“Ambulatory monitoring of electrodermal and cardiac functioning in anxiety and worry”

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By
Sigrun Doberenz, Dipl.-Psych.
Born September 29, 1981 in Karl-Marx-Stadt (now: Chemnitz)

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1st reviewer: Prof. Dr. Walton T. Roth
2nd reviewer: Prof. Dr. Clemens Kirschbaum

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Note

The following chapters of this thesis have already been published or submitted for publication:

**Chapter 4 (referred to as study 1):**


**Chapter 5 (referred to as study 2):**


**Chapter 6 (referred to as study 3):**

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<td>ANS</td>
<td>Autonomic nervous system</td>
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<tr>
<td>CAN</td>
<td>Central autonomic network</td>
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<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
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<tr>
<td>EDA</td>
<td>Electrodermal activity</td>
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<tr>
<td>EEG</td>
<td>Electroencephalogram</td>
</tr>
<tr>
<td>EMA</td>
<td>Ecological momentary assessment</td>
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<tr>
<td>EMG</td>
<td>Electromyogram</td>
</tr>
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<td>EOG</td>
<td>Electrooculogram</td>
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<tr>
<td>ESM</td>
<td>Experience sampling method</td>
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<tr>
<td>FFT</td>
<td>Fast Fourier Transform</td>
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<tr>
<td>GAD</td>
<td>Generalized anxiety disorder</td>
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<tr>
<td>HF</td>
<td>High-frequency (heart rate variability)</td>
</tr>
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<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal (axis)</td>
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<tr>
<td>HR</td>
<td>Heart rate</td>
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<td>HRV</td>
<td>Heart rate variability</td>
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<td>IBI</td>
<td>Interbeat intervals</td>
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<tr>
<td>LF</td>
<td>Low-frequency (heart rate variability)</td>
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<tr>
<td>MSSD</td>
<td>Mean squared successive differences</td>
</tr>
<tr>
<td>N1</td>
<td>First stage of non-rapid eye movement sleep</td>
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<tr>
<td>N2</td>
<td>Second stage of non-rapid eye movement sleep</td>
</tr>
<tr>
<td>N3</td>
<td>Third stage of non-rapid eye movement sleep</td>
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<tr>
<td>NREM</td>
<td>Non-rapid eye movement</td>
</tr>
<tr>
<td>NSF</td>
<td>Non-specific electrodermal activity fluctuations</td>
</tr>
<tr>
<td>ooEMG</td>
<td>Orbicularis-oculi electromyogram</td>
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<tr>
<td>PD</td>
<td>Panic disorder</td>
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<tr>
<td>PNS</td>
<td>Parasympathetic nervous system</td>
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<td>PSG</td>
<td>Polysomnography</td>
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<tr>
<td>PTSD</td>
<td>Posttraumatic stress disorder</td>
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<tr>
<td>REM</td>
<td>Rapid eye movement</td>
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<tr>
<td>RMSSD</td>
<td>Square root of the mean squared differences</td>
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<tr>
<td>RSA</td>
<td>Respiratory sinus arrhythmia</td>
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<tr>
<td>SC</td>
<td>Skin conductance</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>SCL</td>
<td>Skin conductance level</td>
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<td>SCR</td>
<td>Skin conductance response</td>
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<td>SNRI</td>
<td>Selective serotonin and norepinephrine reuptake inhibitor</td>
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<tr>
<td>SNS</td>
<td>Sympathetic nervous system</td>
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<tr>
<td>SSRI</td>
<td>Selective serotonin reuptake inhibitor</td>
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<tr>
<td>TZA</td>
<td>Tricyclic antidepressant</td>
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<tr>
<td>ULF</td>
<td>Ultra-low-frequency (heart rate variability)</td>
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<tr>
<td>vEOG</td>
<td>Vertical electrooculogram</td>
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<tr>
<td>VLF</td>
<td>Very-low-frequency (heart rate variability)</td>
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1 Introduction

Emotions are an integral part of the human experience and their interpretation can provide valuable but also misleading clues about oneself and other people’s state of mind. Negative emotional states can be perceived as uncomfortable and – when experienced chronically – can develop into anxiety and mood disorders. The more pervasive these disorders the more severely they affect and disable a person’s everyday functioning and often their sleep as well.

According to Lang and colleagues (1998), emotions may be expressed verbally, behaviorally, and physiologically, i.e., emotions can be reported, observed, and objectively measured. Each measurement approach provides important, unique, and often conflicting information that can be used in the assessment and treatment evaluation of psychological disorders affecting the emotions. Autonomic measures have been used to indicate the physiological components of emotions, such as those along the worry-anxiety-fear-panic spectrum. Worry has been shown to suppress cardiac responses to imaginal feared material (see Borkovec, Alcaine, & Behar, 2004) and reduce autonomic variability (Hoehn-Saric, McLeod, Funderburk, & Kowalski, 2004; Hoehn-Saric, McLeod, & Zimmerli, 1989). Results for panic and anticipatory anxiety are less conclusive but theoretically these states should go along with increased autonomic arousal. Abnormal autonomic arousal might also be present during sleep as both panic disorder and worrying have been associated with sleeping difficulties. However, most empirical research has been confined to the laboratory where high internal validity is achieved at the cost of poor ecological validity. Thus, the purpose of this doctoral dissertation is to extend and validate laboratory findings on worry, anticipatory anxiety, and panic using ambulatory monitoring. Twenty-four hour monitoring not only can give valuable insights into a person’s daytime emotional experience but also allows observing how these emotions might affect their sleep in their natural environment.

In the following chapter, the reader will be introduced to a conceptual framework that ties together worry, anxiety, fear, and panic, and related anxiety disorders (section 2.1), to autonomic arousal and electrodermal and cardiac arousal in particular (section 2.2), to sleep and its relation to autonomic arousal and anxiety disorders (section 2.3), and to ambulatory monitoring (section 2.4).
After illustrating the aims of this thesis (chapter 3), chapters 4 to 6 present the results of three empirical studies conducted as part of this doctoral research. The first study deals solely with electrodermal monitoring and how it is affected by confounding variables in an ambulatory context (chapter 4). The next study then seeks to investigate the relationship between electrodermal arousal and anticipatory anxiety and panic in a sample of panic disorder patients and healthy controls. The last study focuses primarily on the effect of trait and state worry on subjective and objective sleep and electrodermal and cardiac arousal in a group of high and low worriers. Chapters 7 to 9 summarize and integrate the findings from these three empirical studies, discuss methodological limitations, and provide an outlook into future research.
2 Background

2.1 Worry, anxiety, fear, and panic

In the following sections, the terms worry, anxiety, fear, and panic will be defined, discussed, and integrated into the conceptual framework of perceived threat proximity (Craske, 2003). Central origins and clinical manifestations in different anxiety disorders will be presented.

2.1.1 Definitions and differentiation

Different terms exist to describe anxious states and emotions, such as worry, anxiety, fear, and panic. Although they may be used almost interchangeably in ordinary language, scientific effort has been put into defining and differentiating these terms.

2.1.1.1 Worry

According to Borkovec and colleagues (Borkovec, Robinson, Pruzinsky, & DePree, 1983, p. 10), worry is “a chain of thoughts and images, negatively affect-laden and relatively uncontrollable. The worry process represents an attempt to engage in mental problem solving on an issue whose outcome is uncertain but contains the possibility of one or more negative outcomes. Consequently, worry relates closely to fear process.” The predominance of thought activity during worrying supposedly serves to cognitively avoid perceived threat in the form of (a) negative and more distressing fear imagery and related autonomic arousal, (b) future bad events, and (c) more emotional and anxiety-provoking topics (Borkovec, et al., 2004). Borkovec and colleagues (2004) suggest that – as with any avoidance strategy – worrying is negatively reinforced by a brief reduction in fear or anxiety, but in the long term, it interferes with (corrective) emotional processing during exposure with fear-provoking stimuli and strengthens their anxious meanings. Barlow (2002) also regards worrying as a dysfunctional attempt to cope with anxious apprehension by avoiding it through heightened verbal-linguistic and restricted autonomic activity. Brosschot, Gerin, and Thayer (2006) regard worrying (and ruminating) as a form of perseverative cognition or non-constructive problem-solving regarding the source of anxiety that can prolong or even exacerbate negative affect and concomitant abnormal autonomic activation.
As a repetitive thought process, worrying is similar to obsessing and ruminating but recent scientific effort has been put into distinguishing them. Obsessions are intrusive, unwanted thoughts, images, or impulses typically about violence, sex, or blasphemy and sacrilege (autogenous obsessions; Lee, Lee, Kim, Kwon, & Telch, 2005) that are “not simply excessive worries about real-life problems” (American Psychiatric Association (APA), 1994). Attempts at differentiating worries and obsessions have always found both similarities and differences between the two thought processes (Langlois, Freeston, & Ladouceur, 2000a, 2000b; Turner, Beidel, & Stanley, 1992), prompting some researchers to hypothesize a continuum from autogenous obsessions to worry (with reactive obsessions in between) on dimensions of thought content appraisal and perception of form and triggers (Lee, et al., 2005).

While worrying represents an attempt to cope with anxiety, ruminating is conceptualized as a coping response to depressive mood by passively focusing “one's attention on one's depressive symptoms and on the implication of these symptoms” (Nolen-Hoeksema, 1991, p. 569). As with obsessions, there is a great deal of conceptual overlap, however, and lay concepts of these two thought processes have been shown to differ from these scientific definitions (Hoyer, Gloster, & Herzberg, 2009).

### 2.1.1.2 Anxiety

Anxiety is often defined as an unpleasant mood state (Craske, 2003) rather than an emotion, i.e., it is a longer lasting, more general state of distress (Lang, Davis, & Öhman, 2000). Ambiguity and uncertainty of perceived threat (Izard, 1999) lead to an unspecific and diffuse hyperactivation in peripheral-physiological, central nervous, and subjective systems (Birbaumer & Schmidt, 1999). Higher brain mechanisms and cognition are involved in appraising the threat potential (Izard, 1992). Barlow (2002) introduced the concept of anxious apprehension to emphasize the future-oriented quality of anxiety. According to his view, anxiety is characterized by a rapid attentional shift to the focus of potentially aversive events that are perceived as uncontrollable and unpredictable.

### 2.1.1.3 Fear

In contrast to anxiety, fear is a basic and distinct emotion (Barlow, 2002; Izard, 1992) triggered by present and unambiguous danger (Barlow, 2002; Izard, 1999). It constitutes a more primitive and focused alarm response that mobilizes the organism involving action
tendencies and strong physiological and subjective arousal (Barlow, 2002; Birbaumer & Schmidt, 1999) and usually subsides once the threat is over (Rachman, 2000). Lang and colleagues (2000) further distinguish two types of autonomic and somatic patterns of fear: (1) defensive *immobility* characterized by freezing, hypervigilance, and bradycardia; and (2) defensive *action* describing fight or flight responses.

### 2.1.1.4 Panic

In ordinary language, panic refers to sudden, intense anxiety. Symptoms of panic include physical sensations such as palpitations or heart pounding and racing, sweating, chest pain, feelings of choking, and numbness or tingling sensations, trembling, shortness of breath, nausea, dizziness, hot or cold flashes, and faintness, but also more central cognitive symptoms such as fear of dying or losing control and derealization or depersonalization. According to the DSM-IV-TR (American Psychiatric Association (APA), 1994) criteria, a panic attack is defined as a discrete period of intense fear or discomfort, in which four (or more) of the symptoms above develop abruptly and reach a peak within ten minutes. Several theories exist to explain these attacks (see Roth, Wilhelm, & Pettit, 2005) such as the vicious circle theory of panic where the rapid escalation of symptom number and intensity is caused by a positive feedback loop between anxiety and its effects.

### 2.1.2 Integrating the four terms

Apparently, the terms reviewed above share common features and considerable overlap exists between worry and anxiety, and anxiety and fear. Both anxiety and fear represent protective states that serve the function of detecting and avoiding threat (Craske, 2003) and definitions for panic use both terms almost interchangeably. Consequently, several attempts have been made to integrate these negative moods and emotions into a single model. Based on Bolles’ (1970) model of species-specific defense reactions that assumed innately determined defense behaviors like freezing, fight, or flight for each species, and their own experiments on rats, Fanselow and Lester (1988) proposed rules of response selection that determined which defense behavior would be applied in a given situation. According to the authors, the prey’s perception of predatory imminence would lead to different levels of *fear* that triggered three defensive modes: (1) pre-encounter defenses emerge in areas with some predatory potential and include, for example, protective nest maintenance; (2) post-encounter defenses, such as freezing or a potentiated
startle response are activated as soon as a predator is detected in the present environment; and (3) circa-strike defensive behavior as the last defensive mode is exhibited when contact with the predator is occurring or inevitable and is characterized by fight, flight, or threat displays. Post-encounter behavior seems similar to what Lang and colleagues (2000) call defensive immobility and circa-strike behavior resembles their concept of defensive action. Instantaneous transitions between the three modes based on a changing perception of predatory imminence seem necessary for survival (Fanselow, 1994). Craske (2003) later used the conceptual framework of the animal predatory imminence model and applied it to human emotion (see Figure 1). Instead of assuming different levels of fear, she aligned worry, anxiety, fear, and panic along the predatory imminence continuum which she referred to more generally as proximity to threat. In this view, humans will respond with worry to future, uncertain, or undetected threat (similar to pre-encounter phase) signaled by distal contextual cues. As threat becomes detected (post-encounter phase) and more certain, anxious worry shifts to fear and then to panic. While proximal contextual cues elicit anxiety and anticipatory arousal, explicit (conditioned) threat stimuli result in fear (and freezing). Intense fear and panic are induced by innately (unconditioned) aversive stimuli. Figure 1 represents an attempt by the author to integrate this model with the predatory imminence model although respective levels of defensive behavior and emotional states do not correspond exactly.
2.1.3 Physiological basis

2.1.3.1 Autonomic arousal correlates

Similar to Borkovec et al. (2004) and Barlow (2002), Craske postulates further that while autonomic arousal is inhibited during worry to facilitate cognitive processing and planning in preparation for threat, it is activated once the threat is detected in preparation for fight or flight mobilization and intensifies as threat imminence increases (Craske, 2003).

In their defense cascade model, Lang and colleagues (1997) present a somewhat different pattern of autonomic activation. As shown in Figure 2, both electrodermal and cardiac arousal continue at baseline values (i.e. they are not inhibited) for most of the pre-encounter phase which is associated with worrying and anxiety in Craske’s model (2003). As threat imminence (subjective arousal axis) increases further, the amplitude of skin conductance responses increases correspondingly while heart rate initially decelerates even after the threat is perceived as certain and detected (post-encounter) – almost like an orienting response. Not until orienting turns into defense responding and overt action is imminent during circa-strike, heart rate starts accelerating. Similarly, Fowles (2000) has argued that increased electrodermal activity specifically reflects anxiety anticipating future
threat, while heart rate reflects fear of current threat (for a discussion, see Parente, Garcia-Leal, Del-Ben, Guimaraes, & Graeff, 2005).

Figure 2. Defense response cascade generated by increasingly arousing aversive stimuli (adapted from Lang et al. (1997)).

At first, it may seem counterintuitive that sympathetically driven electrodermal activity increases during post-encounter while parasympathetic influence on the heart (leading to heart rate deceleration) rises as well. Maybe instead of working antagonistically on an autonomic continuum as is generally assumed, optimal defense strategies might necessitate a more complex responding of both branches of the autonomic nervous system to increasing threat imminence. The autonomic space model might offer a better representation of autonomic activity in this context (see chapter 2.2.2). Current evidence on autonomic activation during worrying, anticipatory anxiety, and panic will be reviewed in chapters 5 and 6.

2.1.3.2 Central origins

Using freezing as a proxy for post-encounter behavior and activity bursts as indicators for circa-strike behavior, Fanselow and others conducted a series of experiments on rats to explore the underlying neural structure of these defense modes (for a review, see Fanselow, 1994). According to the authors, critical structures for post-encounter behavior involve the central nucleus of the amygdala and the ventral periaqueductal gray, while circa-strike behavior is mediated by the dorsolateral periaqueductal gray and the superior
colliculus. Concomitant autonomic responses are initiated by the central nucleus of the amygdala with projections to the dorsal medulla (partly) via the hypothalamus. Lang and colleagues (2000) also postulated the central nucleus of the amygdala to be involved in fear (post-encounter behavior) and additionally implicated the bed nucleus of the stria terminalis in anxiety processes (pre-encounter behavior).

2.1.4 Anxiety disorders

“Normal” worry, anxiety, and fear are transient, generally limited in scope, and experienced by the majority of individuals because they might provide an evolutionary advantage. Anxiety and fear represent protective states that serve the function of detecting and avoiding threat (Craske, 2003). Normal worry is thought to be a constructive strategy for dealing with potentially stressful life events, helping to facilitate problem-solving, information seeking, and active coping behaviors that reduce anxiety (Ruscio, Borkovec, & Ruscio, 2001). Pathological levels of worry, anxiety, and fear, however, are associated with considerable psychological dysfunction and can culminate in the development of one or more anxiety disorders. Pathological worry which has been shown to be different in degree and not in kind from normal worry (Ruscio, et al., 2001), is a central feature of Generalized Anxiety Disorder where patients experience chronic, excessive, and uncontrollable worries about a great number of real life issues. Worrying is highly prevalent in other anxiety disorders as well although the range of topics is more limited. For instance, Panic Disorder patients worry about having another panic attack and social phobics about being embarrassed in public. In Specific Phobias, pathological fears can lead to phobic avoidance of the feared object. Panic attacks are the central feature of Panic Disorder but can also occur in other anxiety disorders.

2.1.5 Summary

Worry, anxiety, fear, and panic share common and unique features. They may represent different defensive emotional states along a continuum of perceived threat proximity (Craske, 2003). Different underlying central neural structures seem to be involved that may lead to distinct and complex autonomic responding. Pathological levels of worry, anxiety, fear, and panic may result in the development of one or more anxiety disorders that should show signs of abnormal autonomic arousal.
2.2 Autonomic arousal

The autonomic nervous system (ANS) is a portion of the peripheral nervous system that innervates the smooth muscle of all inner organs and organ systems, the heart, and glands. It regulates vital functions like arterial blood pressure, gastrointestinal motility and secretion, sweating, and body temperature. Because much of this regulation occurs at the level of spinal reflexes, it was originally thought to take place autonomously without necessitating higher level (central) control (Birbaumer & Schmidt, 1999). However, these functions are orchestrated and can be modulated rapidly and intensely by higher centers in the central autonomic network including the hypothalamus, which can coordinate the different visceral functions of the body to meet situational demands (Benarroch, 1993). The following sections will deal with the physiologic anatomy and functioning of the ANS in more detail, give an example of orchestrated ANS activity in the form of the stress response, and elaborate on electrodermal and cardiac activity as two indicators of autonomic arousal.

2.2.1 Physiologic anatomy and characteristics

Next to the enteric nervous system (Benarroch, 2007b), two branches of the autonomic nervous system can be distinguished, the sympathetic (SNS) and the parasympathetic nervous system (PNS) (Benarroch, 2007a; Birbaumer & Schmidt, 1999). Both sympathetic and parasympathetic efferents require a two-neuron pathway, consisting of a (central) preganglionic neuron and a (peripheral) postganglionic neuron, before they synapse onto their respective target organs. Cell bodies of the sympathetic preganglionic neurons lie in the intermediolateral horn of the thoracic and lumbar segments T-1 to L-2 of the spinal cord (Benarroch, 2007a). Their axons leave the spinal cord via spinal nerves through the ventral root and synapse onto cell bodies of postganglionic neurons located in two paravertebral chains of ganglia aligned to both sides of the vertebral column and the prevertebral ganglia (site of ganglionic enteric reflexes independent of the central nervous system; Benarroch, 2007a). Considerable functional convergence and divergence occurs at the ganglionic level between preganglionic and postganglionic neurons of similar function, where one preganglionic neuron can synapse onto many postganglionic neurons in different ganglia, while at the same time many preganglionic neurons can synapse onto one postganglionic neuron (Birbaumer & Schmidt, 1999). Postganglionic fibers then travel to their target organs. As an exception to this rule, preganglionic sympathetic fibers directly innervate the secretory chromaffin cells of the adrenal medulla which are modified neuronal
cells analogous to postganglionic cells. The chromaffin cells release epinephrine and norepinephrine directly into the blood stream where the catecholamines act as “circulating hormones” (Benarroch, 2007a, p. 19). Thus, sympathetic activity is both neural and hormonal.

Parasympathetic preganglionic cell bodies lie in brainstem nuclei (cranial nerves III, VII, IX, X) and the sacral spinal segments (S2 to S4). Cranial nerve X – also referred to as nervus vagus or vagal nerve – represents the majority of all parasympathetic efferent projections (Guyton & Hall, 2006) and originates in the dorsal nucleus of the vagus nerve and the nucleus ambiguus (Benarroch, 2007a). With some exceptions, preganglionic fibers can pass uninterrupted to the target organ as the ganglia of the postganglionic neurons are either very close to the target organs or imbedded in their walls (Benarroch, 2007a). The postganglionic neurons spread their fibers through the substance of the organ (Guyton & Hall, 2006).

Afferent projections from the target organs travel back to the same segments of the spinal cord (via sympathetic or parasympathetic nerves) or to the nucleus of the solitary tract in the brainstem (via cranial nerves) (Jordan & Spyer, 1986). There they either synapse onto corresponding preganglionic neurons via at least one interneuron to close the autonomic reflex arc and/or relay their sensory information to the central autonomic network (Benarroch, 1993).

Both sympathetic and parasympathetic preganglionic neurons and parasympathetic postganglionic neurons are cholinergic, i.e., they mainly use acetylcholine as a neurotransmitter which attaches itself to nicotinic (all ganglia) and muscarinic (postganglionic parasympathetic effectors only) postsynaptic receptors. Postganglionic sympathetic neuroeffectors primarily use norepinephrine and epinephrine as neurotransmitters and are thus called adrenergic (for one prominent exception, see section 2.2.3.2). Pharmacologically, two prominent postganglionic sympathetic postsynaptic receptor types can be distinguished: α-adrenergic (α₁ and α₂) and β-adrenergic (β₁ and β₂) receptors. Other sympathetic and parasympathetic transmitters include neuropeptides (e.g., substance P), adenosine triphosphate (ATP), and nitric oxide (NO) (Benarroch, 2007a).
2.2.2 Autonomic functioning

Apart from some exceptions (e.g., sweat glands) the two branches of the ANS innervate the same organs but have opposite effects. Originally, autonomic activation in dually innervated organs was conceptualized as reciprocal with sympathetic dominance on one end and parasympathetic dominance on the other end of a continuum (Berntson, Cacioppo, Quigley, & Fabro, 1994). Berntson and colleagues (1994) argue that this pattern leads to mutually synergistic, stable, and strong target organ reactivity with the widest range of dynamic control. However, they extended this conceptualization by the autonomic space model (Berntson, et al., 1994) to include instances of independent and even coactive or co-inhibitive activity of both branches, as have been proposed in Lang and colleagues’ (1997) defense cascade model (see Figure 2).

Much of autonomic activity is regulated via autonomic reflexes to maintain homeostasis. To psychophysioologists, however, central regulation of the ANS is of greater importance and ANS activity indicators have been used to probe attentional and emotional processes, for example. The central autonomic network (CAN; Benarroch, 1993) is a group of interconnected areas in the brain that receive viscerceptive, humoral, and exteroceptive information and control (among others) preganglionic sympathetic and parasympathetic outputs (see Table 1). It involves similar structures to those that have been discussed in the context of anxiety, fear, and panic, such as the amygdala and the hypothalamus (see section 2.1.3.2). The latter is central in the initiation and coordination of the stress response, which will be described in more detail in the following.
### Table 1. Components of the central autonomic network (CAN) according to Benarroch (1993)

<table>
<thead>
<tr>
<th>Components of the CAN</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insular, medial prefrontal, other regions of prefrontal</td>
<td>• High-order autonomic control</td>
</tr>
<tr>
<td>cortex</td>
<td></td>
</tr>
<tr>
<td>“Extended amygdala” (central nucleus of the amygdala,</td>
<td>• Autonomic expression of emotional states</td>
</tr>
<tr>
<td>bed nucleus of the stria terminalis)</td>
<td></td>
</tr>
<tr>
<td>Hypothalamic areas (paraventricular nucleus, lateral</td>
<td>• Initiate and coordinate autonomic, neuroendocrine, and behavioral</td>
</tr>
<tr>
<td>hypothalamic area, dorsomedial nucleus)</td>
<td>responses critical for homeostasis and stress responses</td>
</tr>
<tr>
<td>Parallel circuits in the periaqueductal gray matter of the</td>
<td>• Coordinate autonomic, pain-controlling, and motor mechanisms for stress-</td>
</tr>
<tr>
<td>midbrain</td>
<td>related, aggressive, and reproductive behaviors</td>
</tr>
<tr>
<td>Parabrachial Kölliker-Fuse region of the dorsolateral</td>
<td>• Relays viscerosensory information to the forebrain</td>
</tr>
<tr>
<td>pons</td>
<td>• Important component in circuits that control respiration, circulation,</td>
</tr>
<tr>
<td></td>
<td>and vomiting</td>
</tr>
<tr>
<td>Nucleus tractus solitarius</td>
<td>• First relay station of visceromotor afferents carried by glosso-</td>
</tr>
<tr>
<td></td>
<td>pharyngeal and vagus nerves</td>
</tr>
<tr>
<td></td>
<td>• Initiates multiple medullary reflexes controlling cardiovascular,</td>
</tr>
<tr>
<td></td>
<td>respiratory, and other autonomic effectors</td>
</tr>
<tr>
<td></td>
<td>• Provides viscerosensory inputs to all other regions of the CAN</td>
</tr>
<tr>
<td>Intermediate reticular zone of the medulla (including</td>
<td>• Contains premotor autonomic and respiratory neurons and interneurons</td>
</tr>
<tr>
<td>ventrolateral medulla)</td>
<td></td>
</tr>
</tbody>
</table>

### 2.2.2.1 The stress response

Many changes take place in an organism when faced with a threat or stressor during post-encounter and circa-strike defensive behavior (see section 2.1.2). According to Lang and colleagues (1997), a change from orienting to defensive behavior after perceiving a threat (post-encounter phase) will result in increased startle reactivity. The most prominent circa-strike behavior is the “fight-or-flight-reaction” or stress response.

Peripherally, the sympathetic branch and the adrenal medulla are responsible for the quickly responding “fight-or-flight-reaction” (Guyton & Hall, 1996), which enables the body to perform vigorous muscle activity it otherwise would not be capable of. In the course of this response, a series of physiological changes like accelerated respiration, an increase in blood pressure and blood flow to the active muscles, and a decrease in blood flow to internal organs, e.g., the gastrointestinal tract and the kidneys, is observed. The rate of cellular metabolism throughout the body, glycolysis in the liver and the muscles, muscle strength, mental activity, and sweating all increase. Activation of the parasympathetic nervous system produces opposite effects – also called “rest-and-digest” in reference to the sympathetic “fight-and-flight” response – i.e., lower blood pressure and increased
gastrointestinal motility, but does not affect sweating since the sweat glands are innervated only by the sympathetic branch. Preganglionic sympathetic neurons form functional units, such as the cardiomotor or sudomotor units, that allow individual bodily functions to operate independently (Jänig & Häbler, 2003). Central autonomic regulation can address and modulate these units separately or synchronize them (Venables, 1991), and thus lead to refined, specific, and graded responses to different kinds of stressors or threats. Sympathetic activation and parasympathetic withdrawal occur within seconds after the onset of an evoking stimulus but do not provide an adequate long-term defense since these effects wear off after only about 30 minutes unless repeatedly evoked by a stressor.

The stress response also includes a more delayed secretion of cortisol from the adrenal cortex, which will be mentioned here for completeness. This secretion is initiated by neural signals to the paraventricular nucleus of the hypothalamus, which releases corticotropin-releasing hormone (CRH). CRH in conjunction with arginine vasopressin increases production and secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland into the bloodstream. ACTH stimulates the synthesis and release of glucocorticoids (mainly cortisol in humans) from the adrenal gland. The free cortisol (i.e. not bound to proteins in the blood) binds to intracellular steroid receptors resulting in increased cell metabolism and breakdown of glycogen to glucose in the liver, providing an energy source throughout the body. Free cortisol, in addition to promoting an adaptive response to stressors, also suppresses certain immune reactions that underlie inflammation, which can lead to tissue damage and pathologic conditions (Willenberg, Bornstein, & Chrousos, 2000). To end cortisol release from the bottom-up, cortisol in the bloodstream creates a negative feedback loop by suppressing further CRH and ACTH production and secretion. Cortisol is measured non-invasively from hair, urine, or saliva or invasively from blood plasma and serum (Sauvé, Koren, Walsh, Tokmakejian, & Van Uum, 2007).

