the anxiety-prone group both followed a progressively increasing peri-stimulus related time-course, suggesting anticipatory activity. Anticipation of pain engages cognitive systems overlapping with and able to affect cortical nociceptive circuitry {Porro, 2002 #203;Koyama, 2005 #32}. The non-anxious group component exhibited marked activation of brainstem areas capable of modulating transmission of ascending nociceptive signals at the bulbo-spinal level {Fields, 1991 #970;Tracey, 2002 #971}, and of cortical areas known to exert top-down control onto these brainstem structures, like the amygdala {Rizvi, 1991 #974} and OFC {Floyd, 2000 #984}. The anxiety-prone group component demonstrated stimulus-related deactivations in brainstem, cerebellum, SI and left DLPFC. Activity in the DLPFC often signals the value of expected reward besides actual outcome {Lee, 2007 #1117}. This might suggest that the DLPFC, as part of “cognitive” cortico-cerebellar loops {Ramnani, 2006 #1143}{Salmi, #3189} may be able to contextually amend ongoing executive commands (e.g. modulation of SI sensory features), based on comparison of input from cerebellar anti-nociceptive “programs” with actual pain experience.

AMY, Amygdala; BOLD, Blood oxygenation-level dependent; BS, Brainstem; CER, Cerebellum; DLPFC, Dorsolateral prefrontal cortex;
HIP, Hippocampus; IC, Independent component; IFG-PO, Inferior frontal gyrus pars opercularis; INS, Insula; IPL, Inferior parietal lobule; ITG, Inferior temporal gyrus; LOC, Lateral occipital cortex; LOC-S, Superior lateral occipital cortex; MFG, Middle frontal gyrus; MTG, Middle temporal gyrus; PHG, Parahippocampal gyrus; PoCG, Postcentral gyrus; PrCG, Precentral gyrus; PUT, Putamen; SFG, Superior frontal gyrus; SMA, Supplementary motor area; SPL, Superior parietal lobule; STG, Superior temporal gyrus; TP, Temporal pole; THAL, Thalamus; vMPFC, Ventromedial prefrontal cortex.
Suppl. Fig. 4. General linear model contrast between the non-anxious (n = 6) and the anxiety-prone (n = 6) groups during the 49°C experimental series. Series-specific vMPFC activity-related regressors were used to explore brain regions that co-activated with the vMPFC. A propensity of the non-anxious group to co-activate sub-cortical areas, encompassing amygdala and brainstem, was found. However, the anxiety-prone group was inclined to co-engage cortical areas, including somato-sensory processing regions. These findings supported the results of the tensor independent component analysis (Suppl. Fig. 3).

ACC, Anterior cingulate cortex; AMY, Amygdala; BS, Brainstem; CAU, Caudate; CER, Cerebellum; FP, Frontal pole; HIP, Hippocampus; IFG, Inferior frontal gyrus; ITG, Inferior temporal gyrus; LG, Lingual gyrus; MFG, Middle frontal gyrus; MTG, Middle temporal gyrus; NAc, Nucleus accumbens; OcFG, Occipital fusiform gyrus; OFC, Orbitofrontal cortex; PC, Precuneus; PCC, Posterior cingulate cortex; PHG, Parahippocampal gyrus; PoCG, Postcentral gyrus; PrCG, Precentral gyrus; PUT, Putamen; SFG, Superior frontal gyrus; SMA, Supplementary motor area; TFG, Temporal fusiform gyrus; TOFG, Temporo-occipital fusiform gyrus; TP, Temporal pole; THAL, Thalamus.
Suppl. Fig. 5. Average heart rate plotted for the non-anxious (n = 6) and the anxiety-prone (n = 6) individuals by session and stimulus-temperature. During the imaging session, the non-anxious subjects’ heart rate increased in a temperature-dependent fashion. However, the anxiety-prone individuals’ heart rate remained stable across the different experimental conditions. A similar anxiety-specific heart rate pattern was found in the psychophysical session, but did not reach significance. Compared to pre-stimulus, HR increased in the stimulus and post-stimulus periods in both groups during the 49°C experimental series, but this increase was more pronounced in the non-anxious individuals. Exp, Expectation; Stim, Stimulus; PS, Post-stimulus. Error bars: SEM.
**Psychophysiological training session**

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<tr>
<th>Temperature</th>
<th>Group</th>
<th>Heart Rate (beats per minute)</th>
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<tr>
<td></td>
<td>Anxiety-prone</td>
<td>Rest: 60, Exp: 62, Stim: 64, PS: 66</td>
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**ANOVA (non-dichotomized)**

- **Main effect of Anxiety:** F(9, 55.78) = 8.833, p < 0.001
- **Session X Stimulus X Anxiety:** F(10, 2222.60) = 7.619, p < 0.001
- **Session X Period X Anxiety:** F(12, 3008.16) = 1.989, p = 0.022
- **Stimulus X Period X Anxiety:** F(36, 2994.49) = 2.308, p < 0.001
Suppl. Fig. 6. Average respiratory rate during the imaging session plotted for the non-anxious (n = 6) and the anxiety-prone (n = 6) individuals by period. Across periods, the non-anxious subjects exhibited a 15.7% higher respiratory rate than the anxiety-prone individuals did. Exp, Expectation; Stim, Stimulus; PS, Post-stimulus. Error bars: SEM.
ANOVA (non-dichotomized)
Main effect of Anxiety: $F(9, 115.54) = 4.505, p < 0.001$
Period X Anxiety: $F(12, 1296.47) = 3.756, p < 0.001$
References


Chapter III

EFFECT OF ANXIETY-PRONENESS ON HEART RATE VARIABILITY DURING EXPERIMENTAL PAIN AND PRE-EXPERIMENTAL REST


The following manuscript has been completed and is ready for submission. Stylistic specificities are due to the requirements of the journal it was prepared for. Morten Hadsel and Dr. Robert Coghill designed the paradigm. Morten Hadsel, Dr. Alexandre Quevedo and Dr. Yoshitetsu Oshiro collected the data. Dr. Timothy Houle acted as a consultant for statistical and analytical issues. Dr. Robert Kraft was responsible for operating the fMRI scanner. Dr. John Schmidt performed the block-level analyses. Morten Hadsel performed the analyses and prepared the manuscript. Dr. Robert Coghill acted in an advisory and editorial capacity.
Abstract

Background

Elevated heart rate variability, reflecting autonomic flexibility and neurovisceral integration, is linked to stress-resiliency, while low heart rate variability is associated with negative health, including anxiety. Moderate anxiety potentially harbors an adaptive advantage, and we recently showed that anxiety-prone individuals were less sensitive to experimental heat pain than non-anxious subjects. It is unknown if anxiety-prone individuals’ pattern of autonomic and cognitive-emotional processing would be reflected by increased measures of heart rate variability. We hypothesized that anxiety-prone subjects would exhibit higher measures of heart rate variability than non-anxious controls.

Methods

We analyzed physiological recordings from our previous study, where heart rate was recorded from twelve healthy volunteers during rest and while they were experiencing and evaluating innocuous and noxious thermal stimuli. Psychometric assessment divided the group into six anxiety-prone and six non-anxious subjects. Time- and frequency-domain measures of heart rate variability were calculated.
**Results**

Across all experimental conditions, the anxiety-prone subjects exhibited a greater time-domain and high and low frequency heart rate variability, but a lower low- to high-frequency power ratio than the non-anxious individuals.

**Conclusions**

The results confirmed our hypotheses and could indicate that the anxiety-prone subjects possessed an ability to maximally harness neurobiological processing systems to ensure optimal experiential, physiological and behavioral outcomes. Consequently, a non-linear relationship between anxiety and HRV metrics seems to exist. Further characterization of anxiety-proneness is needed, both under varying conditions and through different approaches, including HRV analyses.
Introduction

Heart rate variability (HRV) describes variation in the intervals between consecutive heartbeats (Malik, 1996). It reflects autonomic inputs to the sino-atrial node of the heart and their differential contribution to cardiac chronotropy (Allen et al., 2007). Autonomic influences are the most important determinants of heart rate (HR) (Berntson et al., 1991) with sympathetic activity increasing and parasympathetic activity reducing it (Levy, 1990). HR is determined by sympathetic and vagal activation or withdrawal or inverse activity among them, although vagal function dominates dynamic HR regulation (Saul, 1990). The differential impact of sympathetic and vagal activity on HR can be probed by power-spectral analyses of heart beat-to-beat recordings (Pagani et al., 1986). High frequency (HF) power reflects parasympathetic, while low frequency (LF) power reflects both sympathetic and parasympathetic activity (Japundzic et al., 1990).

Models of visceral and emotional function (Benarroch, 1993; Porges, 2007) have been consolidated with Dynamic Systems Theory into comprehensive models encompassing affective, cognitive, physiological and behavioral components of emotional dynamics. Central to these models is the use of HRV, mainly as a proxy for cardiac vagal control, as an index of autonomic flexibility and neuro-visceral integration (Thayer and Lane, 2000; Friedman, 2007). Autonomic flexibility and neuro-visceral integration reflect the organism's ability to efficiently react and adapt to environmental challenges through dynamic interactions between involved components (Davis, 1958; Friedman, 2007).
Dynamic interactions indicate physiological variability, which is crucial to sustain homeostasis and is a sign of health, while decrease of such variability is associated with pathology (Goldberger, 1997). These concepts are supported by data linking high HRV with stress-resiliency (Fabes and Eisenberg, 1997), and low HRV with risk for cardiovascular disease and mortality (Thayer and Lane, 2007), as well as psychopathology (Rottenberg, 2007). Anxiety, a negative mood condition characterized by rigid cognition and behavior (Friedman et al., 1993; Friedman et al., 2000), has in its episodic, persistent and pathological forms been associated with low HRV and reduced vagal responsiveness (see Friedman, 2007 for review). Low-grade anxiety, however, has been related to increased performance (Yerkes and Dodson, 1908) and life expectancy (Mykletun et al., 2009), compared to more severe and no anxiety. We recently showed that during noxious heat stimulation, anxiety-prone individuals perceived less pain and exhibited lower HR than non-anxious subjects (Hadsel, 2010). We explained these findings as context-dependent adaptive outcomes of a decision-making process involving two competing motivational states (Fields, 2004). The motivational state associated with the anxiety-prone group’s putative preoccupation with threats and worries (Fox et al., 2002) may have outcompeted the pain-related motivations and suppressed the responses to this conflicting event (Bolles and Fanselow, 1980).

It is not known if the putative advantageous characteristics associated with anxiety-prone individuals reflect a pattern of autonomic and cognitive-emotional processing that would be expressed by increased measures of HRV. To explore
this question, we performed HRV analyses on anxiety-prone and non-anxious participants, using physiological recordings from our previous study. We tested the hypothesis that anxiety-prone individuals would exhibit greater measures of HRV compared to a non-anxious control group, both during environmental stress in the form of noxious stimulation and during a pre-experimental rest period.

Materials and Methods

Subjects

Twelve volunteers with an average age of 25.4 years (SD = 1.7) participated in this study. The group had mixed ethnic composition, consisting of 6 Caucasians, 2 African-Americans, 2 Asians, 1 African-Hispanic and 1 Hispanic. All subjects were right-handed and reported being healthy, non-smokers, drug- and pain free with no history of prolonged pain or drug abuse. The seven female participants were scheduled for experimental testing before day eight or after day twenty of their menstrual cycle. Each subject gave written, informed consent for participation in the experiment. All procedures were approved by the Institutional Review Board of Wake Forest University School of Medicine.

Experimental design and stimulus paradigm

A training session familiarized the subjects with all procedures and was followed by the experimental session one week later. Two weeks subsequent to the experimental session, a psychometric evaluation was performed. The experimental session involved functional magnetic resonance brain imaging, the
results of which have been reported elsewhere. The present manuscript reports data related to HRV during the experimental session.

In each session, the participants were placed in a supine position while HR and respiratory rate (RER) were monitored. After HR and RER had reached stable values, five minutes of “resting” activity was recorded before the experiment began. One experimental test series was made up of four contiguous, repeated blocks that each consisted of a stimulus period and a 35°C baseline. Each stimulus period encompassed either highly noxious (49°C), moderately noxious (47°C) or innocuous (25°C) stimuli that were kept at target temperature for 23 seconds, with a rise/fall rate of 4°C per second. Nine experimental test series, three per stimulus temperature, were administered in pseudorandom order. Overall series order was further randomized between the subjects. The subjects were blinded to the order of the stimuli. The stimulus paradigm is depicted in figure 1.

All stimuli were delivered to the participants’ left lower leg by a thermal stimulator (TSA II, Medoc Ltd., Ramat Yishai, Israel), utilizing a 16 x 16 mm contact thermode. The thermode was moved to “naïve” skin areas before each experimental test series to avoid long-term suppression or sensitization of nociceptive afferents (Price and Dubner, 1977), and placed on pre-marked skin sites in a counter-balanced fashion across subjects to avoid order effects.
Visual analog scale use

Following each experimental test series, the subjects rated perceived pain intensity and unpleasantness/pleasantness on sliding VAS scales. Such scales have been validated for sensory and affective ratings of pain and exhibit ratio scale properties (Price et al., 1983; Price et al., 1994). The pain intensity scale had a 0 – 10 range and was anchored by “No pain sensation” and “Most intense pain imaginable”. The unpleasantness/pleasantness scale, with a -10 to +10 range, had a neutral position in the middle and was anchored to the left by “Most unpleasant imaginable” and to the right by “Most pleasant imaginable”. The unpleasantness/pleasantness scale was also used to quantify hedonic change during temperature transitions, data that will be published elsewhere. For simplicity, we employed this scale, rather than a traditional unpleasantness scale, for all affective ratings in the experiment.

