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“Effects of sleep on cardiovascular responses during aversive classical conditioning”

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ACC ..... *anterior cingulate cortex*
ANS ..... *autonomic nervous system*
ARC ..... *activity-regulated cytoskeleton-associated gene*
ASC ..... *Allgemeine Schlafcharakterisierung*
BS ..... *boredom susceptibility*
CAMK4 ..... *calmodulin-dependent protein kinase IV*
CAN ..... *central autonomic network*
CR ..... *conditioned response*
CS ..... *conditioned stimulus*
df ..... *degrees of freedom*
Dis ..... *disinhibition*
DSS ..... *Durchschlafschwierigkeiten*
ECG ..... *electrocardiogram*
EEG ..... *electroencephalography*
EMG ..... *electromyography*
EOG ..... *electrooculography*
ERP ..... *event-related potentials*
ES ..... *experience seeking*
ESS ..... *Einschlafschwierigkeiten*
FFT ..... *fast Fourier transform*
fMRI ..... *functional magnetic resonance imaging*
GABA ..... *gamma-Aminobutyric acid*
GES ..... *Gefühl des Erholtseins nach dem Schlaf*
GSD ..... *Gesamtschlafdauer*
HF ..... *high frequency*
HR ..... *heart rate*
HRD ..... *heart rate deceleration*
HRV ..... *heart frequence variability*
IEG ..... *immediate early gene*
ISI ..... *interstimulus intervals*
LF ..... *low frequency*
LTD ..... *long-term depression*
LTP ..... *long-term potentiation*
MI ..... *myocardial infarction*
NA ..... *negative affect*
NEO-FFI ..... *NEO-five factor inventory*
NMDA ..... *N-methyl-D-aspartate*
OR ..... *orienting reflex*
PA ..... *positive affect*
PCC ..... *posterior cingulate cortex*
PET ..... *positron emission tomography*
PGO ..... *ponto-geniculo-occipital*
pNN50 ..... *proportion of successive beat-to-beat intervals that differ by more than 50 ms divided by the total number of beat-to-beat intervals*
PNS ..... *parasympathetic nervous system*
PPG ..... *photoplethysmograph*
PSD ..... *power spectral density*

PSS ..... *Psychosomatische Symptome in der Schlafphase*
PSYA ..... *Psychische Ausgeglichenheit vor dem Schlaf*
PSYE ..... *Psychisches Erschöpftsein vor dem Schlaf*
PTSD ..... *post-traumatic stress disorder*
PVT ..... *psychomotor vigilance task*
PWA ..... *pulse wave amplitude*
RAAS ..... *renin-angiotensin-aldosterone system*
REM ..... *rapid eye movement*
RM ..... *repeated measures*
RMSSD ..... *root mean square of successive differences*
RSA ..... *respiratory sinus arryhthmia*
SAM ..... *self-assessment manikins*
SCR ..... *skin conductance response*
SD ..... *standard deviation*
SDNN ..... *standard deviation of beat-to-beat intervals*
SF-A/R ..... *Schlaffragebogen-A/revised*
SNS ..... *sympathetic nervous system*
SOA ..... *stimulus-onset-asynchrony*
SQ ..... *sleep quality, sleep quality*
SSS ..... *sensation seeking scale*
STAI ..... *state-trait-anxiety inventory*
SW-R ..... *sharp wave-ripples*
SWS ..... *slow wave sleep*
TAS ..... *thrill and adventure seeking*
UR ..... *unconditioned response*
US ..... *unconditioned stimulus*
VLF ..... *very low frequency*
VLPO ..... *ventral lateral hypothalamic preoptic nucleus*
vmPFC ..... *ventromedial prefrontal cortex*
VZA ..... *vorzeitiges Aufwachen*

1 Introduction

In the following study we investigated human aversive conditioning, comparing conditioned cardiovascular responses, in particular heart rate (HR), heart rate variability (HRV) and the pulse wave amplitude (PWA), before and after a 2-hour-sleep interval and before and after a 2-hour interval of wakefulness on two separate experimental days. We examined behavioural measures, emotional state, vigilance, and sleep quality measures during the experiment. We also tested the relationship between anxiety-related personality traits and conditioned responses. The following presentation provides an overview of the theoretical background and research concerning this study, ending with a brief summary of the setup and the aim of the experiments performed.

1.1 Aversive conditioning

Human conditioned reflex was initially discovered by Edwin B. Twitmyer (1873-1943) at the University of Pennsylvania during his dissertation work on the "knee-jerk" reflex. The participants in this study were asked to verbalize the word "ah" or clench their fists in order to increase the reflex. A bell was struck as a signal to start verbalizing or clenching just before the patellar tendon was tapped. Twitmyer noticed that after repeats a knee-jerk could be provoked by simply ringing the bell without the following tap (Twitmyer, 1905). He performed follow-up studies but psychologists in America did not take much notice of these findings at the time (Clark, 2004).

Meanwhile in Russia, Ivan Petrovich Pavlov (1849-1936) discovered the same phenomenon during research on the salivary glands of dogs. His original work involved implanting fistulas in the oesophagus of dogs. He noticed that these dogs still produced saliva whilst eating, although the food never actually reached the stomach (Pavlov & Thompson, 1902). For his work on the digestive glands he was awarded with the 1904 Nobel Prize in Physiology and Medicine ("The Nobel Prize in Physiology or Medicine 1904," n.d.). The original term for his findings was "psychic secretion", combining his observations with the suspected mental process behind these findings. In the following years he built on these findings which eventually resulted in his famous work on "conditioned reflexes" from 1927 (Clark, 2004; Pavlov, 1927). In psychology the phenomenon is known as classical conditioning or Pavlovian conditioning.

A simple classical conditioning setup consists of several phases. In acquisition there is an unconditioned stimulus (US), an unconditioned response (UR) and a conditioned stimulus (CS). The US is a stimulus that does not need to be learned and causes a UR according to inborn, genetically fixed mechanisms, e.g., food (US) causes salivation (UR) in dogs. The CS is an initially neutral stimulus which is consistently paired with the US (e.g., bell ringing). Through this pairing, a connection between the CS and the US is created. After conditioning, the CS can be presented without the US, causing a conditioned response (CR) which is identical to the UR (when the bell rings, the dog salivates, even if no food is presented). Finally, if the CS is continuously presented without the US, eventually there will no longer be a CR. This phase is called extinction (Lonsdorf et al., 2017)

In the case of aversive conditioning – a subtype of classical conditioning – the US is an aversive stimulus which can cause a UR, fear. Therefore, it is also commonly known as fear conditioning. The US is paired with a neutral CS. During conditioning an association is formed between the US and the CS. Eventually, fear learning takes place, and the CS alone elicits a CR. Aversive conditioning is seen as one of the leading evolutionary mechanisms for animals, teaching them to fear other animals, places or objects or situations which can be necessary for survival. One of the most famous experiments of aversive conditioning was a study on “Little Albert”, conducted by John Watson and Rosalie Rayner in 1920. Albert, a young child, was conditioned to fear a rat, in this case the CS, using an aversive noise as a US (Watson & Rayner, 1920). Although impressive from a scientific point of view, a study of this kind would not be accepted by a modern ethical committee, as the child was in great distress by the end of the experiment (Maren 2001). In recent years there has been an increasing amount of research on aversive conditioning, with a focus on the neurobiological mechanisms involved and its use for understanding pathological conditions such as phobias and post-traumatic stress disorder.

The development of fear or anxiety disorders has been related to aversive conditioning. Panic disorder is believed to be a product of classical conditioning, leading to a fear of fear (Wolpe & Rowan, 1988). According to Keane et al., 1985, post-traumatic stress disorders (PTSD) may also derive from conditioning. It is hypothesized that during an initial traumatic event, a person may become conditioned to various stimuli which were

present in the situation. Stimulus generalization across other stimuli and avoidance of the fear-generating stimuli induces PTSD, leading to a higher level of anxiety in general (Cahill & Foa, 2007; Grillon & Morgan III, 1999; Keane et al., 1985). Specific phobias are also thought to be a result of Pavlovian conditioning, although a specific CS-US pairing often cannot be found (Reiss, 1980). There is evidence that anxiety patients display stronger conditioned responses to aversive conditioning both during acquisition and extinction (Lissek, Powers, et al., 2005). They seem unable to inhibit their feared memories (Davis et al., 2000).

On the other hand, treatment of these disorders is also often based on conditioning. Classical conditioning is the basis of modern behavioural therapy (Franks, 1969; Wolpe & Lazarus, 1966), as well as operant conditioning, the method of learning as first described by B. F. Skinner, in which a behaviour is either reinforced or punished, with the conditioned subject changing its behaviour in response to these consequences (Skinner, 1938).

1.2 Methodology of aversive conditioning studies

In aversive conditioning studies, conditioned stimulus types used are mostly auditory tones, olfactory or visual cues. Visual CSs include pictures of coloured lights, geometric shapes (Meulders et al., 2012), human faces, or animals (Hermans et al., 2002; Lonsdorf et al., 2017). As a US, most commonly tactile electrical stimulation is used. The intensity of the stimulation is usually calibrated to the individual level of discomfort during the stimulation, choosing a point at which the stimulus is “unpleasant, but not painful” (Lipp, 2006). The level of tolerated stimulation has large interindividual variation. Also, from an ethical point of view, using highly aversive US especially in groups like children and severely disabled individuals is problematic (Kotchoubey & Pavlov, 2017; Pavlov & Kotchoubey, 2019). Therefore auditory stimuli provide a good alternative to electrical stimulation, including loud tones (as done in this study), white noise or more complex aversive sounds like human screams (Büchel et al., 1998; Glenn et al., 2012; Hamm et al., 1989). Many other methods such as air-blows to the larynx, painful heat stimuli, painful rectal distension etc. have been tested with different results (Lonsdorf et al., 2017).

Outcome measures of conditioned response in aversive conditioning research mainly focus on (1) verbal reports of the subjects, (2) behavioural changes or (3) physiological changes, with both central and peripheral (vegetative) indices. Verbal reports often use rating scales to quantify the intensity of each stimulus or of the fear (Hermans et al., 2002). Questionnaires can be used to analyse the extent to which the participants have learned the CS-US relationship (Dawson & Reardon, 1973; Lipp, 2006). Common behavioural measures include approach tests, measuring the distance between the participant and the feared object or the time spent near the object (Craske et al., 2003). Attentional bias studies, often using dot probe tasks, show that anxious participants usually focus their attention on more threatening stimuli (Lipp, 2006; Mogg & Bradley, 1999).

Physiological or neurobiological outcome measures are very useful, since they can be measured at the same time the learning occurs and they cannot be consciously influenced in the same way as the previously mentioned methods (Lipp, 2006). Electrodermal activity is a very common method used during aversive conditioning. Also known as the skin conductance response, it measures the resistance of the skin, which is controlled by the sympathetic nervous system. An emotional reaction leads to a sympathetic response of the sweat glands, reducing the electrical resistance of the skin (Dawson et al., 2007). The cardiovascular response towards conditioning is also a sensitive parameter. The simplest method is to examine the heart rate, acquired by counting the number of heart beats per minute. Usually, the UR to a US is the brief acceleration of the heart rate, signalling a defence reaction of the body. The CR to a CS-US-pairing typically results in a brief acceleration of the HR whilst awaiting the US, followed by a deceleration (Brownley et al., 2000; Lipp, 2006). Other cardiovascular measurements include heart rate variability and pulse wave amplitude, as discussed in the following chapters. The mean eye blink amplitude during anticipation of an aversive electrotactile US is potentiated compared to times where an aversive US is not anticipated (Davis et al., 1993). Eye blink, measured with an electromyography (EMG) of the orbicularis oculi muscle, is a reliable method to measure the fear-potentiated startle response during human aversive conditioning.

With the help of positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), the neuronal fear network of the brain has been examined

more closely in recent years. In a systematic review of neuroimaging studies examining human aversive conditioning, Sehlmeyer et al. (2009) narrowed down three main fear-related brain areas which are activated during aversive conditioning; (1) the amygdala, (2) the anterior cingulate cortex (ACC) and (3) the insular cortex. Activation of these areas is particularly strong in patients with anxiety disorders (Etkin & Wager, 2007). These regions are parts of the "core" fear network in response to negative stimuli (Mechias et al., 2010). Also, there is evidence of functional deactivations during aversive conditioning. Deactivation of the ventromedial prefrontal cortex (vmPFC) and the posterior cingulate cortex (PCC), in particular, may be interpreted as a neural correlate of the "safety" signal towards a CS- (Fullana et al., 2016; LaBar et al., 1998). Recently, electroencephalography (EEG) has also been introduced to analyse the effect of aversive conditioning. Averaging techniques of EEG such as event-related potentials (ERP) have been used to elucidate direct responses of the cortical activity towards conditioning (Miskovic & Keil, 2012).