In addition to autonomic and HPA-axis effects, stress can also go along with an increased startle response (Grillon, Duncko, Covington, Kopperman, & Kling, 2007). As part of Lang and colleagues’ (1997) defense cascade model (see Figure 2), this response will be described here as well. The startle response is a cranial-to-caudal wave of flexor movements along the neural axis, elicited by an abrupt sensory event of adequate intensity (Landis & Hunt, 1939). The startle eye blink is the earliest and most reliable component of this
defensive reflex, which apparently acts to avoid organ injury. Its underlying primary neural reflex pathway begins with afferent sensory pathways to the pontine reticular formation in the brain stem, which in turn excites efferent motor neurons. Activation of the amygdala by fear conditioning potentiates the startle response via direct projections to the reticular site (Davis, 1989). The startle eye blink response can be measured non-invasively from the activity of the orbicularis oculi muscle (orbicularis-oculi electromyogram, ooEMG) or from potentials in electrodes above and below the eye (vertical electrooculogram, vEOG).

2.2.3 Electrodermal activity: skin conductance

Several indices have been used to probe autonomic arousal and its underlying central affective control. In the following, electrodermal activity as an indicator of sympathetic arousal will be explored.

2.2.3.1 Measurement of skin conductance

Electrodermal activity (EDA) describes changes in the skin’s ability to conduct electricity and has been measured in different ways, such as skin potential, skin resistance, or skin conductance. After being recommended over skin potential and resistance (Fowles, Christie, & Edelberg, 1981; Lykken & Venables, 1971), over the years, skin conductance (SC) has become the most often used EDA measure. It is an “exosomatic” method of recording sweating (Boucsein, 1992; Venables, 1991), where the electrical conductance of the skin is measured by applying an external direct current at a constant voltage. The usual unit of measurement is µmho or µSiemens. Commonly derived measures include SC level (SCL) and SC variability measures, such as SC fluctuations which can be either non-specific or triggered SC responses and are usually quantified in terms of rate, amplitude, rise time, and half-recovery time.

2.2.3.2 Neuropsychophysiological properties

The vertical and horizontal anatomical structure of the skin determines its electrical properties. In particular, knowledge of the structure and local distribution of the sweat glands is important in order to understand the mechanisms of EDA, and why palmar and plantar sites of the skin have been the most widely used for measuring EDA (Boucsein, 1992).
2.2.3.2.1 Anatomy of the skin and the sweat glands influencing EDA

Vertically, the skin (also called cutis) and the subcutis can be distinguished. The subcutis is composed of loose connective tissue and contains the secretory part of the sweat glands. The cutis consists of two layers, the dermis and the epidermis. Both dermis and subcutis serve as mechanical and thermal insulation layers because of their high content of fatty tissue. Their relatively high electrical conductance is mainly due to their being surrounded by fluid (e.g., blood, the interstitial fluid and the lymph). The epidermis has a specific layering that is particularly important to EDA: as it becomes dryer in the outer layers, its electrical resistance increases. The epidermis’ completely horny outer layer, the stratum corneum, is especially thick on the palms and soles (Ya-Xian, Suetake, & Tagami, 1999).

Horizontally, two types of skin can be found, ridged skin and polygonal skin. The surface of ridged skin is covered with ridges and furrows in a genetically fixed pattern and is only found on the palmar and plantar sites of the hands and feet. It does not contain any scent or sebaceous glands and is completely hairless (also called glabrous skin). The ducts of the sweat glands enter the epidermis at the nadir of the ridges. Polygonal skin, on the other hand, covers the rest of the body and ducts of sweat and scent glands enter the epidermis at the top of the ridges.

Sweat glands are exocrine (eccrine) glands and pour out their excretions directly onto the surface of the skin. Their density differs regionally and is highest on the palms and soles and the lowest on the arms, legs, or the trunk. Sweat glands are completely missing on the lips (Sato, Kang, Saga, & Sato, 1989). A sweat gland consists of a secretory part and the reabsorptive duct. As mentioned before, the secretory part is located in the subcutis and is made up of a tube irregularly coiled into a rounded mass. This tube later turns into the duct and after following a wavelike course reaches the skin surface through a pore.

2.2.3.2.2 Physiology of the sweat glands

Sweat glands are innervated solely by sympathetic, sudorisecretory (also called sudomotor) nerve fibers, not by parasympathetic afferents. Thus, their activity can be regarded as a purely sympathetic measure. The main center for sudorisecretory control is the hypothalamus. From there, the so called hypothalamic-reticular-spinal sympathetic pathway in which sudorisecretory and vasoconstrictory nerve fibers are closely intermeshed, leads to pre- and then postganglionic neurons of the SNS (see section 2.2.1), that synapse
onto the sweat glands, forming a plexus of nerve fibers around each secretory part. Although most postganglionic sympathetic synapses are adrenergic and secrete norepinephrine and epinephrine, adrenergic innervation plays only a minor role in sweating because most of the sudorisecretory synapses are cholinergic and activate muscarinic receptors (see Buono, Tabor, & White, 2011). This has led to speculations about a possible parasympathetic influence at that level, and even though sweating is controlled by nerve fibers that anatomically are distributed through the SNS, some researchers called it a parasympathetic function (Guyton & Hall, 1996). The cholinergically activated secretory process is assumed to be controlled by an electrochemical gradient involving several ion transporters and channels in the secretory portion of the sweat gland (Wilke, Martin, Terstegen, & Biel, 2007). However, the exact mechanisms are still unclear. As a result, sweat in the duct of the sweat gland rises and flows to the surface in a pulsatile manner with pulsations of 12-21 Hz (Nicolaidis & Sivadjian, 1972), before being reabsorbed by the duct. The sweat itself contains mostly water, sodium chloride, potassium, bicarbonate and lactic anions, making it a good conductive medium. The concentration of sodium chloride in the sweat is approximately 0.3%. Higher-level structures of the central autonomic network, like the amygdala and prefrontal areas (see Table 1), can influence the hypothalamus and thereby influence sweating, although much regulation happens on the level of segmental spinal reflexes.

### 2.2.3.3 Functional aspects

The main function of exocrine sweat glands is to regulate body temperature (thermoregulatory sweating). Palmar and plantar sweat glands, however, may be different. By disentangling the effects of ambient temperature and excitement, Kerassidis (1994) found that relaxed subjects in a high temperature environment showed only minimal palmar and plantar EDA while they were sweating profusely from their forehead and chest. Evidence such as this has led many researchers to believe that palmar and plantar EDA might be more closely (and maybe even independently) involved in so called emotional sweating rather than thermoregulatory sweating (Asahina, Suzuki, Mori, Kanesaka, & Hattori, 2003; Storm, 2001; Wilke, et al., 2007). Its biological significance may become clear by regarding it as part of (post-encounter and circa-strike) defensive behavior where hydration of palmar and plantar skin sites keeps the skin flexible and induces increases in frictional contact and

2.2.3.4 Confounding variables

As with other physiological measures, the conductance of the skin is also influenced by factors other than emotional arousal. It is therefore vital for EDA research, especially in an ambulatory context, to control for these variables (e.g. by monitoring them), or if they cannot be controlled for, to be aware of their possible influence. Factors discussed here include sweat gland density, skin temperature, medication, caffeine, or nicotine use, and thyroid disease. Other confounding variables are the focus of chapter 4: length of recording, ambient temperature, physical activity, time of day, sex, age, and race.

2.2.3.4.1 Sweat gland density

SC at the time of testing depends on transient as well as persistent interindividual differences in the skin, and the mediating variables are the degree of hydration of the epidermis (see chapter 4) and the density of sweat glands at the respective skin site. Not all sweat glands are active and there are interindividual and probably also interracial differences in sweat gland density (Rawlings, 2006). Allen, Armstrong & Roddie (1973) found that the amount of sweating is a positive linear function of sweat gland density and can therefore produce differences in SC between different skin sites and probably also between individuals.

2.2.3.4.2 Skin temperature

An increase in skin temperature from 36°C to 37.1°C can lead to significant changes in latency, amplitude and recovery rate of skin conductance responses (SCRs) (Lobstein & Cort, 1978). Boucsein (1992) reported that the water permeability of the stratum corneum increases exponentially with rising temperature.

2.2.3.4.3 Pharmacological agents

Drugs that mimic or block the actions of acetylcholine or neurotransmitters of the central autonomic network (Benarroch, 1993) are likely to affect skin conductance measurements. Candidates are acetylcholine agonists or antagonists that bind to postsynaptic nicotinic or muscarinic receptors.
Fluoxetine – a selective serotonin reuptake inhibitor (SSRI) – blocked nicotinic receptors (Garcia-Colunga, Awad, & Miledi, 1997) potentially affecting autonomic arousal in general. Sertraline – another SSRI – reduced SCL without affecting SC response amplitudes (Siepmann, Grossmann, Mueck-Weymann, & Kirch, 2003). In study 2 (chapter 5), we therefore investigated the use of antidepressants (mostly SSRI’s) on 24-hour SCL in a sample of panic disorder patients and non-anxious controls (Doberenz, Roth, Wollburg, Breuninger, & Kim, 2010). The number of electrodermal non-responders was significantly increased in schizophrenic inpatients taking anticholinergic medication (Schlenker et al., 1995).

Well known is also the effect of local and systemic administration of atropine – a muscarinic antagonist – to reduce or even eliminate sweating during thermal challenge, exercise, or exogenous administration of acetylcholine or its analogs (Foster & Weiner, 1970; Kolka & Stephenson, 1987; Longmore, Bradshaw, & Szabadi, 1985).

2.2.3.4.4 Caffeine

Caffeine – a methylxanthine – is probably the most widely used central nervous system stimulant by man (Nehlig, Daval, & Debry, 1992) consumed mostly in the form of coffee, tea, sodas, or energy drinks. Its stimulatory effects are primarily credited to its antagonistic effects on adenosine receptors and thus blocking adenosine’s inhibiting effects on the central nervous system, including norepinephrine transmission (Barry et al., 2005). Within 30 to 45 min after ingestion, caffeine acutely increased SCL (e.g., Barry, et al., 2005; Bruce, Scott, Lader, & Marks, 1986; Quinlan et al., 2000; Zahn & Rapoport, 1987) and the number of NSFs (Zahn & Rapoport, 1987).

2.2.3.4.5 Nicotine

Nicotine is a tobacco alkaloid present in smoke. It stimulates nicotinic receptors of the autonomic ganglia at low doses and thus has both sympathetic and parasympathetic effects that seem to differ between the sexes. Smoking increased SCR amplitudes to an auditory orienting paradigm with female smokers displaying the highest SCR amplitudes and SCL compared to male smokers and non-smokers (Knott, 1984). In another study, women’s skin resistance levels decreased after smoking while men’s levels increased (Furedy, Algan, Vincent, Demirgoren, & Pogun, 1999). These results suggest increased EDA in women compared to men in response to nicotine.
2.2.3.4.6 Thyroid disease

Deficiency and overproduction of thyroid hormones have been associated with abnormal skin conductance (re)activity. The underlying mechanisms are yet unclear but may involve changes in epidermal thickness, activity of plasma catecholamines, and brain metabolism (see further, Dolu, Süer, Özesmi, Kelestimur, & Esel, 1997; Dolu, Süer, Özesmi, Kelestimur, & Ozcan, 1999). Hypothyroid patients had lower SCL, fewer NSFs, and prolonged SCR onset latencies to repeated acoustic stimulation that were unrelated to psychiatric symptoms (Dolu, et al., 1999). In contrast, hyperthyroidism was related to higher SCL and more NSFs in a similar paradigm (Dolu, et al., 1997; Morakinyo, Aitken, Zealley, & Irvine, 1972). These results suggest a possibly linear relationship between the level of thyroid hormones and EDA.

2.2.4 Cardiac activity: heart rate and heart rate variability

Innervated by both sympathetic and parasympathetic efferents, chronotropic cardiac activity will be presented in the following sections as a common indicator of autonomic arousal.

2.2.4.1 Measurement and quantification

Heart rate (HR) refers to the rate of cardiac contractions and is expressed in beats per minute (bpm). To determine the timing of these beats accurately, an electrocardiogram (ECG) measures electrical potential differences at the skin surface which are caused by depolarization and repolarization of the heart muscle. Instantaneous HR (HRi) is derived from the length of individual interbeat intervals (IBIs, in ms) using the following formula: HRi = 60,000/IBI.

HR varies in a rhythmic manner called HR variability (HRV). Several frequency bands of HRV have been distinguished (Berntson et al., 1997): (a) high frequency (HF) band from about 0.15 Hz to 0.4 Hz, (b) low frequency (LF) band from about 0.05 Hz to 0.15 Hz, (c) very low frequency (VLF) band from about 0.003 Hz to 0.05 Hz, and (d) ultra-low frequency (ULF) band below 0.003 Hz. VLF and ULF phenomena have been investigated much less than HF and LF bands and may reflect thermoregulatory cycles and circadian rhythms (Berntson, et al., 1997). HF and LF bands have been associated with autonomic modulation, with HF-HRV being predominately mediated by vagal influences and LF-HRV by both sympathetic and

HRV can be quantified using either time-domain or frequency-domain methods. Time-domain methods are considered the simpler approaches (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Aggregating across frequencies, they preserve temporal integrity of the signal at the expense of frequency resolution (Berntson, Quigley, & Lozano, 2007). Methods include statistical methods such as the standard deviation of IBIs or the square root of the mean squared differences in successive IBIs (RMSSD), geometric methods such as the HRV triangular index, and the peak-valley method. As the total variance of HRV increases with the length of the analyzed recording, shorter periods are more strongly affected by short-term or high-frequency variability and thus are better measures of HF-HRV.

Frequency-domain methods represent more systematic attempts at parsing the data into specific frequency components. By aggregating across time, they preserve the frequency composition at the expense of temporal resolution (Berntson, et al., 2007). A common approach of decomposing the overall IBI variance into specifiable frequency bands is the Fourier transformation (commonly using the computationally more efficient Fast Fourier Transform, FFT) which “transforms” the time domain representation of variance into a frequency domain representation or spectral density function. Integrating (summing) this function across the respiratory frequency bands yields an estimate of total power (in msec²) within those bands which represents a quantitative estimate of vagal control of the heart (Berntson, et al., 2007). Since it quantifies periodic components of variability, the Fourier transform must assume at least weak stationarity of the signal, i.e., a constant mean and variance over the time interval. A violation of this assumption becomes increasingly likely the longer the time interval and can compromise the analysis and interpretation of the data. Thus, it is advisable to use short analytic periods comprising a minimum of ten respiratory cycles (Berntson, et al., 2007). Other methods have been used in the literature, including those that specifically aim to quantify respiratory influences on the heart like cross-spectral analysis and transfer function analysis (for a review, see Berntson, et al., 1997). Respiration rate and tidal volume affect RSA independent of vagal output fluctuations (Ritz & Dahme,
and algorithms have been developed to account for their effects (Schulz, Ayala, Dahme, & Ritz, 2009).

2.2.4.2 Neuropsychophysiological properties

The heart muscle contains pacemaker tissue which enables it to contract autonomously and spontaneously. In healthy individuals, a spontaneous action potential originates in the sinoatrial node – also called the heart’s pacemaker – located in the right atrium and travels across both atria, which consequently contract. The potential then reaches the atrio-ventricular node from where it spreads to both ventricles via the bundle of His and the Purkinje fibers, causing both ventricles to contract and pump the blood out into the pulmonary circuit and the rest of the body.

2.2.4.2.1 Heart rate

The chronotropic state of the heart is modulated by humoral, hormonal (e.g., adrenomedullary catecholamines), and mechanical factors, but mostly by innervations to the sinoatrial node from both the sympathetic (SNS) and the parasympathetic (PNS) branches of the autonomic nervous system. The SNS innervates the sinoatrial node via upper thoracic (stellate) ganglia, and by releasing norepinephrine at the postganglionic terminals speeds the sinoatrial node rhythm (and increases contractility of the heart) via a $\beta_1$ receptor-mediated second messenger cascade of intracellular signals. In contrast, the PNS slows the rate of sinoatrial depolarization thereby decreasing heart rate (and contractility). It innervates the heart via the vagal nerve whose postganglionic terminals release acetylcholine binding to muscarinic cholinergic receptors and activating a transmembrane potassium channel. Vagal responses are faster with almost no time delay, while sympathetic responses are slower with a 1-2 second time delay (Berntson, et al., 1997).

2.2.4.2.2 HRV at respiratory frequencies or respiratory sinus arrhythmia

Respiratory sinus arrhythmia (RSA) or HRV at respiratory frequencies has been known for over a century but its underlying mechanisms are not completely understood. It describes the normal small heart rate increase during inhalation and heart rate decrease during exhalation. Variability of such high frequency is unlikely to be of sympathetic origin as cardiac sympathetic receptors at the sinoatrial node respond only slowly to stimulation and act as filters of frequencies above 0.15 Hz (Berntson, et al., 1997). Research findings consistently indicate that RSA is mediated almost entirely by fluctuations in vagal cardiac
activity on a breath-by-breath basis as RSA has been shown to be eliminated or substantially attenuated following cervical vagotomy (Anrep, Pascual, & Rossler, 1936; Hamlin, Smith, & Smetzer, 1966), ganglionic blockade (Katragadda, Xie, Puleo, Skatrud, & Morgan, 1997), cholinergic blockade (Hamlin, et al., 1966), and heart transplantation (Bernardi et al., 1989). The magnitude of RSA is generally regarded as a proxy of basal levels of efferent vagal cardiac nerve traffic stemming from (among others) brainstem reflexes aimed at maintaining blood pressure and gas homeostasis, and most importantly for psychophysologists, from central origins indicating a person’s level of emotional arousal. The vagal output fluctuates with respiration as respiratory centers in the brainstem inhibit it during inspiration and “gate” it during expiration (Berntson, et al., 1997), possibly to improve gas exchange at the level of the lungs via efficient ventilation-perfusion matching (Yasuma & Hayano, 2004). This intermittent vagal output has also been termed “vagal brake” (Porges, 2007, p. 9). Other proposed biological mechanisms linking respiratory and heart activity are (a) afferent feedback from stretch receptors in the lungs, and (b) volume and baroreceptor reflexes elicited by alterations in blood flow from changing intrathoracic pressure (Papillo & Shapiro, 1990).

2.2.4.3 Confounding variables

Just like skin conductance, cardiac activity is subject to confounding influences other than central autonomic output related to affect.

2.2.4.3.1 Somatic activity and fitness

Heart rate has been known to increase in anticipation of and during somatic activity to meet heightened metabolic demands (for a review, see Obrist, Webb, Sutterer, & Howard, 1970), resulting in what has been described as cardio-somatic coupling (Roberts & Young, 1971). Regular aerobic exercise and physical fitness produce resting bradycardia possibly due to increased vagal activity. HR recovery time was decreased after physical exercise (Keller & Seraganian, 1984). In a recent meta-analysis, exercise training resulted in HR decreases and HF-HRV increases that were less with age (Sandercock, Bromley, & Brodie, 2005). The concept of “additional heart rate” was introduced about four decades ago (see Wilhelm & Roth, 1998) to disentangle the emotional activation component from the component due to physical metabolic demands. Especially in ambulatory monitoring studies
where subjects are physically active, monitoring of somatic activity and obtaining data on fitness level are necessary to estimate the emotional arousal component in HR and HRV.

2.2.4.3.2 Pharmacological agents

Drugs that mimic (agonist) or block (antagonist) the actions of acetylcholine at postsynaptic nicotinic or muscarinic receptors or neurotransmitters of the central autonomic network (Benarroch, 1993) are likely to affect heart rate measurements – with more specific effects expected the more peripheral the substance’s action. Beta-blockers (β-adrenergic antagonists), for instance, reduce the chronotropic and ionotropic effects of epinephrine on the heart. Sertraline (SSRI) caused a significant reduction in HR while HRV was not affected (Siepmann, et al., 2003). After having found similar results in large samples of clinically anxious (Licht, de Geus, van Dyck, & Penninx, 2009) and depressed patients (Licht et al., 2008), a recent longitudinal study reported on the impact of a variety of antidepressants on cardiac measures (Licht, de Geus, van Dyck, & Penninx, 2010). They found that use of tricyclics (TCAs), selective serotonin and norepinephrine reuptake inhibitors (SNRIs), and SSRIs increased HR and reduced RSA over a 2-year period – an effect that was reversed in participants who discontinued the medications. They concluded that antidepressants had unfavorable cardiovascular effects. The study’s analysis strategy and its conclusion that SSRIs were harmful have been criticized, however, for a number of reasons including the inappropriate use of covariance statistics, collapsing across multiple SSRI medications with varying degrees of anticholinergic effects, and the inclusion of patients with a prior history of cardiovascular disease (Kemp, Quintana, & Malhi, 2011).

2.2.4.3.3 Caffeine

The effects of caffeine – a central nervous system stimulant shown to increase SCL (see section 2.2.3.4.4) – on cardiac functioning have been largely inconsistent: for example, caffeine significantly raised HR within 30 min after ingestion (Buscemi, Mattina, Tranchina, & Verga, 2011) or not until the night following caffeine consumption (Green & Suls, 1996), or had no effects on HR at all (Barry, et al., 2005; Zahn & Rapoport, 1987). HRV 90 min after ingestion was unchanged in a placebo-controlled study with habitual coffee drinkers (Rauh, Burkert, Siepmann, & Mueck-Weymann, 2006). Further research is clearly indicated to unravel the complex effects of caffeine on chronotropic cardiac functioning.
2.2.4.3.4 Alcohol

Ethanol or drinking alcohol is a central nervous system depressant with complex action whose neuropharmacological effects on autonomic functioning, both short-term and long-term, are just beginning to be understood. Alcohol and its first metabolite acetaldehyde act on a variety of neurotransmitter systems, such as GABA\textsubscript{A}, serotonin, and nicotinic acetylcholine receptors, and ion channel systems with direct and indirect effects ranging from disinhibition to sedation (Narahashi, Aistrup, Marszalec, & Nagata, 1999; Vengeliene, Bilbao, Molander, & Spanagel, 2008). Acute alcohol ingestion prolonged the heart’s PR and QTc intervals in healthy individuals (Lorsheyd, de Lange, Hijmering, Cramer, & van de Wiel, 2005). While HR was unaffected by a moderate dose of alcohol (0.7 g/kg), HRV was reduced 30 and 60 min after ingestion in eight healthy volunteers (Weise, Krell, & Brinkhoff, 1986). A higher dose of alcohol (1.12 g/kg), however, led to increases in HR and decreases in RSA (Reed, Porges, & Newlin, 1999). Chronic alcohol consumption was associated with central and peripheral nervous system damage and vagal neuropathy (Malpas, Whiteside, & Maling, 1991).

2.2.4.3.5 Other influences

HR and HF-HRV indices seem to decrease with age and are greater among women than men (Antelmi et al., 2004). This decrease with age was also steeper in men compared to women (Antelmi, et al., 2004) but gender differences were found to disappear beyond the age of 50 (Umetani, Singer, McCraty, & Atkinson, 1998).

As mentioned earlier (section 2.2.4.1), VLF and ULF components of HRV may reflect thermoregulatory cycles and circadian rhythms (Berntson, et al., 1997). Smoking (see section 2.2.3.4.5) has been shown to increase HR (Furedy, et al., 1999; Gilbert, Dibb, Plath, & Hiyane, 2000).

2.2.5 Summary

Apart from some exceptions, both branches (sympathetic and parasympathetic) of the ANS directly innervate and regulate the functioning of the inner organs via autonomic reflexes to maintain homeostasis. The ANS can be modulated and orchestrated by higher centers in the brain, the so-called central autonomic network. It is this higher control that initiates autonomic arousal in response to perceived threat stimuli or stressors and can turn autonomic arousal measures into indicators of defensive emotional states, such as anxiety.
In particular, elevated sympathetic activation as indexed by electrodermal arousal is traditionally thought to indicate defensive states and stress, whereas heightened parasympathetic activation as measured by RSA for example, is supposed to indicate the opposite, i.e. states of relaxation. However, autonomic arousal involves more than mere antagonistic activity of the SNS and PNS, and may include instances of independent and even coactive or co-inhibitive activity of both branches, as have been proposed in Lang and colleagues’ (1997) defense cascade model (see Figure 2). Furthermore, both electrodermal and cardiac arousal are subject to a variety of confounding influences that need to be taken into account in the study of physiological arousal during defensive states such as anxiety. Some of these confounding variables and their effects on electrodermal arousal will be investigated in chapter 4.

All empirical studies reported in this thesis (chapters 4 to 6) will deal with the relationship between psychological (defensive) states and indicators of autonomic arousal while controlling for confounding effects. Current evidence for worry’s and anxiety’s association with electrodermal and cardiac measures during waking will be reviewed in detail. Furthermore, we investigated these associations during sleep in the natural environment – which has rarely been done in the literature.

2.3 Sleep

Sleeping occupies about one third of our time and sleep disturbances are frequently reported in anxiety disorders. Very few studies have investigated how autonomic arousal might be affected during sleep by processes like anxiety or worry that seem to require a conscious mind. In the following, sleep will first be defined based on traditional polysomnographic assessments. However, we will also explore the possibility that measures of electrodermal and cardiac arousal might offer additional insights into sleep that traditional methods might not register. Lastly, sleep disturbances common in anxiety disorders will be described with a special emphasis on worry and panic.

2.3.1 Definition

Scientifically, sleep is defined on the basis of behavioral and physiological criteria. Behavioral criteria in humans include a characteristic posture, immobility or reduced mobility, closed eyes, reduced response to external stimulation, and increased reaction time (Chokroverty, 2010). Physiological criteria are derived from polysomnography (PSG)
encompassing electroencephalographic (EEG), electrooculographic (EOG), and electromyographic (EMG) recordings.

2.3.1.1 Sleep stages

A close examination of PSG recordings has led to a distinction of two sleep states: rapid eye movement (REM) sleep and non-REM (NREM) sleep. In adults, these two states alternate cyclically four to six times during sleep with an average duration of about 90 to 110 min per cycle (Chokroverty, 2010). In healthy human adults, NREM sleep dominates the first third of sleep and accounts for about 75 to 80 percent of sleep, while REM sleep becomes more prominent in the last third of sleep. According to the traditional Rechtschaffen and Kales (R&K) scoring manual for sleep (1968), NREM sleep can be further divided into four stages (NREM stages 1 to 4) while the more recent American Academy of Sleep Medicine (AASM) scoring manual (Iber, Ancoli-Israel, Chesson, & Quan, 2007) only distinguishes between three stages (N1, N2, N3) by combining NREM stages 3 and 4 into N3. Deepening NREM sleep is characterized by progressive slowing and increasing amplitude of EEG activity. Stage N1 is defined polysomnographically as a transition from alpha waves (8-13 Hz) which are common during waking with eyes closed, to theta waves (4-7 Hz). Stage N2 is characterized by sleep spindles (11-16 Hz) and K complexes. During stage N3, delta waves (0.5-2 Hz) with peak-to-peak amplitudes greater than 75 μV constitute at least 20 % of the EEG, which is why it is often referred to as slow-wave sleep. The transition from N1 to N2 to N3 is characterized by progressively decreased muscle tone and loss of consciousness of the external environment. REM sleep criteria include rapid eye movements, muscular atonia, and rapid low-voltage EEG activity that resembles waking activity mixed with distinctive sawtooth waves.

2.3.1.2 Electrodermal arousal during sleep

Electrodermal arousal levels have been observed to drop during sleep (Koumans, Tursky, & Solomon, 1968) and at night during total sleep deprivation (Miró, Cano-Lozano, & Buela-Cazal, 2002). However, according to a series of early studies, the rate of spontaneous EDA fluctuations seems to progressively increase from waking to NREM sleep and as NREM sleep deepens (Broughton, Poire, & Tassinari, 1965; Freixa i Baque et al., 1983; Johns, Cornell, & Masterton, 1969; Johnson & Lubin, 1966; Koumans, et al., 1968; Lester, Burch, & Dossett, 1967; McDonald, Shallenberger, Koersko, & Kinzy, 1976). Furthermore, they
culminate in so called “electrodermal storms” during slow-wave sleep (Lester, et al., 1967). During REM sleep, these rates drop again but it has not been possible to reliably distinguish EEG sleep stages on the basis of this EDA activity (Koumans, et al., 1968). The finding of increased spontaneous EDA activity during sleep prompted Johnson and Lubin (1966) to question its ability to indicate arousal, vigilance, or alertness. However, most of these early studies used skin potential as an EDA measure which has certain disadvantages (Fowles, et al., 1981). Furthermore, sympathetic nerve activity to the skin (Kodama et al., 1998) is lower during NREM sleep than when awake. Thus, chapter 4 will re-examine EDA activity during sleep using skin conductance.