Acquisition of HR and RER

HR was monitored by a pulse-oximeter (Nonin Medical, Inc, Plymouth, MN) attached to the subjects’ left middle finger, while RER was recorded with a respiratory belt transducer (Pneumotrace®, AD Instruments, Inc, Colorado Springs, CO) placed at the level of the abdomen. HR and RER were sampled at 100 Hz with a digital chart recorder (PowerLab/4sp, AD Instruments, Inc, Colorado Springs, CO). Custom IDL programs (IDL version 5.0, ITT Corp., Boulder, CO) generated the HR, inter-beat interval (IBI) and RER time series. Due to technical difficulties, two 25°C and three 47°C HR-series were missing.
(4.1% of all series), and all data pertaining to these series were excluded from the analyses.

**Psychometric assessment**

Anxiety was measured by the anxiety scale of the Brief Symptom Inventory® (BSI) (Derogatis and Melisaratos, 1983; Derogatis, 1993). This scale’s raw scores (range 0 – 4) were used in all statistical analyses. We administered the BSI due to its capability of assessing psychological symptom patterns, a subject that will be addressed elsewhere. Psychometric evaluation took place 14 days subsequent to the experimental session to avoid confounding of the anxiety measures by experimental and scanner-related fear/anxiety (Friday and Kubal, 1990; Murphy and Brunberg, 1997; Sarji et al., 1998). Consequently, during the experimental session the subjects were blinded to the study’s focus on anxiety and the researchers were blinded to the subjects’ anxiety levels. To enable correlation of BSI anxiety scale scores with scores from a unidimensional anxiety scale, we also administered Spielberger’s State-Trait Anxiety Inventory (STAI) (Spielberger, 1983; Spielberger and Vagg, 1984).

**HRV pre-processing and analyses**

Kubios HRV version 2.0 (Biosignal Analysis and Medical Imaging Group, University of Kuopio, Kuopio, Finland) was utilized for pre-processing of the IBI time series and for time- and frequency-domain HRV analyses (Niskanen et al., 2004; Tarvainen, 2008).
Pre-processing

To convert non-equidistantly sampled to equidistantly sampled IBI time series a cubic spline interpolation method (Mateo and Laguna, 2000) with an interpolation rate of 4 Hz (Berntson et al., 1997) was employed. As IBI series artifacts can produce invalid HRV analysis results (Ramanathan and Myers, 1996; Berntson and Stowell, 1998), every inter-beat interval time series was manually inspected. Data points that deviated more than four standard deviations from their time series mean were corrected with a piecewise cubic spline interpolation method (Tarvainen, 2008). A smoothness priors-based detrending procedure, which is an advanced time-varying high pass filter (Tarvainen et al., 2002), was used to remove corrupting low frequency trends in the IBI time series (Weber et al., 1992). The smoothing parameter ($\lambda$) was set to 500, resulting in a cut-off frequency of 0.035 Hz, which was below the frequency ranges examined in this study.

To enable assessment of the low frequency spectrum, a minimum heart data recording of 120 seconds is recommended (Malik, 1996). However, to avoid skin damage (Hardy et al., 1952) the noxious stimulation had to be limited to 30 seconds per block, which rendered a frequency-domain HRV analysis on the period level (baseline vs. stimulation) unfeasible. Further, the fluctuating nature of the thermal stimuli introduced an unwanted low frequency standing wave into the heart data time series. However, preliminary time-domain HRV analyses (SDNN, RMSSD and pnn50) on the block level ruled out trends within series. Further, the frequency of the experimentally induced standing wave equaled
0.0125 Hz, which was well below the frequency ranges of interest. Also, the employed detrending procedure presumably removed the experimentally induced low-frequency wave. Consequently, we concluded that it was justified to perform the HRV analyses on the series level.

**Analyses**

Individual pre-processed IBI time series were subjected to time- and frequency-domain HRV analyses. For definitions of the time-domain statistical variables, please see legend of table 1. For frequency-domain HRV analyses a non-parametric, Fast Fourier Transform (FFT) and a parametric, autoregressive modeling based (AR) method (Berntson et al., 1997) were employed. Welch’s periodogram method was utilized to calculate the FFT spectrum, applying a window width of 256 seconds and a window overlap of 50%. In the AR spectrum estimation we used a model order of 16. Through integration, absolute power was extracted in a HF and a LF band (Tarvainen, 2008). The HF and LF bands were defined as frequencies between 0.15 and 0.4 Hz, and 0.04 and 0.15 Hz, respectively (Malik, 1996).

**Statistical analyses**

SPSS® version 16.0 (SPSS Inc., Chicago, IL) was employed for mixed model analyses of variance (ANOVA) of all data. A two-tailed probability value of 0.05 was used as significance criterion in all analyses. Exact p-values were also reported. Where appropriate, Bonferroni-corrected post-hoc tests were used to
examine significant effects. BSI anxiety score, although representing a continuous variable, was entered as a factor in the analyses due to the fixed number of anxiety score levels. For visualization purposes, the participants were dichotomized into anxiety-prone (BSI anxiety raw scores > 0, n = 6) and non-anxious (BSI anxiety raw scores = 0, n = 6) groups.

All statistical analyses were performed as *a priori* models. In the preliminary HRV analyses, Stimulus (25°C, 47°C or 49°C stimulation), Series (number of uniform acquisition series), Block (number of the within-series baseline-stimulus epochs) and Anxiety (BSI anxiety scores) were entered as factors into the model. For the retrospective VAS ratings, the factors employed were Stimulus, Series, and Anxiety. For the HR, HRV and RER analyses, Stimulus (pre-experimental rest, 25°C, 47°C or 49°C stimulation) and Anxiety were examined, collapsing across series. The rational for collapsing across series was based on uniform retrospective VAS ratings across analogous stimulus series and on absence of a series-effect in the preliminary HRV analyses. To contrast the magnitudes of absolute power between the HF and the LF bands, a factor labeled Frequency was introduced, which encoded power values as dichotomized categories, high or low. Spearman correlation analyses between the subjects’ BSI anxiety scores and their STAI State and Trait scores were also performed.
**Covariates in the HRV analyses**

Respiratory sinus arrhythmia (RSA) reflects respiratory-linked HR variability (Grossman and Taylor, 2007). Disagreement exists about the necessity to take respiration into consideration during HRV analyses (Berntson et al., 1997; Denver et al., 2007; Grossman and Taylor, 2007; Porges, 2007). Methodological aspects of incorporating respiration in statistical analyses of HRV have further been debated (Miller and Chapman, 2001). We followed the recommendations set forth by The Society for Psychophysiological Research’s Task Force (Berntson et al., 1997) by setting the HF bandwidth of the frequency-domain analyses to encompass the actual RER of the participants. Further, as anxiety-related differences in RER were present, we implemented the recommendations of Allen et al. (Allen et al., 2007) to employ a covariance analysis to assess whether significant effects resulting from a standard ANOVA would survive after accounting for variance due to respiration.

An association between obesity and HRV/RSA has been found (Karason et al., 1999). Based on height and weight recordings, Body Mass Index (BMI), as a measure of body fat, was calculated for each participant (National Heart Lung and Blood Institute, Obesity Education Initiative) and also entered into the mixed ANOVA as a covariate of no interest.
Results

Psychometric scores

The mean BSI anxiety raw score was 0.35, which put the participants at the 50th percentile of the normative sample of adult non-patients. The mean BSI anxiety raw score for the anxiety-prone subjects equaled 0.70 and for the non-anxious individuals it was 0.00, corresponding to the 78th (range: 35th% - 97th%) and the 22nd percentile of the reference population, respectively. Strong positive Spearman correlations between the BSI anxiety scores and the state ($r_s = 0.69$, $p = 0.014$) and trait ($r_s = 0.70$, $p = 0.011$) STAI scores (Suppl. Fig. 1) indicated significant coherence between these independent psychometric instruments and supported the use of BSI raw scores as an estimator of anxiety in the context of this study.

Psychophysical VAS ratings

The absolute and relative affective rating patterns and their statistical significance corresponded to those of the pain-intensity ratings. For clarity, only data pertaining to pain-intensity will be presented (Fig. 2). Perceived pain intensity increased with increasing stimulus temperature [main effect Stimulus: $F(2, 15.50) = 22.05$, $p < 0.001$], but the anxiety-prone individuals perceived each noxious stimulus as less intense than the non-anxious subjects [main effect Anxiety: $F(5, 31.17) = 4.60$, $p = 0.003$; Stimulus x Anxiety: $F(10, 15.47) = 2.66$, $p = 0.041$].
Heart rate

Average HR was altogether higher in the non-anxious than in the anxiety-prone subjects [main effect Anxiety: \( F(9, 31.85) = 4.90, p < 0.001 \)]. Across the experimental conditions, the anxiety-prone individuals exhibited stable average HR. During the pre-experimental rest condition no difference between the groups was observed, but due to a temperature-dependent HR increase in the non-anxious group, progressively larger group differences emerged with increasing stimulus temperatures [Stimulus x Anxiety: \( F(23, 1415.68) = 5.32, p < 0.001 \)] (Fig. 3). For HR estimates, please see Table 1.

Heart rate variability

Time-domain

The anxiety-prone subjects exhibited greater heart rate variability for all time-domain measures across all experimental conditions, relative to the non-anxious individuals (Fig. 4). For estimates and statistical results, please see Table 1.

Frequency-domain

Compared to the non-anxious individuals, the anxiety-prone subjects exhibited greater absolute power of both the LF and HF bands across all experimental conditions (main effect of Anxiety for each bands). Average absolute power was larger in the HF than in the LF band (main effect of Frequency). In the non-anxious group, the relationship between LF and HF
absolute power was approximately 2:3, while in the anxiety-prone group this ratio was about 1:2 (Frequency x Anxiety interaction). Substantial correspondence between the outcomes of the FFT and the AR methods was demonstrated (Fig. 5). For estimates and statistical results, please see Table 1.

Covariates

Across all experimental conditions, the non-anxious individuals exhibited 18.9% greater average respiratory rate compared to the anxiety-prone subjects [main effect Anxiety: F(9, 168.15) = 4.87, p < 0.001] (Suppl. Fig. 2). The range of the RER was 14.0 – 20.2 breaths/minute, corresponding to 0.23 – 0.34 Hz, a range clearly encompassed within the HF range (0.15 – 0.40 Hz). The non-anxious individuals exhibited a greater average BMI (26.7) than the anxiety-prone subjects (24.3) [main effect Anxiety: F(5, 106.65) = 3.42, p = 0.007)] (Suppl. Fig. 3). The introduction of RER and BMI as covariates did not change the effects of the original mixed ANOVAs reported above. Consequently, anxiety-differential effects of RER and BMI did not account for the anxiety-specific effects of HRV.

Discussion

The anxiety-prone subjects experienced noxious stimulation as less painful than the non-anxious individuals. HR did not differ between the two groups during rest but with increasing stimulus temperatures, HR increased progressively in the non-anxious, while remaining stable in the anxiety-prone
group. The anxiety-prone subjects exhibited greater measures of HRV than the
non-anxious individuals in the time-domain and within both the HF and LF band
of the frequency-domain HRV analyses. The LF/HF power ratio was increased in
the non-anxious relative to the anxiety-prone group. RER and BMI were greater
in the non-anxious than in the anxiety-prone group, without confounding the
anxiety-specific effects of HRV.

Data examining the relationship between anxiety-proneness and HRV are
sparse. However, clinical research has shown that individuals suffering from
post-traumatic stress disorder (PTSD) exhibit increased thresholds to painful
thermal stimuli (van der Kolk et al., 1989; Geuze et al., 2007). Although these
studies did not evaluate HRV, other data have demonstrated reduced HF power
and greater LF/HF ratios in PTSD sufferers compared to control subjects (Cohen
et al., 1997; Cohen et al., 1998; Cohen et al., 2000b; Cohen et al., 2000a).
Taken together, this might suggest reduced pain sensitivity associated with
lowered cardiac vagal control in individuals with PTSD. In healthy participants,
higher threshold for moderate pain and reduced cold pain unpleasantness were
associated with greater LF resting HRV (Appelhans and Luecken, 2008).
Consequently, the clinically anxious and the healthy individuals represented 2
different populations where reduced pain sensitivity was associated with opposite
measures of vagal control. The anxiety-prone individuals from our study shared
an association between reduced pain sensitivity and increased measures of
cardiac vagal control with the healthy subjects. The anxiety-prone and clinically
anxious individuals shared reduced pain sensitivity, compared to their respective
control groups. Valence and arousal are considered basic dimensions of affect (Christie and Friedman, 2004). The affective states related to threat of, and exposure to, pain in the three different populations presumably all had similar negative affective valence and a certain degree of motivational power, reflecting the amount of arousal necessary to control the environmental perturbation to regain homeostasis (Lang et al., 1990). In the anxiety-prone and clinically anxious subjects, a competing motivation was also present, these individuals’ attentional bias towards threats and worries (Fox et al., 2001; Fox et al., 2002; Verkuil et al., 2009). A decision-making process will putatively favor the affective state able to secure optimal context-dependent adaptation through engagement of appropriate effector systems (Fields, 2004; Fields, 2007). In the anxiety-prone subjects and the PTSD sufferers the anxiety-related emotional state was likely favored, suppressing the responses to the competing noxious event (Hadsel, 2010). Mechanisms of stress-induced analgesia were potentially also engaged in the PTSD group (Pitman et al., 1990). Based on the HRV data from the three populations, we must assume that decision-making and executive processing was defined by rigid action tendencies in the PTSD group (Friedman et al., 2000; Friedman, 2007), but reflected unimpaired dynamic integration and regulation of contextual inputs in the healthy as well as the anxiety-prone individuals (Friedman and Thayer, 1998; Friedman, 2007). Consequently, the relationship between anxiety and HRV seems to depend on the degree of anxiety and not universally demonstrate an inverse correlation.
Anxious subjects exhibit persistent vigilance towards worries and threat cues (Thayer et al., 2000; Fox et al., 2001; Fox et al., 2002; Verkuil et al., 2009) and worries have been associated with low vagal tone in anxious individuals (Thayer et al., 1996). The anxiety-prone subjects demonstrated measures of high vagal tone, making the latter finding seems counterintuitive to our original hypothesis that threat-related motivations outcompeted pain-related motivations. As elevated measures of HRV have been linked to effective emotion regulation (Gross, 1998; Segerstrom and Nes, 2007), emotion regulatory dispositions might modify the HRV outcome. We therefore speculate that the observed increase in HF HRV in the anxiety-prone individuals may be a result of an interaction between attentional and emotion regulatory systems. In the light of the autonomic flexibility-neurovisceral integration model of anxiety and cardiac vagal tone (Friedman, 2007), our HRV analyses results could indicate that the anxiety-prone subjects possessed an ability to maximally harness neurobiological processing systems to ensure optimal experiential, physiological and behavioral outcomes. In general, anxiety-prone individuals may exhibit a “non-pathological” increase in vigilance and fear-reactivity that allows them to avoid harm through precautionary and active behavior. Accordingly, the results support the hypothesis that moderate anxiety harbors an adaptive advantage. This hypothesis has also been supported by an association between low trait-anxiety and increased risk-behavior and reduced help seeking (Lee et al., 2006; Mykletun et al., 2009). However, because of potential relative higher processing
load in the anxiety-prone subjects, the results might simply reflect the response to contextual demands.