1.3 Cardiovascular conditioned responses

1.3.1 Heart rate and heart rate variability

Measuring heart rate (HR) and heart rate variability (HRV) is a method which predates many of today's modern techniques to examine the cardiovascular system. The first books on the pulse originate from about 2500 B.C., attributed to the Chinese Emperor Hoamti, who is said to have been very skilled in the art of feeling the pulse (Bedford, 1951). With the invention of a pulse watch in 1707 the Rev. Stephen Hales was able to note that heart rate varied with respiration (Hales, 1733). Carl Ludwig noticed oscillations of the heart beat during respiration, a phenomenon now known as respiratory sinus arrhythmia (RSA) (Ludwig, 1847). In the second half of the 20th century, the development of modern equipment to analyse cardiovascular parameters resulted in a growing number of studies on HRV (Billman, 2011).

HRV measures the variation of the heart's beat-to-beat intervals, more specifically the amount of heart rate fluctuation around the mean HR. In modern days HRV is measured with common beat detectors like the electrocardiograph (ECG) or a photoplethysmograph (PPG). HR alone is a non-stationary signal which changes in time and therefore might not provide a reliable reflection of the physiological status of the

participant; whereas HRV is a more stable parameter which can directly reflect the changes of the heart beat in response to stimuli (Rajendra Acharya et al., 2006). The heart rhythm is regulated by the autonomic nervous system (ANS) consisting of a parasympathetic (PNS) and a sympathetic system (SNS) as well as other systems such as the renin-angiotensin-aldosterone system (RAAS). Heart rate is under tonic inhibitory control via the parasympathetic vagal nerve (Levy, 1990). Working together, these systems are able to regulate the cardiovascular system rapidly (Akselrod et al., 1981). Therefore, the HRV provides an insight not only in the change of heart rate in time but also the function of the whole cardiovascular system. The polyvagal theory (Porges, 2001) also proposes a close relationship between the heart and the autonomic nervous system. According to the theory, RSA or HRV describes the amount of outflow from the vagal nerve to the heart. Thus, the cardiac vagal tone can be examined by measuring the HRV of the subject.

The most common ways to analyse HRV are time domain and frequency domain methods. Time domain methods are the simplest way of measuring HRV. SDNN, the standard deviation of NN or beat-to-beat intervals, provides an easily-calculated estimate of the overall HRV. Other time domain indices are based on the comparison between successive beats, including the often used root mean square of successive differences (RMSSD) or the proportion of successive NNs that differ by more than 50 ms divided by the total number of NNs (pNN50) (Bilchick & Berger, 2006; Malik et al., 1996).

Using frequency domain methods, HRV can be divided into specific frequency components which, according to some studies, may allow an assessment of the ANS function. Since the first spectral analysis on the frequency of heart rate fluctuations in the 1970s, three distinct spectrums have been identified for the HRV (Saykrs, 1973). Akselrod et al. identified a low frequency (LF) band between 0.04 – 0.15 Hz, which is modulated by both sympathetic and parasympathetic nervous systems, and a high frequency (HF) band between 0.15 – 0.40 Hz, which is regulated by the parasympathetic system only. They achieved their results by performing both a parasympathetic as well as a complete autonomic blockage with the adequate medication (Akselrod et al., 1981). The HF band mainly consists of the vagally mediated oscillations due to RSA (Bilchick & Berger, 2006). During inspiration, the HR

accelerates due to inhibition of the parasympathetic vagal nerve. During expiration, HR decelerates due to increased vagal activity, particularly by stimulating the acetylcholine-rich sinus node of the heart (Grossman & Kollai, 1993). The very low frequency (VLF) band, with a frequency range of <0.04 Hz, is influenced by many factors, including the level of neurohormones, blood volume status or cardiac pump competence (Saul, 1990). Fast Fourier transform (FFT) is used for power spectral density (PSD) estimation by converting the time domain of the HR data into frequency domain (Welch, 1967).

There have been many clinical studies examining the interplay between HRV and cardiologic or endocrinologic disorders. In general, a dysfunctional system is less capable of adapting to changing conditions. Therefore a healthy heart has a higher HRV (Thayer et al., 2012). A number of studies showed that post-myocardial infarction (MI) patients have a decreased HRV (Carney et al., 2001). Vagal activity is reduced after a MI. Regular measurements of HRV might be useful for the estimation of post-MI patients' health situation (Rothschild et al., 1988; Schwartz et al., 1992). HRV is also lower in patients with diabetic neuropathy. RSA of these patients is diminished due to reduced vagal activity (Pfeifer et al., 1982). A reduction of the spectral power of both PNS and SNS can be observed in patients with chronic renal failure, especially those undergoing dialysis (Axelrod et al., 1987). Reduction of all frequencies, but mainly the frequencies >0.04 Hz, can also be observed in patients with congestive heart failure (Saul et al., 1988). Smoking impairs the ANS function, reducing HRV (Hayano et al., 1990).

Research on HRV also plays a growing role in recent psychology and neurology studies. Patients with severe brain damage show a decreased HRV, further aggravated by rising intracranial pressure (Lowensohn et al., 1977). Lower HRV is also common in patients with depression and anxiety disorders (Gillie & Thayer, 2014). HRV is seen as an index to measure stress vulnerability in general. Regulated predominantly by the PNS, a lower HRV is a sign of PNS dysfunctionality (Porges, 2001). HRV can also be used as a tool for predicting emotional behaviour (Appelhans & Luecken, 2006). Performing aversive conditioning experiments has enabled a deeper understanding of the mechanisms behind psychological disorders, as described in the following chapter.

As early as 1867, the great French physiologist Claude Bernard proposed a connection between the heart and the brain (Bernard, 1867). This connection has been investigated during numerous aversive conditioning studies. Results suggest an inhibitory pathway leading from the ventromedial prefrontal cortex to the amygdala. In addition to its major role in fear learning, the central nucleus of the amygdala is also involved in the regulation of cardiovascular, autonomic, and endocrine responses. Through further switching points in the brainstem, an activation of the prefrontal cortical region leads to an inhibition of sympathetic action, resulting in a higher HRV and a lower HR. Reduced activity of the pathways from the prefrontal cortex to the amygdala or the brainstem leads to a disinhibition of the SNS and a reduced HRV and higher HR, as can be found in patients with fear disorders (Thayer et al., 2012; Thayer & Lane, 2009; Wendt et al., 2015). Patients with higher HRV can adapt to the conditions of aversive conditioning more easily. During learning, their initial startle response decreases faster. They also learn faster to recognize stimuli signalling safety. Finally, fear extinction is learned more rapidly (Pappens et al., 2014). Patients with lower HRV struggle to inhibit their defensive reaction to aversive stimuli once activated, especially when the stimuli are unpredictable (Gorka et al., 2013). They show a stronger startle response, including potentiated startle responses to neutral and positive stimuli (Ruiz-Padial et al., 2003). Their startle response shows a delayed decrease compared to high-HRV-patients. Fear extinction as well as fear inhibition is delayed. Strengthening of the cortico-cardiac pathway could help patients with anxiety, e.g. with mental training (Pappens et al., 2014; Wendt et al., 2015).

Direct responses of the cardiovascular system towards aversive stimuli have also been extensively examined. Subjects' conditioned cardiac responses cluster according to three patterns: Accelerators, moderate decelerators and decelerators (Hare, 1972; Hodes et al., 1985). Accelerators show a transient rise of HR following an aversive stimulus, whereas decelerators' HR falls after the same stimulus. It is proposed that, apart from learning that two events are related to each other, accelerators also build up a defensive response to the CS. They also tend to find the aversive stimulus more unpleasant than decelerators do. Their learning effect may be interpreted as a sensory rejection. Decelerators, on the other hand, do not build up any sort of defensive response, they are only aware of the contingency. They have an impaired startle conditioning as well as an

enhanced conditioning of distress (Sevenster et al., 2015). The lowering of the heart beat is a part of an orienting response with an increased awareness of the conditioning (Hamm & Vaitl, 1996; Hare, 1972; Hodes et al., 1985). The response can therefore be seen as a sign of sensory intake or a "transient detecting response" (Bradley, 2009).

1.3.2 Pulse wave amplitude

The pulse wave amplitude is defined as the peak-to-peak difference between the highest amplitude value and the lowest amplitude value of each pulse wave as measured with photoplethysmography. It is a newer, less commonly used, measure which provides additional information on individuals' cardiovascular state.

In general, a decrease in PWA is usually linked to vasoconstriction of the peripheral blood vessels as a part of a sympathetic activation. An increase marks vasodilatation and parasympathetic activation. Research on the orienting reflex (OR) shows that PWA decreases as a part of the response to the stimulus. When the stimulus is presented several times, PWA response is habituated and the drops become less intense (Johnson & Lubin, 1967). PWA also drops as a response to stressful stimuli, such as heat-induced pain or headache (Velzen et al., 2015). It is similar to other peripheral measures of the body like skin conductance, which also decreases during arousal (Hamm et al., 1989). Smoking a single cigarette also decreases PWA as a result of vasoconstriction (Saumet et al., 1986).

1.4 Sleep and memory: definition

Sleep is a state of the body in which animals show a reduced responsiveness to stimuli, a reduced muscle activity and a loss of consciousness. It is repeated periodically and is regulated through an internal circadian clock, combining peripheral information from the body with central nervous mechanisms as well as external factors such as sunlight (Aschoff, 1965). Metabolic, immunologic and other bodily functions can be restored during sleep, but probably could also be adjusted during quiet wakefulness (Knutson et al., 2007; Lange et al., 2010). The main functions of sleep are therefore assumed to be processing or modification of memory and synaptic plasticity as well as detoxication of the brain (Inoué et al., 1995; Rasch & Born, 2013; Tononi & Cirelli, 2006).

Sleep can be subdivided into two neurophysiological stages, simply described as rapid eye movement (REM) sleep and non-REM sleep. Non-REM sleep includes light sleep

stages 1+2 and deep sleep stages 3+4, also named slow wave sleep (SWS). During a typical night's sleep, the first half of the night is mainly filled with SWS, followed by the second half, mainly filled with REM-sleep (Iber et al., 2007; Rechtschaffen, 1968). EEG activity during SWS is characterized through high-amplitude slow oscillations, disrupted by high-frequency spindles and sharp wave-ripples (SW-R) (Rasch & Born, 2013). Cholinergic activity as well as cortisone levels are low during SWS. EEG during REM-sleep shows lower-amplitude, faster theta activity and ponto-geniculo-occipital (PGO) waves. It is more like EEG during wakefulness. Acetylcholine levels and cortisone levels are higher than during SWS and are also similar to wake periods (Diekelmann & Born, 2010).

Memory can be split into several stages: (1) acquisition, (2) consolidation, subdivided into stabilization, enhancement and integration, and, if necessary, (3) retrieval (Abel & Lattal, 2001). Human memory is generally believed to have three stores working in a sequence: (1) sensory memory for initial control of incoming information which degrades within several seconds, (2) short-term memory, sometimes known as working memory, which stores information for seconds to minutes and relies on repeated recalling to be stored in the (3) long-term memory, which can be retained indefinitely (Atkinson & Shiffrin, 1968). Memory types can also be distinguished: (1) declarative memory includes a) memory of autobiographical events, the episodic memory; as well as b) the memory of general facts acquired during life known as the semantic memory. (2) Non-declarative memory includes procedural memory, memory for skills that can be demonstrated without consciously thinking about the underlying process, e.g. walking. Both memory types can be trained implicitly (without being aware of what is learned) or explicitly (being aware of what is learned), although declarative memory is mostly taught explicitly and procedural memory implicitly (Diekelmann & Born, 2010; Graf & Schacter, 1985; Tulving, 1972). In the case of declarative memory, the hippocampus is the fast learning store whereas the neocortex is the long-term storage. This theory is supported by lesion studies, such as the study on the patient HM with bilateral hippocampus lesions, who was unable to form new memories. Implicit memory is not affected by temporal lesions (Corkin, 2002).

1.5 Sleep and memory: mechanisms

Originally, sleep was thought to merely play a passive role in maintaining memory by sheltering the mind from interference (Jenkins & Dallenbach, 1924). Through growing research on sleep and memory it has been shown that sleep plays an active role in the processing of memory in that newly acquired neuronal pathways are retraced. As described by Rasch and Born, 2013, there are three major mechanisms for processing of memory during sleep which will be explained in the following: (1) the dual process hypothesis, (2) the sequential hypothesis and (3) the active system consolidation hypothesis. There is evidence that sleep-dependent memory consolidation is supported by all three mechanisms and that they are not necessarily mutually exclusive.