2.3.1.3 Cardiac arousal during sleep

During NREM stages, HR and sympathetic nerve activity to skeletal blood vessel muscles (Somers, Dyken, Mark, & Abboud, 1993) are lower than when awake, while at the same time, HF-HRV (RSA) increases (Bonnet & Arand, 1997; Otzenberger et al., 1998). These findings suggest sympathetic inhibition and increased vagal cardiac drive during NREM sleep (Bonnet & Arand, 1997; Malliani, Pagani, Lombardi, & Cerutti, 1991). REM sleep shows a similar pattern of predominantly vagal activity which is interrupted however, by sudden bursts of sympathetic activity (Dickerson, Huang, Thurnher, Nearing, & Verrier, 1993; Hornyak, Cejnár, Elam, Matousek, & Wallin, 1991; Kirby & Verrier, 1989; Okada, Iwase, Mano, Sugiyama, & Watanabe, 1991; Somers, et al., 1993) leading to intermittent tachycardia. Because of these sudden changes in vagal and sympathetic output together with irregular respiratory patterns, correct measurement of HRV during REM sleep has been difficult (Murali, Svatikova, & Somers, 2003).

2.3.1.4 Physical activity during sleep – nocturnal actigraphy

Actigraphy is generally considered a useful, cost-effective, non-invasive and portable method for estimating wake-sleep parameters (such as total sleep time, wake time after sleep onset, sleep-onset latency, number of awakenings) in the natural environment of healthy volunteers (Paquet, Kawinska, & Carrier, 2007; Tahmasian, Khazaie, Sepehry, & Russo, 2010). Based on various algorithms that analyze limb movement (usually from the wrist but also the ankle and trunk), it scores consecutive epochs as either wake or sleep from which the wake-sleep parameters are derived. In a study with healthy volunteers, actigraphy showed high sensitivity in detecting sleep epochs (97 to 99 %) but was rather nonspecific,
overestimating sleep with only 34 to 44 % of epochs correctly scored as sleep (while subjects were actually awake during the other 56 to 66 % of the epochs that actigraphy scored as sleep) – which is almost equivalent to chance. Recent reviews and studies concluded that actigraphy cannot replace PSG as a diagnostic tool for all clinical groups (Morgenthaler et al., 2007), especially in disorders with movement, immobility, or wakefulness during sleep (Tahmasian, et al., 2010). Its diagnostic value in patients with fragmented sleep or in situations where the sleep-wake cycle is challenged, such as jet lag and shift work, is still being debated (Morgenthaler, et al., 2007; Paquet, et al., 2007). In a study on patients with insomnia, however, sleep parameters derived from actigraphy (number of awakenings, wake time after sleep onset, total sleep time, sleep efficiency) significantly and highly correlated with measures derived from PSG, while only sleep-onset latency did not (Lichstein et al., 2006).

Despite its limitations, actigraphy has been extensively used in sleep research and is now included as a measure of sleep duration and pattern in the diagnostic criteria for a number of sleep disorders in the second edition of the International Classification of Sleep Disorders (ICSD-2, American Academy of Sleep Medicine, 2005). For example, actigraphy is recommended to document inconsistencies between objective (actigraphy) and subjective measures of sleep timing in paradoxical insomnia (also called sleep state misperception).

2.3.2 Sleep in anxiety disorders

Sleep disturbances are commonly associated with or represent core symptoms of anxiety disorders (Mellman, 2006). For example, panic disorder (PD) patients can experience nocturnal panic attacks, patients with posttraumatic stress disorder (PTSD) report re-experiencing nightmares, while generalized anxiety disorder (GAD) patients suffer from difficulty initiating and maintaining sleep. In the following, sleep disturbances related to worry and panic will be elucidated.

2.3.2.1 Worry and sleep

Research has shown a close relationship between worrying and sleeping difficulties. When recruiting participants for their study aimed at examining the contribution of worry to insomnia, Watts et al. (1994) observed a higher frequency of worried insomniacs (n=28) than of non-worried insomniacs (n=10) despite comparable recruitment efforts and concluded that insomniacs without high worry symptoms might be a minority. Indeed, the majority of
insomniacs attribute their sleeping problems primarily to cognitive activity in bed (Lichstein & Rosenthal, 1980) where their attention is focused on worries and problems (Harvey, 2000), and which includes activities such as rehearsing, planning, problem-solving, and thinking about sleeplessness and its consequences (Harvey, 2000; Watts, et al., 1994; Wicklow & Espie, 2000). These pre-sleep thoughts are perceived as more distressing (Nelson & Harvey, 2003), more occupying, and less intentional than in good sleepers (Harvey, 2000). Chapter 6 will present extensive evidence for associations between worry and subjective and objective sleep parameters (Table 8).

2.3.2.2 PD and nocturnal panic attacks

As elaborated in chapter 5, panic attacks in PD are not confined to the day but also occur at night. It is estimated that between 44 and 71% of PD patients have experienced a nocturnal panic attack at least once (Craske & Tsao, 2005). Craske and colleagues (Craske & Tsao, 2005; Craske & Waters, 2005) propose that nocturnal panic attacks are conditioned responses to perceived changes in physiological state during sleep. Even though asleep, PD patients may process threat cues and react with elevations in anxious arousal that can escalate to panic. Afraid of having another nocturnal panic attack, PD patients consequently might develop difficulties initiating and maintaining sleep and phobic avoidance behavior of going to sleep (Lepola, Koponen, & Leinonen, 1994). However, not all sleep complaints in PD can be explained by nocturnal panic (Overbeek, Van Diest, Schruers, Kruizinga, & Griez, 2005).

2.3.3 Summary

Sleeping difficulties seem to be associated with anxiety disorders, especially panic disorder and patients suffering from pathological worrying such as GAD patients. Although PSG is still the gold standard in sleep assessment, measures of electrodermal and cardiac arousal show characteristic changes during sleep that in combination with nocturnal actigraphy might provide interesting and less intrusive insights into sleeping difficulties among these patient populations – especially in an ambulatory context.

2.4 Ambulatory monitoring

Ambulatory monitoring is being increasingly applied in the study of autonomic, behavioral, and subjective aspects of anxiety and related states or emotions. In the following sections, ambulatory monitoring will be introduced and compared with laboratory
assessments. Study designs, challenges in obtaining ambulatory self-report data, and problems of data analysis will be presented.

2.4.1 Definition

Ambulatory monitoring or assessment has been defined in many ways. Perhaps the most general definition was given by Haynes and Yoshioka (2007, p. 44) who described it as a “diverse set of measurement strategies to acquire minimally disruptive measures of persons engaging in normal activities in their natural environments”. Some authors went further by specifying ambulatory measures as self-reported, behavioral, and physiological (Ebner-Priemer & Trull, 2009; Fahrenberg, Myrtek, Pawlik, & Perrez, 2007). Trull and Ebner-Priemer (2009) pointed out that the terms ambulatory assessment, ecological momentary assessment (EMA), and experience sampling method (ESM) have been used interchangeably despite differences. While EMA and ESM originally targeted self-report and behavior only, ambulatory monitoring primarily involved measuring physiological variables (e.g., heart rate and blood pressure). In recent years, EMA and ambulatory monitoring have started to merge as more and more EMA studies began including physiological variables, and ambulatory monitoring studies put increased focus on supplemental self-report. Due to technological advances, self-report and behaviors are increasingly being recorded by electronic devices, prompting some authors to already define ambulatory monitoring specifically as “the use of computer-assisted methodology” (Ebner-Priemer & Trull, 2009, p. 109; Fahrenberg, et al., 2007, p. 206). While this is certainly true for physiological measurements, the use of paper logs is still quite common in obtaining self-report.

2.4.2 Why ambulatory monitoring? Laboratory vs. ambulatory assessments

Laboratory studies are considered the “gold standard of controlled observation and concise testing of hypotheses under the most stringent possible methodical isolation of the phenomena in question” (Fahrenberg, et al., 2007, p. 211) and therefore to have high internal validity (Fahrenberg, et al., 2007; Wilhelm & Grossman, 2010). The controlled environments and stimulus conditions allow for comparisons of findings across different laboratories and many well established psychological paradigms are available that are readily interpretable (Turpin, 1990). It is this inherent artificiality of the assessment conditions, however, which poses a great risk to both the internal and external validity of laboratory measurements (Wilhelm & Grossman, 2010). According to the authors, the
psychological phenomena under examination might be altered by many factors, such as the subjects’ prior knowledge of the experimental and artificial stimuli, the novel and potentially frightening nature of the laboratory environment, or asking subjects to remain still and immobile for variable lengths of time when they would rather actively avoid. Laboratory experiments are usually short and thus limit the range of activities and measurement time, which reduces the reliability and generalizability of the results. Long-term ambulatory assessments from subjects unrestricted in their range of physical movement and engaged in their usual activities might solve this problem by capturing and averaging a wider range of a person’s moods and emotional reactivity in varying situational contexts. These assessment conditions allow to record and study rare events (such as panic attacks), the variability and circadian profiles of psychological phenomena at times and places not usually available to laboratory studies. All of these factors serve to increase ecological validity of the assessments. Given the relative lack of experimental control, internal validity needs to be increased by monitoring relevant confounding variables either through self-report (e.g., food, alcohol consumption) or automatically (e.g., physical activity using an accelerometer) which can be burdensome and lead to reactivity effects and inaccuracies.

Recent technical advances especially in ambulatory cardiovascular measurement allow for smaller and smaller devices to be attached to the participant’s body. Yet in other areas such as electrodermal measurement or electroencephalography, most devices still necessitate the use of rather long and obtrusive cables making it hard to forget the fact that one is being monitored. Thus, even though participants are technically “unrestricted” and are free to “engage in their usual activities”, they might either not feel comfortable enough to do so (e.g., going out with friends) or unable to do so (e.g., washing dishes with finger electrodes) unless they take off the equipment.

Thus, both ambulatory and experimental approaches have limitations and most researchers consider them complementary and not opposing research approaches that when combined can help validate experimental findings from the laboratory under real-life conditions (Fahrenberg, et al., 2007).
2.4.3 Methodological considerations

2.4.3.1 Study design and assessment strategies

According to Wilhelm and Grossman (2010), ambulatory designs can vary on, at least, five dimensions: (1) naturalness (ranging from fully naturalistic to fully structured), (2) type and number of channels recorded (ranging from one to over 20), (3) degree of situational context awareness (ranging from no context information to broad context awareness by concurrently monitoring confounding variables), (4) sampling mode (ranging from continuous monitoring to time- or event-dependent sampling), (5) assessment duration (ranging from minutes to months).

2.4.3.2 Physiological data: dry versus wet electrodes

Different types of bioelectrodes have been used in clinical studies including wet electrodes and dry electrodes. Wet refers to silver/silver chloride (Ag/AgCl) electrodes that use an electrolytic gel to form a conductive path between the skin and the electrode (Searle & Kirkup, 2000). Reusable or disposable types exist with the latter being recommended for hygienic reasons (Putnam, Johnson, & Roth, 1992). Dry electrodes, on the other hand, are made up of a benign metal (such as stainless steel) and do not utilize a gel (Searle & Kirkup, 2000) which is why they have been suspected of being more susceptible to artifacts than wet electrodes (Godin, Parker, & Scott, 1991). They might offer benefits over wet electrodes especially in long-term electrodermal monitoring, however, because they do not cause skin irritation, such as swelling due to hydration (“raisin skin”). In an experiment comparing wet and dry electrodes, Searle and Kirkup (2000) found that wet electrodes were more affected by moving charge artifacts but less by movement artifacts than dry electrodes – at least initially. Later during the experiment, the effects of movement artifacts on dry electrodes were reduced and resembled those of wet electrodes. However, dry electrodes had twice the contact impedance of wet electrodes. Wet electrodes are still the norm in long-term electrodermal monitoring and until disposable dry electrodes with reasonable signal fidelity become available, wet electrodes will continue to be used. Chapter 4 will investigate the effect of long-term monitoring on electrode site deterioration to determine if 24-h electrodermal monitoring with wet electrodes is feasible.
2.4.3.3 *Questionnaires vs. field diaries*

Questionnaires and interviews rely on retrospective memory recall that requires complex processing, such as retrieving, counting, and averaging relevant information over variable time intervals (Piasecki, Hufford, Solhan, & Trull, 2007). Typically, respondents estimate an answer rather than provide a direct account of facts retrieved from memory (Piasecki, et al., 2007). The validity of these estimations is likely to be affected by several retrospective biases, including (a) forgetting information when the recall period is long and memory retrieval occurs in a different setting from that of memory acquisition, which is typically the case (Wilhelm & Grossman, 2010); (b) enhanced processing of positively valenced material when respondents are in a good mood compared to a bad mood, and vice versa (i.e., the mood congruent memory effect; Kihlstrom, Eich, Sandbrand, & Tobias, 2000); (c) peak experience as well as the most recent experience most heavily influencing recall (peak end rule; Kahneman, Fredrickson, Schreiber, & Redelmeier, 1993), (d) favoring recall of mental representations that fit into idealized self-concepts, social expectations and after-the-fact information, rather than those that represent actual experiences or the target behavior examined (Pohl, 2004). These biases may introduce additional error variance and systematic bias at worst (Wilhelm & Grossman, 2010). Directly assessing experience and behavior ambulatorily or in the laboratory seems to be the better approach especially when immediate experience is the specific focus of the study.

2.4.3.4 *Electronic diaries vs. paper-pencil*

The advantages of diary methods described in the previous section can be undermined when using traditional paper-pencil diaries (Piasecki, et al., 2007). Most of these diaries provide no means of ensuring that entries are made according to the diary protocol specified by the assessor. Back-filling and even forward-filling diary entries can reduce their usefulness to that of other retrospective methods. Electronic diaries can provide a means to reduce non-compliance or at least to record it as all entries are time-stamped. Alert functions (e.g., audible beeps) can follow different sampling strategies (fixed-time, random-time, event-dependent) and can reduce subject burden by serving as reminders to fill in a diary. Methodological reactivity to these prompts can be minimized by allowing the subject to delay, defer, or deny data input when necessary (Fahrenberg, et al., 2007). Electronic diaries allow for efficient branching of questions, on-line data analysis, and direct feedback (for more advantages, see Fahrenberg, et al., 2007). By not being able to review previous
entries, subjects cannot be influenced by them on further entries (Reuschenbach, 2006). Collected data can be easily transferred to a computer for further statistical analysis without running the risk of errors due to manual data entry. However, as with any electronic device, also electronic diaries can fail and make assessor assistance necessary. For more information on current platforms, selecting and configuring electronic diaries, designing a diary protocol, using the data, and barriers to adoption in clinical assessment, refer to Piasecki and colleagues (2007).

2.4.4 Data reduction and analysis

Ambulatory data structure is usually nested as several dependent variables including confounding variables are repeatedly monitored over time, sometimes at unequally spaced time intervals. Furthermore, missing data is common for various reasons such as lacking compliance, movement artifacts, or technical malfunction (Wilhelm & Grossman, 2010). As a first step towards statistical analysis of such vast data sets, artifacts need to be detected and removed before the data can be reduced to a meaningful size. Given the amount of data to be inspected in long-term recordings, this data cleaning is preferably performed either automatically or semi-automatically. When the data is artifact-free, a statistical method needs to be chosen which can appropriately deal with the fact that ambulatory data are often unbalanced, show serial within-subject autocorrelation, and violate the assumption of homoscedasticity (Bolger, Davis, & Rafaeli, 2003; Schwartz & Stone, 1998; Wilhelm & Grossman, 2010). A number of researchers have been advocating multilevel models (also referred to in the literature as linear/non-linear mixed models, hierarchical mixed models, random regression models, for example) to be appropriately addressing most of these issues (Bagiella, Sloan, & Heitjan, 2000; Bolger, et al., 2003; Schwartz & Stone, 1998; Wilhelm & Grossman, 2010). For an easy-to-follow, introductory text book with example code in several common statistical packages such as SPSS and R, I recommend West, Welch and Galecki (2007). These models also offer a convenient way to estimate or control for the effects of confounding variables, such as physical activity and ambient temperature by including them in the model as covariates.

2.4.5 Summary

Ambulatory monitoring allows the acquisition of measures of persons engaging in normal activities in their natural environments. As such it may provide a complementary
approach to laboratory assessments validating experimental findings from the laboratory under real-life conditions and increasing their ecological validity. If possible, self-report and behaviors should be recorded using electronic devices to increase accuracy and validity of the results. The burden of data cleaning and reduction can be quite immense but is facilitated by appropriate hardware and software. Multilevel models are recommended for analyzing ambulatory data.

2.5 Overall summary

Worry, anxiety, fear, and panic may represent different defensive emotional states along a continuum of perceived threat proximity (Craske, 2003) with different underlying central neural structures that lead to distinct and complex autonomic responding. Pathological levels of worry, anxiety, fear, and panic should show signs of abnormal sympathetic and/or parasympathetic arousal both during waking and sleep. However, most empirical research in this context has been confined to the laboratory where high internal validity is achieved at the cost of poor ecological validity.
3 Aims and outline of the present work

This thesis is aimed at investigating the relationships between autonomic arousal and self-reported anticipatory anxiety and worry using long-term recordings in daily life. These recordings in the subjects’ natural environment included periods of waking and sleeping.

First, the effects of potentially confounding variables on ambulatory electrodermal measurements were examined. Confounding variables included epidermal hydration-induced electrode site deterioration, time of day, ambient temperature, physical activity, age, sex, race, and body mass index (study 1).

Next, the effects of trait and state anxiety on 24-hour electrodermal activity were tested in a sample of panic disorder patients and healthy controls. The ability to relax during the day and sleep disturbance at night were investigated (study 2). To the authors’ knowledge, this is the first study to have examined electrodermal arousal ambulatorily during sleep in PD patients.

Similarly, the relations between worry, sleep, and autonomic arousal were the focus of study 3. This time, electrodermal arousal was supplemented by cardiac arousal in terms of heart rate and heart rate variability. Worry variables included trait worry and worry states such as pre-sleep worry, worry during nocturnal awakenings, and worry after waking up in the morning. Both subjective and objective sleep parameters were assessed. Among these measures of sleep disturbance were early morning awakenings and daytime dysfunction, which have so far been neglected in this context, as has been electrodermal activity.
4 Methodological considerations in ambulatory skin conductance monitoring (study 1)

4.1 Introduction

With the exception of measuring hot flashes (for example, Thurston, Blumenthal, Babyak, & Sherwood, 2005), which are more related to thermoregulation than to emotional activation, long-term electrodermal recording has rarely been reported in the literature. Theoretically such recording in a natural setting could contribute to our understanding of emotions and stress in people with and without mental disorders (see also, Turpin, 1990). Fowles (1980) linked electrodermal activity to Gray’s Behavioral Inhibition System, neural pathways related to anxiety, citing numerous papers that show increases in electrodermal activity in response to conditioned stimuli for punishment. Indeed, anxious arousal indicated by increased electrodermal activity and reactivity has been observed in a variety of anxiety disorders, such as panic disorder (Braune, Albus, Froehler, Hoehn, & Scheibe, 1994; Hoehn, Braune, Scheibe, & Albus, 1997; Lader & Wing, 1964) and posttraumatic stress disorder (Blechert, Michael, Grossman, Lajtman, & Wilhelm, 2007). In depression, on the other hand, electrodermal activity is often reduced (Argyle, 1991; Ward, Doerr, & Storrie, 1983).

Ambulatory monitoring can test whether findings recorded for limited time periods or in special settings also are present in daily life. In panic disorder and generalized anxiety disorder, for example, normal skin conductance levels but reduced variance were observed in time limited ambulatory recordings (Hoehn-Saric, et al., 2004), while a more recent 24-hour ambulatory study found chronically elevated skin conductance levels in panic disorder patients (Doberenz, et al., 2010). But ambulatory studies are rare. Although technically feasible, investigators may have been discouraged by the possibility that long-term recordings in daily life might be subject to uncontrollable confounding influences of electrode site deterioration, ambient temperature, physical activity, and incidental environmental stimuli.

To our knowledge, only two studies have been published on the problems of long-term ambulatory electrodermal recording. Turpin et al. (1983) tested 12 subjects for a maximum of 7 h. Epidermal hydration, produced by aqueous electrode solutions with a salt concentration lower than interstitial fluid or sweat or with high percentages of water in the
gel base, had been suspected of negatively affecting long-term skin conductance (SC) measurement by causing swelling of the epidermis and closure of sweat gland pores (Fowles & Venables, 1970). Recording of electrical activity of the heart or brain may benefit by skin changes towards lower resistance under electrodes over time, but for SC such changes lead to inaccuracy. Thus, Turpin et al. (1983) compared hydrating (high water content in base solution) vs. non-hydrating (minimal water content in base solution but similar salt content) electrolyte mediums. Contrary to their prediction of a continuous decrease over three h in SC level (SCL) and in the number and amplitude of SC responses (SCRs) to a reaction time task, no main effect for duration of recording could be found, indicating no differences in these measures between old and freshly applied electrodes. The hydrating medium, however, resulted in significantly fewer and smaller SCRs after three and six h compared to the non-hydrating gel.

Boucsein et al. (2001) obtained reliability estimates of electrodermal responses in different auditory habituation sequences after 24-hour monitoring from 12 female students. They compared freshly applied electrodes to old electrodes that had been worn 24 h. The correlation of SCR amplitudes between a reference set of freshly applied electrodes measured by a laboratory device and freshly applied electrodes at a different site recorded simultaneously by an ambulatory device was 0.96 (n=12), between the reference set and the old electrodes at a different site measured with the ambulatory device was 0.85 (n=3), and between the reference set and a set of freshly applied electrodes at the site of the old electrodes measured with the ambulatory device was 0.90 (n = 5). Despite the low subject numbers and missing statistical comparisons between these different correlations, the authors concluded that the ambulatory recordings lacked both reliability and validity after 24 h, although these correlations seem reasonably high.

Turpin et al. (1983) were also aware of circadian influences on SC although they did not look at the effects of sleep. In part these may be related to changes in body temperature (Hot, Naveteur, Leconte, & Sequeira, 1999; Turpin, et al., 1983), with SCL minima in the morning (5 am to 7 am) and maxima during the evening (7 pm to 9 pm) (Miró, et al., 2002; Venables & Mitchell, 1996). Electrodermal levels and fluctuations have been observed to drop during sleep except during “electrodermal storms” (Lester, et al., 1967) and transitorily during certain sleep stages (Broughton, et al., 1965; Freixa i Baque, et al., 1983; Johns, et al.,
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Recording outside of a temperature-controlled laboratory also poses potential problems. Although the hands are not the principal areas of thermoregulatory sweating, Turpin et al. (1983) found that ambient temperature was positively correlated with the frequency of SCRs, but only between subjects. Temperature did not correlate with SCL. In the laboratory, higher electrodermal activity was seen when exposing male subjects to hot compared to cold air (Lobstein & Cort, 1978; Scholander, 1963). Venables and Mitchell (1996) on the other hand, who examined tonic and phasic SC measures in children and young adults in a laboratory on the island of Mauritius during the hot and cold seasons, did not find seasonal differences in males. In females differences were limited to higher orienting response magnitudes during the hot season.

Physical activity, which is highly correlated with heart rate under ambulatory conditions, might also change SC through its effects on thermoregulation and sweating or through emotional arousal associated with the activity. Turpin et al. (1983) examined the effects of somatic activity on ambulatorily acquired SCL and SCR frequency and amplitude, but found no significant correlations. However, the wrist activity sensors they used could register only imperfectly the metabolic demands of gross body activity. Szpiler and Epstein (1976) found that motor activity (tapping rate) was unrelated to the number of non-specific SC fluctuations (NSFs; \( r = .05 \)) during anticipation of shock in 60 undergraduate male volunteers, while correlations with magnitude of SCRs and SCL were small (\( r = .23 \) and \( r = .27 \), respectively). The relevance of this to motor activity in daily life is tenuous. In an aversive classical conditioning paradigm in 10 male rats, Roberts and Young (1971) examined the relationship between gross body movement, electrodermal activity recorded from the soles of the animals and heart rate (HR) and heart rate variability (HRV). They found that HR positively correlated with movement while SC increased after the conditioned stimulus regardless whether movement increased or decreased. Seventy-seven percent of the SC variability was attributable to habituation but none to movement, leading them to conclude that there was no electrodermal-somatic coupling.

Electrodermal measures may also vary with individual differences in age, sex, race, and body mass index (BMI). If aged skin is drier than young skin, electrodermal activity
should diminish with *age*, which is supported by several studies (Barontini, Lazzari, Levin, Armando, & Basso, 1997; Eis dorfer, Doerr, & Follette, 1980; Gavazzeni, Wiens, & Fischer, 2008; Kronholm, Alanen, & Hyppae, 1993) although testing younger populations has yielded mixed results (Greene, 1976; Shibagaki, Sakamoto, & Furuya, 1994; Venables & Mitchell, 1996). *Sex* may play a confounding role in EDA measurement because of monthly hormonal variations in women (Goldstein et al., 2005). Laboratory studies have been inconclusive, some finding no sex differences in electrodermal levels and responding (Carrillo et al., 2001; Furedy, et al., 1999; Gavazzeni, et al., 2008), and others finding that men have higher SCL (Eisdorfer, et al., 1980; M. Kelly, Forsyth, & Karekla, 2006; Kronholm, et al., 1993), and that women have higher SCR amplitudes to a stressful task (Carrillo, et al., 2001; Eisdorfer, et al., 1980). Some of these differences disappear with age (Eisdorfer, et al., 1980) while others show no sex-age interactions (Gavazzeni, et al., 2008). *Racial* effects on electrodermal measures may be due to a decreasing number of active sweat glands with increasing skin darkness (Boucsein, 1992). African versus Indian descent had no effect on SC measures of children and young adults from Mauritius (Venables & Mitchell, 1996), while some studies indicate that Caucasians show higher electrodermal activity levels than Blacks (for a review of the literature, see Boucsein, 1992). Little data exist on the effect of Asian versus Caucasian race on electrodermal measures. Wesley and Maibach (2003) cited data from unpublished conference abstracts suggesting lower electrodermal activity in Asians compared to Caucasians. *Body mass index* (BMI) did not correlate significantly with a sympathetic activity index (SCL, SCR amplitude, habituation of SCR) (Kronholm, et al., 1993), although Peterson et al. (1988) reported a decrease in several non-electrodermal indicators of sympathetic and parasympathetic nervous system activity with increasing percentage of body fat and higher BMI.

Other influences on electrodermal variables that may be independent of emotional arousal in 24-hour recording include medications with anticholinergic effects (Schlenker, et al., 1995), caffeine intake (Barry, et al., 2005), and smoking (Furedy, et al., 1999; Knott, 1984). Diseases such as hyper- or hypothyroidism (Dolz, et al., 1999) or individual differences in the physical properties of the skin such as sweat gland density (Allen, et al., 1973) also affect SC.
The first goal of the present study was to repeat the observations of Turpin et al. (1983) with a larger number of normal subjects, longer recording times, and a wider variety of SC variability measures. The most frequently obtained SC variability measure in laboratory experiments is non-specific SC fluctuations (number and amplitude), but their detection depends on arbitrary amplitude and rise-time thresholds based on visual impressions rather than on theory (Bach, Friston, & Dolan, 2010). Whether these thresholds are valid in an ambulatory setting is unknown. A measure with fewer assumptions is skin conductance variance, which was used by Hoehn-Saric et al. (2004) in an ambulatory study of panic disorder and generalized anxiety disorder. They found skin conductance variance to be reduced in their sample compared to healthy volunteers, which they interpreted as diminished autonomic flexibility in these patients. SC variance is affected by both number and amplitude of SC fluctuations. Here we report the more familiar SC standard deviation. As this variability measure depends on SCL, we also computed the coefficient of SC variation. Area under curve (AUC) of SC has also been proposed (Bach, et al., 2010) but was not tested here.

From our review of the literature, we predicted that (a) SCL would decline over the course of 24 h due to epidermal hydration-induced electrode site deterioration, (b) all measures of electrodermal activity would decrease during sleep, (c) ambient temperature would be positively related to the number and amplitude of NSFs, (d) physical activity would show no effects on any of the electrodermal measures, (e) age would be negatively related to SCL and NSF number and amplitude, and (f) men would show higher SCL and smaller NSF amplitudes. We were uncertain about the effects of race and BMI.

The second goal of this study was to determine the relationship between electrodermal measures and self-reported arousal and sleep quality after the effects of the confounding variables are removed.