Both the HF and LF findings corresponded to overall greater HR in the non-anxious relative to the anxiety-prone subjects. If both bands were interpreted as representing parasympathetic activity (Sloan et al., 1996), the results were consistent with vagal withdrawal in the non-anxious relative to the anxiety-prone subjects. If, on the other hand, the LF band was seen as harboring sympathetic activity (Pagani et al., 1992), the LF/HF ratio, as an index of sympatho-vagal balance (Lombardi et al., 1996), would reflect greater relative sympathetic HR control in the non-anxious (LF/HF = 0.67) than in the anxiety-prone individuals (LF/HF = 0.53). Surprisingly, no significant Stimulus x Anxiety interaction in the frequency domain HRV analyses was found. Although, HR changes without accompanying changes in HRV have been described (Sloan et al., 1991), a negative correlation between HR and HF HRV is generally observed (Allen et al., 2007). The standing wave that was introduced into the heart data time series by thermal stimulation had a clear Stimulus- and Anxiety-differential effect on HR. Although this wave presumably was removed by the detrending procedure and its frequency lay outside the frequency spectra that were explored in this study, it is still not known to what degree it might have impacted the results of the HRV analyses. If smoothing did not take out all effects of the stimulus-induced LF wave, it could have increased variability, and impacted the statistical power of the analyses. The overall between-group differences were so robust that they survived the putative effect. However, the Stimulus differences might
have been affected, resulting in absence of a significant Stimulus x Anxiety interaction for the HRV measures.

To further elucidate how anxiety-proneness affects behavior in stressful situations and how it relates to measures of HRV, future examinations should employ non-pain aversive stimulation (Lyonfields et al., 1995; Bornas et al., 2005) or prolonged negative stress like mental arithmetic (Sloan et al., 1991). To enable the study of effects of differential perturbations of anxiety-prone and non-anxious subjects’ motivational states, experiments could apply volitional emotion regulation strategies (Gross, 1998) to down-regulate anxiety-related attention in the anxiety-prone group and introduce a competing high-level motivational state of positive affect in the form of a reward task in the non-anxious group (Ashby et al., 1999).

Limitations of the present study involve questions about generalizability of the results. All participants were healthy, young graduate students, and it cannot be ruled out that the anxiety-prone subjects represented a phenotype with moderate levels of anxiety that possessed specific cognitive and emotional-motivational characteristics. Further, our rest condition involved “rest” in the experimental environment and other results might have been obtained from rest in external, private surroundings.

Our findings underscore the use of HRV as a biological marker to evaluate central processing capabilities of cognitive-emotional and physiological systems. With increasing exploration of non-linear aspects of biology, we do anticipate that new knowledge will emerge in the field of HRV, enabling improved understanding
and utility of basic features of emotions and emotion-regulation and of the autonomic nervous system’s control of cardiovascular dynamics. Further exploration of anxiety-proneness is needed through epidemiological studies, but also through the experimental acquisition of a broad range of variables, including HRV metrics.
Fig. 1. Stimulus paradigm. One experimental test series consisted of four contiguous, repeated blocks. Each block comprised a 35°C baseline, and a stimulus that was kept at target temperature for 23 seconds with a rise/fall rate of 4°C per second. Nine series, three per stimulus temperature, were administered in pseudorandom order to each blinded participant per session.
Block 1  |  Block 2  |  Block 3  |  Block 4

- Light blue: Baseline, 35°C, 30 s pre-stimulus duration, 20 s post-stimulus duration
- Magenta: Thermal stimulation, 25°C, 47°C or 49°C
Fig. 2. Retrospective visual analog scale (VAS) ratings of pain intensity during the experimental session. Anxiety-prone subjects (n = 6) perceived noxious thermal stimuli as less painful than the non-anxious individuals (n = 6). Error bars: Standard error of the mean.
ANOVA (non-dichotomized)
Main effect Stimulus: $F(2, 15.50) = 22.05$, $p < 0.001$
Main effect Anxiety: $F(5, 31.17) = 4.60$, $p = 0.003$
Stimulus X Anxiety: $F(10, 15.47) = 2.66$, $p = 0.041$
Fig. 3. Average heart rate during the experimental session. The non-anxious individuals’ (n = 6) heart rate increased with increasing stimulus temperature while the anxiety-prone subjects’ (n = 6) heart rate did not change across conditions. Consequently, gradually increasing group differences arose with progressive increase in stimulus temperature. However, no group difference was found during pre-experimental rest. For estimates and statistics, please see Table 1. Error bars: Standard error of the mean.
Fig. 4. Mean inter-beat intervals and time-domain measures of heart rate variability derived from the experimental session. Compared to the non-anxious individuals (n = 6), the anxiety-prone subjects (n = 6) exhibited greater heart rate variability for all time-domain measures across all experimental conditions. For estimates and statistics, please see Table 1. MeanRR, Mean of the inter-beat intervals; NN50, Number of successive inter-beat interval pairs that differ more than 50 ms; pNN50, NN50 expressed in percent of the total number of inter-beat intervals; RMSSD, Square root of the mean squared differences between successive inter-beat intervals; SDNN, Standard deviation of the inter-beat intervals. Error bars: Standard error of the mean.
Fig. 5. Fast Fourier transform (FFT) and autoregressive (AR) frequency-domain measures of heart rate variability derived from the experimental session. Compared to the non-anxious individuals \((n = 6)\), the anxiety-prone subjects \((n = 6)\) exhibited greater absolute power of both the low and the high frequency bands across all experimental conditions. Average power value was larger in the high than in the low frequency band. In the non-anxious group, the relationship between low and high frequency band power was approximately 2:3, while in the anxiety-prone group this ratio was 1:2. The Fourier transform and the autoregressive method generated analogous results. For estimates and statistics, please see Table 1. LF, Low frequency band \((0.04 – 0.15 \text{ Hz})\); HF, High frequency band \((0.15 – 0.40 \text{ Hz})\). Error bars: Standard error of the mean.
Table 1. Estimates and statistics for heart rate and heart rate variability.

Anxiety, BSI anxiety score; AR, Autoregressive method; FFT, Fast Fourier transform method; Frequency, HF-LF band categories (based on dichotomized absolute power values); HF, High frequency band = 0.15 – 0.40 Hz; LF, Low frequency band = 0.04 – 0.15 Hz; MeanRR, Mean of the inter-beat intervals; NN50, Number of successive inter-beat interval pairs that differ more than 50 ms; pNN50, NN50 expressed in percent of the total number of inter-beat intervals; RMSSD, Square root of the mean squared differences between successive inter-beat intervals; SDNN, Standard deviation of the inter-beat intervals; SEM, Standard error of the mean; Stimulus, Type of acquisition series (Pre-stimulation rest, 25°C, 47°C or 49°C stimulation series).
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<tr>
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<th>NON-ANXIOUS GROUP</th>
<th>ANXIETY-PRONE GROUP</th>
<th>MIXED ANOVA (Non-dichotomized)</th>
<th>Statistical effect</th>
<th>Statistical significance</th>
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<td>Estimate</td>
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<td><strong>Heart Rate (1/min.)</strong></td>
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<td>F(23, 1415.68) = 5.32, p &lt; 0.001</td>
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<td>Mean RR (ms)</td>
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<td>0.53</td>
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<td>Main effect Frequency</td>
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<td>LF/HF</td>
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<td>Frequency X Anxiety</td>
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Suppl. Fig. 1. Strong positive Spearman correlation between Brief Symptom Inventory® (BSI) anxiety scores and State-Trait Anxiety Inventory (STAI) scores for both state and trait anxiety underscored substantial coherence between these psychometric instruments in the context of this experiment. N = 12.
Suppl. Fig. 2. Average respiratory rate during the experimental session. The non-anxious individuals (n = 6) exhibited greater respiratory rate than the anxiety-prone subjects (n = 6) across all experimental conditions. Error bars: Standard error of the mean.
ANOVA (non-dichotomized)
Main effect Anxiety: $F(9, 168.15) = 4.87$, $p < 0.001$
Suppl. Fig. 3. Average body mass index scores by anxiety-group. The anxiety-prone subjects’ (n = 6) average score was in the normal range, while the higher mean score for the non-anxious individuals (n = 6) placed them in the overweight category. Error bars: Standard error of the mean.
ANOVA (non-dichotomized)
Main effect Anxiety: $F(5, 106.65) = 3.42$, $p = 0.007$
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Chapter IV

EFFECT OF ANXIETY-PRONENESS ON CENTRAL HEART RATE-RELATED
BRAIN PROCESSING DURING EXPERIMENTAL PAIN

M. S. Hadsel, A. Quevedo, Y. Oshiro, R. A. Kraft and R. C. Coghill

The following manuscript has been completed and is ready for submission. Stylistic specificities are due to the requirements of the journal it was prepared for. Morten Hadsel and Dr. Robert Coghill designed the paradigm. Morten Hadsel, Dr. Alexandre Quevedo and Dr. Yoshitetsu Oshiro collected the data. Dr. Robert Kraft was responsible for operating the fMRI scanner. Morten Hadsel performed the analyses and prepared the manuscript. Dr. Robert Coghill acted in an advisory and editorial capacity.
Abstract

Integration of central autonomic activity with cognitive-emotional processes coordinates goal-directed behavior. Ventromedial prefrontal cortex has been associated with parasympathetic activity, and the dorsal anterior cingulate cortex and right insula with sympathetic activity. Activity in the latter two areas has been related to cognitive and motor tasks. We recently found pain perception and heart rate to depend on anxiety-proneness. Few investigations have explored central heart rate correlates under conditions of pain and their dependency on anxiety-proneness. We therefore performed functional magnetic resonance imaging of twelve healthy volunteers that experienced and retrospectively rated innocuous and noxious thermal stimuli, while heart rate was recorded. Psychometric assessment was performed two weeks post-imaging and ranked the participants according to level of anxiety-proneness. Our findings associated the ventromedial prefrontal cortex with cardio-vagal regulation during painful conditions and implicated left temporal pole in anxiety-specific vagal heart rate control, and pre-/post-central gyrus in interoception. Lack of central correlates of sympathetic regulation likely resulted from sparse cognitive/motor challenges during the experiment. As overall heart rate reactivity did not correlate with perceived pain, it seems likely that the emotional state associated with individual experimental series, and not perceived pain per se, controlled autonomic responses. The main neural correlates of cardiovascular measures partly overlapped with those involved in pain processing, potentially indicating integration of autonomic with both sensory and cognitive-emotional processes.
The present findings may serve as a basis for further multi-disciplinary and more specific investigations of central heart rate control in painful settings, in particular in the context of coexisting low-grade anxiety.
Introduction

Models of neuro-visceral integration suggest that cognitive and emotional brain processing inter-relate with a central autonomic network (Benarroch, 1993) to coordinate context-dependent adaptive processes (Thayer and Lane, 2000; Critchley, 2005; Friedman, 2007). Vagally mediated prefrontal tonic inhibition of heart rate (HR) has been proposed, as increase in HR resulted from pharmacological prefrontal inactivation (Ahern et al., 2001). Research utilizing positron emission tomography (PET) found a negative correlation between prefrontal activity and HR (Critchley et al., 2000b; Gianaros et al., 2004) and a positive correlation between prefrontal activity and parasympathetic cardiac events (Lane et al., 2009). Functional magnetic resonance imaging (fMRI) has confirmed an inverse relationship between ventromedial prefrontal cortex (vMPFC) activity and HR during handgrip exercise (Wong et al., 2007) as well as during pain and its anticipation (Porro et al., 2003). Retrograde transneuronal labeling in rats has established parasympathetic connections between the heart and brainstem autonomic regions, as well as hypothalamus, anterior cingulate cortex (ACC), insula and frontal cortex (Ter Horst and Postema, 1997). Consequently, cortical vagal effects on HR are likely mediated via brainstem autonomic regulatory centers. Insula and dorsal ACC activity is thought to reflect sympathetically driven complex integration of autonomic with cognitive and motor processes (Augustine, 1996; Williamson et al., 1997; Paus et al., 1998; Critchley et al., 2000b; Critchley et al., 2003).
Several imaging studies have utilized HR as a measure of arousal (Kalisch et al., 2005), reported on its coactivity with other examined parameters (Coghill et al., 1994) or studied its perturbation by physical activity (Wong et al., 2007). However, relatively few investigations have explored the correlates of central HR control under conditions of experimental pain. We therefore employed fMRI and physiological recording methods to delineate areas of HR-related cortical processing in a model employing different levels of thermal noxious stimulation. To limit additional causal influences on HR, apart from retrospective rating of the experienced pain, no other cognitive or physical tasks were performed during the experiment. Our hypothesis predicted an inverse relationship between HR and vMPFC activity in HR-related brain processing, but minor or no insula and dorsal ACC involvement due to putative limited cognitive participation.