The dual process hypothesis maintains that different sleep stages are of use for different types of memory. Hippocampus-dependent declarative memory is thought to be consolidated during SWS, whereas procedural and implicit memory profit from REM sleep (Plihal & Born, 1997). Emotional declarative memories seem to profit from both sleep stages (Wagner et al., 2002).

The sequential hypothesis states that all memory types depend on both sleep stages taking place in an alternating rhythm. SWS in believed to weaken non-adaptive memory traces leaving the remaining memory traces to be consolidated during REM sleep (Giuditta et al., 1995). Learning may depend on the amount of SWS-REM-cycles and memory may be weakened if REM sleep does not follow SWS (Langella et al., 1992). This concept is also supported by the synaptic homeostasis hypothesis assuming that slow waves of SWS downscale the synaptic strength of all connections, nullifying weak connections and leaving the newly learned potentiated synapses to be consolidated during REM sleep (Tononi & Cirelli, 2006).

Finally, the active system consolidation hypothesis states that memory is initially stored in both the short-term hippocampal store and the long-term neocortical store. During SWS, slow oscillations control the repeated reactivation of the hippocampal store and potentiates memory (Marshall et al., 2006). Spindles and SW-R drive the memory into the neocortex, their activity is associated with reactivation of previously learned memories (Bergmann et al., 2012; Ego-Stengel & Wilson, 2010). REM sleep then may stabilize the consolidation process through theta activity and PGO waves (Rasch &

Born, 2013). A shift from hippocampal activity to neocortical activity can be seen on fMRI during memory tests performed before and after sleep (Takashima et al., 2009).

Ultimately, sleeping promotes the formation of new memories leading to a change in the synaptic plasticity. Long-term potentiation (LTP) and long-term depression (LTD) are mechanisms leading to an enhanced or decreased postsynaptic calcium release, respectively, changing local neuronal activity and regulating gene transcription (Cooke & Bliss, 2006). LTP relies on N-methyl-D-aspartate (NMDA) receptors and others; blocking of these during sleep will impair learning (Aton et al., 2009). Since inhibition of gamma-Aminobutyric acid (GABA) receptors contributes to synaptic plasticity, administration of GABA agonists (benzodiazepines) leads to impairment of LTP (Del Cerro et al., 1992).

The neurotransmitter acetylcholine also plays a role in memory formation during sleep. Rasch and Born, 2013, provide a good overview of the topic. As mentioned above, during SWS acetylcholine levels are low; during REM sleep they are higher and similar to levels during wakefulness. Low levels are thought to enable reactivations of the hippocampal networks and help with transformation to the neocortex (Hasselmo, 1999). REM sleep relies on higher levels to improve memory, possibly by inducing the expression of immediate early genes (IEGs) such as the activity-regulated cytoskeleton-associated gene (ARC). Therefore, blocking cholinergic receptors during REM sleep impairs learning (Legault et al., 2006).

Gene expression, mediated by LTP and LTD, is also known to change during post-learning sleep. Sleep deprivation decreases protein levels in the cerebral cortex (Ding et al., 2004). The expression of calmodulin-dependent protein kinase IV (CAMK4) is upregulated during sleep. This gene is known to be involved in synaptic plasticity and long-term memory (Ahn et al., 1999).

1.6 Sleep and emotional learning

Emotional material is memorized more easily than neutral material and is more resistant to forgetting. It is clear that sleep consolidates memory in general, but there is an ongoing discussion about whether sleep has an additional effect on the consolidation of emotional memory compared to neutral material (Lipinska et al., 2019).

In their 2008 study, Payne et al. (2008) showed that memory of emotional scenes was better preserved following a 12-hour night time sleep compared to a 12-hour day time period spent awake, whereas neutral background objects were forgotten in the same term (Payne et al., 2008). In the second study of these authors, two groups were tested 24 hours after learning. The sleep first group, that slept soon after learning, had superior memory for emotional objects compared to the wake first group, that went to sleep 16 hours post-learning. Participants who were tested 12 hours after learning, directly following their 12-hour sleep, also had a better memory, indicating that the sleep itself had a beneficial impact on emotional learning (Payne et al., 2012). A number of studies came to similar conclusions (e.g., Hu et al., 2006). Even a short nap has a benefit on emotional memory compared to staying awake, which was equivalent to the effect of a whole nights' sleep (Payne et al., 2015).

A large number of studies examined the effect of sleep deprivation on emotional learning. Sleep deprivation may affect emotional memories in two different ways. Firstly, the dysregulation model proposes that sleep deprivation actually amplifies responses to negative emotional stimuli (Yoo et al., 2007). This is due to a suppression of amygdala activity, which reduces the ability to regulate emotions (Motomura et al., 2013). Individuals fail to inhibit a learned response to negative stimuli (Anderson & Platten, 2011). Subjective threat expectancies were also enhanced following sleep deprivation in a recent study (Zenses et al., 2019).

Secondly, according to the fatigue model, sleep loss causes a decrease in arousal and emotional intensity since the desire to sleep interferes with motivation and energy to perform (Engle-Friedman, 2014). Sleep deprivation lowers responses to both negative and positive stimuli, suggesting a general reduction in emotional reactivity (Minkel et al., 2011; Schwarz et al., 2013). This increased sleepiness causes a higher intensity of negative moods (Haack & Mullington, 2005). There does not seem to be any emotional benefit of sleep loss, neither in healthy nor in mentally ill patients. An exception may be sleep deprivation following trauma since this may prevent the development of PTSD (Qureshi et al., 2019).

Sleep seems to play a greater role in hippocampal-dependent tasks. In the context of aversive conditioning, contextual fear, which relies on the hippocampal function to

analyse more complex events such as spatial memory, is influenced more than hippocampus-independent cued fear, which mainly relies on the amygdala (Graves et al., 2003; Hagewoud et al., 2010; Hamann, 2001; Phillips & LeDoux, 1992). As a possible explanation, the ventral hippocampus projects to the ventral lateral hypothalamic preoptic nucleus (VLPO) following contextual fear conditioning. The VLPO mediates non-REM sleep and thus may be responsible for an increase in non-REM sleep following contextual fear conditioning (Hellman & Abel, 2007). REM-sleep, on the other hand, also improves fear memory retrieval through synchronized theta oscillations between amygdala and hippocampus (Narayanan et al., 2007). The amount of REM sleep correlated positively with the consolidation of negative objects, probably due to a stronger activation of the amygdala (Payne et al., 2012).

The recall interval for emotional memories is also important. Associations learned under low arousal conditions exhibited high immediate recall, but rapid forgetting. High-arousal associates, on the other hand, exhibited lower immediate recall but higher permanent memory (Kleinsmith & Kaplan, 1964). Higher arousal induces higher amygdala activity, influencing long-term consolidation (McGaugh, 2004).

Not all studies conclude that sleep has an additional benefit for emotional memory, though. A 2019 meta-analysis showed no overall effect for preferential sleep-dependent consolidation of emotional over neutral material. Negative material was memorized better than neutral material after both sleep and wake periods. Positive material was not memorized better than neutral material. Combined positive and negative material had a particularly strong effect on memory, but also with no difference between wake and sleep periods (Lipinska et al., 2019).

In summary, although there is inconsistent existing literature, sleep is believed to be crucial for the consolidation of emotional memory, especially when hippocampus-dependent tasks are involved. REM sleep and Non-REM-sleep both play a role in the consolidation of these memories. The exact mechanisms will be the subject of further research.

1.7 Sleep and conditioned fear responses

The following studies have analysed the effect of sleep on conditioned fear responses using different measures, similar to our study.

Skin conductance response (SCR) is often measured for aversive conditioning paradigms. In an aversive conditioning experiment, skin conductance responses were enhanced after sleep compared to wakefulness during a recall of the aversive CS, indicating an improved consolidation of fearful memories through sleep (Menz et al., 2013). Particularly the second, REM-sleep dominated, half of the night promoted the discrimination between fear-relevant and neutral stimuli for SCRs (Menz et al., 2016). In another study measuring SCR, fear memory was conditioned for two stimuli, accompanied by the extinction procedure for one of the two stimuli. Interestingly, participants also showed a diminished response to the unextinguished stimulus after sleep, suggesting that sleep promotes a generalization of extinction of conditioned fear (Pace-Schott et al., 2009).

In another study by Pace-Schott et al., the effect of a daytime nap on inter-session habituation to aversive visual stimuli was investigated. The setup of the experiment was quite similar to ours. SCR to repeatedly presented highly aversive stimuli showed a greater inter-session habituation following a 2-hour nap compared to a 2 hour period of waking. This was also the case for corrugator EMG responses. EMG responses were augmented in the wake group but not in the sleep group (Pace-Schott et al., 2011). Cardiovascular responses were also analysed and will be presented in chapter 1.8.

In an fMRI study, a sleep group showed an enhanced activation of the basolateral amygdala during a recall of an aversive conditioning task compared to the wakefulness group. The consolidation strength correlated with the amount of REM sleep, during which the amygdala is activated (Menz et al., 2013).

Hagewoud et al, 2011, examined endocrine and neuronal responses in rats. Re-exposure of a group of rats to a conditioned aversive stimulus after sleep deprivation elicited a diminished adrenal corticosterone response to the stimulus compared to a non-sleep-deprived group. An immunohistochemical analysis also showed a reduced activation of typical transcription factors in the amygdala and hippocampus (Hagewoud et al., 2011).

1.8 Sleep and cardiovascular responses

General studies on HRV during sleep show that the spectral power is higher during REM-sleep compared to non-REM-sleep, particularly the LF component (regulated by the PNS and the SNS) and the VLF component (regulated by hormonal mechanisms

such as the RAAS system and others) (e.g., Rajendra Acharya et al., 2006). Conversely, SWS is associated with an increase of the HF component indicating increased parasympathetic influence (Takahashi et al., 1998).

Sleep deprivation leads to an impairment of emotional regulation, as previously mentioned in chapter 1.6, and also decreases HRV (Gruber & Cassoff, 2014). HRV correlates negatively with the amount of sleep disturbances in patients with panic disorder (Hovland et al., 2013). A decrease in HRV is seen as a sign of an imbalanced emotional system or as a result of sleep loss.

As mentioned in chapter 1.3.2, a PWA decrease is linked to vasoconstriction of the peripheral blood vessels as a part of sympathetic activation. This is the case in REM sleep that is associated with a peripheral vasoconstriction, marking a sympathetic activation (Lavie et al., 2000). Sleep studies on patients with obstructive sleep apnoea syndrome showed an initial PWA increase at the beginning of an obstructive event, as a sign of vasodilatation. This is followed by an intense vasoconstriction, leading to PWA reaching its lowest overall values about ten cardiac cycles after the end of an event (Haba-Rubio et al., 2005; Ramirez et al., 2013).

All kinds of disturbances can cause a PWA drop during sleep, such as auditory tones (Catcheside et al., 2002). The PWA decrease as a component of the OR as mentioned in chapter 1.3.2 is also particularly intense when the stimulus is presented during REM sleep (Johnson & Lubin, 1967). Recurring PWA drops can also be measured during undisturbed sleep - especially during REM sleep - marking a change in cortical activity with significant increases in EEG power density in all EEG frequency bands (Delessert et al., 2010).

Pace-Schott et al, 2011, examined the habituation of heart rate deceleration (HRD) during presentation of aversive stimuli before and after a 2-hour nap and wake period, respectively. The 2-hour nap actually prevented the habituation of HRD, leading to a lower HRD in the wake group, but not in the sleep group.

1.9 Setup and aim of the current study

In the following study we analysed the effect of sleep on the conditioning of heart rate, heart rate variability and pulse wave amplitude during a classical conditioning setting

with aversive unconditioned stimuli. We also analysed emotional reactions towards the stimuli. After initial aversive conditioning, one group of participants was permitted to sleep for 2 hours. The second group stayed awake for the same time period. After this period, the aversive conditioning task was performed again.

The measures we extracted can be categorized as follows:

(1) Cardiovascular measures (HR, HRV, PWA)

We believe that cardiovascular responses to sleep or wakefulness were never investigated in the framework of aversive conditioning. Our aim was to see whether sleep would modify the response to the aversive conditioning in comparison to wakefulness, and in what way the response would be altered. From previous studies, we expected HR to decrease and HRV to increase for the sleep group, since this would indicate a potentiated learning effect of the aversive stimulus during sleep and as a result, an enhanced adaption to the aversive stimulus and better emotional regulation during post-sleep conditioning.

(2) Self-assessment measures (arousal, valence)

The aim of the self-assessment measures was to analyse the changes in emotional reaction, especially towards the conditioned stimuli, between the three blocks of each procedure, as well as the general changes between each test time. We expected behavioural measures to change for the sleep group compared to the wake group, showing that sleep has a modifying effect on behavioural memory.