4.2 Materials and Methods

4.2.1 Participants

Forty-eight healthy volunteers (37.3 ± 11.8 years, age range 19 – 64 years, 67% women) were recruited from the local community by advertisement for three separate but methodologically similar studies. Their mean BMI was 23.1 ± 3.2 kg/m² and the majority of
participants were self-identified as Caucasian (48%) or Asian (44%), while four identified themselves as of other races (8%, 2 American Indian/Alaska Native, 1 Black, 1 more than one race). There were no significant correlations among age, sex, BMI, or race. Exclusion criteria for all participants were substance abuse or dependence in the past year and a history of, or current, DSM-IV axis I disorders as diagnosed by the Structured Clinical Interview for DSM-IV TR Axis I Disorders (First, Spitzer, Gibbon, & Williams, 2001). Also excluded were participants who reported a history of severe cardiovascular, lung, or neurological disease, uncontrolled thyroid problems, or who showed signs of cognitive impairment during the phone screening and direct interactions with the interviewers. Eligible participants were allowed to continue on stable doses of medicines prescribed by physicians, but were excluded if they were taking psychoactive drugs or drugs with substantial anticholinergic effects, which have direct effects on SC. Participants received monetary compensation for being tested.

4.2.2 Procedure

This investigation was carried out in accordance with the latest version of the Declaration of Helsinki. The study design was reviewed and approved by the Stanford Institutional Review Board. Individuals who passed the phone screening were invited to an appointment where they gave written informed consent for further assessment. This began with a Structured Clinical Interview for DSM-IV-TR Axis I Disorders (First, et al., 2001) conducted by graduate students in psychology who were trained and supervised by a clinical psychologist. If no grounds for exclusion were found, they were asked to wash their hands with soap in preparation for the application of skin conductance electrodes (Dawson, Schell, & Filion, 1990; Venables & Christie, 1980). Next, they underwent testing in a laboratory room where numerous autonomic and respiratory measures were recorded while they were trying to relax and to change their breathing according to several different instructions. The procedure and results of this testing have been reported elsewhere (Conrad et al., 2007; Roth et al., 2008; Wollburg, Meuret, Conrad, Roth, & Kim, 2008). After the laboratory session, the ambulatory monitoring device was connected at 1:17 pm on average. Participants wore it continuously until they returned the next day. Length of recording ranged between 18:42 and 28:51 with a mean of 24:02 (hr:min) during which the SC electrodes were applied to their non-dominant hand, secured with medical tape for the duration of the recording. All participants were supplied with additional tape to secure the
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electrodes if they seemed to be coming unattached. As soon as the participants returned to
the laboratory, a set of fresh SC electrodes was attached to the same hand. After 20 min, an
SCL reading was first obtained from the old SC electrodes and immediately afterward (within
20 sec) from the freshly applied electrodes using the same digital recorder.

During the 24-hour monitoring, subjects were asked to fill out a short questionnaire
at 4 pm and 8 pm the first day, as well as at 8 am the second day. This questionnaire
assessed their emotional state over the last four h (4 pm and 8 pm) or since they woke up in
the morning (8 am) on a subjective units of distress scale from 0, “not at all”, to 10,
“extremely” for such items as “excited” and “sleepy”. Self-reported measures of sleep
including the number of awakenings during sleep, sleep duration, and whether the sleeper
felt rested after sleep were obtained on the morning of the second day of recording. In
addition, participants were given a packet of questionnaires to return completed the next
day, which included the Beck Anxiety Inventory (BAI; Beck, Epstein, Brown, & Steer, 1988) to
be answered for the past week and the Pittsburgh Sleep Quality Index (PSQI; Buysse,
Reynolds III, Monk, Berman, & Kupfer, 1989) to be answered for the past month. The Beck
Depression Inventory (BDI; Beck, Ward, Mendelson, Mock, & Erbaugh, 1961) was given to all
but 19 participants who instead received the short version of the Beck Depression Inventory
(Abdel-Khalek, 2001).

4.2.3 Physiological Assessment

Physiological data were recorded with a 3-channel ambulatory digital recorder
(3991x/3 BioLog, UFI, Moro Bay, CA, USA) worn in a handbag or waist pack. The device
measures 3.3 x 7.1 x 12.7 cm and weighs 230 g with its battery. Channels were (1) skin
conductance measured by applying 0.5 V DC to electrodes on the middle or lower phalanges
of digits 2 and 3 (digits 4 and 5 for the freshly applied electrodes if there was not enough
space on digits 2 and 3) of the non-dominant hand. Skin conductance in the range 0.01–
39.95 μS/iemens was sampled with ±0.01 μS resolution at 10 Hz and digitally low-pass
filtered at 0.5 Hz. Commercial disposable electrodes with a circular contact area of 1 cm
diameter were used (EL507, Biopac Systems, Inc., Goleta, CA, USA). They were pre-filled with
isotonic wet gel (by weight 0.5% saline and 75% water), which was supplemented by
additional gel of the same kind applied to the center of the electrode. Gel of such high water
content is likely to hydrate the skin under it. (2) Bodily activity was sampled at 1 Hz from a
UFI 1110 Jitterbug Actigraph accelerometer attached to the participant’s ankle on the non-dominant side. This device responds to motion in all directional axes. The absolute deviation of its voltage oscillations is a measure of leg activity in arbitrary units, which is representative of general body movement. (3) Ambient temperature was sampled with an accuracy of 0.1 degrees Celsius at 0.1 Hz from a sensor in the handbag or waist pack where it was exposed to ambient air but insulated from body heat and direct sun exposure. The recorder had an event-marker button, with which participants could indicate time of getting into bed and turning out the lights, and of getting up in the morning. The nature of the event was written on a paper log along with the time the button was pressed.

4.2.4 Data Reduction

Physiological recordings were analyzed offline with customized software written by the second author in Matlab® (MathWorks, Natick, MA, USA). First, following a standardized set of rules, data were examined visually for artifacts which were excluded from further analysis. Manual editing excluded time periods when electrodes were detached or that contained spikes in skin conductance (SC) greater than 0.5 μS, which represented movement artifacts or loose electrodes. Automatic editing excluded SC values below 0.5 μS. After editing, the SC data were filtered using a zero-phase order 10 low-frequency Butterworth filter with a cutoff frequency of 0.05 Hz. The data were then segmented into waking and sleeping by locating two times: getting into bed and turning out the lights, and getting up, both based on self-report, event markers, and an examination of the activity channel for cessation and resumption of activity.

The edited data were then analyzed in 1-min epochs during each of three periods (waking on day 1, sleeping, waking on day 2) resulting in, for example, 360 epochs (i.e., 6 h) for one person’s sleep recording. Skin conductance (SC) for each 1-min epoch was measured as: (1) SC level (SCL) from the mean of filtered SC; (2) SCL standard deviation (SCLstd) from the standard deviation of filtered SC; (3) Coefficient of SC variation from SCLstd divided by SCL; (4) Number of non-specific skin conductance fluctuations (NSFs). NSFs were detected as rises greater than 0.01 μS between consecutive samples of the first derivative of filtered SC. The detection program skipped forward 5 s before looking for the next rise; (5) Amplitude of these NSFs, calculated as the difference between the minimum SCL within 3 s before a rise
and the maximum SCL within 3 s after a rise. Physical activity and ambient temperature were averaged over each 1-min epoch.

Psychological measures included retrospective ratings of recent emotional states (the adjectives “excited” and “sleepy”) taken at three prescribed times during the 24-hour measurement period. The ratings at 4 pm and at 8 pm on the first day were used as an approximation for the emotional state on day 1, whereas the ratings of how subjects felt since waking up the morning of day 2 (taken approximately at 8 am) were used to estimate mood on the second day. To measure sleep quality, an ad hoc composite score was calculated as sleep duration - the number of awakenings during sleep + 1 if the individual felt rested after sleep. This score was scaled by multiplication by 1/3. Thus, a higher score indicates a better sleep quality.

4.2.5 Statistical analysis

**Within-subject** analyses were based on individual 1-min epochs as described above (continuous data), while for **between-subject** analyses these epochs were averaged for each of the three time periods (waking on day 1, sleeping, waking on day 2), resulting, for example, in one SCL value for sleeping.

4.2.5.1 Between-subject analyses

Psychophysiological data were analyzed using linear mixed models as suggested by Bagiella et al. (2000), following a top-down strategy (see West, et al., 2007). Marginal models (without any random effects specified) were fitted by maximum likelihood with an unstructured covariance matrix for the residuals using SPSS 17.0 (SPSS Inc., Chicago, IL).

(1) The extent of electrode site deterioration was determined in two ways: First, SCL was compared from the old and the freshly applied electrodes (electrode effect: old vs. fresh), while controlling for **between-subject** effects of the categorical variables sex (male, female) and race (Asian, Caucasian, Other), and for the covariates age, body mass index, mean temperature (averaged over the whole 24-hour period), mean activity (averaged over the whole 24-hour period), and length of recording using two-sided t-tests (contrasts). Second, a 15 min period at the beginning of the 24-hour monitoring was averaged and compared to an averaged 15 min period at around the same time 24 h later. Ambulatory SC variables from these two 15-min time periods (time effect: beginning of recording, 24 h
later) were compared while controlling for *between-subject* effects of the categorical variables sex (male, female) and race (Asian, Caucasian, other), and the covariates age, body mass index, mean temperature during each 15 min period, and mean activity during each 15 min period using two-sided t-tests (contrasts). Data with a time difference of exactly 24 h between those two time periods were available from 29 subjects and data with at least a 23-hour difference from another 4 subjects. Comparable time periods from the remaining 15 subjects were not available because of equipment failure shortly before the monitoring ended (1 subject), detachment of the activity sensor rendering the data invalid (1 subject), and stopping the recording for scheduling convenience earlier than the full 24 h (13 subjects). For both methods, a percentage change index was calculated as \[
\frac{(\text{old electrode} - \text{fresh electrode})}{\text{fresh electrode}} \times 100.
\]

(2) All ambulatory SC variables averaged over time of day periods were tested for the effects of time of day (Waking on day 1, Sleeping, Waking on day 2), sex (male, female), race (Asian, Caucasian, Other), and the covariates age, body mass index, temperature (averaged over time of day period), and activity (averaged over time of day period). Effect size for each variable was calculated as Cohen’s d using t values and degrees of freedom \[d=2t/\sqrt{v(df)}\]. Variables with p-values greater than 0.1 were omitted from the models unless the model fit changed significantly.

(3) Daytime residuals (day 1 and day 2, without sleeping) of the models described in (2) were subsequently entered as dependent variables into new models with several subjective self-report variables as covariates (e.g., excitement on day 1 and day 2, and sleep quality) to test their effect on the electrodermal variables after time of day and other variables had been accounted for.

(4) To determine the relationships among the various SC measures, *between-subject* Spearman correlation coefficients (on averaged data) were calculated for all ambulatory SC variables for each time period separately (day 1, sleeping, day 2). To test whether they are differentially related to each other, these correlation coefficients were then converted into Pearson correlation coefficients based on formula (4) in Rupinski and Dunlap (1996), so they could be statistically compared while taking their dependence into account. The method employed here is based on Cohen and Cohen (1983), p. 57, whose formula yields a t-statistic with \(n - 3\) degrees of freedom that tests for a significant difference in the correlation...
between variables X and Y and V and Y, e.g., SCL and SCLstd and number of NSFs and SCLstd. We did not adjust these analyses for Type I errors from multiple testing, so these results need to be interpreted with caution.

4.2.5.2 Within-subject analyses

(5) The within-subject effects of continuously recorded physical activity and ambient temperature on the electrodermal indices (1-min epochs) were calculated by correlating the measures with each other for each subject, t-testing the Fisher’s z-transformed correlation coefficients of the whole sample, and calculating Cohen’s d effect sizes as described above.

(6) To determine the relationships among the various SC measures, within-subject correlations as described above (see section 5) were calculated for all ambulatory SC variables for each time period separately (day 1, sleeping, day 2).

4.3 Results

(a) Electrode site deterioration. Comparing manual SCL readings from the old and freshly applied electrodes at the end of the 24-hour monitoring (first method), electrode sensitivity declined significantly by an average of 20% (n=46) over 24 h (F(1,46)=9.0, p<0.01) with the old electrodes registering a mean SCL of 9.4 μS just before they were replaced, compared to 11.8 μS for the freshly applied electrodes. The difference between old and fresh could not be accounted for by age, sex, BMI, race, total 24-hour ambient temperature, total 24-hour physical activity, or the length of recording. The second method compared 15-min SC measurements from the same electrodes taken 24 h apart. Electrode sensitivities did not change significantly (average change = 0%, n=33) with the electrodes registering a mean SCL of 6.6 μS both during first 15-min period (freshly applied) and 24 h later (old). The differences in SCLstd, the coefficient of SC variation, amplitude or number of NSFs were not significant, indicating no significant change in electrode sensitivity.

(b) Time of day showed consistently lower electrodermal activity in all measures during sleep than during waking. SCL declined significantly from day 1 to day 2 whereas the coefficient of SC variation (SCLstd/SCL) showed the opposite effect (see Figure 3.).
(c) **Ambient temperature.** As shown in Table 2, ambient temperature significantly increased the number of NSFs but did not affect other electrodermal indices between-subjects. Within-subjects, however, significant positive relationships for all electrodermal indices were observed on both days, with effect sizes ranging from 1.0 for SCL to 1.5 for SCLstd on the first day and 0.7 for SCL and 0.8 for all others on the second day (see Table 3). The only exception was the coefficient of SC variation, which was not significantly related to temperature on the second day (d=0.5). During sleeping, on the other hand, none of the electrodermal measures showed significant within-subject relationships with ambient temperature (d’s ranging from 0.0 for NSF amplitude to 0.5 for the number of NSFs).
Table 2. Effect sizes and significance levels of between-subject relationships of confounding variables and electrodermal activity measures

<table>
<thead>
<tr>
<th>Confounding variables</th>
<th>SCL</th>
<th>SCLstd</th>
<th>SCLstd/SCL (coefficient of SC variation)</th>
<th>NSF (number)</th>
<th>NSF (amplitude)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Continuous</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>(0.4)</td>
<td>(0.2)</td>
<td>(0.0)</td>
<td>positive</td>
<td>(0.6*)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity</td>
<td>(0.0)</td>
<td>(0.0)</td>
<td>(0.0)</td>
<td>positive</td>
<td>(0.3*)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>negative</td>
<td>negative</td>
<td>(0.1)</td>
<td>negative</td>
<td>-(0.3)</td>
</tr>
<tr>
<td></td>
<td>(-0.6*)</td>
<td>(-0.5*)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index</td>
<td>(-0.4)</td>
<td>(-0.4)</td>
<td>(0.0)</td>
<td>negative</td>
<td>-(0.6*)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Categorical</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>W1&gt;W2&gt;S</td>
<td>W1=W2&gt;S</td>
<td>W2&gt;W1&gt;S</td>
<td>W1=W2&gt;S</td>
<td>W1=W2&gt;S</td>
</tr>
<tr>
<td></td>
<td>(W1&gt;W2: 0.8**)</td>
<td>(W1=W2: 0.1)</td>
<td>(W2&gt;W1: 0.7*)</td>
<td>(W1=W2: 0.8**)</td>
<td>(W1=W2: 0.3)</td>
</tr>
<tr>
<td></td>
<td>(W1&gt;S: 1.7***</td>
<td>(W1=S: 1.8**</td>
<td>(W1=W2: 1.4***</td>
<td>(W1=W2: 1.4***</td>
<td>(W1=S: 1.1***</td>
</tr>
<tr>
<td></td>
<td>(W2&gt;S: 1.4***</td>
<td>(W2=S: 1.4**</td>
<td></td>
<td></td>
<td>(W2&gt;S: 0.8**)</td>
</tr>
<tr>
<td>Sex</td>
<td>(0.2)</td>
<td>(-0.3)</td>
<td>Men &lt; Women</td>
<td>(-0.4)</td>
<td>Men &lt; Women</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td>Asian&lt; Caucasian</td>
<td>(0.4)</td>
<td>Asian&lt;Caucasian</td>
<td>(0.8**)</td>
<td>Asian&lt;Caucasian</td>
</tr>
<tr>
<td></td>
<td>(-0.6*)</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Note. Values given in brackets represent Cohen’s d effect sizes (based on t statistics and degrees of freedom from contrast comparisons); * = p-value <0.05, ** = p-value<0.01, *** = p-value <0.005;
Abbreviations. SCL, skin conductance level; SCLstd, SCL standard deviation; NSF, non-specific fluctuations; W1, Waking on day 1; W2, Waking on day 2; S, Sleeping.

Table 3. Within-subject correlations between physical activity, ambient temperature, and electrodermal measures

<table>
<thead>
<tr>
<th>Confounding variables</th>
<th>SCL</th>
<th>SCLstd</th>
<th>SCLstd/SCL (coefficient of SC variation)</th>
<th>NSF (number)</th>
<th>NSF (amplitude)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ambient temperature</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W1</td>
<td>1.0**</td>
<td>1.5***</td>
<td>1.3***</td>
<td>1.3***</td>
<td>1.2***</td>
</tr>
<tr>
<td>S</td>
<td>0.3</td>
<td>0.5</td>
<td>0.2</td>
<td>0.5</td>
<td>0.0</td>
</tr>
<tr>
<td>W2</td>
<td>0.7*</td>
<td>0.8**</td>
<td>0.4</td>
<td>0.8**</td>
<td>0.8**</td>
</tr>
<tr>
<td><strong>Physical activity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W1</td>
<td>2.1***</td>
<td>1.9***</td>
<td>1.6***</td>
<td>2.4***</td>
<td>1.0**</td>
</tr>
<tr>
<td>S</td>
<td>0.7*</td>
<td>1.7***</td>
<td>2.6***</td>
<td>1.5***</td>
<td>0.7*</td>
</tr>
<tr>
<td>W2</td>
<td>2.2***</td>
<td>1.9***</td>
<td>2.4***</td>
<td>2.4***</td>
<td>-0.3</td>
</tr>
</tbody>
</table>

Note. Values represent Cohen’s d effect sizes (based on t statistics and degrees of freedom from testing Fisher’s z-transformed correlation coefficients); * = p-value<0.05, ** = p-value<0.01, *** = p-value <0.005;
Abbreviations. SCL, skin conductance level; SCLstd, SCL standard deviation; NSF, non-specific fluctuations; W1, Waking on day 1; W2, Waking on day 2; S, Sleeping.

(d) Physical activity also increased the number of NSFs between-subjects, although its effect was smaller than for ambient temperature and did not reach significance. As shown in Table 3, within-subject physical activity was positively related to all electrodermal indices (effect sizes between 1.6 and 2.4 on both days) except for the amplitude of NSFs, which actually decreased with higher levels of activity (effect size -1.0 on day 1 and -0.3 on day 2).
During sleeping, however, only positive correlations were observed, which were large for SCL standard deviation (d=1.7), the coefficient of SC variation (d=2.6), and the number of NSFs (d=1.5), and somewhat smaller for SCL and the NSF amplitudes (0.7).

\((e)\) With the exception of the coefficient of SC variation (SCLstd/SCL), age was consistently negatively related to all electrodermal indices. This relationship was significant for the number of NSFs, whereas SCL and SCL standard deviation only tended to decrease with age. \((f)\) Sex significantly affected NSF amplitudes and the coefficient of SC variation such that men had lower values than women.

Asians and Caucasians were the only two racial groups in our sample large enough for comparison. Asians had lower SCL and fewer NSFs than Caucasians, but higher coefficients of SC variation. No other differences were observed. Body mass index (BMI) significantly lowered the number of NSFs and was negatively but not significantly related to SCL and SCL standard deviation with small to medium effect sizes.

**Intercorrelations of electrodermal measures.** During both the first and the second waking period, all electrodermal measures were highly intercorrelated between subjects with correlation coefficients ranging from 0.6 to 0.9, and NSF amplitudes accounting for the lower values (see Table 4). The only exceptions were the correlations between SCL and the coefficient of SC variation which were lower than 0.6 and became non-significant on the second day. Statistically testing the relative strength of these correlations using t-tests confirmed that during waking, SCL was more closely related to SCLstd and the number of NSFs than to their amplitude. It was least related to the coefficient of SC variation, as were SCLstd and the number of NSFs. The most closely related to the coefficient was NSF amplitude. During both waking periods and sleeping, the number of NSFs was least related to the amplitude of NSFs. During sleeping, correlations between SCL, SCL standard deviation and the number of NSFs still ranged from 0.8 to 0.9, while all correlations with NSF amplitude and between the coefficient of SC variation and SCL standard deviation became non-significant. The coefficient of SC variation correlated negatively with SCL and the number of NSFs. Statistical comparisons of these correlations showed that NSF amplitude was unrelated to the other measures. SCL, SCLstd, and the number of NSFs showed equally strong correlations with each other. The coefficient of variation was more closely related to
SCL than to the other measures. *Within subjects*, all Fisher’s z-transformed inter-correlations were significantly positive at all times.

**Table 4. Intercorrelations of electrodermal measures**

<table>
<thead>
<tr>
<th>Electrodermal measures</th>
<th>SCL</th>
<th>SCLstd</th>
<th>NSF (number)</th>
<th>NSF (amplitude)</th>
<th>SCLstd/SCL (coefficient of SC variation)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SCL</strong></td>
<td>Between-S</td>
<td>W1: 0.9**</td>
<td>W1: 0.9**</td>
<td>W1: 0.7**</td>
<td>W1: 0.3*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S: 0.8**</td>
<td>S: 0.8**</td>
<td>S: 0.1</td>
<td>W2: 0.2</td>
</tr>
<tr>
<td></td>
<td>Within-S</td>
<td>W2: 0.9**</td>
<td>W2: 0.8**</td>
<td>W2: 0.6**</td>
<td>W2: 0.2</td>
</tr>
<tr>
<td><strong>SCLstd</strong></td>
<td>Between-S</td>
<td>W1: 0.9**</td>
<td>W1: 0.9**</td>
<td>W1: 0.7**</td>
<td>W1: 0.7**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S: 0.9**</td>
<td>S: 0.9**</td>
<td>S: 0.2</td>
<td>W2: 0.8**</td>
</tr>
<tr>
<td></td>
<td>Within-S</td>
<td>W2: 0.9**</td>
<td>W2: 0.8**</td>
<td>W2: 0.6**</td>
<td>W2: 0.6**</td>
</tr>
<tr>
<td><strong>NSF (number)</strong></td>
<td>Between-S</td>
<td>W1: 8.1***</td>
<td>W1: 8.7***</td>
<td>W1: 0.7**</td>
<td>W1: 0.6**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S: 4.0***</td>
<td>S: 4.7***</td>
<td>S: 0.2</td>
<td>S: -0.3*</td>
</tr>
<tr>
<td></td>
<td>Within-S</td>
<td>W2: 8.0***</td>
<td>W2: 8.9***</td>
<td>W2: 0.6**</td>
<td>W2: 0.5**</td>
</tr>
<tr>
<td><strong>NSF (amplitude)</strong></td>
<td>Between-S</td>
<td>W1: 12.6***</td>
<td>W1: 1.9***</td>
<td>W1: 0.8**</td>
<td>W1: 0.8**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S: 2.3***</td>
<td>S: 4.2***</td>
<td>S: 1.3***</td>
<td>S: -0.0</td>
</tr>
<tr>
<td></td>
<td>Within-S</td>
<td>W2: 2.6***</td>
<td>W2: 8.0***</td>
<td>W2: 1.7***</td>
<td>W2: 1.7***</td>
</tr>
<tr>
<td><strong>SCLstd/SCL (coefficient of SC variation)</strong></td>
<td>Between-S</td>
<td>W1: 4.9***</td>
<td>W1: 9.3***</td>
<td>W1: 12.8***</td>
<td>W1: 12.8***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S: 0.8**</td>
<td>S: 6.0***</td>
<td>S: 3.2***</td>
<td>S: 4.0***</td>
</tr>
<tr>
<td></td>
<td>Within-S</td>
<td>W2: 5.1***</td>
<td>W2: 9.2***</td>
<td>W2: 7.5***</td>
<td>W2: 6.7***</td>
</tr>
</tbody>
</table>

Note. Between-subjects correlations represent Spearman’s rho correlation coefficients; within-subject values represent Cohen’s d effect sizes (based on t statistics and degrees of freedom from testing Fisher’s z-transformed correlation coefficients); * = p-value<0.05, ** = p-value<0.01, *** = p-value<0.005;

Abbreviations. SCL, skin conductance level; SCLstd, SCL standard deviation; NSF, non-specific fluctuations; Within-S, within-subject correlations; Between-S, between-subjects correlations; W1, Waking on day 1; W2, Waking on day 2; S, Sleeping.

**Electrodermal residuals and self-report.** Subjects reported normal values on trait measures of depression (BDI: 1.9 ± 3.5, n=48) and anxiety (BAI: 2.5 ± 3.0, n=33), and low levels of excitement (“feeling excited”: 0.9 ± 1.0, n=47) and daytime sleepiness (“feeling sleepy”: 2.6 ± 1.6, n=47) on the days of testing. In this group of healthy subjects, there were no significant relationships between the self-report measures above and any of the electrodermal daytime residuals. Information on subjective sleep characteristics is limited due to missing data. On the nights of testing, subjects reported waking up 1.8 ± 1.8 times (n=25), sleeping for 7.6 ± 1.0 h (n=26), and feeling rested in 72% of cases (n=25). Their calculated score on the composite sleep quality measure was 2.1 ± 0.7 (n=23), and their habitual sleep quality was normal (PSQI: 3.8 ± 1.8, n=23). Measures of subjective sleep quality (PSQI total score, calculated measure of sleep quality on night of testing) did not co-vary with any of the residuals at night.
4.4 Discussion

The results for electrode site deterioration were contradictory. The first method that involved comparing single SCL values from old and freshly applied electrodes at the end of the monitoring resulted in a significant SCL decrease over 24 h and suggests electrode site deterioration. The second method, however, which compared 15-min periods at the beginning of the recording with 15-min periods about 24 h later from the same pair of electrodes, could not detect significant differences in any of the SC measures. The first method raises the question whether individual differences in emotional activation induced by the experimenter switching from the old to the new electrodes might have caused higher SCL values from the freshly applied electrodes. That SCL on the second day was lower than on the first day appears most likely to be a circadian time of day effect. Electrodermal activity has been reported to be lowest in the morning (Miró, et al., 2002; Venables & Mitchell, 1996), which in our study was on the second day. Measures of electrodermal variability, however, were not lower on the second day. Coefficient of skin conductance (SC) variation was actually higher, probably because the drop in SCL on the second day increased the ratio of standard deviation to level. This is in contrast to Turpin et al.’s findings (1983) of both fewer and smaller SC responses in hydrating electrodes, but again, they only measured SC for up to 6 h. Considering the methodological problems of the first method, we think that the results of the second are more accurate, and that electrode site deterioration has only a minimal effect on 24-hour SC measurements.

As predicted, both tonic and phasic electrodermal activity were much higher during waking than sleeping. This fits with regarding sleep as a low point on a SC arousal continuum (Koumans, et al., 1968). We did not find evidence of greater night-time compared to waking rates of non-specific electrodermal fluctuations that had been previously reported (e.g., Broughton, et al., 1965; Johnson & Lubin, 1966). Such “storms” (Lester, et al., 1967) were usually seen with skin potential rather than skin conductance.

Ambient temperature had little impact on any of the between-subject measures except number of NSFs. This finding is similar to that of Turpin et al. (1983) who found no temperature effects on SCL but increased frequencies of NSFs with rising temperature. In the laboratory, increased palmar SCLs at hotter air temperatures have been observed (Scholander, 1963) which could be expected even though thermoregulatory sweating on the
palms and soles is less prominent than on other skin areas. We observed, however, strong within-subject effects in all SC measures during the first day (and all but the coefficient of SC variation on the second day). Turpin et al. (1983) did not find the positive within-subject effects of temperature that we observed, possibly because their data pool was limited to mean values from 12 hourly intervals of a few minutes each. Non-significant temperature relationships at night might have been confounded by inconsistency in the location of the sensor. In bed, subjects removed the waist pack with the sensor in it, some putting the pack under the covers and some outside the covers. Thus, the effects of temperature on SC data cannot be generalized to entire 24-hour periods, but at the least waking and sleeping periods need to be considered separately. Averaging over longer time periods might obscure or remove the effects of possible relationships between ambient temperature and SC data.

Previous research had indicated that physical activity does not affect electrodermal activity (Roberts & Young, 1971; Turpin, et al., 1983). In the current study, none of the electrodermal measures were significantly affected between subjects by the level of physical activity, although activity tended to increase the number of NSFs. Within subjects, however, physical activity increased electrodermal activity both during day and night, with the exception of NSF amplitude, which was negatively related to physical activity during the day. Activity may raise SCL and increase fluctuations because of associated emotional activation, or in the case of sustained or strenuous activity, because of increases in body temperature. Like for ambient temperature, shorter data segments might be necessary to show relationships between activity and SC most clearly.

Age was negatively related to the number of NSFs and also its relationships with SCL and SCL standard deviation were in the expected direction (e.g., Eisdorfer, et al., 1980; Gavazzeni, et al., 2008) and of medium size. Coefficient of SC variation has less dependence on mean levels, and it did not show an age effect, recommending this measure for populations of diverse ages.