We recently found anxiety-prone subjects to perceive noxious stimuli as less painful and exhibit lower HR than non-anxious individuals, presumably as part of a motivationally induced prioritization of worry/anxiety- over pain-related behavior (Hadsel, 2010b). As the perigenual division of the ACC has been implicated in emotional (George et al., 1995; Kulkarni et al., 2005) and autonomic processing (Vogt, 2005), we predicted that in addition to the vMPFC, the perigenual ACC would exhibit anxiety-specific HR-related activity.

Porro et al. (2003) found a population of HR- and pain-related brain activation clusters that exhibited comparable changes in activity during noxious stimulation and anticipation. These clusters were distributed across the medial
brain and were thought to represent close spatial overlap between different neuronal populations, providing a link between cognitive and emotional processing. We predicted that the pain-related activations would demonstrate a partial spatial overlap with neural correlates of HR in regions of higher-order processing in the prefrontal cortex.

Materials and Methods

Subjects

Twelve volunteers (seven females) with an average age of 25.4 (SD = 1.7) participated in this study. The group had mixed ethnic composition, consisting of 6 Caucasians, 2 African-Americans, 2 Asians, 1 African-Hispanic and 1 Hispanic. All subjects were right-handed, and according to self-report, healthy, drug- and pain free, had no history of a prolonged pain condition or drug abuse and were non-smokers. The female participants were scheduled for experimental testing before day eight or after day twenty of their menstrual cycle. All subjects gave written, informed consent, acknowledging that they would experience painful stimuli, that all methods and procedures were clearly explained, and that they were free to withdraw from the experiment at any time without prejudice. All procedures were approved by the Institutional Review Board of Wake Forest University School of Medicine.
Experimental design and stimulus paradigm

A psychophysical training session acquainted the participants with all experimental procedures and was succeeded by an experimental/imaging session seven days later. A psychometric assessment was performed 14 days subsequent to the imaging session. In each session, the participants rested in a supine position. The experimental test series were delivered to the subjects' left lower leg after their HR-values had reached stable levels. One experimental test series encompassed four contiguous, repeated blocks that each consisted of a stimulus period and a 35°C baseline. Each stimulus period involved either highly noxious (49°C), moderately noxious (47°C) or innocuous (25°C) stimuli that were kept at target temperature for 23 seconds, with a rise/fall rate of 4°C per second. Three test series were applied per stimulus temperature for a total of nine series. The series were administered in pseudorandom order to blinded participants, with overall series order additionally randomized between subjects. The stimulus paradigm is illustrated in figure 1.

A thermal stimulator (TSA II, Medoc Ltd., Ramat Yishai, Israel) with a 16 x 16 mm contact thermode was used to deliver all stimuli. In order to prevent long-term suppression or sensitization of nociceptive afferents (Price and Dubner, 1977), the contact thermode was moved to “naïve” skin locations before each test series. To avoid order effects, the thermode was placed on pre-marked skin sites in a counter-balanced fashion across subjects. Suppression of warm afferents in skin areas exposed to noxious heat can last up to several minutes (LaMotte and Campbell, 1978). To ensure that our results would not be distorted
by such suppression in potentially overlapping border-zones of the stimulated skin regions, we utilized a cool (25°C) rather than a warm stimulus as the non-noxious control in this study.

**Visual analog scale (VAS) use**

Following each experimental test series, the subjects assessed perceived pain intensity and unpleasantness/pleasantness. Real-time ratings, including cold sensation during the 25°C test series, were additionally carried out during the training session. All ratings were performed with VAS sliding scales. Such scales have been well validated for sensory and affective ratings of pain and exhibit ratio scale properties (Price et al., 1983; Price et al., 1994). The pain intensity and cold sensation scales both had a 0 – 10 range and were anchored by “No pain sensation” and “Most intense pain imaginable” and by “No cold sensation” and “Painfully cold”, respectively. The unpleasantness/pleasantness scale, with a -10 to +10 range, had a neutral position in the middle and was anchored to the left by “Most unpleasant imaginable” and to the right by “Most pleasant imaginable”. This scale was also used for quantification of hedonic change during temperature transitions, data that will be addressed elsewhere. To avoid confusion, we employed the unpleasantness/pleasantness scale, rather than a traditional unpleasantness scale, for all affective ratings in our experiment.

**Acquisition of HR, respiratory CO₂ and real-time VAS ratings**

HR was monitored by a pulse-oximeter (Nonin Medical, Inc, Plymouth,
MN) attached to the subjects’ left middle finger. CO$_2$ is a vasodilator and changes in blood CO$_2$ concentrations affect regional cerebral blood flow and the fMRI signal (Rostrup et al., 1994; Rostrup et al., 2000). There is a high correlation between partial CO$_2$ pressure in arterial blood gas measurements and end-expiratory air (Corbo et al., 2005). Throughout our experiment, the non-anxious individuals exhibited greater respiratory rate than the anxiety-prone subjects (Hadsel, 2010b). Therefore, to exclude potential group differential effects of CO$_2$-induced vascular changes on the fMRI signal, we utilized capnography to create CO$_2$ time-series that were entered in the imaging analysis as regressors of no interest. The participants were instructed to breathe through the nose, as respiratory air was sampled via nasal cannulas (Salter Labs, Arvin, CA). Continuous analysis of CO$_2$ concentration in the respiratory air was conducted with a capnograph (Capstar-100, CWE Inc., Ardmore, PA), and together with HR and real-time VAS ratings sampled at 100 Hz with a digital chart recorder (PowerLab/4sp, AD Instruments, Inc, Colorado Springs, CO). Custom IDL programs (IDL version 5.0, ITT Corp., Boulder, CO) were utilized to resample the different time series to the frequency of the functional imaging data (0.5 Hz) to create series-specific HR, CO2 and VAS regressor files. Due to technical difficulties, two 25°C and three 47°C HR-series were missing (4.6% of all series), and all data pertaining to these series were excluded from further analyses.
**Psychometric assessment**

Anxiety was assessed by the anxiety scale (0 – 4 range) of the Brief Symptom Inventory® (BSI) (Derogatis and Melisaratos, 1983), and this scale’s raw scores were used in the statistical analyses of this study. We utilized the BSI due to its multi-dimensional nature, aspects of which we will address elsewhere. To avoid confounds related to experimental and scanner-related fear/anxiety (Friday and Kubal, 1990; Murphy and Brunberg, 1997; Sarji et al., 1998), the psychometric instrument was administered 14 days after the imaging session. As a result, the researchers were blinded to the participants’ anxiety levels and the participants were blinded to the focus on anxiety during the experiment.

**Image acquisition and image processing**

Functional MRI data were acquired on a 1.5T General Electric Twin-Speed LX scanner with a birdcage quadrature head coil (General Electric Medical Systems, Milwaukee, WI). For functional imaging, two-dimensional blood oxygenation level-dependent (BOLD) images of the whole brain were acquired continuously by an echo-planar technique [40 ms echo time (TE); 2s repetition time (TR); 28 x 5-mm-thick slices; 3.72 x 3.75 mm in-plane resolution; 90° flip angle; no slice gaps] (Ogawa et al., 1990). Each functional acquisition series lasted 350 seconds (175 volumes), during which the subjects were requested to keep their eyes closed. High-resolution structural images were acquired using a three-dimensional spoiled gradient-echo sequence (600 ms inversion time; 9.1 ms TR; 20° flip angle; 1.98 ms TE; 256 x 196 matrix; 1.5 mm section thickness
The functional image analysis package FSL version 4.0 [Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (Center for FMRIB, University of Oxford, Oxford, UK)] (Smith et al., 2004) was employed for image processing and statistical analysis. The following pre-processing was applied to the input data: movement correction; masking of non-brain voxels (Smith, 2002); spatial smoothing by 5 mm with a 3D isotropic Gaussian kernel; scaling of each functional image by its mean global intensity; and nonlinear high-pass temporal filtering with a cutoff period identical to 1.5 x the block length. The functional images were registered to the corresponding structural data using a 7-parameter linear 3D transformation and converted into standard stereotaxic space (as defined by the Montreal Neurological Institute) using a 12-parameter linear 3D transformation (Talairach and Tournoux, 1988; Jenkinson and Smith, 2001; Jenkinson et al., 2002). As signal loss and fMRI artifacts (Devlin et al., 2000; Gorno-Tempini et al., 2002) might result from magnetic field inhomogeneities caused by proximity of air-filled spaces to the ventral prefrontal brain, pre-registration thresholding was employed to remove areas of signal loss from the activation maps. Cardiac pulsatility artifacts are mostly seen related to the brainstem and the ventricles and can impede signal detection in the analysis of BOLD fMRI, especially when applying HR-related regressors (Dagli et al., 1999; Friese et al., 2004). To reduce this cardiac-induced noise in the BOLD signal, we used a parsimonious approach. Results from within-subject second
level analyses that revealed cardiac pulsatility artifacts were discarded, and the constituent first level individual series results were revisited. 4D image files that corresponded to first level analysis results clearly indicating cardiac noise were subjected to independent component analyses (Beckmann and Smith, 2004). Independent components exhibiting artifacts were identified by visual inspection and removed from the 4D image file by a denoising algorithm, which is part of the FSL analysis package. Denoised files were then utilized for re-analyses.

**Imaging analyses**

Statistical imaging analyses were performed with general linear models. For the thermal stimulus-related brain activity, a boxcar regressor (stimulus-periods = +1; rest-periods = -1) was applied, while individual, series-specific time-series comprising continuous HR were used as regressors to examine the HR-related brain activations. To reduce confounding effects of CO\textsubscript{2}-induced vascular changes on the fMRI signal, series-specific recordings of continuous respiratory CO\textsubscript{2} from the imaging session were entered as regressors of no interest. The regressors were convolved with a gamma-variate model of the hemodynamic response function (mean lag: 6 sec.; SD: 3 sec.) and temporally filtered with the same parameters as the fMRI data. Clusters of voxels with a Z-score > 2.3 and a p < 0.05 were considered statistically significant (Worsley et al., 1992). Fixed-effects second level analyses contrasted intra-subject series and were also used to explore the conjunction of first level temperature-related contrasts. On the third level, anxiety-scores were introduced as an additional regressor and mixed-
effects models explored inter-subject variance.

Pain-related emotions are associated with pain-related autonomic regulation (Rainville et al., 2005) and both increase and decrease in HR has been demonstrated subsequent to different elicited emotions (Bauer et al., 2002). To address a potential need to regress out the perceptual aspects of pain in the present HR-related imaging investigation, we performed a correlation analysis of perceived pain intensity and stimulus-induced HR changes. [In our data, perceived pain intensity was linearly related to perceived pain unpleasantness, i.e. the affective/emotional component of pain (Fig. 2)]. As no significant correlations were demonstrated (Fig. 3), we refrained from regressing out the perceptual aspects of pain in the HR-related imaging analysis.

To compare spatial features and visualize potential spatial overlaps of the thermal- and heart rate-related activations, their activation-clusters were binarized and co-presented in joint brain maps by means of a custom IDL program (IDL version 5.0, ITT Corp., Boulder, CO).

Outliers were defined as BOLD activity values beyond three inter-quartile ranges above or below the upper or lower quartile, respectively. One outlier-series (0.92% of all series) was detected and excluded in toto from all analyses.

Processing and statistical analyses of the psychophysical and physiological data

SPSS® version 15.0 (SPSS Inc., Chicago, IL), was employed for mixed model analyses of variance (ANOVA) of all non-imaging data. A two-tailed
probability value of 0.05 was used as significance criterion in all analyses. Exact p-values were also reported. Where appropriate, Bonferroni-corrected post-hoc tests were utilized to examine significant effects. Although anxiety score represented a continuous variable, it was entered as a factor in the analyses due to the fixed number of anxiety score levels. For visualization purposes, subjects were dichotomized into anxiety-prone (BSI anxiety raw scores > 0, n = 6) and non-anxious (BSI anxiety raw scores = 0, n = 6) groups. To simplify the management of the ANOVA raw data, the time series were aggregated to reflect average activity across each period.

HR was examined with an a priori model, entering Stimulus (type of experimental series), Period (within-block pre-stimulus, stimulus or post-stimulus period) and Anxiety as factors into the analysis, collapsing across series and blocks. The rationale for collapsing across series was based on uniform retrospective VAS rating behavior across analogous stimulus series. The rationale for collapsing across blocks was based on uniform real-time VAS rating behavior across blocks during the training session and the absence of a block-effect in previous HR analyses of this material (Hadsel, 2010b). Spearman-correlations between subjects’ average retrospective VAS scores and their BSI anxiety scores were also performed.
Results

Psychometric scores

The results for the BSI anxiety raw scores were previously reported (Hadsel, 2010b). Compared to a normative sample of adult non-patients, the anxiety-prone subjects (n = 6) were located at the 78th (range: 35th% - 97th%) and the non-anxious individuals (n = 6) at the 22nd percentile, with the overall mean score at the 50th percentile. Strong Spearman correlations between these BSI anxiety scores and state \( r_s = 0.69, p = 0.014 \) and trait \( r_s = 0.70, p = 0.011 \) anxiety scores obtained by Spielberger’ State-Trait Anxiety Inventory (Spielberger, 1983; Spielberger and Vagg, 1984) have also been reported earlier (Hadsel, 2010a).