(3) Emotional state measures (STAI state, PANAS)

The emotional state measures aimed at testing whether aversive conditioning influences state anxiety according to the State-Trait Anxiety Inventory (STAI) – the temporary state of nervousness or discomfort at the exact moment of the questionnaire – as well as the current positive and negative emotions according to the Positive and Negative Affect Schedule (PANAS) of the participants. We expected positive emotions to increase after a 2-hour nap, but to decrease as a result of conditioning, and wanted to analyse whether the intervention had a modifying effect on the measures.

(4) Vigilance measures (Stanford Sleepiness, Vigilance Test)

The aim of the vigilance tests was to gain knowledge of the current state of sleepiness (Stanford sleep scale) and vigilance (Vigilance Test) and be able to see which effect the intervention had on the participants' attention. We expected higher vigilance following a sleep period.

(5) Personality traits (STAI trait, SSS, NEO-FFI)

Personality questionnaires were analysed to see whether there was a correlation between participants' day to day trait anxiety, their sensation seeking levels, their neuroticism levels and the results of the self-assessment measures. A higher general level of stress or anxiety as measured with the STAI trait questionnaire and the neuroticism scale of the Big Five questionnaire was expected to correlate positively with the intensity of reactions in the conditioning experiment. Also, we expected to observe a weaker emotional response to US in individuals who scored higher in the Sensation Seeking Scale (SSS).

(6) Sleep measures (SF-A/R)

For the sleep questionnaires, we wanted to see whether aversive conditioning would change sleep quality or sleep time on the day after the experiment and whether there was a difference between the sleep day and the control day.

2 Methods

2.1 Participants

The study included 18 participants with an average age of 24.7 ± 3.18 years. A total of 9 female and 9 male participants were acquired through a student email distributor and notices in the local area. All but 2 participants were students at the University of Tübingen. No subjects reported any physical or psychological disabilities, and no medication was taken that might have impaired test performance. All participants were non-smokers. All participants but one were right-handed. All participants were German native speakers. Three participants (P02, P08, P09) were excluded from the PPG analysis due to incomplete or incorrect recording data, resulting in a group of 15 participants for the analysis. For the PWA data only, P12 was also excluded from the analysis for the same reason, resulting in a group of 14 participants for this analysis.

The study was approved by the Ethical Committee of the University of Tübingen (project number 417/2014B01). All participants gave written consent and were paid for their involvement in the study.

2.2 Procedure

Each participant visited the laboratory for a total of three afternoons. During the first "adaptation" day, participants answered several questionnaires and underwent a number of experiments that were not analysed in this dissertation. Also, participants had a two-hour-nap to adapt to sleeping in a new environment. Separated by at least one week, the second and third days then took place, which consisted of a "sleep" and a "control" day, respectively. The order of these days was counterbalanced between participants. The tests performed on these two days were identical; the only difference was the intervention: on sleep days, participants were permitted to sleep for 2 hours between the tasks. On control days, participants stayed awake for the same time period whilst watching a silent film or playing a computer game. The activities changed every 30 min. Electroencephalography (EEG), electromyography (EMG) and electrooculography (EOG) data were collected to assure that participants were, indeed, asleep on the sleep day and did not sleep on the control day. On both days, participants arrived at 1:00 pm, were seated in a comfortable chair and attached to the physiological monitoring.

General design of the experiment is depicted in Figure 1.

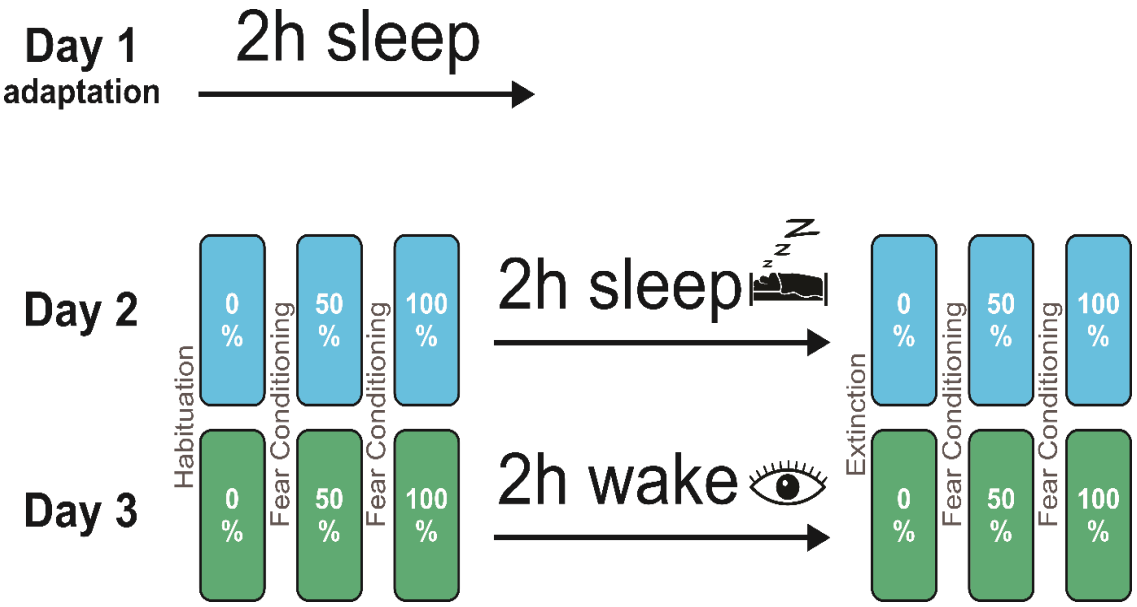

Figure 1 – General design of the experiment

Before and after the intervention (sleep/wakefulness) the participants underwent an aversive conditioning experiment, as well as a second experiment which was not analysed in this dissertation. During the aversive conditioning experiment, they were asked to stay awake, sit still, and attentively listen to the stimuli with closed eyes. All the stimuli were presented auditorily. Participants heard three harmonic tones presented by means of pneumatic earphones (3M E-A-RTONE). One of them (Standard) consisted of five frequencies with the main frequency (f1) randomly chosen between 320 and 500 Hz, and f2 = f1*2, f3 = f2*2, f4 = f3*2, and f5 = f4*2. The other two tones were referred to as Deviant 1 and Deviant 2. One of the deviant tones had the main frequency 25% higher, and the other one 25% lower than main frequency of the standard tone. The frequency assignment was random.

The duration of the standard tone and the deviant tones was 100 ms and 400 ms respectively, with 5 ms fade-in and fade-out. One of the deviants, randomly selected, was presented to the left ear and the other one was presented to the right ear.

The deviant tones played the roles of conditioned stimuli (CS+ and CS-) paired with an aversive unconditioned stimulus (US+) and neutral sound (US-, mildly pleasant). The stimulus-onset-asynchrony (SOA) between CS and US was fixed to 500 ms. SOA for unpaired CSs was 800±50 ms. The aversive stimulus was a 1000 Hz sine tone embedded into a burst of white noise (95 dB, 500 ms). Initially, the US+ was presented 3 times and participants were asked to score the sound on arousal and valence scales (SAM). Afterwards, three blocks of 280 standard, 60 CS+ and 60 CS- stimuli were presented with different reinforcement rates (0%, 50% and 100%). Thus, CS+ and CS- were not paired with any US in the first block. In the second block, half of the CS+ and CS- were paired with their US+/-. In the final block, all CS+ and CS- were paired with their US+/-. After each block, each stimulus was presented individually once again and participants rated them using the SAM. The same task was repeated identically after the intervention. For an overview, see Figure 2.

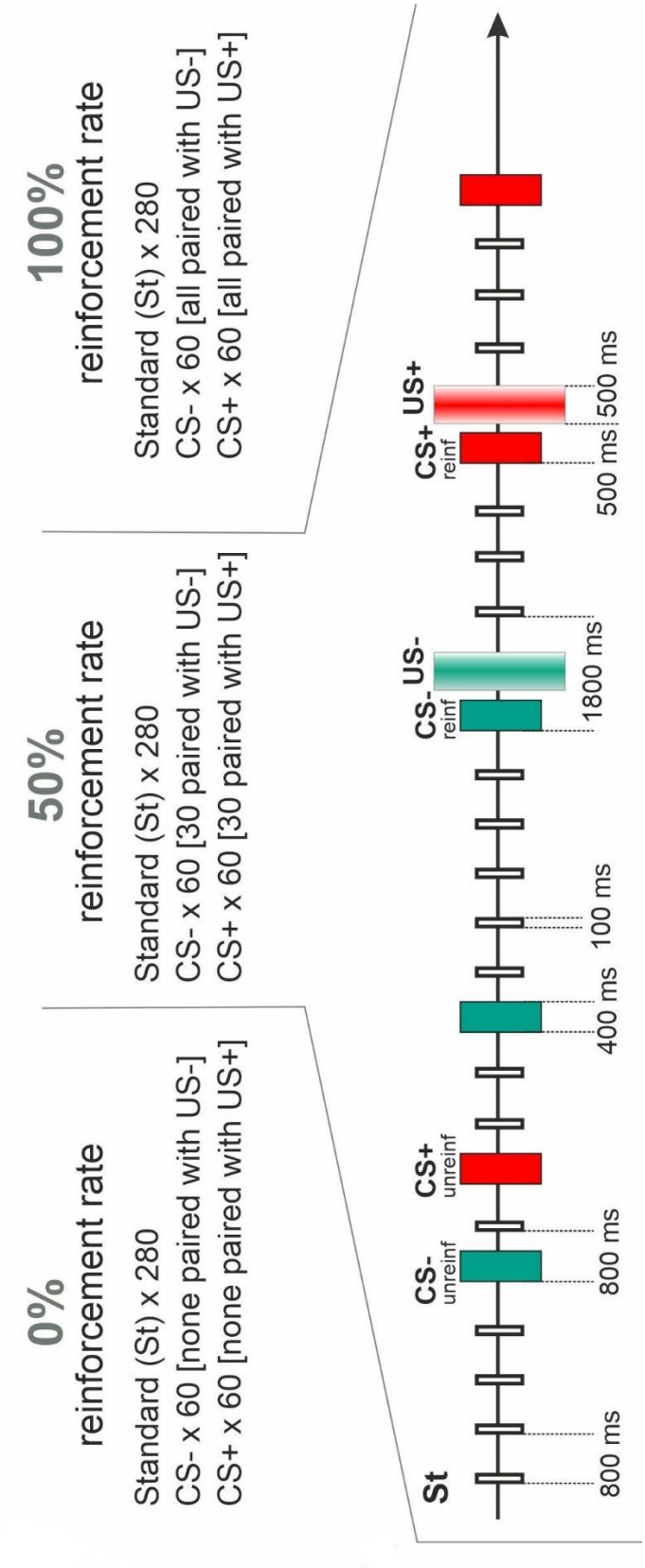

Figure 2 – above: presented sounds for each block during aversive conditioning experiment, below: stimulus onset-asynchrony (SOA) and duration of presented sounds

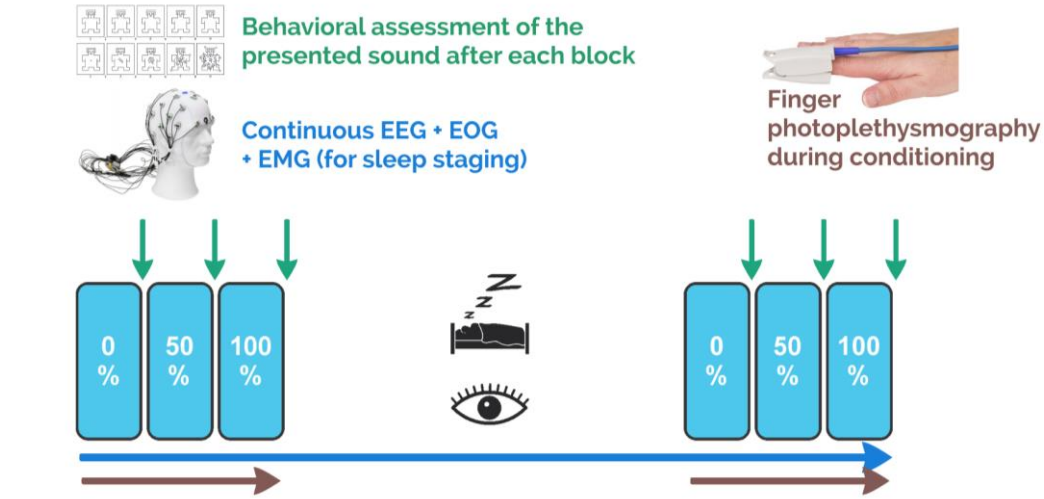

Figure 3 – apparatus used during the procedure and behavioural assessment times

2.3 Cardiovascular measures

The pulse wave was measured continuously from finger plethysmography during all experiments. Three measures were extracted from the data:

(1) Heart rate (HR): number of systolic maximum pulse wave amplitudes, representing the number of heart beats per minute
(2) Heart rate variability (HRV): the amount of variability around the heart rate
(3) Pulse wave amplitude (PWA): the mean amplitude between systolic maximum and minimum of each pulse wave

The pulse wave form was generated with MATLAB (MATLAB and Statistics Toolbox Release 2018b, The MathWorks, Inc., Natick, Massachusetts, United States). The data from each conditioning block was extracted and a high-pass filter of 0,5 Hz was applied using the EEGLAB MATLAB Toolbox (Delorme & Makeig, 2004). The MATLAB App HRVTool v1.03 (Marcus Vollmer, University of Greifswald, Germany, 2019) was used to analyse mean HR, HRV and PWA for each conditioning block. Mean HR and mean PWA over each conditioning block was calculated. The root mean square of successive differences (RMSSD) was used as a common and simple time domain

measure of HRV, recommended in the international guidelines (Malik et al., 1996). PWA was calculated by subtracting the negative peak amplitude from the positive peak amplitude.