As predicted, men showed smaller NSF amplitudes and overall smaller coefficients of variation than women but their SCL was only slightly higher and did not reach significance. This is contrary to previous reports of significantly higher SCL in men (Eisdorfer, et al., 1980; M. Kelly, et al., 2006; Kronholm, et al., 1993), but consistent with Furedy et al. (1999). Significant findings were obtained in large samples (N>95) in which quite small differences
became significant. In the current study, the other SC variability indices, SCL standard deviation and the number of NSFs, also showed small to medium albeit non-significant effect sizes in the direction of SC variability being higher in women.

*Asians* exhibited lower SCL and a trend to fewer NSFs than *Caucasians*. To the authors’ knowledge, this is the first time these effects have been looked at ambulatorily in these two racial groups. However, the coefficient of SC variation was higher in Asians compared to Caucasians indicating that once the effect of mean levels is reduced, Asians have higher SC variability. This variability is different from the kind indexed by the number of fluctuations.

*Body mass index (BMI)* decreased the number of NSFs without having a significant influence on other measures. These findings confirm the results from Kronholm et al.’s (1993) sample of 199 subjects, which did not show a relationship between BMI and a sympathetic index including SCL and SCR amplitudes (but not the number of NSFs). Effect sizes for SCL and SCL standard deviation in our study were of medium size and in the direction of higher BMI being associated with less electrodermal activity, and might have been significant in a sample as large as Kronholm et al.’s (1993). Peterson et al. (1988) also found a decrease in other sympathetic measures with increasing BMI. These findings indicate a negative relationship between BMI and some indicators of electrodermal activity. Coefficient of SC variation and NSF amplitudes on the other hand, do not seem to be influenced by BMI.

Overall, our results show that the various electrodermal indices we measured are not equally affected by the confounding variables. Two clusters of measures emerge. One comprises SCL, SC standard deviation, and the number of NSFs, which showed similar susceptibility to confounding variables and were correlated more closely with each other than with two other measures. The other cluster was NSF amplitude and coefficient of SC variation, which were less correlated with other measures. The coefficient of SC variation was the only measure that was greater in Asians than in Caucasians. These differences imply that not all electrodermal measures index a unitary activation dimension. A recent functional magnetic resonance imaging study found that different brain structures regulate tonic and phasic SC activation (Nagai, Critchley, Featherstone, Trimble, & Dolan, 2004). SCL, a tonic measure, had a closer relationship with brain regions associated with anticipatory...
anxiety (for a discussion, see Parente, et al., 2005) than did SC variability. A recent 24-hour monitoring study in panic disorder patients found SCL to be chronically elevated in these anxious patients compared to normal controls (Doberenz, et al., 2010) while measures of phasic SC activation were not.

Note that our between-subject results are based on averaging data over several h, and thus are relatively insensitive to common variance that may be present in shorter epochs. Only with shorter epochs did relationships with ambient temperature and physical activity emerge. Similarly, longer analysis epochs may have obscured more transient relationships between mood and physiology. After the effects of the confounding variables were removed, none of the residuals of the averaged between-subject electrodermal measures showed significant relationships with self-reports of daytime arousal or subjective sleep quality. Relatively small variations and floor effects in emotional activation in our sample of healthy volunteers may also have contributed to a lack of relationships.

Our study is limited in several ways. First, the relatively small sample size did not allow us to test for interactions between the different confounding factors. We tested quite a few measures in a relatively small subject sample. Second, we could have said more about the relationship of our measures to emotional activation if we had measured additional physiological channels such as heart rate and heart rate variability. Technological advances make multichannel recording less and less cumbersome and intrusive. Third, electrode deterioration using the first method could have been measured more accurately if fresh electrodes had been applied near the old electrodes and measurements made simultaneously for the two sets rather than sequentially, avoiding the possibility that emotional activation changed between measurements. We did not use a standardized challenge to assess skin conductance reactivity after 24-h as other studies have done, which would have allowed us to compare the differences between old and fresh electrodes in electrodermal reactivity for the other SC measures as well.

We hope that our results will encourage greater use of ambulatory electrodermal monitoring in the study of emotional activation during waking activities and sleep. Corrections for the effects of confounding variables are quite feasible, for example, by including them as covariates in statistical analyses. While this might not be necessary for ambient temperature and physical activity at the between-subject level, it is advisable at the
within-subject level. In clinical studies using electrodermal measures, additional factors such as the effects of psychotherapeutic medication must also be taken into account as we discovered in our 24-hour panic disorder study (Doberenz, et al., 2010). Even relatively healthy populations in developed countries are frequent users of over-the-counter and prescription medications with autonomic or central nervous system effects.
5 Twenty-four hour Skin Conductance in Panic Disorder (study 2)

5.1 Introduction

Anxiety in panic disorder (PD) is not confined to panic attacks but is present at a lower intensity before and after attacks. This kind of anxiety in PD is often called anticipatory because theoretically it results from exteroceptive and interoceptive conditioning to threat cues associated with panic attacks (Bouton, Mineka, & Barlow, 2001). With interoceptive conditioning, bodily sensations experienced during panic attacks become conditioned stimuli, eliciting anxiety that later may eventuate in panic. External cues become conditioned stimuli for anxious avoidance of places and situations. Catastrophic interpretations of bodily sensations may be a cognitive aspect of this conditioning (D. M. Clark, 1986).

It is unclear, however, whether anticipatory anxiety would also lead to sustained physiological activation in PD patients. One way to elicit physiological expressions of anticipatory anxiety is to threaten healthy humans with electric shock – a stimulus that is as aversive as a panic attack to a PD patient. Increase in skin conductance level (SCL) and in the number of non-specific fluctuations are observed in these subjects as part of a pattern of an emotional activation (e.g., Gaebelein, Taylor, & Borden, 1974; Lanzetta, Cartwright-Smith, & Kleck, 1976; Monat, Averill, & Lazarus, 1972). The activation is expressed in multiple physiological systems including the brain, where blood flow increases in the left insula, right temporal sulcus, left fusiform gyrus, and left anterior cingulate cortex (Chua, Krams, Toni, Passingham, & Dolan, 1999). Electrodermal measures may express this activation better than cardiovascular ones. Fowles (2000) has argued that increased electrodermal activity specifically reflects anxiety anticipating future threat such as would be the next panic attack, while heart rate reflects fear of current threat (for a discussion, see Parente, et al., 2005). Indeed, skin conductance measures have been elevated in PD patients when cardiovascular ones were normal (Braune, et al., 1994; Hoehn, et al., 1997; Parente, et al., 2005).

Consistent with these theoretical expectations, many studies have reported greater skin conductance activation in PD patients than in comparison groups under diverse conditions, ranging from resting baselines to watching a panic attack video (see Table 5). The findings, however, are far from consistent. In the one ambulatory study where SC was
recorded during daily activities, SCL was not higher in PD patients than in non-anxious controls, either during non-anxious periods or periods of stress and panic (Hoehn-Saric, et al., 2004). Instead these authors found lower skin conductance variability (mean squared successive differences in SC, MSSD) values in PD patients, which led them to assert that PD is characterized by "diminished autonomic flexibility." Reported laboratory studies (see Table 5) vary methodologically in several ways that might contribute to their inconsistency: (a) PD was diagnosed using different versions of the DSM. (b) PD severity varied. (c) Agoraphobic fears were not always assessed. Such fears are especially likely to affect the results of testing in small rooms or chambers, if the subject is alone, and if tethered with electrode leads or tubes to stationary equipment (Roth et al., 1986). (d) Variations in depression could have been important since depression can lower electrodermal activity (Argyle, 1991; Ward, et al., 1983). (e) Psychoactive medication or any medication with autonomic effects could have influenced outcomes. For example, it is noteworthy that the two studies that allowed the use of benzodiazepines did not show significant differences in SCL or the number of NSFs (Argyle, 1991; Birket-Smith, Hasle, & Jensen, 1993). The ambulatory study of Hoehn-Saric and colleagues (2004) prohibited medications affecting the central or autonomic nervous system, but did not assess possible effects of agoraphobia or depression on their electrodermal measures.
Table 5. Laboratory studies measuring electrodermal activity in panic disorder patients.

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample</th>
<th>Medications allowed</th>
<th>Setting</th>
<th>Procedure</th>
<th>SCL (except as noted)</th>
<th>Results</th>
<th>Other measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lader &amp; Wing</td>
<td>20 PD (pre-DSM-III, 3 with AG sx), 20 HC</td>
<td>?</td>
<td>alone in dimly lit sound-attenuated room</td>
<td>(a) resting baseline (11 min)</td>
<td>log SCL (a) At min 11: PD &gt; HC (b) At min 8 to 21: PD &gt; HC</td>
<td>(a,b) PD &gt; HC</td>
<td>(b) 1st log SCR: PD = HC Subsequent log SCRs: PD &lt; HC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(b) auditory startle (20 min) + 1 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lader &amp; Mathews</td>
<td>3 PD (pre-DSM-III, AG sx not established)</td>
<td>?</td>
<td>?</td>
<td>recording during spontaneous panic attacks</td>
<td>marked and rapid increase, slow decline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1970)</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Freedman et al.</td>
<td>8 PD (DSM-III, AG dx not established), 9 HC</td>
<td>?</td>
<td>connected to IV line</td>
<td>3 sessions (5-10 days apart) of panic attack provocation:</td>
<td>(a,b,c,d) PD = HC; (e) PD &gt; HC; sessions with panic attacks = sessions without</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1984)</td>
<td></td>
<td></td>
<td></td>
<td>(a) resting baseline (10 min)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(b) IV dextrose (10 min)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(c) IV infusion of sodium lactate/isoproterenol/placebo (randomized) until panic attack occurred or 20 min</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(d) IV dextrose (10 min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(e) resting baseline (10 min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roth et al.</td>
<td>37 AG with panic attacks (DSM-III), 19 HC</td>
<td>no: TCAs or MAOIs</td>
<td>sound-attenuated room, 24 AG alone, 13 AG with companion</td>
<td>(a) resting baseline (15 min)</td>
<td>log SCL (a,d) AG &gt; HC, habituated (b) AG &gt; HC, habituated faster in HC (c) AG &gt; HC, habituated faster in HC</td>
<td>(a,d) AG &gt; HC</td>
<td>SCR: (b) total number: AG &gt; HC (c) magnitude, recovery: AG = HC</td>
</tr>
<tr>
<td>(1986)</td>
<td></td>
<td></td>
<td></td>
<td>(b) habituation (10 min, to 18 identical tones, 1 different tone)</td>
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<td></td>
<td>(c) startle white noise bursts of different intensities (10 min)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>(d) resting baseline with 20 white noise bursts during min 3 (5 min)</td>
<td></td>
<td></td>
<td></td>
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</table>

60
<table>
<thead>
<tr>
<th>Study</th>
<th>Sample</th>
<th>Medications allowed</th>
<th>Setting</th>
<th>Procedure</th>
<th>SCL (except as noted)</th>
<th>Results NSFs</th>
<th>Other measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>McNally et al. (1989)</td>
<td>24 PD</td>
<td>?</td>
<td>?</td>
<td>(a) resting baseline (10 min)</td>
<td>(a,b,d) PD = HC</td>
<td>(c) PD (exercise), HC (quiet sitting) &gt; PD (quiet sitting), HC (exercise)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(DSM-III-R, 18 with AG), 24 HC</td>
<td></td>
<td></td>
<td>(b) word rating task</td>
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<td></td>
<td>(c) exercise or quiet sitting</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>(d) recall task (3 min)</td>
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</tr>
<tr>
<td>Roth et al. (1990)</td>
<td>38 PD</td>
<td>no: benzos, TCAs</td>
<td>alone in sound-attenuated room</td>
<td>(a) several tasks including hyperventilation</td>
<td>(a) not reported</td>
<td>(b) PD &gt; HC</td>
<td>(b) time to habituation: PD &gt; HC</td>
</tr>
<tr>
<td></td>
<td>or AG</td>
<td></td>
<td></td>
<td>(b) habituation of orienting SCR to auditory tones (10 min)</td>
<td>(b) PD &gt; HC (log SCL)</td>
<td></td>
<td>(b) slope of SCL decline: PD &lt; HC</td>
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</tr>
<tr>
<td>Argyle (1991)</td>
<td>66 PD</td>
<td>yes: benzos and stable TCAs (7 patients)</td>
<td>alone in big or small room</td>
<td>resting baselines (16 min each) in (a) big room</td>
<td>(a, b) min 15, 16: PD = HC</td>
<td>(a) log SCL min 15, 16: MD &lt; non-MD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(DSM-III, 37 with AG, 21 with MD), 10 HC</td>
<td></td>
<td></td>
<td>(b) small room (all HC + 10 PD) in randomized order</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hoehn-Saric et al. (1991)</td>
<td>18 PD</td>
<td>no to all</td>
<td>in dimly lit room, alone?</td>
<td>(a) resting baseline (10 min)</td>
<td>(a,b,c,d) PD = HC</td>
<td>range of SCL: (b,c) PD &lt; HC (a,d) PD = HC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(DSM-III-R, AG dx not established), 18 HC</td>
<td></td>
<td></td>
<td>(b) divided attention task (5 min)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(c) risk taking task (5 min)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>(d) resting baseline (5 min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birket-Smith et al. (1993)</td>
<td>12 PD</td>
<td>yes: benzos, TCAs, SSRIs, and antipsychotics</td>
<td>quiet room, alone?</td>
<td>(a) habituation to auditory stimuli</td>
<td>(b) SRL: PD = AG = HC, medicated = not medicated</td>
<td>(a) PD = AG = HC</td>
<td>(a) SRR habituation rate: PD &gt; HC</td>
</tr>
<tr>
<td></td>
<td>(DSM-III-R), 11 AG with panic attacks, 12 HC</td>
<td></td>
<td></td>
<td>(b) resting baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Braune et al. (1994)</td>
<td>27 PD</td>
<td>no to all</td>
<td>quiet, well-lit room, alone?</td>
<td>resting baseline (15 min)</td>
<td>PD &gt; HC</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(DSM-III-R, 12 with AG), 10 HC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Sample</td>
<td>Medications allowed</td>
<td>Setting</td>
<td>Procedure</td>
<td>SCL (except as noted)</td>
<td>Results NSFs</td>
<td>Other measures</td>
</tr>
<tr>
<td>-----------------------</td>
<td>--------</td>
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<td>---------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>Hoehn et al. (1997)</td>
<td>33 PD (DSM-III-R, 19 with AG), 10 HC</td>
<td>no to all</td>
<td>room?, alone?</td>
<td>(a) resting baseline (15 min), (b) 4x stress exposure reactivity: frightening video, mental arithmetic, panic attack video, improvised speech (3-4 min) (c) 4x recovery (3 min) (d) final resting baseline (17 min)</td>
<td>(a-d) PD &gt; HC</td>
<td></td>
<td>(b, panic attack video) SCR: PD &gt; HC</td>
</tr>
<tr>
<td>Roth et al. (1998)</td>
<td>14 PD (AG dx not established), 15 HC</td>
<td>no to all</td>
<td>alone in small sound-attenuated room</td>
<td>relaxing (10 min) (after talking for 4 min)</td>
<td>PD = HC</td>
<td></td>
<td>fractional SCL decline: PD &lt; HC goodness of fit over log time (inverse of variance): PD &lt; HC</td>
</tr>
<tr>
<td>Wilhelm et al., (2001)</td>
<td>16 PD (DSM-III-R, AG dx not established), 19 HC</td>
<td>no to all</td>
<td>alone in large, quiet temperature controlled room</td>
<td>quiet sitting (30 min)</td>
<td>PD = HC</td>
<td>PD = HC</td>
<td></td>
</tr>
<tr>
<td>Alpers et al. (2005)</td>
<td>21 driving phobics (11 with history of panic attacks, AG dx not established), 17 HC</td>
<td>no to all</td>
<td>quiet sitting (a, e) alone in small room</td>
<td>(a) quiet sitting (7 min) (b) approaching highway (10 min) (c) driving exposure (60 min) (d) return to lab (10 min) (e) quiet sitting (7 min)</td>
<td>(a-e) phobic &gt; HC</td>
<td>(a-e) phobic &gt; HC</td>
<td></td>
</tr>
<tr>
<td>Parente et al. (2005)</td>
<td>14 PD, 16 medicated sx-free PD, 16 HC</td>
<td>yes: SSRIs, clomipramine</td>
<td>?</td>
<td>simulated public speaking</td>
<td>PD &gt; medicated PD = HC</td>
<td>PD = HC</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Sample</td>
<td>Medications allowed</td>
<td>Setting</td>
<td>Procedure</td>
<td>Results</td>
<td>Other measures</td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
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<td>--------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
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<td></td>
</tr>
<tr>
<td>Blechert <em>et al.</em> (2007)</td>
<td>26 PD (AG dx not established, no MD), 32 HC</td>
<td>yes: SSRIs, SNRIs</td>
<td>alone in small temperature and sound-controlled room</td>
<td>(a) quiet sitting (5 min)</td>
<td>electrodermal sympathetic index= ESI (SCL, NSFs, SCR amplitude): (a,b) PD = HC</td>
<td>medicated = not medicated</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>no: benzos,</td>
<td></td>
<td>(b) threat of shock (5 min)</td>
<td>(b) ESI reactivity: PD = HC</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>beta-blockers,</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>sympatho-mimetic drugs,</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>antipsychotic,</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TCAs</td>
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</tbody>
</table>

**Abbreviations:** PD = Panic disorder patient; HC = healthy control; AG = Agoraphobia; MDE = Major Depressive Episode Disorder; dx = diagnosis; sxs = symptom; TCAs = tricyclic antidepressants; MAOIs = monoamine oxidase inhibitors; benzos = benzodiazepines; SSRIs = selective serotonin reuptake inhibitors; SNRIs = serotonin-norepinephrine reuptake inhibitors; IV = intravenous; SCL = skin conductance level; NSFs = number of non-specific fluctuations; SCR = skin conductance response; SRR = skin resistance response; ?= no information.
Thus, although theoretical considerations and experimental evidence give us reason to believe that PD patients would exhibit elevated SCL and a higher rate of NSFs during normal daytime activities, data supporting this hypothesis are inconsistent and come exclusively from laboratory environments where anxiety levels may be more a reaction to an artificial, restrictive setting than representative of a PD patient’s daily reality. The one ambulatory study failed to find elevated SCL or higher rates of NSFs in PD. In fact, a measure of fluctuations (MSSD) was lower. That study, however, was limited to daytime recording of 6 out of every 30 minutes for a total of 6 hours.

Besides abnormal daytime electrodermal activation, PD patients might also be expected to differ from healthy controls during sleep for at least two reasons. First, panic attacks in PD are not confined to the day but also occur at night. Epidemiological data on nocturnal panic attacks is limited, but it is estimated that between 44% and 71% of PD patients have experienced a nocturnal panic attack at least once (Craske & Tsao, 2005). Craske and colleagues (Craske & Tsao, 2005; Craske & Waters, 2005) propose that nocturnal panic attacks are conditioned responses to perceived changes in physiological state during sleep. Even though asleep, PD patients may process threat cues and react with elevations in anxious arousal that can escalate to panic. Second, PD is frequently comorbid with depression, which is strongly associated with sleep disturbances (for a summary of these disturbances, see Benca, 2005). PD patients have more sleep complaints than controls, even in the absence of either reported nocturnal panic attacks or comorbid depression (Overbeek, et al., 2005), although polysomnographically measured sleep parameters are usually normal (e.g., Arriaga et al., 1996; Landry, Marchand, Mainguy, Marchand, & Montplaisir, 2002; Stein, Enns, & Kryger, 1993b). In one study, measures of heart rate variability were lower in PD patients during sleep (McCraty, Atkinson, Tomasino, & Stuppy, 2001), which is consistent with less parasympathetic and more sympathetic activation, although heart rates did not differ. Heart rate and blood pressure are not elevated in PD during sleep (Abramson, Keshavan, & Sitaram, 1989; D. B. Clark et al., 1990) unless patients have comorbid major depression (Abramson, et al., 1989). As far as the authors know, skin conductance has never been reported for PD patients during sleep.

The study here is unique in measuring skin conductance, temperature, and activity ambulatorily for a 24 hour period in PD patients and matched non-anxious controls. We had
three *a priori* hypotheses: First, that during waking periods, skin conductance levels (SCL) and measures of non-specific skin conductance fluctuations (rate of non-specific fluctuations, 1-min SC standard deviation, and mean square successive difference) would be higher in the panic patients than in non-anxious controls. In spite of mixed empirical findings, it is compelling theoretically that PD patients would be more sympathetically activated by anxiety from cues that had previously caused attacks or from attacks themselves. Second, that during waking periods, PD patients would be less able to relax than controls when told to sit quietly and relax and that this would manifest itself in less negative SCL slopes, higher SCL and more non-specific skin conductance fluctuations (both rate of non-specific fluctuations and 1-min SC standard deviation) during our Ambulatory Relaxation Test (ART). Slower deactivation would be consistent with findings from studies reviewed above (Table 5) using a similar procedure in the laboratory. Third, that the differences postulated for waking would also be present in some form during sleeping periods since PD patients often complain of poor sleep. For the same reason, night-time physical activity should be higher in PD patients than controls. For hypotheses that were confirmed, we planned exploratory analyses of possible relationships between skin conductance and state anxiety, panic attacks, agoraphobic symptoms, state depression, and subjective sleep disturbance. Furthermore, we planned to explore an alternate index of skin conductance activation, namely run lengths of ascending or descending SC activation.

5.2 Methods

5.2.1 Participants

Participants from the local community were recruited by advertisement for studies of the effectiveness of breathing training for anxiety disorders. Twenty-two panic disorder (PD) patients (42.7 ± 15.0 yrs, 64% women) were matched with 29 healthy controls (42.1 ± 12.3 yrs, 66% women) on age, gender, race, and body mass index (BMI). Patients met DSM-IV criteria for PD with or without agoraphobia, with panic attacks of frequency and severity that were at least mild on the Panic Disorder Severity Scale (PDSS - Shear et al., 1997). They were willing to undergo a 5-session course of breathing training and to accept the possibility of an 8-week treatment delay if assigned to the waiting list group. The outcome of that study will be reported elsewhere. Healthy controls were selected not to have a current diagnosis or history of any anxiety disorder.
Exclusion criteria for all participants were a history of, or current, psychosis, cognitive impairment (e.g., a prior diagnosis of dementia, brain damage, or mental retardation or clear evidence of impaired memory or understanding during interactions with the interviewers), or substance abuse or dependence in the past year. They were allowed to continue on stable doses of medicines prescribed by physicians, but were excluded if they were taking drugs with substantial anticholinergic effects, such as tricyclic antidepressants, which have direct effects on skin conductance. They were also told not to take benzodiazepines prescribed “as needed” on the day of testing. However, no blood tests were performed on the day of testing to confirm adherence.

5.2.2 Procedure

This investigation was carried out in accordance with the latest version of the Declaration of Helsinki. The study design was reviewed and approved by the Stanford Institutional Review Board. Individuals who passed the phone screening were invited to an appointment where they gave written informed consent for further assessment after the nature of the procedures had been fully explained. The assessment began with a Structured Clinical Interview for DSM-IV-TR Axis I Disorders (First, et al., 2001). If no grounds for exclusion were found, they were asked to wash their hands with soap in preparation for the application of skin conductance electrodes (Dawson, et al., 1990; Venables & Christie, 1980). Next they underwent testing in a laboratory room where numerous autonomic and respiratory measures were recorded while they were trying to relax and to change their breathing according to several different instructions. The results of this testing have been reported elsewhere (Conrad, et al., 2007; Roth, et al., 2008; Wollburg, et al., 2008).

After the laboratory session, the ambulatory monitoring device was connected. Participants wore it continuously until they returned the next day. Recording usually started and ended at around 14:00 with the length of recording ranging between 18:40 and 25:50 with a mean of 23:40 (h:min). During that time, participants performed four identical ambulatory relaxation tests (ARTs). For each test they first walked at a normal pace for 3 min to establish comparable baseline activation levels in each participant during a standard, non-stressful activity. They then sat quietly without talking or moving for 8 min, during which they were instructed to relax. Test instructions and timing had been recorded on a portable cassette recorder, which subjects played at the prescribed times of each test: the first in the
hospital at a mean time of 14:25 hours, the second in the afternoon after leaving the hospital (mean 18:40), the third the next morning (mean 9:40) before returning to the hospital, and the fourth back at the hospital (mean 13:25). In the hospital, the quiet sitting took place in a spacious room without the experimenter present; outside the hospital subjects were to find a quiet, undisturbed place to sit. Subjects filled out a short questionnaire each time before and after they completed the 8 min relaxation part of the ART, and also at 16:00 and 20:00 the first day, as well as 8:00 the second day. This questionnaire assessed their current emotional state and their emotional state during the last min of relaxation (e.g., “anxious”, “worried”), respectively on a subjective units of distress scale from 0, “not at all”, to 10, ”extremely”. Of most interest for this report is the adjective “anxious”. Furthermore, participants were asked to report the number of panic attacks during the day, as well as during the night. On the morning of the second day of recording, subjects reported the number of awakenings during sleep, subjective sleep duration, and whether they felt rested after sleep.

In addition, participants were given a packet of questionnaires on the first day, which they returned completed the next day. These included the Beck Anxiety Inventory (Beck, et al., 1988) to be answered for the past week (internal consistency, α = .92), the Mobility Inventory for Agoraphobia (Chambless, Caputo, Jasin, Gracely, & Williams, 1985) with no time frame given (α = .91-.97), and the Pittsburgh Sleep Quality Index (Buysse, et al., 1989) to be answered for the past month (α = .83). The Beck Depression Inventory (Beck, et al., 1961) to be answered for the past week (α = .86), was also given to all participants except for 8 controls who received the short version of the Beck Depression Inventory (α = .91, Abdel-Khalek, 2001).

5.2.3 Physiological Assessment

Physiological data were recorded with a 3-channel ambulatory digital recorder (3991x/3 BioLog, UFI, Moro Bay, CA, USA) worn in a handbag or waist pack. The device measures 3.3 x 7.1 x 12.7 cm and weighs 230 g with its battery. Channels were (1) skin conductance measured by applying 0.5 V DC to electrodes on the middle or lower phalanges of the index and middle finger of the non-dominant hand. Skin conductance in the range 0.01–39.95 μS(iemens) was sampled with ± 0.01 μS resolution at 10 Hz and digitally low-pass filtered at 0.5 Hz. Disposable electrodes with a circular contact area of 1 cm diameter
Twenty-four hour Skin Conductance in Panic Disorder (study 2) pre-filled with isotonic gel were used (EL507, Biopac Systems, Inc., Goleta, CA, USA). In order to maintain contact for 24 hours, additional Biopac isotonic gel (Gel 101) was applied to the center of the electrode. (2) Bodily activity was sampled at 1 Hz from a UFI 1110 Jitterbug Actigraph accelerometer attached to the participant’s ankle on the same side as the non-dominant hand. This device responds to motion in all directions and axes with oscillations around 0 V. (3) Ambient temperature was sampled with an accuracy of 0.1 degrees C at 0.1 Hz from a sensor in the pack. The recorder had an event-marker button, with which participants could indicate the beginning of the ART, periods of emotional arousal, time of getting into bed and turning out the lights, getting up in the morning, or unusual events. The nature of the event was recorded in a paper log along with the time the button was pressed. Bodily activity and temperature were measured to account for their possible influence on skin conductance measures (Boucsein, 1992; Turpin, et al., 1983).

5.2.4 Data Reduction

Physiological recordings were analyzed offline with customized software written by the second author in Matlab® (MathWorks, Natick, MA, USA). First, they were examined visually for artifacts which were excluded from further analysis. The record was then segmented into waking and sleeping, both on the basis of self-report, event markers, and an examination of the activity channel for cessation and resumption of activity. Waking periods were further divided into periods of usual activities and periods when the ART was performed. The results presented below are based on 1-min epochs of measurements from each channel (skin conductance, ambient temperature, physical activity) during each of these three periods (waking-usual activities, waking-ART, sleeping) unless otherwise indicated.

Raw skin conductance (SC) data was filtered using a zero-phase, order 10 low-frequency Butterworth filter with a cutoff frequency of 0.05 Hz. Skin conductance (SC) yielded four measurements: (1) mean skin conductance level based on 1-min means of filtered SC, (2) mean skin conductance variability based on within-1-min standard deviations of filtered SC, (3) mean number of non-specific skin conductance fluctuations (NSFs) in 1-min epochs defined as +0.01 µS(iemens) inflections in the first derivative of filtered SC between consecutive data points, and (4) mean square successive differences (MSSD) based on the raw SC data sampled down to 4 Hz (throughout time periods of waking-usual
activities and sleeping) in an attempt to replicate Hoehn-Saric’s (2004) ambulatory findings who calculated MSSD separately for every epoch of 6 minute raw SC data sampled at 4 Hz. The last three measures represent different SC variability measures. Number of NSFs best reflects traditional practice but fails to take into account amplitude, whereas 1-min SC standard deviations and MSSD are affected by both number and amplitude of SC fluctuations. The Spearman correlations between these different SC variability measures were all significant at the 0.01 level, Spearman’s rhos ranging from 0.70 to 0.98. Because of this redundancy, we included as primary measures only the customary NSFs measure (3) and the MSSD (4) to replicate the abovementioned study, and not the SC standard deviation measure (2).