Psychophysical pain ratings

Anxiety-specific ratings of the noxious stimuli were observed (Fig. 2). This was demonstrated by a negative Spearman correlation between average retrospective VAS pain intensity ratings and BSI® anxiety scores during 49°C stimulation \( (r_s = -0.73, p = 0.008) \). Further, the retrospective VAS pain unpleasantness/pleasantness ratings revealed that with increasing anxiety, highly noxious thermal stimuli were perceived as less unpleasant \( (r_s = 0.73, p = 0.007) \). Results from the mixed ANOVA regarding the retrospective and real-time psychophysical thermal and pain ratings have previously been reported (Hadsel, 2010b) and confirmed the outcomes of the Spearman correlation analyses.
Heart rate and respiratory CO\textsubscript{2}

The non-anxious individuals’ heart rate was on average 11\% greater than that of the anxiety-prone subjects [Main effect Anxiety: F(8, 18.06) = 4.19, p = 0.005] and increased with increasing stimulus temperature [Stimulus x Anxiety: F(16, 1060.53) = 6.03, p < 0.001]. Post-hoc tests revealed no statistically significant differences between HR during the two lower temperature conditions in the non-anxious group or across any experimental series in the anxiety-prone subjects. Neither could any group difference be identified during the 25°C experimental condition. For either group, heart rate did not vary across periods within any experimental condition [Stimulus x Period x Anxiety: F(16, 1101.86) = 1.53, p = 0.080]. However, during the 49°C stimulus series, both groups exhibited a tendency for accelerated heart rate during the stimulus/post-stimulus periods compared to the pre-stimulation period (Fig. 4).

Average respiratory CO\textsubscript{2} concentrations varied with level of anxiety [Main effect Anxiety: F(8, 136.07) = 16.01, p < 0.001]. However, these concentrations were confined within the norm range (4\%–6\%) in both groups, and measured 5.4\% (SEM: 0.04) and 5.1\% (SEM: 0.04) in the anxiety-prone and the non-anxious groups, respectively.

Imaging

Thermal stimulus-related brain activity (Fig. 5)

Previously reported brain activation patterns were observed during noxious stimulation (Derbyshire et al., 1997; Coghill et al., 1999; Peyron et al.,
2000; Treede et al., 2000). Thalamus, insulae, mid-dorsal anterior cingulate cortex, cerebellum, and contralateral pre- and post-central gyri exhibited pain-related activations. The precuneus, posterior cingulate and ventromedial prefrontal cortices deactivated during noxious stimulation.

Heart rate-related brain activity (Fig. 6)

Across stimulus conditions, involvement of several brain areas implicated in central autonomic and cognitive-emotional processing was observed. A cluster encompassing the ventromedial prefrontal and pregenual anterior cingulate cortices, as well as the temporal poles exhibited lower heart rate-related activity during the 49°C series when compared to both the 47°C and the 25°C conditions. The precuneus/posterior cingulate cortex also demonstrated lower heart rate-related activity during the 49°C than during the 47°C experimental series. During the 49°C condition, right insula and the opercular part of the inferior frontal gyrus activity correlated with heart rate, as did sensory areas involving thalamus and the right pre- and post-central gyri. HR-related cerebellum activity was observed during both noxious conditions. Anxiety correlated positively with heart rate-related left temporal pole activity during the 49°C experimental condition. That relationship was also verified in the anxiety-specific 49°C to 47°C comparison, as no or a very weak such correlation existed during the 47°C series. However, a similar, but statistically non-significant, correlation must have been present in the 25°C condition, as no significant differences emerged in the 49°C to 25°C contrast. Anxiety correlated negatively with heart rate-related activity in bilateral
pre- and post-central gyri during the 49°C experimental series. The statistical contrasts revealed that this correlation was stronger during the 49°C condition than during the 47°C and the 25°C conditions, but mainly for left-sided pre- and post-central gyrus heart rate-related activity.

Spatial overlap between heart rate- and thermal stimulus-related brain activity (Fig. 7)

Brain areas exhibiting HR- and thermal stimulus-related activity during noxious stimulation partially overlapped in several sensory and cognitive-emotional processing regions. Brain regions that exhibited less HR- and thermal stimulus-related activity in the 49°C than in the 47°C and the 25°C temperature conditions displayed partial spatial overlap in the vMPFC. During the 49°C experimental condition, HR- and thermal stimulus-related activations of the left pre- and postcentral gyri were inversely correlated with anxiety scores, and incorporated an area of overlapping activity. However, no overlap was evident between any of the statistically temperature-contrasted anxiety-specific activation maps.

Discussion

The non-anxious individuals’ heart rate increased with increasing stimulus temperature and was on average greater than the anxiety-prone subjects’ heart rate, which was stable across stimulus conditions. Overall HR increased slightly with rise in stimulus temperature. HR-related vMPFC activity was lower during
49°C than during 47°C and 25°C stimulation but did not differ between the two lower temperature conditions. In light of previous data (Ahern et al., 2001; Gianaros et al., 2004; Lane et al., 2009) and basic autonomic physiology, it seems reasonable to assume that the HR-related vMPFC activity correlated with parasympathetic activity. During affective states related to noxious stimulation, the parasympathetically related vMPFC activity was therefore reduced to enable increase in HR. This is identical to previously demonstrated cardio-vagal events during physical activity (Wong et al., 2007). The cluster of HR-related vMPFC activity also encompassed perigenual ACC areas. Presumably, this reflected analogous processing of these two regions, as the subgenual/ventral ACC has been associated with visceromotor integration (Vogt, 2005) and parasympathetic activity (Matthews et al., 2004; Tang et al., 2009).

According to the Z_{max} coordinates, both the main vMPFC cluster reflecting HR-related and the main vMPFC cluster reflecting pain-related processing were located more ventro-caudal during the 49°C than during the 47°C experimental condition. Also when comparing the main HR- with the main pain-related processing locus within noxious experimental series, it was evident that HR-related processing was located more ventro-caudal than the comparable cluster of pain-related processing (Table 1). During the 49°C condition the main loci of HR- and pain-related vMPFC processing extended ventrally into the left temporal pole. These observations suggested differential autonomic- and pain-related spatial involvement of the vMPFC dependent upon how aversive the context was perceived. Our findings, at least partly, corresponded to findings of autonomic
processing in the prefrontal region being associated with its ventromedial part and the posterior orbitofrontal cortex (Hoff et al., 1963; Hall et al., 1977).

Overall HR-related TP activity was reduced during the 49°C condition, compared to the 47°C and 25°C experimental states. However, HR-related left TP activity correlated with anxiety during 49°C stimulation, demonstrating that the anxiety-prone subjects deactivated the area less than the non-anxious individuals and putatively exhibited a higher relative level of parasympathetic activity. The TP has been linked to affective-sensory integration in primates (Markowitsch et al., 1985; Morán et al., 1987), and to social and emotional processing (Ding et al., 2009) and fear-/anxiety-related autonomic regulation (Kimbrell et al., 1999; Masaoka and Homma, 2001) in humans. A PET study of social phobics during anticipatory fear revealed reduced blood flow in the left TP accompanied by increased HR (Tillfors et al., 2002). Presumably, the anxiety-prone individuals directed their attention towards non-pain worry/anxiety (Hadsel, 2010b), which might have caused less anticipatory fear towards pain, compared to the non-anxious subjects. Consequently, this could explain the anxiety-prone individuals’ relative higher level of left TP activity and their lower HR. A positive correlation between trait anxiety and parasympathetic activity has also been reported in a study of meditation (Murata et al., 2004).

Pre- and post-central gyrus HR-related activity correlated negatively with anxiety during the 49°C experimental series. The correlation was stronger for left-sided activity during the 49°C condition than during the 47°C and the 25°C conditions. This brain region has been implicated in interoception (Cameron and
Minoshima, 2002; Pollatos et al., 2007) and the somato-sensory/-motor activity presumably reflected that the anxiety-prone subjects perceived and possibly maintained lower HR than the non-anxious individuals. Anatomical connections from the primary somato-sensory/-motor cortices to the brainstem have been established in rodents (Desbois et al., 1999). Consequently, it could be speculated that this would allow putative efferent signals from pre-/post central gyri to modulate brainstem autonomic activity.

Ventral PCC is associated with self-relevance assessment of contextual events and is reciprocally connected to the visceromotor peri-genual ACC region (Vogt and Pandya, 1987). PCC activity correlated negatively with parasympathetic activity during emotional elicitation (O'Connor et al., 2007), and positively with exaggerated blood pressure reactions to behavioral stressors (Gianaros et al., 2005) and HR during noxious stimulation (Porro et al., 2003). In our data a part of the ventral PC/PCC exhibited less HR-related activity during the 49°C than during the 47°C experimental condition and presumably therefore conflicted with previous findings. However, in the same statistical contrast, ventral PC/PCC and perigenual ACC activity correlated, potentially reflecting information-exchange between autonomic and self-relevance assessment processes.

Autonomic events in dorsal ACC and right insula are biased towards sympathetic activity (Williamson et al., 1997; Critchley et al., 2000a; Critchley et al., 2003). Insula contains both sympato-excitatory and tonically active sympato-inhibitory neurons (Cechetto and Chen, 1990; Yasui et al., 1991) and
right insula has been associated with stress-related sympathetic activity (Fechir et al., 2009). In our data right insula demonstrated HR-related activity during the 49°C experimental series, although no such activity emerged in the contrast between the different experimental conditions. The HR-related activity probably reflected sympathetic output operating in conjunction with vMPFC vagal withdrawal to increase HR, may be reflecting the stressful features of highly noxious stimulation. Dorsal ACC activity has been correlated with task difficulty (Paus et al., 1998), and insula activity with “effortful” events (Williamson et al., 1997), implicating these areas in cognitive/emotional-autonomic integration (Augustine, 1996; Critchley et al., 2000b). In our study, no continuous tasks were conducted during scanning, only retrospective rating of the stimulus was performed. Due to the participants’ knowledge of uniform stimuli across individual experimental series, and their experiences from the training session, the rating behavior might have become “automated”, further reducing the associated cognitive “effort”. These might be reasons we saw no HR-related dorsal ACC and only sparse HR-related insula engagements. Our results did not confirm those of Porro’s study (2003), where pain-ratings were partly performed during the experiment and HR-related mid/dorsal ACC activity during noxious stimulation was found.

Cerebellum exhibited HR-related activity during noxious stimulation, with no differences in activity between the experimental conditions. Specific cerebellar participation in visceral processes has been suggested based on observed correlations between cerebellar activity and HR (Critchley et al., 2000b;
Tillfors et al., 2002; Wong et al., 2007; Napadow et al., 2008) as well as anatomical connectivity between cerebellum and hypothalamus (Dietrichs et al., 1994). However, analogous to cerebellum’s role in motor execution and control by the use of internal, forward models, “cognitive” cortico-cerebellar loops could be the basis for cerebellar cognitive-emotional and autonomic learning, and for execution of routine commands (Ramnani, 2006).

Cerebral hemispheric lateralization in central cardiac control generally relates sympathetic activity to the right and parasympathetic activity to the left hemisphere (Oppenheimer et al., 1992; Williamson et al., 1997; Yoon et al., 1997; Critchley et al., 2000b; Critchley et al., 2000a; Williamson et al., 2001). Due to dual sympathetic/parasympathetic innervations, inferences about such laterality from assessment of end-organ output might be flawed (Wittling et al., 1998). Our study putatively found the insula, vMPFC and the anxiety-specific TP 49°C activations to agree with the general concept of autonomic lateralization.

Shared variability between central noxious- and HR-related processing, as expressed by their partial spatial overlaps, was localized both to brain regions linked to sensory processing and higher-order coordination of goal-directed behavior. The observed overlaps might simply represent co-variation between the two types of processing, as frequently noxious stimuli perturb autonomic activity (Harper et al., 2000). However, the overlaps could imply interactions between noxious- and HR-related processing, as has been hypothesized within a central autonomic network (Benarroch, 2006) and in activation clusters across the medial brain (Porro et al., 2003). Also, individual differences in electrodermal
reactivity partly reflected differences in pain-evoked brain responses, identifying areas of dual pain and autonomic processing (Dube et al., 2009). Central noxious- and HR-related processing might also undergo common pharmacological modulation, e.g. endogenous opioids can generate analgesic and bradycardic responses (Kiritsy-Roy et al., 1989; McCubbin et al., 1993; Pollo et al., 2003; Bodnar, 2008). Accordingly, endogenous opioid action could, apart from explaining the disparity in HR and perceived pain between the non-anxious and the anxiety-prone groups, also be related to co-localized noxious- and HR-related processing areas.

The results demonstrated that overall HR did not correlate with perceived pain, but did increase for the experimental series as a whole with increase in related stimulus temperature. Although this relationship was not statistically significant, the anxiety-specific effects were, demonstrating both between group and within non-anxious group effects. It has been shown that pain-related emotions are able to modulate associated autonomic activity (Rainville et al., 2005). It therefore seems likely that the emotional state associated with individual experimental series, and not perceived pain per se controlled autonomic responses during the experiment. Accordingly, such an emotional state might be more broadly linked to the painful stimuli through additional anticipatory and other fear/anxiety-related events.