Cardiovascular data was measured on each experimental day (intervention: sleep and control). Each day consisted of two conditioning experiments (test time: before and after). Each conditioning experiment consisted of three blocks, in which the CS+ and CS- were paired with rising reinforcement rates (block 1: 0%; block 2: 50%; block 3: 100%) (see Figure 3). We performed a 3 (reinforcement rate) x 2 (test time) x 2 (intervention) repeated measures analysis of variance (ANOVA) for each of the three measures.

2.4 Self-assessment manikins (SAM): Arousal and valence

SAM were used as a non-verbal method to evaluate emotional responses towards the stimuli during the conditioning experiment (Bradley & Lang, 1994). Before the conditioning the US+ was presented three times and participants were asked to score the sound on arousal and valence scales. Then SAM were presented after each sound presentation following each of the three reinforcement blocks (see Figure 3). Our SAM consisted of two emotional rating dimensions, arousal, and valence. Both measures have 5 pictures each, an ‘x’ can be placed on each manikin or in between two manikins, resulting in a 9-point rating scale for each dimension (see Figure 4). Mean arousal and valence for the initial US+ presentation was calculated. We also performed 2 (intervention: sleep vs. control) x 2 (time: before vs. after the intervention) x 2 (condition: CS+ vs. CS-) x 3 (reinforcement rate: 0, 50, 100 %) repeated measures ANOVAs for both valence and arousal scores.

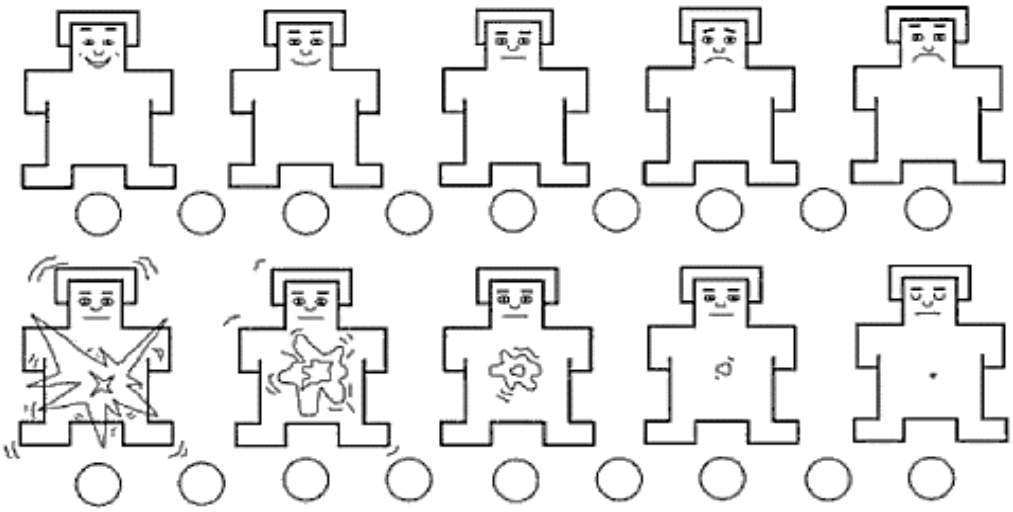

Figure 4 – Image of the Self-Assessment Manikins (SAM), representing “valence” (top panel) and “arousal” (lower panel)

2.5 Emotional state measures

2.5.1 STAI (Spielberger S): state questionnaire

The STAI by Spielberger et al. (Spielberger, 1970) contains two scores of 20 items each that were separated in this study. The Spielberger S / State Anxiety questionnaire was one of these scores with 20 questions and was completed 4 times on each intervention day, before and after each conditioning experiment. In the test, higher scores indicate higher anxiety levels. We performed a 2 (intervention: sleep vs. control) x 2 (time: before vs. after intervention) x 2 (conditioning: pre- vs. post-conditioning) repeated measures ANOVA.

2.5.2 Positive and Negative Affect Schedule (PANAS)

The PANAS consists of two 10-item scales to measure participants’ current state of positive and negative affect (Watson et al., 1988). The rating scale ranges between 1 (not at all) and 5 (very much). Participants were asked to complete the test before and after each conditioning experiment, resulting in a total of 4 tests per intervention day. We performed 2 (intervention: sleep vs. control) x 2 (test time: before vs. after intervention) x 2 (pre- vs. post-conditioning) repeated measures ANOVA for the positive affect ratings (PA) and negative affect ratings (NA).

2.6 Vigilance measures

2.6.1 Stanford Sleepiness Scale

The Stanford sleepiness scale is a single rating scale ranging from 1 (wide awake) to 8 (fast asleep) to assess the participants' sleepiness (Hoddes et al., 1972). It was measured 6 times during each experimental day (before experiment I, before and after conditioning; before and after intervention). Test time no.3 (pre-intervention) was compared with test no. 4 (post-intervention) for both intervention days (sleep vs. control). A 2 (intervention: sleep vs. control) x 2 (test time: pre-intervention vs. post-intervention) repeated measures ANOVA was performed.

2.6.2 Vigilance test / psychomotor vigilance task (PVT)

Participants were asked to perform a psychomotor vigilance task (PVT) a total of 4 times on each experimental day; before and after each conditioning and the intervention, respectively. The test was constructed as a 5-minute computer game, a further development of the originally purely visual PVTs (Wilkinson, 1968). In this vigilance test, the participant is asked to place a finger on two selected buttons of a computer keyboard. A round figure repetitively appears on either the left (first button) or on the right side (second button) of a dark screen. Subsequently the participant has to press the correct button as fast as possible. Lower scores indicate faster reaction times. Reaction times were averaged for each completed test; mean reaction times for test 2 (pre-intervention) and 3 (post-intervention) were calculated. A 2 (intervention: sleep vs. control) x 2 (test time: pre-intervention vs. post-intervention) repeated measures ANOVA was performed.

2.7 Personality traits

For all personality trait scores, we calculated Spearman rank-order correlations with cardiovascular responses and emotional responses.

2.7.1 STAI (Spielberger T): trait questionnaire

The Spielberger T / Trait anxiety test was the second of the STAI scores evaluated, consisting of 20 questions. It was completed once during the preparation day. Higher scores indicate a higher trait anxiety level.

2.7.2 Sensation Seeking Scale (SSS)

The SSS (Beauducel et al., 2003; Zuckerman et al., 1964) was presented twice during the recovery time following each intervention. It consists of 4 subscales (Thrill and Adventure Seeking (TAS), Disinhibition (Dis), Experience Seeking (ES), and Boredom Susceptibility (BS)) with 10 items each, adding up to 40 items in total. We used the results of the questionnaire following the 2-hour sleep. The results of all subscales were added up to one complete SSS score. Higher scores indicate higher sensation seeking levels.

2.7.3 Big Five Personality Traits (NEO-Five Factor Inventory)

The NEO-Five Factor Inventory (NEO-FFI) is a questionnaire containing a total of 60 statements (Costa & McCrae, 1989). Participants were asked to rate each statement. As a result, an analysis of the "Big Five Personality Traits" was possible, which are:

(1) openness to experience (inventive/curious vs. consistent/cautious)
(2) conscientiousness (efficient/organized vs. easy-going/careless)
(3) extraversion (outgoing/energetic vs. solitary/reserved)
(4) agreeableness (friendly/compassionate vs. challenging/detached)
(5) neuroticism (sensitive/nervous vs. secure/confident)

The test was performed in the recovery period following each intervention, twice in total. Higher scores indicate higher levels of each personality trait.

2.8 Sleep questionnaires (SF-A/R: Schlaffragebogen)

As a part of the initial demographic data collection, participants were asked to estimate their average sleep time on a daily basis. Participants were asked to wake up 1 hour earlier than usual the night before each experimental day. Mean sleep times were calculated.

The SF-A/R (Schlaffragebogen, „sleep questionnaire"-A, revised) (Görtelmeyer, 2011) was used to evaluate the participants' sleep quality the night before and after each intervention day and also following the 2-hour nap on the experimental day. Participants completed the SF-A/R regarding the previous night during the session, the SF-A/R concerning the night after was completed the morning after each session and sent back to the principal investigator. The questionnaire contains a total of 25 questions. The interpretation sheet allows the coding of a number of categories testing different aspects

of sleep quality as can be seen in Table 1. We assessed the effect of (1) intervention (results on the sleep day vs. results on the control day) and (2) time (results for the night before each intervention vs. results for the night after each intervention) on sleep duration (in h) and sleep quality. Sleep quality (SQ) was calculated as described in the manual as the following calculation of scales: (18 – ESS – DSS – VZA + ASC) / 4. We also calculated mean SQ for the nap and all four measured nights.

A 2 (intervention: sleep vs. control) x 2 (time: pre-intervention night vs. post-intervention night) repeated measures ANOVA was performed for each category of the SF/A-R questionnaire.

Table 1 – Categories of sleep in the SF-A/R sleeping questionnaire (German/English) (Görtelmeyer, 2011)

ESS	Einschlafschwierigkeiten - difficulties falling asleep (1: no difficulties / 5: very difficult)
DSS	Durchschlafschwierigkeiten - difficulties during sleep (1: no difficulties / 5: very difficult)
VZA	vorzeitiges Aufwachen - waking up too early (1: did not wake up early / 5: woke too early)
ASC	Allgemeine Schlafcharakterisierung - general sleep characteristics (1: bad / 5: good sleep characteristics)
GSD	Gesamtschlafdauer - total sleep time (hours, minutes in decimal system)
SQ	Schlafqualität - sleep quality (1: low / 5: high sleep quality)
GES	Gefühl des Erholtseins nach dem Schlaf - feeling of recovery after sleep (1: not recovered / 5: recovered)
PSYA	Psychische Ausgeglichenheit vor dem Schlaf – psychological balance before sleep (1: imbalanced / 5: balanced)
PSYE	Psychisches Erschöpftsein vor dem Schlaf – psychological exhaustion before sleep (1: not exhausted / 5: exhausted)
PSS	Psychosomatische Symptome in der Schlafphase – psychosomatic symptoms during sleep (1: none / 5: many)

2.9 Statistical evaluation methods

Microsoft Excel (2019) was used to create data tables with the collected data. This data was exported to JASP Version 0.10.2.0(2019) for first analysis. The main statistical analyses were performed with IBM SPSS Statistics for Windows, Version 26.0 (2019). Tables and statistical graphs were created in SPSS. Photoplethysmography data was analysed with R (R Core Team, 2013). R was also used for all correlation analyses.

All data included into the ANOVAs were checked for sphericity, the Greenhouse-Geisser epsilon was used for correction in the cases of violation of sphericity.

3 Results

3.1 Cardiovascular measures

In the repeated measures (RM) ANOVA, there was a significant Intervention x Test Time interaction on participants' heart rate (see Table 3). As shown in Figure 5, a period of wakefulness as opposed to sleep caused a significantly stronger decrease in HR when comparing test times. Mean HR in the experimental block directly after the 2-hour nap was 70.516 beats per minute (SD = 8.05), whereas mean HR directly after the 2-hour wakefulness was 63.678 beats per minute (SD = 5.915) (see Table 2). This effect was replicated in PWA and HRV (see Table 5 and Table 7), although HRV moved in the opposite direction resulting in a higher HRV after the wakefulness period (see Figure 6). As can be seen in Table 4, mean HRV in the experimental block directly after the 2-hour nap was 39.816 (SD = 15.774), whereas mean HRV directly after the 2-hour wakefulness was 56.929 (SD = 22.628). As shown in Table 6, mean PWA after the 2-hour nap was 12.531 µV (SD = 6.849), after the 2-hour wakefulness it showed a stronger suppression: 8.580 µV (SD = 6.028). No effect of the reinforcement rate was observed in HR and HRV, but PWA demonstrated suppression in the 50% and 100% blocks compared with 0% reinforcement rate, with no difference between the reinforced blocks (see Figure 7). This effect can be seen particularly well in Figure 8, where the 0% reinforcement rate was taken as a baseline for the second and third block.