Psychological measures included ratings on the current emotional state taken at three time points during the 24-hour measurement period as well as before and during the last min of each of the four repetitions of the ART. The within-subject mean ratings on the current emotional state questionnaire for the adjectives “anxious”, “sad”, “relaxed”, “sleepy”, “worried”, and “tense” were calculated for waking-usual activities and waking-ART (except for “sad” which was not available for the ART) separately.

5.2.5 Statistical analysis

Psychophysiological data were analyzed using linear mixed models as recommended by Bagiella, Sloan, and Heitjan (2000), following a top-down strategy (see West, et al., 2007). Data were analyzed from the three measurement periods: waking-usual activities, waking-ART, and sleeping. Marginal models (without any random effects specified) were fitted by maximum likelihood with an unstructured covariance matrix for the residuals. Primary measures were tested using one-tailed and secondary measures using two-tailed significance tests. SPSS 16.0.2 (SPSS Inc., Chicago, IL) was used for these computations.

Demographic and self-report measures. Study participants were compared on several demographic and clinical variables. Continuous and normally distributed measures were analyzed using independent-sample t tests. If the assumption of normality was violated, non-parametric Wilcoxon rank-sum tests were calculated. Differences in categorical variables were tested with χ² tests or Fisher’s exact tests. Feeling “anxious” was a primary psychological measure since we hypothesized that PD patients would feel more anxious
during waking than controls. Other measures, such as feeling “sad” or “worried” were secondary, exploratory measures.

**Psychophysiological measures.** Both pooled and sequential analyses were made. (A) Within-subjects means were calculated based on all 1-min measurement epochs within the waking and within the sleeping periods (except for the MSSD which were based on the re-sampled raw SC for these periods). Primary variables for hypothesis testing were the means of SCL, number of NSFs, and MSSD. These variables were tested for (a) period differences (waking-usual activities vs. sleeping), and (b) group differences using marginal models with main effects for group and time, and adjusting for the effects of age, gender, mean temperature, mean activity and the use of antidepressant medication. Group by period interactions were included in the model if they significantly improved the fit (tested by performing ML-based likelihood ratio tests). In addition, activity, another primary variable, was tested (one-tailed) for group differences during sleeping, adjusting for the same covariates except for activity. Bonferroni correction was applied when necessary. Cohen’s \(d\) was calculated using \(t\) values and degrees of freedom \([d=2t/\sqrt{(df)}]\).

(B) Sequential analyses took into account the temporal sequence of SCL within subjects. (1) Ascending and descending run lengths of continuous epoch-by-epoch ascent or descent for 1-min epochs were calculated for waking-usual activities and sleeping. The \(n+1^{st}\) epoch was considered a continuation of an ascending (descending) run if its value was greater (less) or equal than the value of the \(n^{th}\) epoch. Once a next value interrupted this run, the length in min (derived from the number of epochs) of this run was calculated and the run length counter was reset to zero. The ascending run length was considered an indicator of prolonged periods of steadily increasing sympathetic activation or panic-like symptoms, while descending run lengths might indicate steadily decreasing activation or relaxation. (2) Linear and higher order regressions for consecutive 1-min mean SCL were calculated for sleeping. (3) The slopes of decline in the SCL 1-min means over the 8-min sitting periods of the ART combined over the four repetitions were calculated for each subject.

### 5.3 Results

As shown in Table 6, groups did not differ in age, gender, BMI, or race. PD patients reported having experienced 4.5 full-blown panic attacks and 17.4 limited symptom attacks
during the past month, and a total PDSS sum score of 14.7 (± 4.2). Compared to controls, they were more depressed by the Beck Depression Inventory (BDI), more anxious by the Beck Anxiety Inventory (BAI), more agoraphobic when alone or accompanied as indicated by the Mobility Inventory for Agoraphobia (MIA), and had more sleeping problems by the Pittsburgh Sleep Quality Index (PSQI). Smaller sample sizes for the questionnaire data were mostly due to missing data on more than an acceptable number of individual items in the respective questionnaires (carefully following the guidelines given by the authors) that prevented the calculation of total scores.

Table 6. Demographics and self-report measures

<table>
<thead>
<tr>
<th>Demographics</th>
<th>PD (n=22)</th>
<th>Controls (n=29)</th>
<th>Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>42.7 ± 15.0</td>
<td>42.1 ± 12.3</td>
<td>t(50) = -1.54 (NS)</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td>14 (64)</td>
<td>19 (66)</td>
<td>χ² (1,38) = 0.02 (NS)</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>26.1 ± 4.1</td>
<td>24.5 ± 4.2</td>
<td>t(50) = -1.34 (NS)</td>
</tr>
<tr>
<td><strong>Racial Category</strong></td>
<td></td>
<td></td>
<td>p=0.29, FET(NS)</td>
</tr>
<tr>
<td>Whites</td>
<td>11 (50)</td>
<td>20 (69)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>2 (9)</td>
<td>4 (14)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>4 (18)</td>
<td>3 (10)</td>
<td></td>
</tr>
<tr>
<td>Missing or declined</td>
<td>5 (23)</td>
<td>2 (7)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Questionnaires</th>
<th>PD n</th>
<th>Controls n</th>
<th>Wilcoxon W</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDI (0-63)</td>
<td>13.5 ± 11.0 (18)</td>
<td>1.0 ± 4.0 (28)</td>
<td>421.5***</td>
</tr>
<tr>
<td>BAI (0-63)</td>
<td>20.5 ± 21.3 (14)</td>
<td>2.0 ± 5.0 (20)</td>
<td>245.0***</td>
</tr>
<tr>
<td>MIA alone (1-5)</td>
<td>2.4 ± 1.3 (18)</td>
<td>1.1 ± 0.3 (27)</td>
<td>408.5***</td>
</tr>
<tr>
<td>MIA accompanied (1-5)</td>
<td>1.9 ± 1.0 (19)</td>
<td>1.0 ± 0.1 (27)</td>
<td>407.5***</td>
</tr>
<tr>
<td>PSQI (0-21)</td>
<td>7.0 ± 5.0 (11)</td>
<td>4.0 ± 2.5 (21)</td>
<td>277.0**</td>
</tr>
</tbody>
</table>

Note. Values are expressed as mean ± SD or number (%) for the demographic variables, and as median ± interquartile range for the self-report measures. Group sizes vary for the self-report measures due to missing data.

Abbreviations. NS = not significant, ** = p-value<0.01, *** = p-value<0.005, FET = Fisher’s Exact Test; BDI = Beck Depression Inventory, BAI = Beck Anxiety Inventory, MIA alone = Mobility Inventory for Agoraphobia (subscale “when alone”), MIA accompanied = Mobility Inventory for Agoraphobia (subscale “when accompanied”), PSQI = Pittsburgh Sleep Quality Index; PD = Panic disorder group.

Fifty-nine percent of the PD group were diagnosed as PD with Agoraphobia. Forty-one percent of the PD group had other comorbid diagnoses: Major Depressive Disorder, 4; Generalized Anxiety Disorder, 3; Specific Phobia, 2; Posttraumatic Stress Disorder, 2; Obsessive Compulsive Disorder, 1. None of the controls had a history of PD or any other axis I disorder.

Four PD patients were taking benzodiazepines regularly at the time of testing and two as needed, from which they were discouraged during the monitoring. On a regular basis,
5 were taking selective serotonin reuptake inhibitors (SSRI’s), 2, other antidepressants (venlafaxine, mirtazapine), 1, buspirone, and 1, a beta-blocker. None was taking tricyclic antidepressants or antihistamines. None of the control group was taking a psychoactive drug and none of the subjects in either group was taking sleeping aids. In the following, subjects described as taking “antidepressant medication” are the 5 PD patients who were regularly taking SSRI’s and the 2 who took other antidepressants (including 1 subject also taking buspirone).

5.3.1 Tests of our hypotheses

Mean SCL during day 1 was significantly higher than during day 2 but there was no group by day interaction in waking mean SCL. The data from both days were then collapsed.

![Graph showing SCL across PD and control subjects for each 1-min epoch synchronized by onset of sleeping period. At least 10 valid observations per group were required for included time epochs.]

Hypothesis 1 (waking differences). As shown in Table 7, the mean skin conductance levels were significantly higher in the PD group than in the control group and this effect further increased after adjusting for the effects of gender, age, mean temperature, mean
activity, and antidepressant medication. In the adjusted model, mean SCL was significantly decreased by age \([F(1,47.8)=5.3, \ p<0.05]\) and the use of antidepressant medication \([F(1,46.6)=4.8, \ p<0.05]\). Women tended to have higher mean SCL \([F(1,47.4)=3.6, \ p=0.06]\), whereas mean temperature and activity did not affect mean SCL. The mean numbers of NSFs and the MSSD sampled at 4 Hz as in Hoehn-Saric’s (2004) paper did not differ significantly between the groups, although the number of NSFs tended to be higher in the PD patients after adjusting for the covariates. PD patients reported being more anxious than controls when performing usual activities \((p<0.005)\).

Hypothesis 2 (ART). During the ART on the other hand, neither the mean nor the slope of SCL \([PD=C=-0.3, F(1,49)=0.0, \ p>0.05]\) differed between the groups. The same was true for the number of NSFs. PD patients reported being more anxious than controls when trying to relax \((p<0.005)\).

Hypothesis 3 (sleeping differences). Since both waking and sleeping periods were tested in the same model with period as a factor (factor levels were waking and sleeping), the results described above under hypothesis 1 also apply to the sleeping period except for the self-report, which only was available for waking periods. There was no interaction between Group and Period (levels waking and sleeping). Mean activity during the night was significantly higher in the PD group than the control group, which weakened after adjusting the model for age, mean temperature, gender and antidepressant medication.
Table 7. Estimated marginal means (SE) of primary electrodermal measures and activity by period (model 1: waking and sleeping, model 2: ART, model 3: sleeping only)

<table>
<thead>
<tr>
<th></th>
<th>Period</th>
<th>PD (n = 22)</th>
<th>Controls (n = 29)</th>
<th>t</th>
<th>df</th>
<th>p</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>mean SCL</strong></td>
<td>Unadjusted</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>waking</td>
<td>7.8 (0.6)</td>
<td>6.9 (0.6)</td>
<td>-2.0*</td>
<td>49.6</td>
<td>&lt;0.05</td>
<td>-0.6</td>
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<tr>
<td></td>
<td>sleeping</td>
<td>3.2 (0.3)</td>
<td>2.3 (0.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted</td>
<td>waking</td>
<td>7.8 (0.6)</td>
<td>6.5 (0.6)</td>
<td>-2.9***</td>
<td>47.9</td>
<td>&lt;0.01</td>
<td>-0.8</td>
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<td></td>
<td>sleeping</td>
<td>3.7 (0.4)</td>
<td>2.4 (0.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>ART</td>
<td>6.9 (0.6)</td>
<td>6.0 (0.5)</td>
<td>-1.2</td>
<td>49</td>
<td>&gt;0.05</td>
<td>-0.3</td>
</tr>
<tr>
<td>Adjusted</td>
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<td>6.0 (0.5)</td>
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<td>49</td>
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<tr>
<td><strong>mean nNSFs</strong></td>
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<tr>
<td></td>
<td>waking</td>
<td>1.9 (0.2)</td>
<td>1.9 (0.2)</td>
<td>-0.4</td>
<td>48.9</td>
<td>&gt;0.05</td>
<td>-0.1</td>
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<tr>
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<td>1.9 (0.2)</td>
<td>1.7 (0.2)</td>
<td>-1.6</td>
<td>48.6</td>
<td>0.06</td>
<td>-0.5</td>
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<td></td>
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<td>0.5 (0.1)</td>
<td>0.3 (0.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>ART</td>
<td>1.2 (0.2)</td>
<td>0.9 (0.1)</td>
<td>-1.3</td>
<td>49</td>
<td>&gt;0.05</td>
<td>-0.4</td>
</tr>
<tr>
<td>Adjusted</td>
<td>ART</td>
<td>1.2 (0.2)</td>
<td>0.9 (0.1)</td>
<td>-1.0</td>
<td>49</td>
<td>&gt;0.05</td>
<td>-0.3</td>
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<tr>
<td><strong>MSSD (4Hz)</strong></td>
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<tr>
<td></td>
<td>waking</td>
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<td>0.0 (0.0)</td>
<td>-1.2</td>
<td>90.9</td>
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<td>0.0 (0.0)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>sleeping</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>-1.0</td>
<td>101.1</td>
<td>&gt;0.05</td>
<td>-0.2</td>
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<tr>
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<td>1.4 (0.1)</td>
<td>-1.9</td>
<td>48</td>
<td>&lt;0.05</td>
<td>-0.5</td>
</tr>
<tr>
<td>Adjusted</td>
<td>sleeping</td>
<td>1.6 (0.1)</td>
<td>1.4 (0.1)</td>
<td>-1.3</td>
<td>48</td>
<td>&gt;0.05</td>
<td>-0.4</td>
</tr>
</tbody>
</table>

**Abbreviations:** SE, standard error; PD, panic disorder; p, p-value (one-tailed); SCL, skin conductance level; nNSFs, number of non-specific fluctuations; MSSD, mean square successive differences in SC; ACT, activity; ART, ambulatory relaxation test; d, Cohen’s d.

- Based on t statistics for contrast comparison.
- Adjusted for gender, age, mean temperature, mean activity, and antidepressant medication.
- Adjusted for gender, age, mean temperature, and antidepressant medication.
- Convergence not achieved. Results were based on the last iteration. Thus the validity of model fit is uncertain.

### 5.3.2 Exploratory tests

During **waking**, PD patients were also less relaxed (p<.05) and more worried (p<.005), tense (p<.005), and sad (p<0.05) than controls. To examine whether waking state anxiety ("feeling anxious") or state depression ("feeling sad") might explain the positive mean SCL findings, marginal model analyses were conducted in the PD group for each time period separately, adjusting for the effects of antidepressant medication, gender, age, mean temperature, and activity. Higher state anxiety was associated with higher mean SCL during waking [F(1, 21)=7.2, p<0.05, Cohen’s d=1.2]. Higher levels of daytime state depression were associated with higher mean SCL during sleeping [F(1,21)=5.8, p<0.05, Cohen’s d=1.0]. Both relationships represent large effects.

When performing similar analyses in the PD group that aimed to elucidate the role of agoraphobia, no significant relationships with mean SCL could be found – neither when
Twenty-four hour Skin Conductance in Panic Disorder (study 2) comparing PD patients with and without agoraphobia (mean Cohen’s d=-0.7 for waking and sleeping), nor considering MIA questionnaire scores (when accompanied: mean Cohen’s d=-0.7, when alone: mean Cohen’s d=-0.6). Although not significant, all these effects were of medium size and pointed towards an inverse relationship between the level of agoraphobia and SCL, i.e., more agoraphobic symptoms corresponded to a lower SCL.

Next, the influence of acute panic symptoms (as opposed to tonically elevated anticipatory anxiety) on mean SCL was examined. Self-report data on panic attacks during waking and sleeping and subjective sleep disturbance was limited due to missing data. Only 15 PD patients (68%) and 19 controls (66%) provided self-reports on the number of panic attacks. During waking, 3 out of these 15 PD patients reported having had 1 panic attack, 1 reported 2, and 1 reported 3 attacks. One PD patient experienced a nocturnal panic attack, while none of the controls reported having had panic attacks during either sleeping or waking. The number of daytime attacks was significantly higher in PD patients (p<.05, two-tailed Fisher’s exact test). Within the PD group, no significant relationships could be found between the number of daytime panic attacks and waking mean SCL (Cohen’s d=0.7) or the number of nocturnal panic attacks and sleeping mean SCL (Cohen’s d=-0.3).

To determine whether subjective accounts of sleep disturbance could have influenced the mean SCL, self-report data on sleep were examined, but here as well, missing data limits generalizability. There were no differences in reported sleep duration, number of nocturnal awakenings, the restfulness of sleep, or daytime sleepiness. Length of the sleeping periods did not differ between groups, with a mean of 515 min in the PD group and 495 min in the control group. PSQI scores within the PD group were not associated with mean SCL during any time period (mean Cohen’s d=-0.5 for waking and sleeping) after adjusting for age, gender, mean temperature, activity, and antidepressant medication.

Finally, mean SCL was examined for periods of steadily increasing or decreasing activation which could indicate abnormal modulation of sympathetic arousal in PD patients. During waking, groups did not differ in either ascending or descending run lengths of 1-min epochs of SCL. Both groups had a steep decline in SCL over the first 30 min of the sleeping period, and then a slower decline. These declines were approximately parallel in the two groups as confirmed by regression analyses. However, an almost significant group by period interaction [F(1,47.3)=3.6, p=0.06] was related to shorter descending run lengths of SCL in
PD patients during *sleeping* (post-hoc test $[F(1,49.2)=4.5, \ p<0.05, \ \text{Cohen's } d=0.6]$). In other words, PD patients had more frequent interruptions in the downward descent of SCL measures than controls.

### 5.4 Discussion

Our hypothesis that during waking periods, skin conductance levels (SCL) and measures of non-specific skin conductance fluctuations would be higher in PD patients was partly confirmed in that mean SCL was elevated during waking (large effect), but measures of SC variability were not. Self-reports documented that these patients experienced more anxiety which itself was associated with higher mean SCL within the PD group (large effect). Figure 4. indicates that this difference in mean SCL disappeared on the second day of recording although no significant interaction between group and day was found. The finding of higher mean SCL during waking anxious states is in agreement with several laboratory studies (e.g., Braune, et al., 1994; Hoehn, et al., 1997; Parente, et al., 2005) but not with the ambulatory study of Hoehn-Saric et al. (2004), and several laboratory studies (e.g., Argyle, 1991; Blechert, et al., 2007).

Methodological differences that may have contributed to the inconsistent findings of previous laboratory studies were explored in our data. In our PD group, although more depressed than controls, there was no significant relationship between state depression and mean SCL during waking, unlike in older studies (Argyle, 1991; Ward, et al., 1983). Similarly, the level or diagnosis of agoraphobia did not significantly influence mean SCL. The observed relationship was inverse, which could be interpreted as the more agoraphobic PD patients successfully reducing their anxiety by avoiding anxiety-provoking situations. Unfortunately, our self-report data were inadequate for testing this interpretation. Taking antidepressant medication had the effect of reducing the mean SCL in PD patients, while state anxiety and daytime panic attacks, increased mean SCL, although the last result was not significant. Thus, some discrepancies between previous studies may have been caused by varying levels of agoraphobia and different medication policies. The former might also account for the null findings in Hoehn-Saric’s ambulatory study (2004) since they did not assess for comorbid agoraphobia, which might have reduced the mean SCL of their PD patients.

Despite a trend in the number of NSFs (medium-sized effect), measures of SC variability did not significantly differ between the groups: neither the number of NSFs nor
the MSSD, which in Hoehn-Saric et al.’s (2004) ambulatory study was significantly lower in the PD group. Our MSSD result must be interpreted with caution because of non-convergence of the statistical model. A failure to find significantly higher SC variability in the presence of higher mean SCL was reported previously (Parente, et al., 2005). According to a recent functional magnetic resonance imaging (fMRI) study, different brain structures regulate tonic and phasic SC activation (Nagai, et al., 2004). SCL, a tonic measure, had a closer relationship with brain regions associated with anticipatory anxiety (for a discussion, see Parente, et al., 2005). Alternatively, the lack of significant SC variability differences may be a consequence of high, uncontrolled SC variance unrelated to anxiety. Unexpected events, interpersonal engagement, and positive and negative emotions of all kinds may affect SC. These factors also may have interrupted descending runs of SC, a possible measure of relaxation during daily activities, and obscured waking differences in the ability of patients and controls to relax.

However, even when these uncontrolled factors were minimized during the ART, during which patients sat quietly and tried to relax, no group differences were found in either mean SCL or its rate of decline. This is contrary to our second hypothesis that during waking periods, PD patients would be less able to relax and physiologically deactivate than controls. These findings can be interpreted as meaning that PD patients can relax just as well as non-anxious controls or that skin conductance is not a valid measure of relaxation. Results might have been different at higher anxiety levels: although more anxious than the control group, almost 90% of our PD patients rated the intensity of their state anxiety in the lower one-third of the scale both during the ART and usual activities. On the other hand, 5 of our 22 PD patients reported a panic attack sometime while being monitored, which is quite a few considering that investigations of PD usually have an entrance criterion of no more than one attack per week. That we found higher mean SCL under daily-life conditions but no differences during a short and relatively standardized test of quiet sitting and relaxing, again calls into question the validity of the ART.

Our third hypothesis, that the differences postulated for waking would be present in some form during sleeping periods, was confirmed. During sleep, PD patients had higher mean SCL than controls. Both groups had steep declines in SCL from waking levels during the first 30 min followed by slower parallel declines afterwards. Exploratory analyses
revealed that the failure of PD patients to achieve lower levels could be related to more frequent interruptions of deactivation as evidenced by shorter descending SCL runs. They also pointed to the potentially aggravating role of comorbid daytime depression, which increased the mean SCL within the PD group on the night of the recording (a large effect). Waking state anxiety and agoraphobia did not significantly affect mean SCL. PD patients rated themselves on the PSQI as having slept more poorly during the past month, but PSQI scores were uncorrelated with mean SCL on the recording night. In spite of SC evidence of poor sleep, PD patients did not rate themselves as sleeping more poorly on the night of recording; both results, however, were weakened by missing data. Higher activity levels during sleep in the PD group indicated sleep disturbance, but this difference disappeared after adjusting for gender, age, mean temperature, and antidepressant medication. Perhaps processing of conditioned interoceptive threat cues during sleep gave rise to bursts of sympathetic autonomic activation as part of a conditioned anxiety response, and interrupted SCL descent. Such bursts could be the basis of nocturnal panic attacks, but if so, they were generally below awareness in that patients did not report poorer sleep than controls on the recording night, and only 1 of 22 PD patients reported having had a panic attack.

This study is limited in several ways. First, the advantages of ecologically valid ambulatory physiological measures must be weighed against the imperfect control of disturbing factors such as activating interpersonal interactions. Better control of these factors might have allowed additional group effects to emerge. Second, we measured only skin conductance to assess anxiety in PD. Heart rate, respiratory sinus arrhythmia, respiratory variability, and end-tidal pCO2 might have informed us whether the skin conductance findings were part of a general pattern of stress activation. Polysomnography would have been helpful, since we cannot determine how much of the sleep period our subjects actually slept and whether the interruptions we observed were associated with EEG changes in specific sleep stages. Skin conductance has rarely been recorded during sleep, and it is conceivable that it can register sleep disturbances in anxiety disorders that are missed by conventional polysomnography. Thirdly, restricting our patient sample to patients not taking any medications would have excluded their possible interfering effects but would have impaired the representativeness of any findings, since in developed countries unmedicated patients can be an atypical subgroup. Apparently, the antidepressants taken by our PD patients lowered their SCLs without producing a clinical remission in panic. We did
not do blood or urine testing to confirm patients’ medication use, which might have revealed reporting failures. Fourthly, since we did not examine patients with other anxiety disorders, we do not know the extent to which our results are specific to the diagnosis of PD. Perhaps increased SCL is a general characteristic of anxiety disorders or is associated with elevated levels of anxiety regardless of diagnosis. Furthermore, as our sample was relatively small, its statistical power for our primary hypotheses was limited. With more participants, statistical tests at a trend (e.g., number of NSFs) probably would have been significant. This particularly applies to our self-report data, which was often missing.

In summary, individuals with current PD show evidence of sustained sympathetic activation, both during the day during their usual activities and at night during sleep. Elevated activation while awake is in accordance with observations that many PD patients suffer from sustained anxiety between panic attacks, which has been ascribed to anxious anticipation of future attacks. Elevated SC activation during sleep is in accordance with patient complaints of disturbed sleep, which have been hard to document with conventional polysomnography not measuring skin conductance. Recording skin conductance outside the laboratory in natural settings shows promise for the assessment of PD severity and could be useful in therapy outcome studies as a supplement to structured interviews and questionnaires. Future ambulatory studies should control for the potentially confounding effects of depression, agoraphobia, and medication.
6 Worried sleep: 24-hr monitoring in high and low worriers (study 3)

6.1 Introduction

A recent review of epidemiological studies of insomnia (Ohayon, 2002), estimated that one-third of the general population reports at least one DSM-IV (American Psychiatric Association (APA), 1994) insomnia symptom from the list of prolonged sleep-onset latency, difficulty in maintaining sleep, experiencing non-refreshing or poor sleep, and impairment of daytime functioning, which includes reduced alertness, fatigue, exhaustion, and dysphoria. Insomnia is often comorbid with depression (Ohayon, 2002) or an anxiety disorder. One such anxiety disorder is Generalized Anxiety Disorder (Mellman, 2006), whose hallmark is excessive and uncontrollable worry.

Research relating worrying and insomnia has focused primarily on establishing a link between pre-sleep cognitive activity and sleep-onset latency. However, even for this sleep parameter, findings are inconsistent (see Table 8 for a review). Interpretation of this research is complicated by whether trait (habitual) or state (last night) estimations of pre-sleep cognitions or worry were measured, what pre-sleep cognitions were evaluated, how sleep was measured, and over what time period. For example, trait worry or intrusive thoughts may not be the largest contributor to variance in daily worry (Verkuil, Brosschot, & Thayer, 2007) or to intrusive thoughts at sleep onset and during nocturnal awakenings, respectively (Hall, Buysse, Reynolds III, Kupfer, & Baum, 1996). A person high in trait pre-sleep arousal or worrying does not necessarily exhibit this behavior on the night(s) he/she is monitored which in some studies was just one. Thus, studies using only state measures may give different results from studies that include trait measures.
Table 8. Studies reporting on the relationship between worry and sleep parameters

<table>
<thead>
<tr>
<th>First author (year)</th>
<th>Worry/ cognitions</th>
<th>Sleep parameter (e.g., SOL)</th>
<th>Relationship</th>
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</thead>
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<td>Trait</td>
<td>Worried</td>
<td>State</td>
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<td>Trait</td>
<td>Worry proneness</td>
<td>Trait</td>
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<td>State</td>
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<td>State</td>
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<td>Trait</td>
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<tr>
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<td>Trait</td>
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<td>State</td>
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<td>Trait</td>
<td>Pre-sleep arousal</td>
<td>Trait</td>
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<td>Omvik (2007)</td>
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<td>State</td>
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<td>Trait</td>
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<td>Speech after wake-up</td>
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<td>Hall (1996)</td>
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Worried sleep: 24-hr monitoring in high and low worriers (study 3)

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<tr>
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<td>Worry</td>
<td>Trait</td>
<td>Questionnaire</td>
<td>HW = LW</td>
</tr>
<tr>
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<td>Worry</td>
<td>State</td>
<td>Sleep logs</td>
<td>HW = LW</td>
</tr>
<tr>
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<td>Trait</td>
<td>Worry</td>
<td>State</td>
<td>Sleep log</td>
<td>HW &lt; LW</td>
</tr>
<tr>
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<td>Trait</td>
<td>Worry</td>
<td>State</td>
<td>Actigraphy</td>
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<tr>
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<td>State</td>
<td>All-night PSG</td>
<td>-</td>
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<tr>
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<td>State</td>
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<td>-</td>
</tr>
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<td>Questionnaire</td>
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<td>Trait</td>
<td>Interview</td>
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<td>Pre-sleep intrusions</td>
<td>State</td>
<td>Sleep logs</td>
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<tr>
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<td>State</td>
<td>Actigraphy</td>
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<td>All-night PSG</td>
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<td>State</td>
<td>All-night PSG</td>
<td>-</td>
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<td>State</td>
<td>Sleep log</td>
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</tr>
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<td>Gross (1982)</td>
<td>State-i</td>
<td>Speech after wake-up</td>
<td>State</td>
<td>Nap PSG</td>
<td>-</td>
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<td>Trait</td>
<td>Worry</td>
<td>Trait</td>
<td>Questionnaire</td>
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<td>Pre-sleep arousal</td>
<td>Trait</td>
<td>Questionnaire</td>
<td>+</td>
</tr>
</tbody>
</table>

**Note.** Results based on group comparisons are presented using inequality (<,>) and equality (=) signs; results based on correlational analyses are presented using + (positive correlation), - (negative correlation), 0 (no correlation).