The strategy of a priori collapsing across blocks and series in the HR analyses was based on the findings that average real-time pain rating behavior was unchanged across blocks and that average retrospective ratings were stable
across analogous experimental series. Additionally, no block-effects were observed in a previous HR analysis on the same material (Hadsel, 2010b). However, subtle and potentially systematic changes in HR within and across analogous experimental series can not be ruled out. Collapsing across blocks and series in the statistical HR analysis, as well as the aggregation of the raw data to reflect average activity across each period might have deflated the effects of HR dynamics and led to the absence of a significant main effect for Stimulus. On the other hand, the individual time series regressors used in the imaging analysis reflected the original resampled recordings. Consequently, the imaging results could have a greater fidelity in representing the genuine physiological events.

Our findings associated the vMPFC with cardio-vagal regulation during painful conditions and implicated left TP in anxiety-specific vagal HR-regulation, and postcentral gyrus in interoception. Lack of central correlates of sympathetic regulation likely resulted from sparse cognitive/motor challenges during the experiment. The main neural correlates of cardiovascular measures partly overlapped with those involved in pain processing, which might indicate integration of autonomic with both sensory and cognitive-emotional processes. The present findings may serve as a basis for further multi-disciplinary and more specific investigations of central HR control in painful settings, in particular in the context of coexisting low-grade anxiety.
Fig. 1. Stimulus paradigm. One experimental test series consisted of four contiguous, repeated blocks. Each block comprised a 35°C baseline, and a stimulus that was kept at target temperature for 23 seconds with a rise/fall rate of 4°C per second. Three test series were applied per stimulus temperature for a total of nine series. The test series were administered in pseudorandom order to blinded participants.
Baseline, 35°C, 30 s pre-stimulus duration, 20 s post-stimulus duration

Thermal stimulation, 25°C, 47°C or 49°C
Fig. 2. Average retrospective visual analog scale (VAS) pain intensity scores (A) and pain unpleasant-/pleasantness scores (B) plotted as a function of Brief Symptom Inventory® (BSI) anxiety scores. Spearman correlations demonstrated that with increasing anxiety, highly noxious stimuli were perceived as less painful and less unpleasant. N = 12.
Fig. 3. Retrospective visual analog scale (VAS) pain intensity scores plotted as a function of average changes in heart rate between stimulus and baseline conditions for each experimental test series. No significant correlations between perceived pain and stimulus-induced heart rate changes were demonstrated. $N = 34$ (49°C), $N = 33$ (47°C) and $N = 31$ (25°C). bpm, beats per minute.
A scatter plot showing the relationship between VAS Pain Intensity score and Stimulus-related heart rate change (bpm) at different temperatures:

- **49°C**: $r = -0.16$, $p = 0.353$
- **47°C**: $r = -0.23$, $p = 0.195$
- **25°C**: $r = 0.15$, $p = 0.427$
Fig. 4. Average heart rate during the experimental session. Overall, the non-anxious individuals (n=6) exhibited greater heart rate than the anxiety-prone subjects (n = 6). The non-anxious individuals’ heart rate increased with increasing stimulus temperature while the anxiety-prone subjects’ heart rate did not change across experimental conditions. Pre., Pre-stimulus period; Stim., Stimulus period; Post., Post-stimulus period. Error bars: Standard error of the mean.
ANOVA (non-dichotomized)
Main effect Period: $F(2, 1107.51) = 27.89, p < 0.001$
Main effect Anxiety: $F(8, 18.06) = 4.19, p = 0.005$
Stimulus X Period: $F(4, 1101.66) = 16.27, p < 0.001$
Stimulus X Anxiety: $F(16, 1060.53) = 6.03, p < 0.001$
Period X Anxiety: $F(8, 1109.17) = 2.94, p = 0.003$
Stimulus X Period X Anxiety: $F(16, 1101.86) = 1.53, p = 0.080$
Fig. 5. Thermal stimulus-related brain activity identified with general linear model analyses, using stimulus-related regressors. Typical brain activation patterns were observed during noxious stimulation. Thalamus, insulae, mid-dorsal anterior cingulate cortex, cerebellum, and contralateral pre- and post-central gyri exhibited pain-related activations. The precuneus, posterior cingulate and ventromedial prefrontal cortices deactivated during noxious stimulation. N = 12. AMY, Amygdala; CAU, Caudate; CER, Cerebellum; HIP, Hippocampus; INS, Insula; ITG, Inferior temporal gyrus; LOC, Lateral occipital cortex; mACC, Mid anterior cingulate cortex; MTG, Middle temporal gyrus; OFC, Orbitofrontal cortex; OL, Occipital lobe; PC, Precuneus; PCC, Posterior cingulate cortex; pgACC, Pregenual anterior cingulate cortex; pg/sg-ACC, Pre-/subgenual anterior cingulate cortex; PHG, Parahippocampal gyrus; Pr/Po-CG, Pre-/Postcentral gyri; PUT, Putamen; SFG, Superior frontal gyrus; SMA, Supplementary motor area; S/M-TG, Superior/Middle temporal gyri; STG, Superior temporal gyrus; TFG, Temporal fusiform gyrus; THAL, Thalamus; TOFG, Temporo-occipital fusiform gyrus; vMPFC, Ventromedial prefrontal cortex.
Thermal stimulus-related brain activity

Temperature

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<td>CAU</td>
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<td>CER PHG THAL OL vMPFC/pgACC</td>
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Left side of image = right brain
Fig. 6. Heart rate-related brain activity identified with general linear model analyses, using individual continuous heart rate time series as regressors. Across stimulus conditions, involvement of several brain areas implicated in central autonomic and cognitive-emotional processing was observed. Main findings included lower heart rate-related activity in the ventromedial prefrontal cortex during the 49°C series when compared to both the 47°C and the 25°C conditions. Anxiety correlated positively with heart rate-related left temporal pole and negatively with pre- and post-central gyrus activity during the 49°C experimental condition. N = 12. AMY, Amygdala; BS, Brainstem; CER, Cerebellum; IFG-PO, Inferior frontal gyrus pars opercularis; INS, Insula; NAc, Nucleus accumbens; OFC, Orbitofrontal cortex; PC, Precuneus; PCC, Posterior cingulate cortex; pgACC, Perigenual anterior cingulate cortex; pg/sg-ACC, Peri-/Subgenual anterior cingulate cortex; Pr/Po-CG, Pre-/Postcentral gyri; sgACC, Subgenual anterior cingulate cortex; SPL, Superior parietal lobule; THAL, Thalamus; TP, Temporal pole; vMPFC, Ventromedial prefrontal cortex.
Heart rate-related brain activity

<table>
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<tr>
<th>Temperature</th>
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<th>49°C</th>
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- vMPFC/OFC
- vMPFC
- Pr/Po-CG
- TP
- Pr/Po-CG
- Pr/Po-CG
- Pr/Po-CG
- vMPFC
- sACC
- CER
- SPL
- INS/IFG-PO
- pgAcc/ACC
- pgACC/vMPFC
- PC/PCC
- vMPFC
- CER
- sgACC/NAc
- pgACC/vMPFC
- pgACC/vMPFC

Left side of image = right brain

-5.5 -2.3 2.3 4.8
Fig. 7. Spatial overlap between binarized general linear model-derived areas of heart rate- (Fig. 6) and thermal stimulus-related (Fig. 5) brain activity. The ventromedial prefrontal cortex represented an area of partial spatial overlap between the heart rate- and thermal stimulus-related deactivations during 49°C relative to the 47°C and the 25°C experimental conditions. No overlap was found between any of the statistically contrasted anxiety-specific activation maps. N = 12. AMY, Amygdala; CER, Cerebellum; HIP, Hippocampus; INS, Insula; MTG, Middle temporal gyrus; OL, Occipital lobe; PC, Precuneus; PCC, Posterior cingulate cortex; pgACC, Pregenual anterior cingulate cortex; PrCG, Precentral gyrus; Pr/Po-CG, Pre-/Postcentral gyri; RSC, Retrosplenial cortex; sgACC, Subgenual anterior cingulate cortex; THAL, Thalamus; TP, Temporal pole; vMPFC, Ventromedial prefrontal cortex.
# Spatial overlap of heart rate- and thermal stimulus-related brain activity

<table>
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<tr>
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<th>Anxiety-specific effect</th>
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- **Heart rate-related brain activity**
- **Overlap of heart rate- and thermal stimulus-related brain activity**
- **Thermal stimulus-related brain activity**

Left side of image = right brain
Table 1. Imaging statistics for the spatially most extensive deactivation cluster of heart rate- and thermal stimulus-related brain activity encompassing the ventromedial prefrontal cortex (vMPFC) and the left temporal pole (TP).
<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Cluster size (voxels)</th>
<th>( Z_{\text{max}} ) location</th>
<th>( Z_{\text{max}} )</th>
<th>( Z_{\text{max}} ) coordinates (mm)</th>
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| Stimulus-related       |                      |                               |
| 47°C                   |                      |                               |
| 49°C                   |                      |                               |
References


Chapter V

DISCUSSION

The results of our study demonstrated that throughout three different VAS rating procedures, with stimulation at two different body sites, and from recordings during two different sessions, anxiety-prone subjects perceived noxious thermal stimuli as less painful than the non-anxious individuals. During the 49°C experimental series, vMPFC hypo-activity was observed in the anxiety-prone relative to the non-anxious group. ICs encompassing peri-stimulus related vMPFC activity accounted for considerably more variability in brain activation in the anxiety-prone than in the non-anxious group. Among qualitative group differences in those ICs was a tendency towards cortical processing in the anxiety-prone and sub-cortical processing in the non-anxious individuals. Stimulus-related right insular and dorsomedial prefrontal activity emerged in the anxiety-prone, relative to the non-anxious subjects.

HR did not differ between the two groups during rest, but with increasing stimulus temperatures HR increased progressively in the non-anxious, while remaining stable in the anxiety-prone group. The anxiety-prone subjects exhibited greater measures of HRV than the non-anxious individuals in the time-domain and within both the HF and LF band of the frequency-domain HRV analyses. The LF/HF power ratio was increased in the non-anxious relative to the anxiety-prone group. RER and BMI were greater in the non-anxious than in the anxiety-prone group, without confounding the anxiety-specific effects of HRV. Our findings also revealed lower heart rate-related activity in the ventromedial...
prefrontal cortex during the 49°C series when compared to both the 47°C and the 25°C conditions. Anxiety correlated positively with heart rate-related left temporal pole and negatively with pre- and post-central gyrus activity during the 49°C experimental condition. The main neural correlates of cardiovascular measures partly overlapped with those involved in pain processing.

We interpreted the psychophysical findings as in support of the perceptual-defensive-recuperative model of fear and pain (Bolles and Fanselow, 1980; Fanselow, 1986) and recent publications on state-dependent opioid analgesia (Fields, 2004; Fields, 2007). The emotional-motivational state associated with putative worries (Dahlin et al., 2005; Brimstone et al., 2007) in the anxiety-prone group may have outcompeted the pain-related motivations and led to their perception of less pain compared to the non-anxious individuals (Miller, 1948; Leknes et al., 2008; Leknes and Tracey, 2008). This demonstrates in an experimental paradigm that a putative, more persistent, mood state can act as a motivator to generate differentiated pain modulatory effects as part of goal-directed behavior. To my knowledge, this is the first time such events have been explored with functional neuroimaging. Diversity in behavioral outcomes from noxious stimulation in healthy subjects undergoing fear- and anxiety-induction (Malow, 1981; Cornwall and Donderi, 1988; Rhudy and Meagher, 2000) might, apart from differential relevance of fear/anxiety to pain, be explained by the existence of disparate innate mood tendencies among the participants.

Maladaptive attentional and emotional mechanisms of pain processing such as pain hypervigilance, pain-related anxiety and pain catastrophizing play
an important role in the development and maintenance of chronic pain conditions. Dysphoric emotions, including anxiety, contribute to this aberrant pattern of behavior (Huber et al., 2010). One might speculate that chronic pain behavior, at least partly, may be explained by synergistic effects between anxiety/fear linked to pain and to pain-associated states. Consequently, our findings not only underscore the positive effect of distraction on pain (Bushnell et al., 1999), but would encourage the use of cognitive treatment approaches involving distraction (McCaul and Malott, 1984) and cognitive restructuring (Gil et al., 1990) to re-appraise the experience of pain-associated states into non-pain related events. The outcome of such procedures has been linked to reduced perception of pain and decreased functional disability (Jensen and Karoly, 1991; Tota-Faucette et al., 1993). At least indirectly, our findings also point to the power of motivational states and suggest the exploitation of such powers in the treatment of clinical conditions (Levensky et al., 2007; Brennan et al., 2008; Wade et al., 2009).

Relative vMPFC hypo-activity was observed in the anxiety-prone subjects during the 49°C experimental series and could reflect that the associated decision-making process was biased to facilitate anxiety-related behavior. This putative irregular affect-guided decision-making process (Davidson, 2003) might have parallels to emotional dysregulation in PTSD. Relative hypo-activity of the vMPFC has also been observed in PTSD and has been implicated in dysfunctional antecedent emotion regulation (Etkin and Wager, 2007). Additionally, PTSD sufferers exhibit relative hypo-activity of prefrontal brain areas
in response to aversive (Shin et al., 2005), and stress-induced hypo-/analgesia (SIA) subsequent to noxious stimulation (Pitman et al., 1990). SIA is traditionally reported in association with physiological arousal (Pitman et al., 1990; Rhudy and Meagher, 2003). As our data demonstrated less physiological arousal in the anxiety-prone than in the non-anxious individuals, SIA does not seem relevant to our findings. Further, the analgesic effect observed in SIA is reportedly exerted through the descending pain modulatory system (Martenson et al., 2009). Our data instead indicated a tendency for cortico-cortical pain processing in the anxiety-prone group, at least relative to the non-anxious subjects.