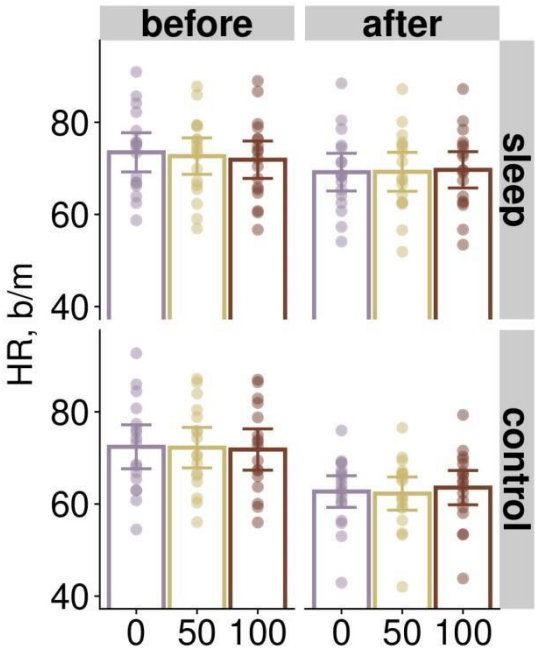

Figure 5 – Heart rate (HR) in beats per minute (b/m), before and after the sleep vs. control intervention, for each reinforcement block (0%, 50% and 100%)

Table 2 – Descriptive statistics: Heart rate (HR) in beats per minute (b/m), before and after the sleep vs. control intervention, for each reinforcement block (0%, 50% and 100%)

	N	Min	Max	*M*	*SD*
sleep: before (0)	15	62.458	90.930	74.314	8.632
sleep: before (50)	15	59.063	87.769	73.153	8.219
sleep: before (100)	15	60.426	88.992	72.564	8.713
sleep: after (0)	15	57.301	88.445	70.516	8.050
sleep: after (50)	15	56.587	87.196	69.874	8.003
sleep: after (100)	15	56.708	87.228	70.439	8.158
control: before (0)	15	54.478	85.960	70.727	8.986
control: before (50)	15	56.072	86.436	70.789	8.738
control: before (100)	15	56.010	86.975	70.383	8.977
control: after (0)	15	53.023	75.950	63.678	5.915
control: after (50)	15	53.274	76.537	63.666	6.192
control: after (100)	15	53.402	79.326	63.987	6.681

Table 3 – Test of Within-Subjects Effects: Heart rate (HR), repeated measures ANOVA.

Notes: df – degrees of freedom.

	df	F	P	η^2
intervention	1; 14	15.004	.002**	.517
time	1; 14	21.419	<.001***	.605
reinforcement	2; 28	.875	.428	.059
intervention * time	1; 14	5.555	.034*	.284
intervention * reinforcement	2; 28	1.190	.319	.078
time * reinforcement	2; 28	2.321	.117	.142
intervention * time * reinforcement	2; 28	.305	.739	.021

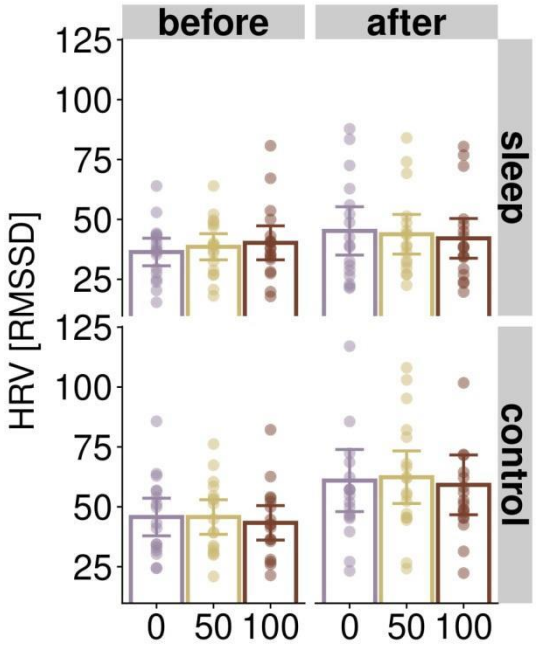

Figure 6 – Heart rate variability (HRV), the root mean square of successive differences (RMSSD), before and after the sleep vs. control intervention, for each reinforcement block (0%, 50%, 100%)

Table 4 – Descriptive statistics: Heart rate variability (HRV), the root mean square of successive differences (RMSSD), before and after the sleep vs. control intervention, for each reinforcement block (0%, 50%, 100%)

	N	Min	Max	*M*	*SD*
sleep: before (0)	15	15.411	52.949	35.390	10.065
sleep: before (50)	15	18.093	63.974	39.202	12.313
sleep: before (100)	15	17.846	80.738	41.716	16.421
sleep: after (0)	15	21.361	72.521	39.816	15.774
sleep: after (50)	15	22.514	84.024	42.594	16.410
sleep: after (100)	15	19.726	80.424	39.769	17.754
control: before (0)	15	24.337	85.700	47.344	16.979
control: before (50)	15	20.960	76.168	47.588	15.221
control: before (100)	15	21.398	82.158	44.862	15.390
control: after (0)	15	23.231	117.058	56.929	22.628
control: after (50)	15	24.210	108.048	59.524	22.552
control: after (100)	15	22.335	101.722	55.232	17.730

Table 5 – Tests of Within-Subject Effects: Heart rate variability (RMSSD), repeated measures ANOVA

	df	F	p	η^2
intervention	1; 14	9.269	.009**	.398
time	1; 14	12.888	.003**	.479
reinforcement	2; 28	1.842	.177	.116
intervention * time	1; 14	6.037	.028*	.301
intervention * reinforcement	2; 28	2.210	.128	.136
time * reinforcement	2; 28	.818	.452	.055
intervention * time * reinforcement	1.4; 19.56	1.135	.322	.075

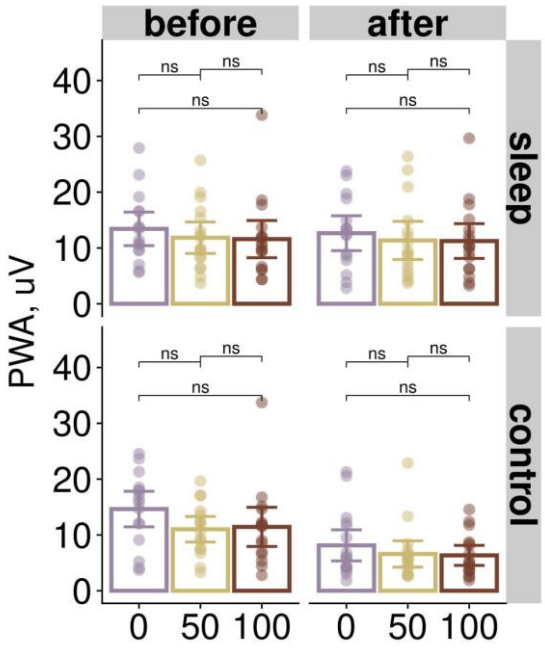

Figure 7 – Pulse wave amplitude (PWA) in µV, before and after the sleep vs. control intervention, for each reinforcement block (0%, 50%, 100%). ns – not significant

Table 6 – Descriptive statistics: Pulse wave amplitude (PWA) in µV, before and after the sleep vs. control intervention, for each reinforcement block (0%, 50%, 100%)

	N	Min	Max	*M*	*SD*
sleep: before (0)	14	5.625	27.907	13.626	6.533
sleep: before (50)	14	3.653	25.721	11.883	6.340
sleep: before (100)	14	4.355	33.805	11.444	7.552
sleep: after (0)	14	2.810	23.766	12.531	6.849
sleep: after (50)	14	3.688	26.374	11.947	7.233
sleep: after (100)	14	3.251	29.648	11.375	7.229
control: before (0)	14	4.113	24.540	15.602	6.170
control: before (50)	14	3.257	19.658	11.451	4.835
control: before (100)	14	2.774	33.724	12.079	7.431
control: after (0)	14	2.967	21.277	8.580	6.028
control: after (50)	14	2.535	22.868	6.872	5.373
control: after (100)	14	1.881	14.540	6.587	3.984

Table 7 – Tests of Within-Subject Effects: Pulse wave amplitude (PWA), repeated measures ANOVA

	df	F	p	η^2
intervention	1; 13	2.094	.172	.139
time	1; 13	13.799	.003**	.515
reinforcement	1.3; 17	5.904	.020*	.312
intervention * time	1; 13	8.882	.011*	.406
intervention * reinforcement	1.18; 15.39	1.464	.250	.101
time * reinforcement	1.2; 15.65	.698	.442	.051
intervention * time * reinforcement	2; 26	.136	.873	.010

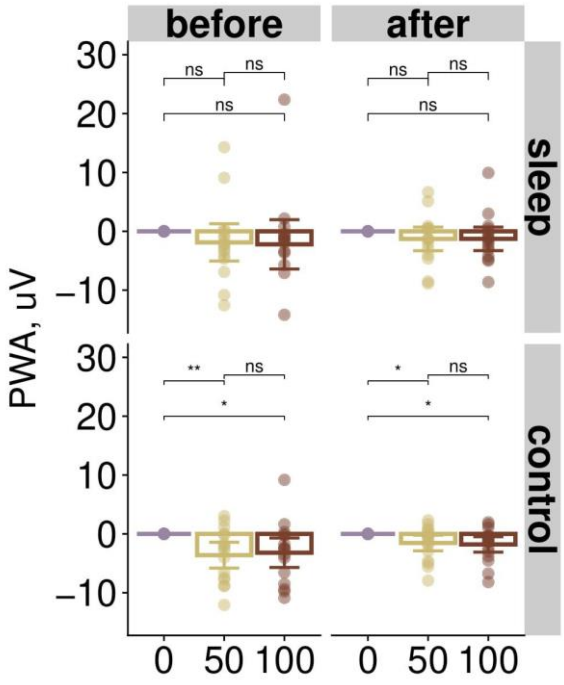

Figure 8 – Pulse wave amplitude (PWA) in µV, before and after the sleep vs. control intervention, for each reinforcement block (0%, 50%, 100%), baselined from the first block of each conditioning session. ns – not significant, * p < .05, ** p < .01

3.2 Self-assessment measures (arousal, valence)

Table 8 shows valence and arousal data for the aversive sound (maximum arousal = 1, minimum arousal = 9; maximum positive valence = 1, maximum negative valence = 9).

Table 8 – Descriptive statistics: initial arousal and valence towards the aversive sound before each experimental day

	Arousal before sleep	Valence before sleep	Arousal before control	Valence before control
Mean	4.00	6.94	2.89	7.72
N	18	18	18	18
Std. Deviation	1.782	1.162	1.183	1.074
Minimum	1	5	1	6
Maximum	8	9	5	9

As can be seen in Figure 9, Table 9, Table 10 and Table 11, valence scores for the CS+ and CS- were almost the same in the first block with a reinforcement rate of 0%. After the second block with a reinforcement rate of 50%, valence scores of the CS+ instantaneously increased significantly in comparison to the CS- scores, which decreased at the same time. In block 3 with 100% reinforcement rate, valence ratings did not differ significantly from the scores in block 2. There were similar, but non-significant effects for the arousal ratings (Figure 10, Table 12, Table 13, Table 14). There was no effect of Intervention and no effect of Test time.