**Abbreviations.** PSG = polysomnography, HW = high worriers, LW = low worrier, State-i = induced state.

### 6.1.1 Worry and autonomic arousal

Measures of autonomic arousal have often been used to assess a person’s stress level or reactivity if self-report needs corroboration or if obtaining it would disturb the process of interest, for example sleep. Heart rate (HR) is affected by both sympathetic and parasympathetic afferents whereas heart rate variability (HRV) in the respiratory frequency range – also known as respiratory sinus arrhythmia – is predominantly mediated by parasympathetic influences (Berntson, et al., 1997), which increase during relaxation. Electrodermal activity on the other hand is a purely sympathetic measure, which increases in an anxious state (Fowles, 1980).
During daytime laboratory measurements of baseline activity, high *trait* worriers (HWs) have had higher HRs (Knepp & Friedman, 2007; Thayer, Friedman, & Borkovec, 1996) and lower HRV (Delgado et al., 2009; Lyonfields, Borkovec, & Thayer, 1995), although some studies could find no such differences (e.g., Borkovec, et al., 1983; Hoehn-Saric, et al., 1989; Wilhelm, et al., 2001). In an ambulatory monitoring study, during the day marginally higher HRs and significantly lower HRVs were observed in patients with Generalized Anxiety Disorder compared to non-anxious controls (Hoehn-Saric, et al., 2004). This study also found significantly reduced skin conductance variance in these patients, while skin conductance levels were the same. Skin conductance levels are also scarcely affected by *trait worry* in the laboratory setting (Hoehn-Saric, et al., 1989; Wilhelm, et al., 2001).

*State* worry has repeatedly been positively associated with higher HR and lower HRV during waking (Brosschot & Thayer, 2003; Brosschot, Van Dijk, & Thayer, 2007; Pieper, Brosschot, Van der Leeden, & Thayer, 2007) even several hours after the worry has passed (Pieper, Brosschot, van der Leeden, & Thayer, 2010) and in the ensuing sleep period (Brosschot, et al., 2007; Yoshino & Matsuoka, 2009) – leading some authors to suggest “unconscious stress-related cognition” (Pieper, et al., 2010, p. 570) or “unconscious worry” (Brosschot, et al., 2007, p. 45).

Chronic primary insomnia has been called a “disorder of 24-h hyperarousal” (Riemann et al., 2010, p. 29) where chronic sleep loss due to the insomnia may partially mask this hyperarousal and thus explain the symptoms of daytime fatigue and occasional nights of good sleep otherwise inconsistent with hyperarousal. Sleep-onset insomniacs have repeatedly shown increased HR during a pre-sleep period (Haynes, et al., 1981; Nelson & Harvey, 2003) and at sleep-onset (Freedman & Sattler, 1982). Chronic insomniacs had increased HRs and decreased high-frequency power of HRV during all stages of sleep compared to matched normal sleepers (Bonnet & Arand, 1998). In a thorough review of the literature, Riemann et al. (2010) found some evidence for autonomic hyperarousal but noted that studies were limited by small sample sizes and differing definitions of insomnia. Measures of electrodermal activity – a purely sympathetic index – have failed to distinguish insomniacs from good sleepers (Freedman & Sattler, 1982). Espie’s psychobiological model of insomnia (Espie, 2002) posits a failure to “de-arouse” during the transition from wakefulness to bedtime as a cause of insomnia. A recent cognitive model of insomnia
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Maintenance (Harvey, 2002) may be able to link excessive worrying during the day and in bed to symptoms of insomnia and hyperarousal. In what seems like a vicious cycle, worrying is linked to increased autonomic arousal and emotional distress, selective attention to sleeping difficulties, distorted perception of a sleep deficit, counterproductive safety behaviors, and erroneous beliefs about sleep. The longer the individual is trapped in this cycle, escalating anxiety and worry may lead to an actual sleep deficit and daytime dysfunction.

6.1.2 Hypotheses

The study reported here was designed to investigate relations between worry, sleep, and autonomic arousal in terms of heart rate and electrodermal activity. Worry variables include trait worry and worry states such as pre-sleep worry, worry during nocturnal awakenings, and worry after waking up in the morning. Both subjective and objective sleep parameters are assessed. Among these measures of sleep disturbance are early morning awakenings and daytime dysfunction, which have so far been neglected in this context, as has been electrodermal activity. From our review of the literature, we predicted that high trait worry would lead to habitually prolonged sleep-onset latency (SOL), an increased number and duration of nocturnal awakenings, lower sleep efficiency and sleep quality, and shorter total sleep time. We predicted that only high state worry on the monitoring night would affect sleep parameters for that night, delaying physiological de-arousal after sleep onset, and increasing HR and decreasing HRV during sleep.

6.2 Methods

6.2.1 Participants

Fifty-six participants from the local community were recruited with the following advertisement in flyers and on the internet: “How much do you worry? Do you find it easy to dismiss worrisome thoughts? OR Do you worry all the time? Would you like to find a way of dealing with your worries?” Identical phone screens including the Penn State Worry Questionnaire (PSWQ; Meyer, Miller, Metzger, & Borkovec, 1990) were used for both groups to avoid response tendencies as both high worriers (PSWQ ≥ 56) and low worriers (PSWQ ≤ 42) were eligible. A total of 157 people completed the phone screening which contained questions regarding shift work, current medications and medical history (including sleep apnea and other sleep disturbances), current and past alcohol or substance use, and
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psychosis. Exclusion criteria for all participants were substance abuse or dependence in the past year and a history of, or current, DSM-IV psychotic disorder as diagnosed by the Structured Clinical Interview for DSM-IV-TR Axis I Disorders (SCID; First, et al., 2001) conducted on the first day of testing. Healthy controls were selected not to have a current diagnosis or history of any anxiety or mood disorder. Also excluded was any participant who reported a history of severe cardiovascular, lung, or neurological disease, uncontrolled thyroid problems, or who showed signs of cognitive impairment during the phone screening and direct interactions with the interviewers. Eligible participants were allowed to continue on stable doses of medicines prescribed by physicians, but were excluded if they were taking psychoactive drugs or drugs with substantial peripheral effects (even on an as-needed basis). Seventy-four people were eligible of which 57 decided to participate in the study. One participant was excluded after the first 24-hr monitoring because of reported as-needed use of benzodiazepines during the recording period. Participants received monetary compensation for being tested.

6.2.2 Procedure

This investigation was carried out in accordance with the latest version of the Declaration of Helsinki. The study design was reviewed and approved by the Stanford Institutional Review Board and the VA Palo Alto Veterans Affairs Research Compliance Office. Eligible individuals were invited to an appointment where they gave written informed consent for further assessment. The assessment began with two interviews conducted by graduate students in psychology who were trained and supervised by a clinical psychologist: the SCID (First, et al., 2001) and a sleep interview based upon the research diagnostic criteria for insomnia (Edinger et al., 2004). If no grounds for exclusion were found, they were asked to wash their hands with soap in preparation for the application of skin conductance electrodes (Dawson, et al., 1990; Venables & Christie, 1980) and the ambulatory monitoring devices were connected. Next, participants underwent laboratory testing where they performed posture exercises and a worry induction, the results of which will be reported elsewhere.

After the laboratory session, participants were introduced to the electronic diary and reminded not to drink more than one glass of alcohol during the following 24 hours and not to take benzodiazepines if they had reported taking them on an as-needed basis. They were
instructed to wear the ambulatory monitoring devices continuously until they returned the next day. Length of recording ranged between 19:04 and 26:48 with a mean of 22:10 (hr:min). During monitoring, subjects were prompted by an alarm to fill out a short electronic diary (using Pendragon Forms v5.1, Pendragon Software Corporation) on a Palm® handheld (Palm® Tungsten E2; secured with TealLock Enterprise Edition v6, TealPoint Software) every two hours during waking. The Palm handheld contained an event log which recorded the exact times at which participants reported (a) going to sleep and waking up (including nocturnal awakenings and daytime naps), (b) taking medications, drinking alcohol or caffeinated beverages, or smoking a cigarette, (c) beginning and ending significant physical exercise, and (d) detachment and replacement of any or all of the sensors. In addition, participants filled out questionnaires on a laboratory computer running ACASI v2.5 NOVA Research Company software when they returned the next day.

6.2.3 Psychological assessment

Electronic diary. A questionnaire on the electronic device assessed the participant’s current state every two hours on a subjective units of distress scale from 0, “not at all”, to 10, “extremely” for the items “sweating”, “heart racing or pounding” (autonomic arousal), and “tired or fatigued” (daytime dysfunction). When they filled out this state questionnaire upon first waking in the morning, additional questions were asked: if and when they worried while in bed (before falling asleep, during the night, after waking up in the morning), when they went to bed, when they first intended to go to sleep (lights off), time to fall asleep (sleep-onset latency), the number and total duration of nocturnal awakenings, total time asleep, when they finally awoke, when they got up, and sleep quality (from 0 to 10). From these answers we calculated the duration of early morning awakening as the period from the final awakening to when they got out of bed, and sleep efficiency as the percentage of time in bed spent asleep. These sleep parameters were based on recommendations for standard assessment in insomnia (Buysse, Ancoli-Israel, Edinger, Lichstein, & Morin, 2006).

Electronic questionnaires. The questionnaire packet included the PSWQ to be answered for what is their typical worry behavior and the Pittsburgh Sleep Quality Index (PSQI; Buysse, et al., 1989) to be answered for their sleep in the past month.
6.2.4 Physiological Assessment

Physiological data were recorded with a customized 3-channel ambulatory digital recorder (3991x/3-SIT BioLog, UFI, Moro Bay, CA, USA) worn in a waist pack. The device measures 3.3 x 7.1 x 12.7 cm and weighs 230 g with its battery. Channels were (1) skin conductance (SC) measured by applying 0.5 V DC to electrodes on the middle phalanges of digits 3 and 4 of the non-dominant hand. Skin conductance in the range 0.1–39.9 μS (siemens) was sampled with ±0.01 μS resolution at 10 Hz. Commercial disposable electrodes with a circular contact area of 1 cm diameter were used (EL507, Biopac Systems, Inc., Goleta, CA, USA). They were pre-filled with isotonic wet gel (by weight 0.5% saline and 75% water). (2) Interbeat intervals (IBIs) were recorded using a proprietary “Fetrode” input assembly provided with the BioLog system that was connected to disposable electrodes (Cleartrace 1700-030, CONMED, Utica, NY, USA) placed on the sternum and 15 cm below the clavicle on the anterior auxiliary line after the area was wiped with an alcohol pad. One of the SC electrodes was used as a reference. IBIs were based on detecting QRS peaks sampled at 1000 Hz. IBIs in the range of 250-1999 ms were resampled at 10 Hz in parallel with the SC data, the last IBI being carried forward until the next beat occurred. (3) Ambient temperature was sampled with an accuracy of 0.1 degrees Celsius at 1 Hz and the resampled at 10 Hz from a sensor attached to the outside of the waist pack where it was exposed to ambient air but insulated from body heat and direct sun exposure.

Subjects also wore an Actiwatch 64 omnidirectional accelerometer (Mini-Mitter Respironics Company, Inc., Bend, OR, USA) attached to the participants’ left ankle that recorded body movement every 15 sec. The Actiwatch was synchronized with the BioLog device at the beginning and the end of the 24-hr assessment using marker buttons. During the 24-hr monitoring, some data loss occurred because twice the BioLog device stopped before the end of recording after a memory error, twice the SC cable ripped, once the IBI cable was removed and incorrectly replaced, once the Actiwatch started recording too late, and once the participant took the Actiwatch off and forgot to put it back on.

6.2.5 Physiological Data Reduction

Physiological recordings were analyzed offline with customized software written by the first author in Matlab® (MathWorks, Natick, MA, USA). First, the data from the two ambulatory devices (BioLog and Actiwatch) were synchronized and the body movement data
was upsampled to 10 Hz. Data were then displayed in consecutive 10-minute time frames and examined visually for artifacts which were excluded from further analysis without interpolating the missing data. Manual editing excluded time periods (a) when electrodes were detached, (b) that contained spikes in skin conductance (SC) greater than 0.5 µSiemens, which represented movement artifacts or loose electrodes, (c) sharp rises and falls in the interbeat intervals (IBI) most likely representing ectopic heart beats, missed beats, or beat detection failures. Automatic editing excluded IBI values outside the range of 0.5 to 1.5 times the mean of that time frame. Intervals of physical exercise, daytime naps, and nocturnal awakenings were determined and excluded from further analyses. By channel, the percentages of data epochs removed by editing were 14.7 % (SC), 14.9 % (IBI), 7.3 % (body movement, and 9.3 % (ambient temperature).

After editing, the SC data were digitally filtered using a zero-phase, order 10, low-frequency Butterworth filter with a cutoff frequency of 0.05 Hz. No filters were applied to the other channels. The data were then segmented into waking and sleeping segments as described below. The edited physiological data were analyzed in 1-min epochs for each of three periods (waking on day 1, sleeping, waking on day 2). For example, one person’s sleep recording might total 360 epochs (6 hours). Skin conductance (SC) for each 1-min epoch was measured as (1) mean filtered SC level (SCL) and (2) number of non-specific skin conductance fluctuations (NSFs) defined as having a rise of 0.01 µS(iemens) within 1 sec after a positive zero crossing in the first derivative of SCL, followed by a rise of 0.05 µS within 3.5 sec from the first rise. From IBI data for each 1-min epoch the following were calculated: (1) heart rate (HR) as 60*1000/IBI; (2) high-frequency power of heart rate variability (HRV), also called respiratory sinus arrhythmia (RSA) by applying MatLab’s pwelch fast Fourier algorithm to detrended IBI data, segmenting it into 8 sections of equal length, each with 50% overlap, then windowing each segment with a Hamming window of the same length as the segment. The power spectral density between 0.15 and 0.4 Hz was averaged. Because missing IBI data were not interpolated, the percentage of invalid segments ranged from 23% (during sleeping) to 32% (during waking on day 2). (3) As an alternate HRV measure, the root mean squared successive differences (RMSSD) between consecutive IBIs were calculated for each 5-min epoch. For 120 min after sleep onset, the duration (in min) and slope of decline for SCL and HR data was calculated by determining the number of epochs before the first zero-crossing of the first derivative of the data occurred and then calculating
the slope of change from sleep onset to that first interruption of decline. Body movement and ambient temperature were averaged over each 1-min epoch.

For the statistical analyses reported in this paper, the 1-min or 5-min epochs were further averaged for each of the time periods (waking on day 1, sleeping, waking on day 2). For example, 360 epochs would be averaged to one value for sleeping.

6.2.6 Derivation of objective sleep measures

Within the patient reported period of time in bed (TIB, “lights off” to “get up”), additional sleep measures were derived: (a) sleep-onset latency (SOL) as the time period from the reported intention to go to sleep (“lights off”) to sleep onset, (b) the number and total duration of nocturnal awakenings (WASO), (c) total sleep time (TST); and (d) sleep efficiency as the percentage ratio of TST to TIB.

**Defined by multiple channels and self-report.** Onset and offset times of the major sleep period, naps, and nocturnal awakenings, were based on a continuous decline in SCL and HR for at least 10 minutes during which no body movement occurred (sleep onset), and an abrupt increase in SCL and HR accompanied by a resumption of body movement and a change in ambient temperature (sleep offset).

**Defined by actigraphy.** Sleep onset was determined from the actigraphic raw data as the first consecutive 10 immobile minutes (activity count = 0) where no more than one 15-sec epoch was scored as mobile. Sleep offset was set as the end of the last consecutive 5 immobile minutes with no more than one epoch scored as mobile, before the participant reported getting up. Epochs during TIB were scored as wake by the Actiware program with a wake threshold set to medium (40 activity counts).

6.2.7 Statistical analysis

**Demographic, self-reported, and confounding variables (analyses without covariates).** Continuous and normally distributed demographic and clinical variables were analyzed using independent-sample t tests. If the assumption of normality was violated, non-parametric Wilcoxon rank-sum tests were calculated. Differences in categorical variables were tested with $\chi^2$-tests or Fisher’s exact tests.
Analyses with covariates. Other self-report and psychophysiological data were analyzed using linear mixed models as suggested by Bagiella, Sloan, and Heitjan (2000), following a top-down strategy (see West, et al., 2007). Marginal models (without any random effects specified) were fitted by maximum likelihood using SPSS 18.0 (SPSS Inc., Chicago, IL). All ambulatory variables were tested for the effects of group (high vs. low worrier, worried vs. not worried in bed) while controlling for the possible effects of the intake of caffeine (yes, no), alcohol (yes, no), cigarettes (yes, no), anti-anxiety or antidepressant medication (yes, no), and sleep medicine (yes, no; for sleep measures only). All psychophysiological variables were additionally controlled for the possible effects of sex, race, age, body mass index, ambient temperature, and body movement. To obtain the most parsimonious solution for each outcome, covariates with p-values greater than 0.05 were omitted from the models unless the model fit changed significantly as determined by χ²-tests of the two competing -2 log likelihood criteria. Effect size was calculated as Hedges’ g, which uses weighted and pooled standard deviations and is recommended for groups dissimilar in size (Ellis, 2009). As with Cohen’s d, effect size is expressed in terms of standard deviation.

6.3 Results

6.3.1 Sample description

Trait and state worry groups. As the main trait worry measure, the PSWQ was re-administered as part of the questionnaire packet after already having been given during the phone screening. It is noteworthy, that on this second administration 15 participants in the high worry (HW) group scored below the PSWQ cut-off score of 56 for this group and would have not been eligible (minimum PSWQ = 48), whereas one participant from the low worry (LW) group scored above the PSWQ cut-off of 42 score for this group (PSWQ = 43). They were not excluded, however, but were assigned an average score from the two administrations. As seen in Table 9, the HW group still scored much higher on the PSWQ than the LW group. To examine spontaneous state worry in bed during the monitoring night above and beyond the effects of trait worry, only the HW group was considered. It was divided into two groups based on whether the participant reported having worried in bed before falling asleep, during nocturnal awakenings, and after waking up in the morning or none of those times (10 HW+ vs. 12 HW-). There were no significant PSWQ differences
between HW+ and HW-, indicating that the occurrence of state worry during monitoring was not related to trait worry severity during the past month.

**Demographics.** As shown in Table 9, HWs and LWs did not differ in age, sex, BMI, race, marital status, education, or work status. There were no significant differences between HW+ and HW- in age, BMI, and sex, although HW+ tended to be older than HW- [U = 31.0, p<.06].

**Diagnoses.** Members of the HW group met criteria for the following current DSM-IV diagnoses: 9 had 1, and 1 had 2, mood disorders; 14 had 1 and 14 fulfilled criteria for more than 1 anxiety disorder (22 Generalized Anxiety Disorder); 3 met criteria for a somatoform disorder; 2 for an eating disorder; 24 met criteria for insomnia during the past month (11 had secondary insomnia with 8 of these (73%) attributing their insomnia to worrying). One LW was given a lifetime diagnosis of an eating disorder, and one LW was diagnosed with primary insomnia. The HW+ and HW- state worry groups did not differ in current anxiety, mood, somatoform, eating, or insomnia diagnoses.

**Cigarettes, medication, alcohol, and caffeine intake.** Twenty-four HWs reported drinking caffeinated beverages during the 24-hr monitoring compared with 13 LWs; none smoked cigarettes compared with 2 in the LW group; 5 drank alcohol compared with 1 LW. None of these differences was significant. There were no significant group differences in the reported intake of any category of medication during the 24-hr monitoring: in the HW group, 5 reported taking psychoactive drugs to relieve symptoms of anxiety or depression (1 GABA reuptake inhibitor, 2 Norepinephrine-dopamine reuptake inhibitors, 2 selective serotonin reuptake inhibitors, 3 serotonin-norepinephrine reuptake inhibitors), 2 took medication to improve sleep (1 zolpidem, 1 herbal), 2 took antipsychotics, 3 took pain medicine, 1 took allergy medicine, 7 took oral contraceptives, and 12 took other medications from other categories. In the LW group, 2 took contraceptives, and 1 another medication. The state worry groups (HW+ and HW-) did not differ significantly in the intake of medication, caffeine, or alcohol during the 24-hr monitoring.
Table 9. Sample characteristics

<table>
<thead>
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<th></th>
<th>HW (n=40)</th>
<th>LW (n=16)</th>
<th>Statistic</th>
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</tr>
<tr>
<td>Age</td>
<td>37.1 ± 11.7</td>
<td>36.1 ± 12.0</td>
<td>F(1,56) = 0.1, ns</td>
</tr>
<tr>
<td>Sex (females)</td>
<td>33 (83)</td>
<td>12 (75)</td>
<td>FET: p = 0.7, ns</td>
</tr>
<tr>
<td>BMI</td>
<td>24.0 ± 4.3</td>
<td>22.5 ± 2.4</td>
<td>U = 273.0, ns</td>
</tr>
<tr>
<td>Race</td>
<td></td>
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<tr>
<td>Whites</td>
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<td>11 (68.8)</td>
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</tr>
<tr>
<td>Asian</td>
<td>5 (12.5)</td>
<td>4 (25)</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Declined to report</td>
<td>4 (10)</td>
<td>1 (6.2)</td>
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<tr>
<td><strong>Marital status</strong></td>
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</tr>
<tr>
<td>Married or living with someone</td>
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<td>6 (37.5)</td>
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</tr>
<tr>
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<td>6 (15)</td>
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<tr>
<td>Separated</td>
<td>1 (2.5)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Never married</td>
<td>25 (62.5)</td>
<td>10 (62.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Education</strong></td>
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</tr>
<tr>
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<td>1 (6)</td>
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</tr>
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<td>2 (13)</td>
<td></td>
</tr>
<tr>
<td>2-yr college</td>
<td>2 (5)</td>
<td>1 (6)</td>
<td></td>
</tr>
<tr>
<td>4-yr college</td>
<td>14 (35)</td>
<td>4 (25)</td>
<td></td>
</tr>
<tr>
<td>Part graduate school</td>
<td>4 (10)</td>
<td>3 (19)</td>
<td></td>
</tr>
<tr>
<td>Graduate school</td>
<td>13 (33)</td>
<td>5 (31)</td>
<td></td>
</tr>
<tr>
<td><strong>Work status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full-time</td>
<td>23 (58)</td>
<td>7 (44)</td>
<td>FET: p=0.3, ns</td>
</tr>
<tr>
<td>Part-time</td>
<td>6 (15)</td>
<td>2 (13)</td>
<td></td>
</tr>
<tr>
<td>Unemployed</td>
<td>6 (15)</td>
<td>3 (19)</td>
<td></td>
</tr>
<tr>
<td>Retired</td>
<td>0 (0)</td>
<td>2 (13)</td>
<td></td>
</tr>
<tr>
<td>Student</td>
<td>4 (10)</td>
<td>2 (13)</td>
<td></td>
</tr>
<tr>
<td><strong>Questionnaires</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSWQ combined (16-80)</td>
<td>66.4 ± 6.8</td>
<td>28.7 ± 5.7</td>
<td>F(1,56) = 381.4***</td>
</tr>
<tr>
<td>PSQI total score (0-21)</td>
<td>7.7 ± 3.4</td>
<td>2.8 ± 1.8</td>
<td>U(56) = 51.5***</td>
</tr>
</tbody>
</table>

Note: Values are expressed as mean ± standard deviation or number (%) for the demographic variables, and as median ± interquartile range for the self-report measures. Group sizes vary for the self-report measures due to missing data.

Abbreviations. HW = high worriers, LW = low worriers; ns = not significant, ** = p-value<.01, *** = p-value <.001; FET = Fisher’s Exact Test; U = Mann-Whitney U-Test; BMI = Body Mass Index; PSWQ combined = Penn State Worry Questionnaire (scores averaged from phone screening and questionnaire packet), PSQI = Pittsburgh Sleep Quality Index.

6.3.2 Trait worry groups (HWs vs. LWs)

On the PSQI, HWs reported longer SOL, shorter total sleep time and lower sleep quality during the past month than LWs (Table 10). PSQI daytime dysfunction was also significantly higher in HWs (7.0 ± 1.3) than LWs [9.0 ± 1.5; U=128.5, p<.001, g=1.5]. In the sleep interview, more HWs than LWs met quantitative criteria for insomnia as recommended by Lichstein and colleagues (Lichstein, Durrence, Taylor, Bush, & Riedel, 2003) on difficulty
falling asleep (13 vs. 0; FET p<.05), difficulty maintaining sleep (16 vs. 1; FET p<.05), and poor sleep quality (21 vs. 0; FET p<.05), whereas the number of participants with prolonged early morning awakenings was higher in HWs than in LWs (10 of 40 vs. 1 of 16), but this was not significant by the FET. During the 24-hr monitoring, HWs reported shorter total sleep time, lower sleep quality, and lower sleep efficiency compared to LWs. No significant differences between the groups were found during the monitoring in how tired and fatigued participants felt after waking up (g=0.3) or on any objective sleep measure (all g's<±0.5). However, on the first day of the monitoring HWs reported being more worried and more often that their heart was racing, which was corroborated by higher heart rates during both the day and the subsequent night (Table 11 and Figure 5). After sleep onset, LWs had steeper SCL deactivation [HWs: -0.0 ± 0.0, LWs: -0.1 ± 0.1; F(1,52)=7.3, p<.01, g=2.0] and their HR tended to decline uninterrupted for a longer period of time [HWs: 2.5 ± 1.5 min, LWs: 3.1 ± 2.1 min; F(1,52)=3.3, p<.08, g=0.4] than did HWs. During the night, LWs were more physically active than HWs.

Table 10. Self-reported trait (PSQI and sleep interview) and state (sleep log) sleep parameters in high worriers (HW) and low worriers (LW)

<table>
<thead>
<tr>
<th></th>
<th>HW (n=38)</th>
<th>LW (n=16)</th>
<th>U</th>
<th>g</th>
<th>HW (n=40)</th>
<th>LW (n=16)</th>
<th>F</th>
<th>g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time in bed (in min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>487.2 ± 80.2</td>
<td>501.3 ± 52.4</td>
<td>ns</td>
<td>-0.2</td>
<td></td>
<td>482.6 ± 59.3</td>
<td>500.2 ± 61.1</td>
<td>ns</td>
<td>-0.3</td>
</tr>
<tr>
<td><strong>SOL (in min)</strong></td>
<td>18.3 ± 13.6</td>
<td>8.9 ± 6.2</td>
<td></td>
<td>171.5*</td>
<td>20.6 ± 19.0</td>
<td>12.1 ± 10.8</td>
<td>ns*</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Nocturnal awakenings</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(number)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Nocturnal awakenings</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>duration (in min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Early morning awakening</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>duration (in min)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total sleep time</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(in min)</td>
<td>402.0 ± 66.0</td>
<td>456.0 ± 66.0</td>
<td>163.0***</td>
<td>-0.8</td>
<td>409.1 ± 79.1</td>
<td>466.6 ± 70.1</td>
<td>8.1**</td>
<td>-0.7</td>
</tr>
<tr>
<td><strong>Sleep efficiency (%)</strong></td>
<td>83.1 ± 15.9</td>
<td>90.3 ± 8.5</td>
<td>ns</td>
<td>-0.5</td>
<td>84.9 ± 12.8</td>
<td>93.0 ± 4.1</td>
<td>6.4*</td>
<td>-0.7</td>
</tr>
<tr>
<td><strong>Sleep quality (1-10)</strong></td>
<td>6.5 ± 2.0</td>
<td>9.0 ± 1.5</td>
<td>116.0***</td>
<td>1.3</td>
<td>5.1 ± 1.8</td>
<td>7.1 ± 1.8</td>
<td>13.9*</td>
<td>-1.1</td>
</tr>
</tbody>
</table>

**Note.** Values are expressed as mean ± standard deviation; 1 = PSQI subscale sleep quality (and daytime dysfunction in the text) was converted from a 0 to 3 scale (higher values indicating greater difficulty) to a 1 to 10 scale for better comparability with the sleep log result [new value = (4 - old value)*10/4];

**Abbreviations.** ns = not significant; * = p-value <.05, ** = p-value <.01, *** = p-value <.001, § = residuals not normally distributed (interpret results with caution; subsequent non-parametric U-test not significant); PSQI = Pittsburgh Sleep Quality Index, SOL = sleep-onset latency; g = Hedges’ g (effect size).
**Worried sleep: 24-hr monitoring in high and low worriers (study 3)**

Table 11. Effects of trait worry on physiological parameters and self-reported physiological arousal and worry in high worriers (HW) and low worriers (LW)

<table>
<thead>
<tr>
<th></th>
<th>HW (n=39 to 40)</th>
<th>LW (n=16)</th>
<th>F</th>
<th>g</th>
<th>HW (n=37 to 39)</th>
<th>LW (n=14 to 16)</th>
<th>F</th>
<th>g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SCL</strong></td>
<td>5.9 ± 3.0</td>
<td>5.9 ± 2.3</td>
<td>ns</td>
<td>0.0</td>
<td>2.5 ± 1.2</td>
<td>2.4 ± 1.3</td>
<td>ns</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>NSF rate</strong></td>
<td>2.7 ± 1.4</td>
<td>2.8 ± 1.5</td>
<td>ns</td>
<td>-0.1</td>
<td>0.5 ± 0.5</td>
<td>0.7 ± 0.7</td>
<td>ns</td>
<td>-0.4</td>
</tr>
<tr>
<td><strong>HR</strong></td>
<td>83.5 ± 9.3</td>
<td>76.1 ± 7.2</td>
<td>11.6**</td>
<td>0.8</td>
<td>66.2 ± 9.0</td>
<td>60.8 ± 5.2</td>
<td>8.6**</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>RMSSD</strong></td>
<td>26.9 ± 17.3</td>
<td>32.0 ± 14.4</td>
<td>ns</td>
<td>-0.3</td>
<td>39.6 ± 23.6</td>
<td>39.6 ± 18.2</td>
<td>ns</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>HF-HRV</strong></td>
<td>1570.7 ± 1970.0</td>
<td>1877.7 ± 2118.6</td>
<td>ns</td>
<td>-0.2</td>
<td>2622.3 ±</td>
<td>2232.1 ±</td>
<td>ns</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>ACT</strong></td>
<td>72.8 ± 33.6</td>
<td>66.6 ± 32.6</td>
<td>ns</td>
<td>0.2</td>
<td>1.8 ± 1.5</td>
<td>2.8 ± 2.2</td>
<td>4.6*</td>
<td>-0.6</td>
</tr>
<tr>
<td><strong>Worried</strong></td>
<td>3.1 ± 2.1</td>
<td>0.6 ± 1.0</td>
<td>21.3***</td>
<td>1.4</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><strong>Sweating</strong></td>
<td>0.8 ± 1.1</td>
<td>0.5 ± 0.8</td>
<td>ns&lt;sup&gt;§&lt;/sup&gt;</td>
<td>0.3</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td><strong>Heart racing or pounding</strong></td>
<td>1.4 ± 1.8</td>
<td>0.4 ± 1.1</td>
<td>ns&lt;sup&gt;§&lt;/sup&gt;</td>
<td>0.6</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

**Note.** Values are expressed as mean ± standard deviation; 1 = Sample sizes vary due to missing data.

**Abbreviations.** ns = not significant, * = p-value<.05, ** = p-value<.01, *** = p-value <.001, § = residuals not normally distributed (interpret results with caution; subsequent non-parametric U-test not significant unless indicated otherwise); SCL = skin conductance level, NSF = non-specific skin conductance fluctuations, HR = heart rate, RMSSD = root of the mean squared successive differences in the interbeat intervals, HF-HRV = high-frequency power of HR variability, ACT = physical activity.