It is known that “global” mood-states are linked to activity in subcortical brain structures (Panksepp, 1998). Therefore, to address the question of what subcortical brain areas could be part of a circuitry involved in putative mood-dependent behavioral choice, a ROI analysis of Nucleus Accumbens (NAc) activity in the present study was performed (not included in the dissertation). NAc is a brain area thought to be involved in novel stimulus detection and identification (Redgrave et al., 1999; Redgrave et al., 2008) as well as reward-related processing (Drevets et al., 2001; Schultz, 2007). Preliminary results from our ROI analysis demonstrated that during non-noxious stimulation NAc deactivation took place in block 1 on the side contralateral to the stimulation. Such activity could potentially be related to detection of novel events. When novelty was paired with saliency, as during noxious stimulation, an anxiety-differentiated NAc response was evident. This response encompassed marked contralateral NAc deactivation only in the anxiety-prone group and only in block
1. No significant NAc signal change occurred subsequent to block 1 or on the side ipsilateral to the stimulation at any time. NAc might therefore be part of an emotional-motivationally biased action selection circuit, activating appropriate effector systems that in the present context suppressed the anxiety-prone group’s responses to its motivationally conflicting noxious event. Our results suggest that NAc activity is central to the relationship between motivational aspects of dispositional affective states and the selection and generation of goal-directed action (Hadsel et al., 2010). NAc has further been implicated in cognitive re-appraisal/emotion regulation (Wager et al., 2008; Kober et al., 2010). Consequently, NAc could also represent a neurobiological substrate involved in pre-attentive emotion regulation, able to shape experience and behavior.

The networks encompassing the vMPFC during 49°C stimulation accounted for substantially more variability in brain activation in the anxiety-prone than in the non-anxious subjects. We suggested that this might reflect a greater extent of dynamic interactions within cognitive-emotional networks in the anxiety-prone individuals, as opposed to the non-anxious subjects. It must be emphasized here that this suggested explanation is speculative. Although intuitively, the finding would suggest a notable difference in brain processing between the two groups, its interpretation might pose a challenge, at least partly, due to our incomplete knowledge of neuronal network dynamics (Hyvarinen, 2009). However, the suggested interpretation would conform to the outcome of the HRV analyses, indicating increased cognitive flexibility and neuro-visceral integration in the anxiety-prone versus the non-anxious subjects.
Both tICA and GLM analyses of the 49°C experimental series demonstrated that networks encompassing the vMPFC in the non-anxious subjects were implicated in top-down regulation of nociceptive activity at the bulbo-spinal level (Bingel et al., 2006), while analogous circuitry in the anxiety-prone group suggested cortico-cortical pain modulation (Lorenz et al., 2003). Cortico-cortical pain modulation might entail modification of the pain experience itself. However, due to the peri-stimulus involvement of somato-sensory and prefrontal areas in the anxiety-prone subjects, such pain modulation might also encompass short-term, dynamic adaptation of representational maps in SI (Iguchi et al., 2001; Schaefer et al., 2005b), including tuning of sensory receptive fields (Quevedo and Coghill, 2007). A prefrontal-cortical gating system has been suggested to underlie these SI regulatory processes (Staines et al., 2002; Schaefer et al., 2005a). Our findings also correspond well with existing data demonstrating that cortico-cortical brain networks represent substrates for affect-related processing of pain (Villemure and Bushnell, 2002; Rhudy et al., 2004).

The dorsomedial prefrontal cortex exhibited greater stimulus-related activity in the anxiety-prone than in the non-anxious group during the 49°C experimental condition, potentially reflecting autonomic modulatory processes (Napadow et al., 2008). Increased activity in the dorsomedial prefrontal cortex has also been associated with error-feedback processing during response inhibition (Greening et al., 2010), which might indicate overriding of putative antinociceptive programs and an impeded decision-making process in the anxiety-prone individuals. The anxiety-prone individuals additionally demonstrated peri-
stimulus related dorsolateral prefrontal cortex activity. This activity could reflect
cognitive emotion regulation (Kalisch et al., 2005) that, in addition to the putative
subconscious behavioral inclinations of the anxiety-prone subjects, might have
led to a reappraisal of their experimental experiences.

Right insula/frontal operculum exhibited greater stimulus-related activity in
the anxiety-prone than in the non-anxious group during the 49°C condition. Right
insula might integrate sensory and cognitive aspects of pain (Kong et al., 2006;
Starr et al., 2009), tune arousal levels (Critchley et al., 2000), and also signal
safety (Christianson et al., 2008). Such a safety signal could reflect the anxiety-
prone group’s tendency to suppress novel stimuli. Insula hyperactivity has also
been described in anxiety-proneness and anxiety disorders during conditions of
uncertainty, like in affective ambiguity (Simmons et al., 2008). Ambiguity might
have arisen in the anxiety-prone group during the 49°C experimental series, as
pain-related affect potentially started to compete with anxiety/worry-related affect.
In our experiment, we observed a negative correlation between pain-related ACC
activity and anxiety-scores. However, pain-related fear has been shown to
positively correlate with ACC activity (Ochsner et al., 2006). The ACC has been
implicated in affective processing, also related to pain (Rainville et al., 1997;
Vogt, 2005). According to our hypothesis, compared to the non-anxious
individuals, the anxiety-prone subjects exhibited increased attention towards non-
pain threats, which could alternatively be conceptualized as lower fear of the
experimental pain and might explain the differential ACC findings. In Ochsner’s
study, the Fear of Pain Questionnaire was utilized and no significant correlation
between Fear of Pain and STAI-trait scores was found (Ochsner et al., 2006). In our study, a strong correlation between the BSI® scores that we utilized and STAI-trait scores was established. Therefore, the difference between a phobic and a putatively more generalized type of anxiety might also be reflected in associated ACC activity. As ACC activity may indicate conflict-monitoring and -signaling (Carter et al., 1998; Botvinick et al., 1999; MacDonald et al., 2000), the ACC findings could alternatively reflect lower probability of processing-conflicts in the anxiety-prone than in the non-anxious subjects. This could be explained by the anxiety-prone subjects’ putative dispositional and preemptive set-up of processing functions to secure hypo-/analgesia and facilitate awareness of non-pain events. Further, macaque ACC neurons encode information about the probability of a reward (Rushworth et al., 2007). It might be that the prospect of pain reduction reflected the contextual reward for the non-anxious subjects, while due to their pervasive anxiety/worry-related concerns, the anxiety-prone individuals did not view reduced pain as a reward during the experiment.

The anxiety-prone subjects exhibited greater measures of HRV than the non-anxious individuals in the time-domain and within both the HF and LF band of the frequency-domain HRV analyses. These results could indicate that the anxiety-prone subjects possessed an ability to maximally harness neurobiological processing systems to ensure optimal experiential, physiological and behavioral outcomes. In general, anxiety-prone individuals may exhibit a “non-pathological” increase in vigilance and fear-reactivity that allows them to avoid harm through precautionary and active behavior. Accordingly, the results support the
hypothesis that moderate anxiety harbors an adaptive advantage. This hypothesis has also been supported by an association between low trait-anxiety and increased risk-behavior and reduced help seeking (Lee et al., 2006; Mykletun et al., 2009). Anxious subjects exhibit persistent vigilance towards worries and threat cues (Thayer et al., 2000; Fox et al., 2001; Fox et al., 2002; Verkuil et al., 2009), and worries have been associated with low vagal tone in anxious individuals (Thayer et al., 1996). As the anxiety-prone subjects demonstrated measures of high vagal tone, the latter finding seems counterintuitive to our original hypothesis that threat-related motivations outcompeted pain-related motivations. A strong relationship has been established between hyperventilatory symptoms and anxiety (Holt and Andrews, 1989) and hyperventilation has been associated with decreased HF HRV (Penttila et al., 2001). As the anxiety-prone group exhibited lower respiratory rate than the non-anxious group and elevated measures of HRV also have been linked to effective emotion regulation (Gross, 1998; Segerstrom and Nes, 2007), we speculated that the observed increase in HF HRV in the anxiety-prone individuals might be a result of an interaction between attentional systems, respiratory patterns and emotion regulatory dispositions. Interestingly, in children the ability to sustain attention and avoid distraction relates to measures of high vagal tone (Friedman and Thayer, 1998) – a finding that would reconcile the observed high HF HRV metrics in the anxiety-prone subjects with their putative hypervigilance towards threat cues (Thayer et al., 2000; Fox et al., 2001; Fox et al., 2002; Verkuil et al., 2009). Future studies of the interplay between the central
nervous system and the body need to address the individual and collaborative role of nervous and humoral factors in both afferent and efferent aspects of emotional-motivational processing. This will be necessary to further unravel the dynamics of goal-directed behavior, and to elucidate pathophysiological aspects of both somatic and psychosomatic disorders (Cameron, 2009).

Our findings associated the vMPFC with cardio-vagal regulation during painful conditions and implicated left TP in anxiety-specific vagal HR-regulation, and postcentral gyrus in interoception. Lack of central correlates of sympathetic regulation likely resulted from sparse cognitive/motor challenges during the experiment. The role of vMPFC activity in cardio-vagal control has previously been demonstrated during physical activity (Wong et al., 2007). The main neural correlates of cardiovascular measures partly overlapped with those involved in pain processing. These overlaps might indicate integration of autonomic with both sensory and cognitive-emotional processes, which has been suggested in earlier studies (Porro et al., 2003; Dube et al., 2009). To better dissect out specific autonomic effects and correlate these with functional brain activity, it will be important for future studies to employ additional methods to e.g. record muscle sympathetic nerve activity (Wong et al., 2007) and/or skin conductance (Dube et al., 2009).

Reconciling apparent conflicting results

In chapter II, a relative vMPFC hypo-activity was associated with a putative overriding of different aspects of a decision-making process to facilitate
anxiety-related activity and to secure hypoalgesia in the anxiety-prone group. However, in chapter IV, relative vMPFC hypo-activity was related to decreased central parasympathetic activity, leading to disinhibition of HR in the non-anxious group. To reconcile the appearing conflicting views, it is important to point out that the vMPFC is a large region of the forebrain that encompasses a myriad of domains and networks. These domains, although interdependent and cooperative in nature, will ultimately be biased toward specialized functions (Fuster, 2008). In chapter II, ICs reflecting artifacts as well as physiological activity were excluded, while in chapter IV, specific and individual HR-related regressors were employed to characterize central correlates of HR regulation. Accordingly, it seems plausible that the brain correlates discussed in the two papers were distinct functional entities.

Experienced pain and HR measurements were lower in the anxiety-prone than in the non-anxious subjects during both the training and the imaging session. However, the absolute levels of the pain ratings decreased from training to imaging in both groups, while the non-anxious individuals’ HR increased between the two time points. These disparate experiential and autonomic findings in the non-anxious group seem counterintuitive. Two factors likely play a role in reconciling the findings: scanner-related fear and habituation. Principally, the scanner environment was the only element present in the imaging session that was absent during training. In human imaging, HR increase is normally observed when subjects are exposed to a scanner environment (Friday and Kubal, 1990; Murphy and Brunberg, 1997; Sarji et al., 1998). HR is not subject to
long-term habituation (Mauss et al., 2003; Martin-Soelch et al., 2006), but painful stimuli are (Bingel et al., 2007; Rodriguez-Raecke et al., 2010). Consequently, a habituation effect is likely to explain the decrease in experienced pain in both groups from the training to the imaging session. However, such an effect would not be expected for the associated HR measurements per se. Through the previously discussed putative worry-related motivational bias in the anxiety-prone group, these subjects presumably directed their attention towards non-pain objects with resulting hypoalgesic responses. This mechanism (Fields, 2004) alone or in combination with associated antecedent emotion regulatory processes (Gross, 1998) might have suppressed experiential, autonomic and behavioral responses to an additional scanner-related fear/anxiety. On the other side, the non-anxious individuals probably did not regulate their scanner fear, but might even have interpreted this fear as pain-related. Therefore, in the non-anxious individuals, fear of the scanner environment, as part of the experimental set-up, might, together with the noxious impact, have led to a synergistic effect (Weisenberg et al., 1984; al Absi and Rokke, 1991) that increased the experiences of pain and led to an increase in associated HR. Consequently, these effects might explain the divergent perceptual and HR findings in the non-anxious group between the two testing sessions. It can not be ruled out that this may also have slightly inflated the group differences in VAS ratings during the imaging session. Careful inspection of the retrospective pain intensity ratings (Fig.2 C, Chapter II) supports such an assumption, as the relative reduction in the ratings between training and imaging were slightly larger in the anxiety-prone
than in the non-anxious group.

Finally, it must be presumed that scanner-experienced subjects would demonstrate less contextual fear in the experimental situation, resulting in lower HR reactivity. Consequently, skewed distribution of scanner-experienced participants across groups could influence the results. Fortunately, this was not the case in our study, as 10 subjects were scanner-naïve, and one scanner-experienced participant was found in each of the two experimental groups.