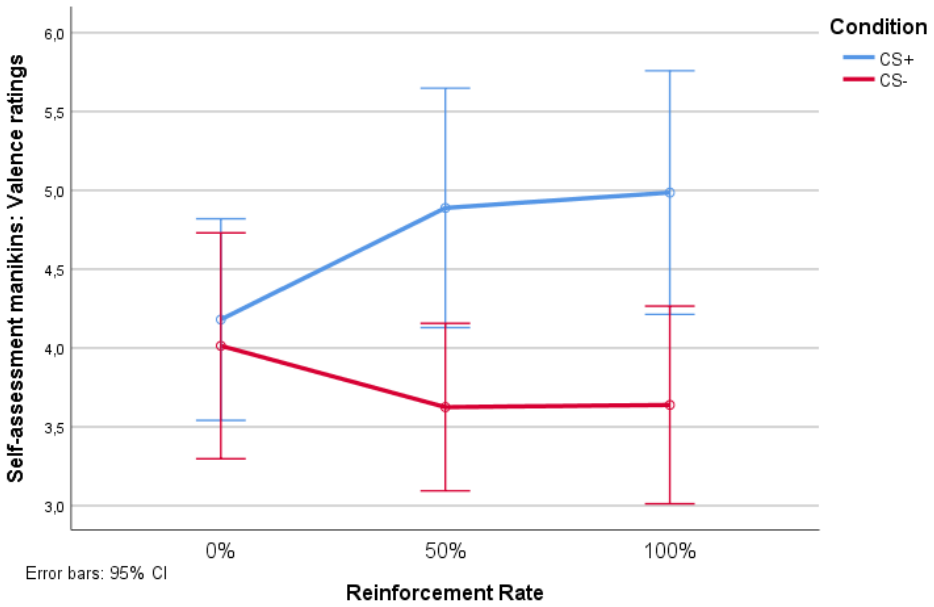

Figure 9 – Self-assessment manikins: Valence ratings of the CS+ and CS- condition, for each reinforcement rate

Table 9 – Descriptive statistics: Self-assessment manikins: Valence ratings of the CS+ condition, before and after the sleep vs. control intervention, after each reinforcement block

	N	Min	Max	*M*	*SD*
sleep: before (1)	18	2	7	4.17	1.689
sleep: before (2)	18	2	7	4.72	1.602
sleep: before (3)	18	1	8	4.89	2.193
sleep: after (1)	18	1	7	3.94	1.955
sleep: after (2)	18	1	9	4.83	2.229
sleep: after (3)	18	1	9	4.78	2.130
control: before (1)	18	1	6	4.11	1.605
control: before (2)	18	1	9	4.89	1.844
control: before (3)	18	3	9	5.11	1.641
control: after (1)	18	2	7	4.50	1.383
control: after (2)	18	2	7	5.11	1.530
control: after (3)	18	2	7	5.17	1.383

Table 10 – Descriptive statistics: Self-assessment manikins: Valence ratings of the CS-condition, before and after the sleep vs. control intervention, after each reinforcement block

	N	Min	Max	*M*	*SD*
sleep: before (1)	18	1	7	4.17	1.757
sleep: before (2)	18	1	7	3.50	1.465
sleep: before (3)	18	1	6	3.44	1.542
sleep: after (1)	18	1	7	3.89	1.906
sleep: after (2)	18	1	6	3.56	1.423
sleep: after (3)	18	1	5	3.50	1.505
control: before (1)	18	1	7	3.94	1.589
control: before (2)	18	1	6	3.50	1.465
control: before (3)	18	1	6	3.67	1.372
control: after (1)	18	1	7	4.06	1.552
control: after (2)	18	2	7	3.94	1.434
control: after (3)	18	1	7	3.94	1.798

Table 11 – Tests of Within-Subject Effects: Self-assessment manikins: Valence ratings, repeated measures ANOVA

	df	F	p	η^2
intervention	1; 17	1.541	.231	.083
time	1; 17	.665	.426	.038
reinforcement	2; 34	3.193	.054	.158
condition	1; 17	14.874	.001**	.467
intervention * time	1; 17	1.574	.227	.085
intervention * reinforcement	2; 34	.583	.564	.033
time * reinforcement	2; 34	.855	.434	.048
intervention * time * reinforcement	2; 34	.313	.734	.018
intervention * condition	1; 17	.053	.821	.003
time * condition	1; 17	.034	.855	.002
intervention * time * condition	1; 17	.006	.938	.000
reinforcement * condition	1.23; 20.98	4.607	.037*	.213
intervention * reinforcement * condition	2; 34	.479	.624	.027
time * reinforcement * condition	2; 34	.724	.492	.041
intervention * time * reinforcement * condition	1.33; 22.66	.063	.869	.004

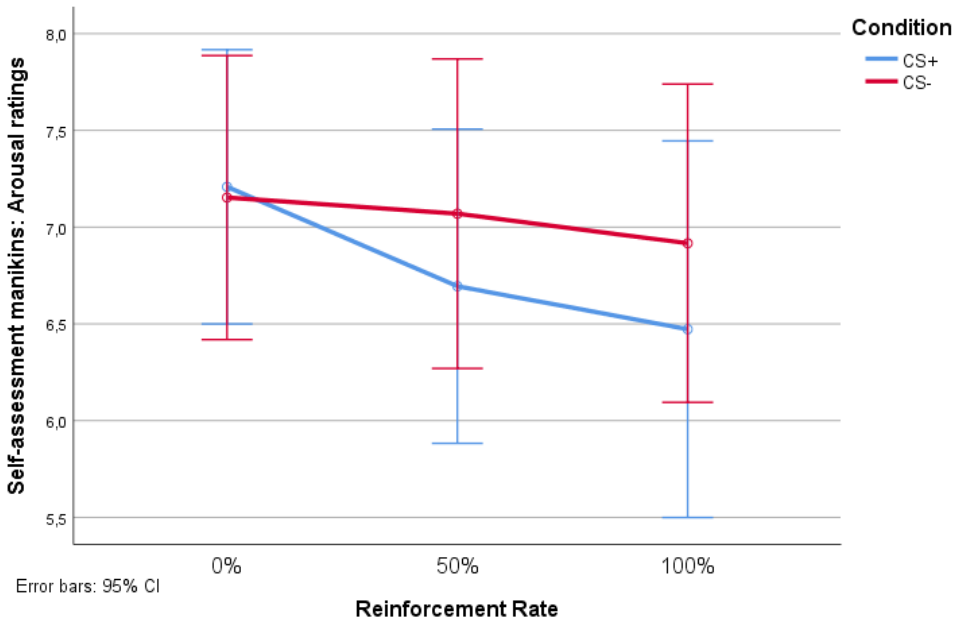

Figure 10 – Self-assessment manikins: Arousal ratings of the CS+ and CS- condition, for each reinforcement rate

Table 12 – Descriptive statistics: Self-assessment manikins: Arousal ratings of the CS+ condition, before and after the sleep vs. control intervention, after each reinforcement block

	N	Min	Max	*M*	*SD*
sleep: before (1)	18	3	9	7.11	1.844
sleep: before (2)	18	3	9	6.67	2.114
sleep: before (3)	18	3	9	6.22	2.157
sleep: after (1)	18	4	9	7.06	1.552
sleep: after (2)	18	2	9	6.56	2.202
sleep: after (3)	18	1	9	6.39	2.380
control: before (1)	18	4	9	7.11	1.605
control: before (2)	18	3	9	7.00	1.970
control: before (3)	18	1	9	6.83	2.149
control: after (1)	18	5	9	7.56	1.504
control: after (2)	18	1	9	6.56	2.121
control: after (3)	18	1	9	6.44	2.281

Table 13 – Descriptive statistics: Self-assessment manikins: Arousal ratings of the CS-condition, before and after the sleep vs. control intervention, after each reinforcement block

	N	Min	Max	*M*	*SD*
sleep: before (1)	18	4	9	7.00	1.534
sleep: before (2)	18	4	9	6.72	1.674
sleep: before (3)	18	4	9	6.89	1.676
sleep: after (1)	18	3	9	7.11	1.967
sleep: after (2)	18	2	9	6.89	2.026
sleep: after (3)	18	3	9	7.00	1.847
control: before (1)	18	4	9	7.06	1.893
control: before (2)	18	4	9	7.28	1.638
control: before (3)	18	1	9	6.67	2.679
control: after (1)	18	4	9	7.44	1.756
control: after (2)	18	4	9	7.39	1.720
control: after (3)	18	3	9	7.11	1.811

Table 14 – Tests of Within-Subject Effects: Self-assessment manikins: Arousal ratings, repeated measures ANOVA

	df	F	P	η^2
intervention	1; 17	.719	.408	.041
time	1; 17	.407	.532	.023
reinforcement	1.33; 22.68	3.499	.064	.171
condition	1; 17	1.385	.255	.075
intervention * time	1; 17	.016	.900	.001
intervention * reinforcement	2; 34	.669	.519	.038
time * reinforcement	2; 34	1.147	.330	.063
intervention * time * reinforcement	2; 34	.934	.403	.052
intervention * condition	1; 17	.009	.924	.001
time * condition	1; 17	1.309	.269	.071
intervention * time * condition	1; 17	.478	.499	.027
reinforcement * condition	2; 34	2.355	.110	.122
intervention * reinforcement * condition	2; 34	1.598	.217	.086
time * reinforcement * condition	1.34; 22.69	.433	.574	.025
intervention * time * reinforcement * condition	2; 34	.658	.525	.037

3.3 Emotional state measures (STAI state, PANAS)

As can be seen in Figure 11 and Figure 12, there seems to be a slight increase of anxiety levels following the pre-intervention experiments in the STAI state questionnaire, whereas the anxiety levels did not rise as much after the post-intervention experiments; Intervention and Test time also appear to influence the scores. Still these results are not significant (see Table 15 and Table 16). Conditioning led to a slight increase in anxiety levels according to STAI.

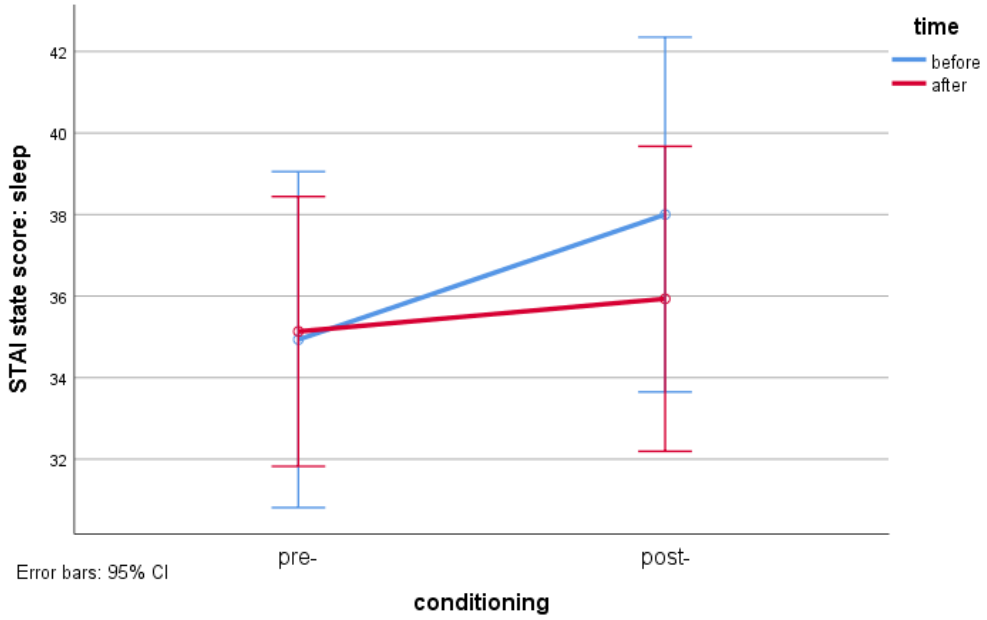

Figure 11 – STAI state score: ratings on the sleep day, before and after the intervention, pre-conditioning vs. post-conditioning

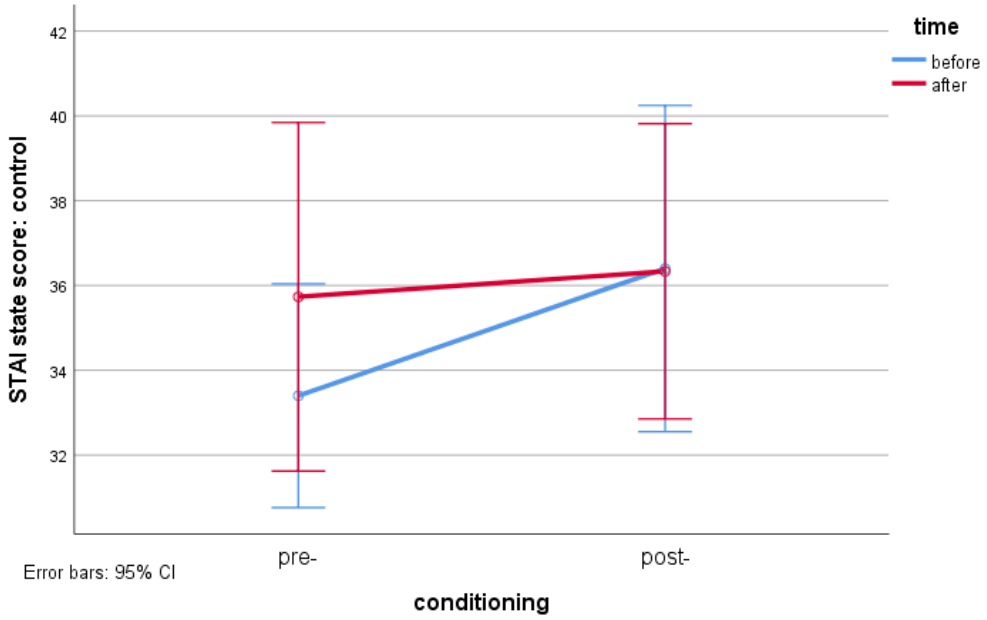

Figure 12 – STAI state score: ratings on the control day, before and after the intervention, pre-conditioning vs. post-conditioning