![Figure 5. Mean heart rates across high worriers and low worriers for each 1-min epoch synchronized by sleep onset. At least 10 valid observations per group were required for included time epochs.](image-url)
6.3.3 State worry groups (HW+ vs. HW-)

HW+ and HW- did not differ in any of the subjective sleep measures (PSQI) or in how many HWS met quantitative criteria for insomnia during the past month (sleep interview), indicating no trait differences that could have moderated state sleeping difficulties during monitoring. On the night of monitoring HW+ reported longer WASO than HW- [46.2 ± 37.2 min vs. 16.4 ± 18.3 min; F(1,22)=6.6, p<0.5, g=1.0] and had lower sleep efficiency as measured by actigraphy [84.6 ± 12.3 % vs. 89.2 ± 4.4 %; F(1,22)=10.7, p<.01, g=0.5]. No other differences were found in subjective or objective sleep measures, or self-reported fatigue after waking up in the morning. During the first day, HW+ reported being more worried but that they sweated less (non-significant trend) and that their hearts raced less than HW- (Table 12). Both then and during the subsequent sleeping period, SCL and the number of NSFs were higher in the HW+ group. During the night, the HW+ group had a higher HR and lower RMSSD. The HW+ group had a shorter [11.4 ± 10.0 min vs. 21.7 ± 22.9 min; F(1,20)=4.8, p<.05, g=-0.5] and steeper decline in SCL [-0.05 ± 0.05 vs. -0.02 ± 0.02; F(1,19)=7.1, p<.05, g=-0.9] during the first two hours after sleep onset, while no differences in the duration or slope of heart rate decline were found.

Table 12. Effects of state worry on physiological parameters and self-reported physiological arousal and worry in high worriers who worried in bed before falling asleep, during the night, and after waking up in the morning (HW+) and those high worriers who did not worry in bed at all (HW-)

<table>
<thead>
<tr>
<th></th>
<th>Waking day 1</th>
<th>Sleeping</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HW+ (n= 9 to 10)</td>
<td>HW- (n= 11 to 12)</td>
<td>F</td>
<td>g</td>
<td>F</td>
</tr>
<tr>
<td>SCL</td>
<td>6.2 ± 3.6</td>
<td>5.3 ± 2.3</td>
<td>6.2*</td>
<td>0.3</td>
<td>3.1 ± 1.2</td>
</tr>
<tr>
<td>NSF rate</td>
<td>2.8 ± 1.9</td>
<td>2.5 ± 1.0</td>
<td>5.0*</td>
<td>0.2</td>
<td>0.5 ± 0.4</td>
</tr>
<tr>
<td>HR</td>
<td>85.1 ± 10.5</td>
<td>82.1 ± 8.8</td>
<td>ns</td>
<td>0.3</td>
<td>69.6 ± 8.5</td>
</tr>
<tr>
<td>RMSSD</td>
<td>18.8 ± 6.0</td>
<td>27.3 ± 12.3</td>
<td>ns</td>
<td>-0.9</td>
<td>25.4 ± 11.3</td>
</tr>
<tr>
<td>Hf HRV</td>
<td>690.7 ± 371.2</td>
<td>1438.6 ± 1550.5</td>
<td>ns</td>
<td>-0.6</td>
<td>1100.3 ± 811.9</td>
</tr>
<tr>
<td>ACT</td>
<td>78.0 ± 20.0</td>
<td>71.8 ± 35.8</td>
<td>ns</td>
<td>0.2</td>
<td>2.2 ± 2.4</td>
</tr>
</tbody>
</table>

|                  |                   |                  |                |                  |                |
| Worried          | 3.2 ± 1.7         | 1.9 ± 1.0        | 5.1*           | 0.9               | --             | --             | --     | --               |
| Sweating         | 0.2 ± 0.3         | 0.8 ± 1.1        | 3.9*           | -0.7              | --             | --             | --     | --               |
| Heart racing or pounding | 0.5 ± 0.8   | 1.5 ± 1.0        | 7.6*           | -1.1              | --             | --             | --     | --               |

Note. Values are expressed as mean ± standard deviation; 1 = Sample sizes vary due to missing data.
Abbreviations. ns = not significant, †= p-value<.07, * = p-value<.05, ** = p-value<.01, *** = p-value<.001; SCL = skin conductance level, NSF = non-specific skin conductance fluctuations, HR = heart rate, RMSSD = root of the mean squared successive differences in the interbeat intervals, Hf HRV = high-frequency heart rate variability, ACT = physical activity.
6.4 Discussion

As expected, high trait worriers (HWs) reported that during the past month they had difficulty falling asleep, staying asleep, shorter and lower quality sleep, and more daytime dysfunction. Not all of these were present on the night of the monitoring, however, where HWs reported similar SOL but lower sleep efficiency compared to LWs. Some non-significant effects were of medium size; in a larger LW sample they might have become significant. Interestingly, no differences between HWs and LWs in any of the objective sleep parameters could be found on the night of monitoring. Similarly, self-reported and objective electrodermal activity largely failed to distinguish HWs from LWs except for the slope of SCL decline during the first two hours after sleep onset. This slope was less steep in HWs indicating slower deactivation. This finding is consistent with Espie’s psychobiological model of insomnia (Espie, 2002) that posits a failure to “de-arouse” during the transition from wakefulness to bedtime as a cause of insomnia. While measures of heart rate variability did not differ between the groups, the HW group, however, had higher heart rates both during waking and the subsequent sleeping period, which could not be accounted for by greater body movement. These results indicate cardiovascular hyperarousal during both waking and sleep, which is in accordance with previous studies (Hoehn-Saric, et al., 1989; Knepp & Friedman, 2007; Thayer, et al., 1996) and partly replicates the findings of a previous ambulatory study in patients with Generalized Anxiety Disorder (Hoehn-Saric, et al., 2004). Yet, several laboratory studies failed to find such differences during baseline recordings (Borkovec, et al., 1983; Hoehn-Saric, et al., 1989; Wilhelm, et al., 2001). Inconsistency between reported results in some cases could have resulted from assessing only trait worry rather than state worry in bed at the time of testing. Yet finding higher HR in HWs than in LWs would not be unexpected since the majority of HWs (n=28) worried at least once in bed and only a few HWs did not worry in bed at all (n=12).

We examined the effect of state worry in separate analyses including only HWs who worried whenever lying awake in bed and those who did not worry in bed at all. There were no significant differences between these two HW subgroups on any of the habitual subjective sleep parameters or the PSWQ, indicating that the occurrence of state worrying in high trait worriers while being monitored is rather a matter of chance. Differences in sleep
or arousal observed on the night of the monitoring also must depend on factors other than the severity of trait worry or the severity of sleep disturbance in the past month.

Worrying in bed led to longer self-reported wake time after sleep onset (WASO) and lower actigraphic sleep efficiency. Consistent with higher autonomic arousal, worrying in bed was associated with higher heart rates (HR) and lower HR variability (HRV measured as RMSSD) during sleep and with elevated skin conductance levels (SCL) and higher rates of non-specific SC fluctuations (NSFs). These differences cannot be explained by longer WASO or more body movement, since data during nocturnal awakenings were excluded and the effects of body movement were statistically accounted for by including it as a covariate. While awake, HWs who would later worry in bed did not yet have higher HRs but did show elevated electrodermal activity and reported increased levels of worrying. However, inconsistent with autonomic hyperarousal, they tended to report less sweating and perceived less heart racing and pounding than those HWs who later would not worry in bed.

Overall these findings indicate that both trait and state worry affect sleep and physiological arousal in a synergistic manner that fits Spielman’s “3 P model” of insomnia (1986). Following this model, trait worry would serve as a predisposing factor which by itself cannot cause insomnia but can make a person more vulnerable to it. State worrying on the other hand, might be regarded as a precipitating factor that coincides with the insomnia onset and makes it apparent. Worrying about sleeplessness as a specific type of worrying might work as a perpetuating factor in the model which will fuel and maintain a transient insomnia and turn it into a persistent insomnia.

This study was limited in some ways. First, the number of LWs in this sample was rather low and might have reduced the representativeness of their data. By being ambiguous about our worry severity cut-offs during the phone screening, we wanted to avoid including volunteers who reported their worry symptoms to be less severe than they really were in order to be eligible and be paid for participation. Second, sleep evaluation with full polysomnography would have increased the validity of the results but had the disadvantage of possibly overwhelming our worried volunteers and reducing participation and compliance. Ambulatory monitoring with fewer, less intrusive, data channels as we did increases the ecological validity of the results, since standard polysomnographic recordings in the laboratory create an atypical sleep environment. Some participants in the HW group
were included in the sample although they taking medications, that in some studies may have slightly increased heart rate and decreased respiratory sinus arrhythmia (Licht, et al., 2009; Licht, et al., 2008). Excluding anyone on any medication in a group wanting treatment for excess worry would leave an atypical sample, and for that reason we included some volunteers taking non-tricyclic antidepressants but controlled for any effects by including medication use as a confounding variable in the statistical analyses.

In summary, high trait worriers showed evidence of subjective sleep disturbance both during the past month and on the night of the monitoring which were in part exacerbated by bedtime worrying. Cardiovascular arousal seemed both affected by trait and state worrying while electrodermal arousal only varied with state worrying. Future research should carefully distinguish between trait and state worry and their distinct effects on sleep.
7 Summary of main findings

7.1 Methodological considerations in ambulatory skin conductance monitoring (study 1)

The first study was aimed at investigating how much ambulatory skin conductance (SC) recordings from the fingers are affected by confounding factors such as electrode site deterioration, ambient temperature (TMP), or physical activity (ACT), or by age, sex, race, or body mass index. We intended to repeat the observations of Turpin et al. (1983) with a larger number of normal subjects, longer recording times, and a wider variety of SC variability measures. SC, TMP, and ACT were recorded in 48 healthy control subjects for a 24-hour period, and SC level (SCL), its standard deviation, the coefficient of SC variation, and frequency and amplitude of non-specific SC fluctuations (NSFs) were calculated.

One method of assessing electrode site deterioration (comparing readings from old electrodes with those from electrodes freshly applied after 24 h of recording) showed an average decline of 20% in SCL, while a second method (comparing readings from 15 min epochs at beginning and after 24 h of recording) found no significant change in either SCL or any of the SC variability measures after 24 h of ambulatory SC recording. As expected, all SC measures were higher during waking than sleep. Two clusters of SC measures emerged which were highly inter-correlated and responded similarly to confounding variables at the between-subjects level: SCL, SC standard deviation, and the number of NSFs on the one hand (cluster A), and the amplitude of NSFs and the coefficient of SC variation on the other (cluster B). Ambient temperature affected only the number of NSFs (part of cluster A) at a between-subjects level. Within subjects, ambient temperature was positively correlated with all SC measures during both recording days but not at night. Unexpectedly, similar results were obtained for physical activity except that it also exhibited positive correlations during sleep. Age and BMI were mostly negatively related to SC measures from cluster A and men had lower cluster B values. Caucasians scored higher on cluster A than Asians, but lower on cluster B measures. After the effects of confounding variables were removed at the between-subjects level, SC measures residuals were not related to self-reported trait depression or anxiety, state excitement or sleepiness on the days of testing, or trait and state subjective sleep quality in this sample of healthy controls.
Thus, 24-hour SC recording outside the laboratory is feasible without encountering issues of electrode site deterioration, but measures need to be corrected for the influence of confounding variables—some more than others—especially when looking at within-subject relationships. The associations with self-report measures may need to be investigated in clinically more diverse samples with more variability in these measures than was present in this sample of healthy controls, to detect significant variation with physiological measures.

7.2 Study 2: Twenty-four hour skin conductance in panic disorder

In the second study, we looked at the relationship between anticipatory anxiety and electrodermal activity in a clinical sample. Skin conductance, physical activity, ambient temperature, and mood were recorded ambulatorily for 24 hours in 22 panic disorder (PD) patients and 29 healthy controls. During the day, subjects performed standardized relaxation tests (ARTs). We hypothesized that tonically elevated anticipatory anxiety in PD during waking and sleeping would appear as elevated skin conductance level (SCL) and greater skin conductance (SC) variability.

PD patients exhibited higher mean SCL and marginally higher mean numbers of NSFs during both usual waking activities and sleeping, but not during the ARTs. SC variability in the form of MSSD did not differ between groups, perhaps because of variance unrelated to anxiety. Further analyses indicated that in the PD group, antidepressant medication reduced mean SCL whereas state anxiety and panic attacks during the day increased it, although the latter medium-sized effect was not significant. More agoraphobic symptoms were associated with lower mean SCL (non-significant medium-sized effect) — possibly because PD patients avoided anxiety-provoking situations during the monitoring. Daytime depressive symptoms were related to elevated mean SCL on the night of the recording. The rate and extent of SCL deactivation over the night was equal in the two groups. However, PD patients had more frequent interruptions of deactivation that could have arisen from conditioned arousal in response to threat cues during sleep.

In summary, anticipatory anxiety and panic are associated with heightened electrodermal arousal that is reduced by antidepressant medication and agoraphobic symptoms. Comorbid depressive symptoms elevate arousal at night.
7.3 Study 3: Worried sleep. 24-hr monitoring in high and low worriers

Chronic worrying and sleep complaints are frequently reported by individuals suffering from anxiety disorders. Thus, the third study investigated worrying and its effects on cardiac and electrodermal arousal and sleep. Electrocardiographic interbeat intervals, skin conductance, ambient temperature, and body movement were recorded for 24 h in high (HW) and low worriers (LW) who maintained a log of worrying and sleep characteristics.

Relative to LW, HW had higher heart rates (HR) during waking and sleeping, which was corroborated by more frequent reports of heart racing and pounding during the day. HW reported shorter total sleep time, lower sleep efficiency, and poorer sleep quality both during the previous month and on the recording night. Self-reported sleep-onset latency was longer in HW compared to LW during the past month but not on the night of the monitoring. Among HW, worry in bed on the night of the recording was associated with longer self-reported wake time after sleep onset, lower actigraphic sleep efficiency, elevated electrodermal arousal (mean SCL and number of NSFs), higher HR, and lower HR variability compared to those who did not worry in bed on the night of the recording – even after wake times during bed were removed from the physiological data.

Thus, both trait and state worry influence cardiac arousal and sleep, while electrodermal arousal seems only to be influenced by state worry.
8 General discussion

8.1 Integrating our main findings

The study of worry, anxiety, and panic has been mostly confined to the laboratory where high internal validity is achieved at the cost of poor ecological validity. As a complementary approach, ambulatory monitoring may remedy this situation.

8.1.1 Implications for long-term ambulatory monitoring

In our first study (chapter 4), we found that long-term electrodermal monitoring is feasible with little to no electrode site deterioration due to epidermal hydration over the course of 24 hours. This result shows that the use of wet electrodes for periods of at least 24 hours can still yield valid results which has been questioned in the literature (Searle & Kirkup, 2000). It remains to be shown, however, if that is still the case for longer assessment periods and how these will affect both the accuracy of the data and the comfort of the research participant. From our own experience with wet electrodes in electrodermal measurements, we find it highly doubtful that longer recording periods will be acceptable to the participants unless these periods include intermittent change of electrode sites or electrode-free intervals.

Inferences about (defensive) emotional states may be drawn from the physiological data when possible confounding influences have been taken into account. Chapter 4 investigated the influences of different confounding variables and found that they affected various skin conductance level and variability measures differentially, and that in the case of ambient temperature and physical activity this influence seemed to increase with growing resolution of the data. Diurnal variations in electrodermal arousal have been found in all three studies and study 3 (chapter 6) also indicated cardiac arousal changes with sleep (see Figure 5) although this observation was not tested statistically. Chapter 5 found that antidepressant medication reduced SCL which is consistent with previous findings (Siepmann, et al., 2003). All of these results underline the importance of controlling for such confounding effects and also that this control can be achieved by entering these variables as covariates into mixed model analyses as previously advocated (Bagiella, et al., 2000; Bolger, et al., 2003; Wilhelm & Grossman, 2010).
Another methodological consideration pertains to the choice of self-report medium in ambulatory monitoring. Studies 1 and 2 (chapters 4 and 5) solely relied on paper-pencil diaries and exhibited a lot of missing data. While this may have been prevented by more careful checking of the returned logs and requests to fill in the missing data, this kind of back-filling of diary entries would have reduced their usefulness to that of other retrospective methods (Piasecki, et al., 2007). Study 3 (chapter 6) therefore used electronic diaries which produced almost no missing data and because entries were time-stamped their momentary character ( ecological momentary assessment) could be verified.

8.1.2 Autonomic arousal in worry, anxiety, and panic (during waking and sleeping)

Ambulatory monitoring showed that PD patients had chronically elevated sympathetic arousal as indicated by electrodermal arousal measures both during the day and at night compared to non-anxious volunteers. This elevated EDA was positively associated with state anxiety and panic within PD patients. Furthermore, state worrying in bed at night produced similar electrodermal results during sleep in HW compared to LW. It would be interesting to know whether HW and PD patients can be placed on a continuum of increasing EDA as has been suggested (see section 2.1.3.1). Indeed, SCL during waking and sleeping was higher in PD patients than HW but we are unable to statistically compare the EDA results of the two groups (see section 8.2.6). Our results indicate, however, that state worry, anxiety, and panic are accompanied by heightened EDA. If worrying is considered a pre-encounter response and anxiety a post-encounter response (Craske, 2003), they can at least partially conform with Lang and colleagues’ defense cascade model (1997) which predicts elevations in EDA with ratings of increased subjective arousal in response to a perceived aversive stimulus (see Figure 2). They are also in line with Fowles (2000) who argued that increased EDA specifically reflected anxiety anticipating future threat.

Fowles (2000) also proposed that HR reflected fear of current threat. Unfortunately, cardiac results are only available from study 3 where both trait worry and state worry in bed were associated with higher HR and the latter also with lower HRV. HW even reported higher HR during waking. Together with the increases in EDA that we found, these findings are contrary to the common notion that worrying is accompanied by an inhibition of autonomic arousal (Borkovec, et al., 2004) and instead favors the perseverative cognition.
hypothesis (Brosschot, et al., 2006). This hypothesis states that worrying as a perseverative cognition about the source of anxiety prolongs or even exacerbates negative affect and concomitant heightened autonomic activation.

In summary, these results show that both worrying and anticipatory anxiety are associated with increased sympathetic and (in the case of worry) reduced parasympathetic arousal. Interestingly, PD patients also reported being more worried than non-anxious controls, indicating that the boundaries between different defensive states are blurred and/or that research participants may not be aware of their scientific distinction.

8.1.3 Autonomic arousal during sleep

In all three studies, measures of autonomic arousal differed between waking and sleeping. After sleep onset, PD patients and HW had more frequent interruptions in the downward descent of SCL measures than controls. Despite the increased EDA during sleep in PD patients compared to non-anxious controls, there were no differences in reported sleep duration, number of nocturnal awakenings, the restfulness of sleep, or daytime sleepiness. But the large number of missing data on paper-pencil diaries limits the generalizability of these results. Both trait and state worrying were associated with sleeping difficulties during the past month and on night of monitoring that were accompanied by abnormal sympathetic and parasympathetic arousal during sleep. This abnormal autonomic arousal during sleep in both highly anxious PD patients and HW might point to the usefulness of autonomic measures in exploring these defensive emotional states (or traits). Autonomic measures might be able to add to the traditional study of sleep using PSG.

8.2 Methodological limitations

The three ambulatory monitoring studies presented here were limited in several ways that will be described in the following.

8.2.1 Sample size

Samples sizes in all three studies were relatively small, especially in the low worrier sample of study 3. Reasons include issues of recruitment (being involved in a 24-h experiment is a rather long commitment that not everyone was willing to undertake) and equipment failure. Low sample sizes limited our analyses in several ways by reducing statistical power, not allowing us to test for interactions between different independent and
confounding variables, and restricting the representativeness of our results. To estimate the impact of low subject numbers on our results, we instead calculated effect sizes in all three studies and found that on several occasions marginally significant effects were of medium size (such as the number of NSFs in study 2) and would have been significant in a larger sample.

8.2.2 Additional physiological channels

The first two studies only measured electrodermal and physical activity. The assessment of emotional activation and concomitant autonomic arousal was thus limited to sympathetic arousal. Additional physiological channels such as heart rate and heart rate variability were added in the third study and improved our understanding of the differentiated action of autonomic arousal related to emotion. Technological advances make multichannel recording less and less cumbersome and intrusive and further channels such as respiratory variability, and end-tidal pCO₂ might have informed us whether the electrodermal and/ or cardiac findings were part of a general pattern of emotional activation. PSG during sleep is still considered the gold standard in sleep assessment and might have increased the validity – both internal and ecological – of our results. Ambulatory PSG might be preferable to standard PSG recordings in the laboratory as it is performed in the subject’s natural sleep environment. However, more channels have the disadvantage of possibly overwhelming study volunteers and reducing participation and compliance. Long-term ambulatory monitoring with fewer, less intrusive, data channels increases the ecological validity of the results as participants might forget that they are being monitored.

8.2.3 Internal vs. external validity

As discussed in section 2.4, the advantages of ecologically valid ambulatory physiological measures must be weighed against the imperfect control of disturbing factors such as activating interpersonal interactions. Better control of these factors might have allowed additional group effects to emerge in studies 2 and 3.

8.2.4 Medication use

Restricting our sample to subjects not taking any medications would have excluded their possible interfering effects but would have impaired the representativeness of any findings, since in developed countries unmedicated patients can be an atypical subgroup. Apparently, the antidepressants taken by our PD patients lowered their SCLs without
producing a clinical remission in panic. In some studies, these medications have been shown to increase heart rate and decrease respiratory sinus arrhythmia (Licht, et al., 2009; Licht, et al., 2008). Excluding anyone on any medication would leave an atypical sample, and for that reason we included some volunteers taking non-tricyclic antidepressants but controlled for any effects by including medication use as a confounding variable in the statistical analyses. We did not do blood or urine testing, however, to confirm patients’ medication use, which might have revealed reporting failures.

8.2.5 Missing self-report data on paper-pencil diaries

Self-report diaries in the first two studies were restricted to paper-pencil diaries that turned out to have a lot of missing data. This data loss may have reduced the power of statistical analyses and the representativeness of self-report data. As discussed in section 2.4.3.4, electronic diaries have several advantages over traditional paper-pencil diaries, which is why we turned to them for the third study.

8.2.6 Comparing across anxiety continuum

Direct comparisons between PD patients and high worriers would have been interesting as they may have told us more about the nature of the anxiety continuum according to Craske’s (2003) view of the threat proximity continuum (see section 2.1.2). This kind of comparison was not possible, however, because different equipment was used in these separate studies, which rendered the actigraphy data incompatible.

8.3 Errata

Study 1 (chapter 4): The first citation in the second paragraph of the discussion section should read: Johnson & Lubin, 1966 instead of Koumans et al., 1968.
9 Outlook and future research

Technical advances in ambulatory monitoring technology and equipment will in time overcome some of the obstacles we have encountered in our studies. Research effort should particularly be put into the development of devices that (a) limit the necessity of electrode cables and focus on wireless technology, (b) are lighter and less obtrusive, (c) are compatible with disposable dry electrodes or sensing fabrics (Fletcher et al., 2010; Valenza et al., 2010), (d) directly interface with electronic self-report devices allowing for more complex data entries beyond signal buttons. Unobtrusive devices will allow for the simultaneous recording of data from multiple physiological, behavioral, and self-report channels without interfering with a person’s normal routine. Unaware of being monitored, behavior will not be modified to accommodate the device, to avoid equipment damage or embarrassment. This will also facilitate recruitment efforts and lead to larger subject samples with more internally and ecologically valid data. The future of self-report lies in electronic diaries which, in the age of smart phones, research participants are becoming more and more familiar with. They will eliminate some of the disadvantages associated with paper-pencil diaries. Especially in the field of long-term electrodermal monitoring, the development of unobtrusive dry electrodes or sensing fabrics with high fidelity will allow for longer recording periods, thus increasing the generalizability of results. With such equipment at hand, ambulatory monitoring will reach its full potential.

Areas of application for ambulatory psychophysiological monitoring are numerous. In a clinical setting, for example, ambulatory blood pressure monitoring has been used to diagnose a variety of hyper- and hypotension conditions, such as white-coat hypertension, for a few decades already (Kanbay, Turkmen, Ecder, & Covic, 2011). Other autonomic indices, like EDA, have been applied in the study of attention outside of healthcare where they might be used in novel human-computer interaction paradigms, such as physiologically-triggered bookmarking in audio books, for example (Pan et al., 2011).

Future ambulatory monitoring studies in the field of defensive emotional states should directly compare worry, anxiety, fear, and panic also during sleep. To the author’s knowledge, there is only one ambulatory monitoring study which compared PD and GAD patients during the day (Hoehn-Saric, et al., 2004) and showed increasing cardiac arousal in
PD compared with GAD, possibly indicating different degrees of autonomic abnormalities along a threat proximity continuum (Craske, 2003). Yet, this study was limited to a few intermittent recording epochs during waking. Our ambulatory studies are a first step in the direction of longer continuous multi-channel recordings that include sleep as well. Future investigations should aim to obtain data from even longer periods of time, preferably several days.
10 References


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Versicherung

Erklärung gemäß § 5 (1) Punkt 5 der Promotionsordnung der Fakultät Mathematik und Naturwissenschaften der Technischen Universität Dresden

Die vorliegende Arbeit

“Ambulatory monitoring of electrodermal and cardiac functioning in anxiety and worry“

wurde an der Stanford University School of Medicine unter der Betreuung von Prof. Dr. Walton T. Roth und der Professur für Biopsychologie im Fachbereich Psychologie der Technischen Universität Dresden unter der Betreuung von Prof. Dr. Clemens Kirschbaum angefertigt.

Hiermit versichere ich, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe; die aus fremden Quellen direkt oder indirekt übernommenen Gedanken sind als solche kenntlich gemacht.

Die Arbeit wurde bisher weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt.

Amberg, den 25.07.2011

(Sigrun Doberenz)