**Individual differences**

Traditionally, variability in statistical data has been treated as unwanted noise, including variability attributed to differences between subjects. Increasingly over the recent years researchers have understood that subject variability can and must be harnessed to enable the full understanding of observed effects in the data (Thompson-Schill et al., 2006). This is and will be important in a translational perspective to facilitate the improvement of diagnostic and treatment procedures in “personalized medicine” (Curigliano, 2010; Hong and Oh, 2010; Naylor and Chen, 2010; Davies, 2006; Vargas, 2009). E.g., clinical conditions with different etiologies might involve similar pathophysiology (Martin et al., 2003; Otto et al., 2003), while clinical presentations with similar etiology might harbor different pathophysiological mechanisms (Martin et al., 2003). Also, multiple mechanisms may coexist in one specific individual and may additionally change over time. Therefore, to improve and to be able to tailor treatment to individual patients it will be necessary to explore those individual
differences to enable mechanism-based stratifications of disease, like has been advocated for neuropathic pain (Woolf et al., 1998; Hansson, 2003).

Our findings suggested that individual differences in emotional-motivational state might significantly influence behavior and associated brain processing. Among elements defining the state of the emotional-motivational circuitry are inherent cognitive-emotional attributes, like mood/anxiety-proneness in our data. The results are in accordance with those of previous publications demonstrating inter-individual differences in pain experiences and related brain correlates (Coghill et al., 2003), and individual differences in emotion processing (Hamann and Canli, 2004). Individual differences in opioid system function have been demonstrated (Zubieta et al., 2003; Nagashima et al., 2007; Smith, 2008) and might also underlie effects of anxiety-proneness on pain perception and autonomic function.

In animal research, marked differences between e.g. separate strains of rodents have been known for a while. These differences are to a large extent both taken into account and exploited when performing experiments (Andrews, 1996; Kacew and Festing, 1996; Kacew et al., 1998; Giorgi et al., 2007). However, it seems that in human experimental research individual differences among the study population are not always addressed in a proper manner. To a large degree this might be attributable to limited amount of resources that makes elaborate pre-experimental testing and sub-grouping unfeasible. Nevertheless, subject variability needs to be addressed to either remove variability during sampling, thereby creating more homogenous study groups or to be harnessed
to improve explanatory power. Our results underscore the importance of interindividual differences in the study population as a source for variability. The findings point to a significant effect of such differences even when they are manifested to a very low degree and not by themselves are being clinically relevant.

A factor regarding individual differences that was not discussed in this work was effect of gender. Data on female sensitivity to noxious stimuli across the menstrual cycle are ambiguous (Riley et al., 1999), and depends on stimulus quality, tissue type and body site stimulated (Giamberardino et al., 1997). The female participants were scheduled for experimental testing before day eight or after day twenty of their menstrual cycle to create a uniform test period, during which the sensitivity to noxious stimuli would be the least perturbed (not increased) (Riley et al., 1999). Women are generally shown to demonstrate higher pain sensitivity than men, often explained by psychosocial or hormonal factors (Berkley, 1997). However, the magnitude of this gender effect in experimental pain is low (Berkley, 1997). Therefore, and also due to the relative low number of participants, we refrained from further analysis of gender in this study. However, this is a topic that can be addressed in future investigations. It must also be pointed out that in a neuroimaging study similar to the present investigation, no statistically significant gender differences on any measure could be demonstrated (Ochsner et al., 2006).
Methodological comments

Although, in this dissertation discrete brain areas have been associated with specific neurobiological processes, and specific neurobiological processes have been treated as separate entities, it is of utmost importance to emphasize that such simplifications are done for didactic reasons and that in real life the organism is and acts as one unit. Consequently, different brain areas and functions are, at least to some degree, interdependent and cooperative (Fuster, 2008), i.e. different functions share common brain areas and networks (Pessoa, 2008).

In the present study we employed participants with innate differences in anxiety and thereby avoided experimental fear-/anxiety-induction. This might have circumvented potential methodological problems. E.g., using a generic picture library to induce anxiety/fear might involve non-successful elicitation of desired emotions (Davidson, 1993) and lack of personal relevance (Kalisch et al., 2005). Also, delivery of physical stimuli to elicit anxiety/fear concurrent with noxious stimulation would potentially have a synergistic effect that might inflate the pain ratings or result in rating of overall distress instead of pain itself (Vowles et al., 2006).

On purpose, the subjects in our investigation were not informed or interrogated about anxiety and their emotional-affective state during the training or the experimental session, as this might have influenced them to ruminate about and introspect their “mental” state during imaging, which could have influenced the results.
It has been demonstrated that ICA is a sensitive brain-imaging tool, able to separate functionally meaningful patterns of spatio-temporal brain activity (Malinen et al., 2007). However, ICA is a relatively novel analysis method in fMRI and particularly in the field of pain. Consequently, comparison of results derived by ICA approaches with those of more traditional GLM procedures would be important as the body of reported ICA imaging analyses are growing. Therefore, we wanted to perform such a comparison on the present data. The outcome of this comparison revealed that the GLM imaging analyses results supported both the stimulus and peri-stimulus related tICA findings. Hence, we interpreted this consistency between the two methods as validation of tICA for use in pain- and affective-related fMRI studies.

**Future studies**

The findings described in this dissertation do encourage further investigations. First of all it would be necessary to repeat the present experiment with a larger study population, in particular to address methodological issues discussed in chapter III and IV. However, several other aspects should also be addressed. As part of the data was of explorative character, it will be important to create and to test specific hypotheses in separate experiments.

In the present investigation we utilized a training session to familiarize the subjects with the experimental set-up and rating procedures, to minimize misunderstanding and errors during the experimental imaging session. However, the participants learned from the training about the uniformity of stimuli across
individual experimental series and about the intensity of applied stimuli. Very likely, this learning effect influenced both behavior and associated brain activity in both groups during the imaging session. The participants probably set up their action tendencies partly in a preemptive fashion, and partly when they became aware of the quality of the stimulus during block 1 in each experimental series. The latter scenario is supported by the ROI data (Fig. 6C), showing that rest-period vMPFC activity is lower during uncertainty about the quality of upcoming stimuli at the beginning and end of an experimental series. Nevertheless, the learning effect from the training session limits the generalizability of the results to be applied to real life situations where uncertainty and novelty are prevailing features of environmental events. Consequently, it would be desirable for future studies to use naïve subjects that will only receive an explanation of the upcoming experiment and not participate in a training session (Porro et al., 2003). Further, the use of a block design with non-uniform blocks or an event-related design would additionally remove all predictability involved in the experiment. The processing of novel stimuli, including decision-making, would presumably require an increased amount of neuronal resources. The interesting question is if both groups would change their vMPFC activity and also the behavioral and physiological parameters in a coordinated fashion, or if the group differences would decline in this new paradigm. The latter outcome might be suggested by findings of uncertainty’s dampening effect on emotions (van Dijk and Zeelenberg, 2006).
To specifically test our main hypothesis that the motivationally most powerful condition directs behavior and to experimentally examine the basic concept of the perceptual-defensive-recuperative model, i.e. that a dominant non-pain event will result in hypo-/analgesia, an experimental paradigm is necessary where the motivational strength of delivered stimuli can be manipulated. To enable such investigation of effects of differential perturbations of anxiety-prone and non-anxious subjects’ motivational states, experiments could apply volitional emotion regulation strategies (Gross, 1998) to down-regulate anxiety-related attention in the anxiety-prone group and introduce a competing high-level motivational state of positive affect in the form of a pleasant stimulus (e.g. erotica) or a reward task in the non-anxious group (Ashby et al., 1999). Under these circumstances one would hypothesize that a convergence between the experimental parameters in the two groups would be observed.

Mechanisms of stress-induced analgesia were putatively underlying the relative hypo-/analgesia that was observed in subjects with clinical anxiety subsequent to noxious stimulation, but did not seem to be the primary mechanism responsible for relative hypo-/analgesia in our study. To evaluate these presumptions it would be necessary to add a patient cohort to the existing experimental design. Then putative differential autonomic measures of arousal could be assessed along the behavioral and brain imaging parameters. To avoid the introduction of potential confounding factors it would be important to select clinically anxious subjects without co-occurring pain or chronic pain. This might be challenging, as a high comorbidity between anxiety and chronic pain

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conditions has been reported (Asmundson and Katz, 2009). Further challenges would include practical problems related to a constricted scanner environment, as well as the creation of a homogenous patient group, taking into consideration type, etiology/development, duration, comorbidities and treatment of the anxiety disorder (Sher and Trull, 1996).

Another interesting question regards what the participants consciously experience during the experiment and what takes place on an unconscious level. I.e., what do the participants attend to and how do our findings relate to the concept of distraction? Data suggest that at least the underlying mechanisms of emotional and attentive actions on pain processing are independent (Villemure et al., 2003; Villemure and Bushnell, 2009) and that anxious subjects exhibit an attentional bias towards threat-related stimuli (Fox et al., 2001; Fox et al., 2002). Nevertheless, it seems reasonable that, in light of the moderate level of anxiety exhibited by the anxiety-prone subjects, they were not consciously preoccupied with their “anxiety” during the experimental sessions. As emotional-affective processing is assumed to take place to a large extent at the unconscious level (Ohman, 1988; Esteves et al., 1994; Katkin et al., 2001; Berridge and Winkielman, 2003; Berridge, 2003; Ohman et al., 2007), the anxiety-prone subjects might have been subconsciously more vigilant towards non-contextual cues than the non-anxious individuals. Additional analyses performed on our data, but not included in this dissertation, showed relative increase in PC/PCC activity in the anxiety-prone group (Hadsel et al., 2008), suggesting that the necessary brain circuits were set up to support such activity. Also supporting
these conjectures is the attributional model of pain and fear that would presume a hypoalgesic outcome when fear/anxiety is directed at a non-pain event or object (Weisenberg et al., 1984; al Absi and Rokke, 1991), a scenario corresponding well to the observed behavior in the anxiety-prone group. Our hypothesis, as previously discussed, would assume that the anxiety-prone subjects preemptively set up their processing machinery to down-regulate or down-prioritize competing input to the decision-making machinery, thereby biasing goal-directed behavior. Such pre-attentive processing is supported by data suggesting goal pursuit (Custers and Aarts, 2010) and emotion regulation (Gross, 1998; Etkin and Wager, 2007) on the unconscious level. Consequently, in the broadest sense one could conceptualize the differential pain experience in the two study groups, at least partly, as an effect of “distraction”. Future studies to address these questions might employ the use of overt and subliminal or backwardly masked contextual and non-contextual stimuli (Katkin et al., 2001; Ohman et al., 2007) to help dissect out group-differential tendencies of attention/distraction both on the conscious and subconscious level, and the effect that would have on both behavioral and brain processing parameters. Immediate post-experimental interviewing is another method often utilized to learn about participants’ conscious experiences during testing, but unfortunately, retrospective reports of hedonic experiences are often unreliable (Kahneman et al., 1993; Redelmeier and Kahneman, 1996).

The tripartite model of anxiety posits that genetic predispositions constitute a generalized biological vulnerability. Early life experiences might
under certain conditions contribute to a generalized psychological vulnerability. Finally, early learning experiences can focus anxiety on distinct life circumstances and could manifest as a specific psychological vulnerability, especially in the development of some particular anxiety disorders (Barlow, 2000). In this context it would be interesting to know if anxiety-prone subjects represent a phenotype harboring only a genetic diathesis for anxiety, leaving them “stable” over the course of life, or if they have an additional generalized psychological vulnerability that would increase their probability of developing clinical anxiety some time down the line. Longitudinal epidemiological data has demonstrated that adolescent anxiety-proneness was associated with reduced accidents and accidental death until age 25, but with higher rates of non-accidental mortality thereafter (Lee et al., 2006). It has been speculated that this effect, as well as the association between elevated measures of anxiety and lower mortality (Mykletun et al., 2009), is due to that low-grade anxiety promotes earlier identification and treatment of potentially life-threatening disease (Carney et al., 2002) and/or a decreased risk behavior associated with non-disease mortality. An interesting hypothesis to explore would be that the putative constant hypervigilant behavior confer an adaptive advantage onto anxiety-prone subjects during young age, but that a chronic stress effect from constant utilization of neuronal and metabolic resources at some point results in a reversal of advantageous into unfavorable outcomes. Research to unravel these questions, as well as the role of anxiety-proneness in the development of anxiety disorders, would necessitate comprehensive and coordinated efforts involving
epidemiological/survey methods combined with genetic screenings and different imaging modalities, both as a prospective and multi-age cross-sectional design. A catechol-o-methyl transferase (COMT) val158met polymorphism has been linked to several clinical anxiety conditions (Domschke et al., 2008; Hettema et al., 2008; Wray et al., 2008) and to pain sensitivity in humans (Diatchenko et al., 2005). Consequently, an exploration of COMT polymorphisms in anxiety-prone and non-anxious subjects would be a natural part of the efforts to further characterize the anxiety-prone state.

In light of the results from the heart rate variability analysis, it would be interesting to further compare the level of cognitive flexibility in the anxiety-prone versus the non-anxious individuals. This could be done by employing a Wisconsin card sorting test and a Stroop task to the participants in the two groups (Stemme et al., 2007).

**Conclusion**

Our results underscore that pain-modulating systems do not respond in a linear fashion to noxious stimuli, but will be engaged according to the overall contextual goal of the organism and guided by competing motivational inputs. Inherent low-grade anxiety is one such input that, if potent enough, might suppress painful experiences and shape associated processes. Consequently, our data suggested that anxiety-prone individuals exhibit unique experiential, behavioral, physiological and brain processing features that set them apart from both non-anxious and clinically anxious subjects. Accordingly, individual
differences in such features are important and must be taken into account both when designing experiments and when their results are interpreted. More research will be needed to further characterize anxiety-proneness and to allow the involved emotional-motivational and cognitive mechanisms to be fully harnessed for use in clinical situations.
References


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VWA, German Business Association for Physicians
Norwegian Dental Association

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