Table 15 – Descriptive statistics: STAI state score, before and after the sleep vs. control intervention, pre-conditioning (1) vs. post-conditioning (2)

	N	Min	Max	*M*	*SD*
sleep: before (1)	15	25	53	34.93	7.440
sleep: before (2)	15	28	58	38.00	7.856
sleep: after (1)	15	26	46	35.13	5.975
sleep: after (2)	15	26	49	35.93	6.756
control: before (1)	15	25	42	33.40	4.763
control: before (2)	15	26	53	36.40	6.947
control: after (1)	15	25	47	35.73	7.421
control: after (2)	15	26	49	36.33	6.287

Table 16 – Tests of Within-Subject Effects: STAI state score, repeated measures ANOVA

	df	F	p	η^2
intervention	1; 14	.205	.658	.014
time	1; 14	.010	.921	.001
conditioning	1; 14	4.372	.055	.238
intervention * time	1; 14	3.187	.096	.185
intervention * conditioning	1; 14	.010	.923	.001
time * conditioning	1; 14	1.818	.199	.115
intervention * time * conditioning	1; 14	.002	.964	.000

In the PANAS questionnaire, ANOVA for PA showed a significant main effect of Conditioning with post-conditioning mean scores decreasing by a mean of 2.5 points compared to pre-conditioning (see Figure 13). There was also a significant Intervention x Time interaction: after the sleep intervention, PA scores increased whereas PA scores following wakefulness decreased (see Figure 14). Finally, there was a significant interaction effect between Time and Conditioning: before the intervention, PA scores were much lower than after the conditioning. After the intervention, the effect of conditioning on PA values was less strong; post-conditioning scores did not differ as much from pre-conditioning values (see Figure 15). Descriptive statistics can be seen in Table 17. The ANOVA results can be seen in Table 18.

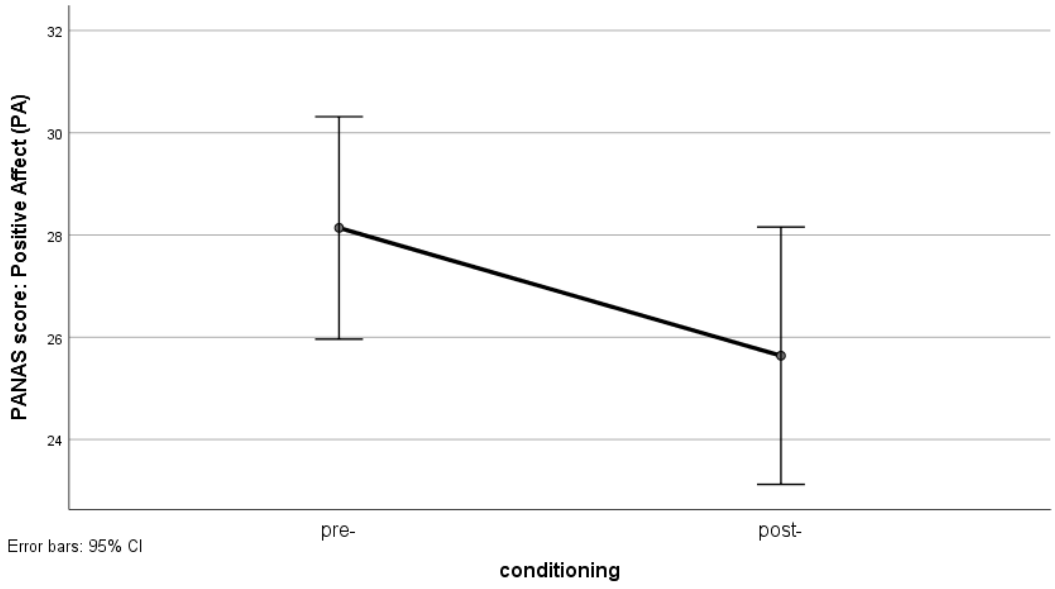

Figure 13 – PANAS score: Positive Affect (PA) ratings of pre-conditioning vs. post-conditioning

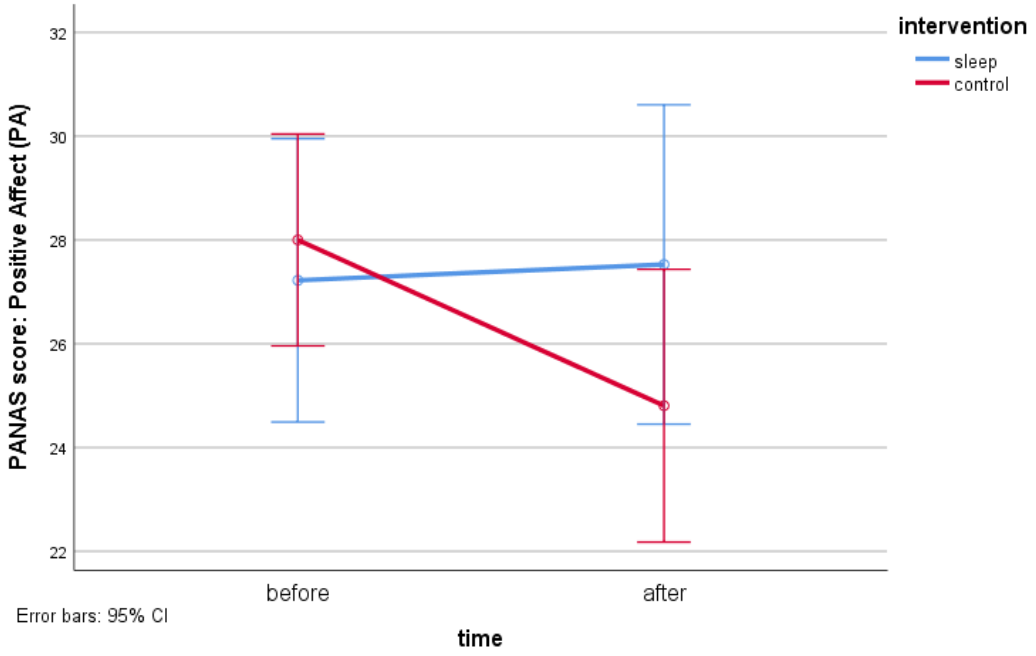

Figure 14 – PANAS score: Positive Affect (PA) ratings of the sleep and control day, before and after each intervention

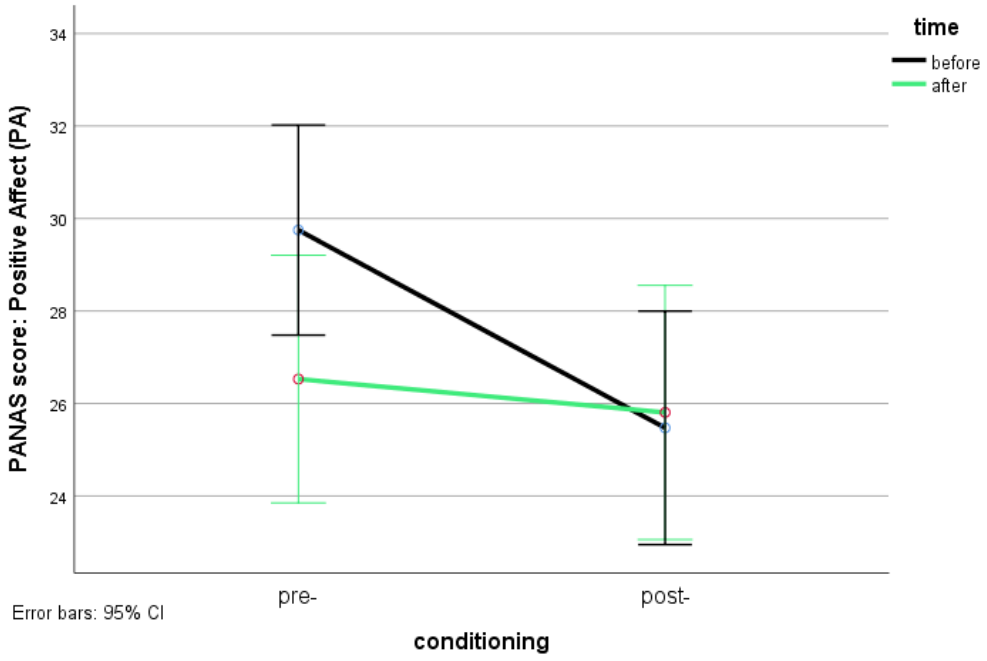

Figure 15 – PANAS score: Positive Affect (PA) ratings before and after the intervention, pre-conditioning vs. post-conditioning

Table 17 – Descriptive statistics: PANAS score: Positive Affect (PA) ratings, before and after the sleep vs. control intervention, pre-conditioning (1) vs. post-conditioning (2)

	N	Min	Max	*M*	*SD*
sleep: before (1)	18	20	40	29.72	5.245
sleep: before (2)	18	10	37	24.72	6.728
sleep: after (1)	18	14	38	27.83	6.138
sleep: after (2)	18	16	40	27.22	6.567
control: before (1)	18	21	41	29.78	5.185
control: before (2)	18	15	32	26.22	4.660
control: after (1)	18	15	33	25.22	5.786
control: after (2)	18	14	33	24.39	5.575

Table 18 – Tests of Within-Subject Effects: PANAS: Positive Affect (PA), repeated measures ANOVA

	df	F	p	η^2
intervention	1; 17	1.201	.288	.066
Time	1; 17	3.183	.092	.158
conditioning	1; 17	15.104	.001**	.470
intervention * time	1; 17	10.679	.005**	.386
intervention * conditioning	1; 17	.527	.478	.030
time * conditioning	1; 17	11.760	.003**	.409
intervention * time * conditioning	1; 17	.670	.424	.038

On the other hand, negative affect scores (Table 19, Table 20) decreased significantly in the second conditioning experiment (see Figure 16). This decrease followed the sleep intervention but not wakefulness (see Figure 17).

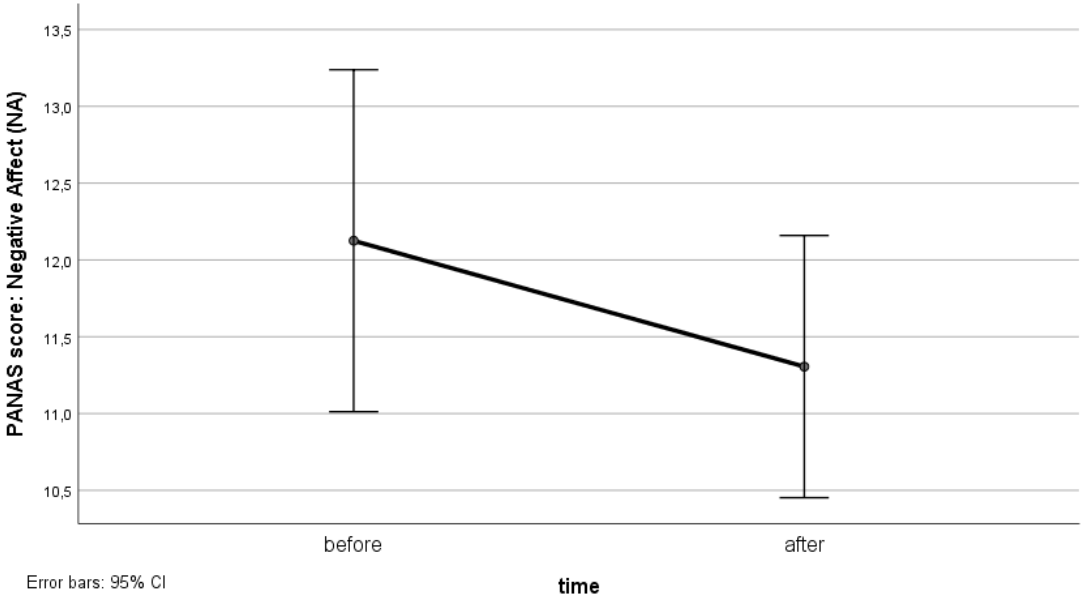

Figure 16 – PANAS score: Negative Affect (NA) ratings before and after the intervention

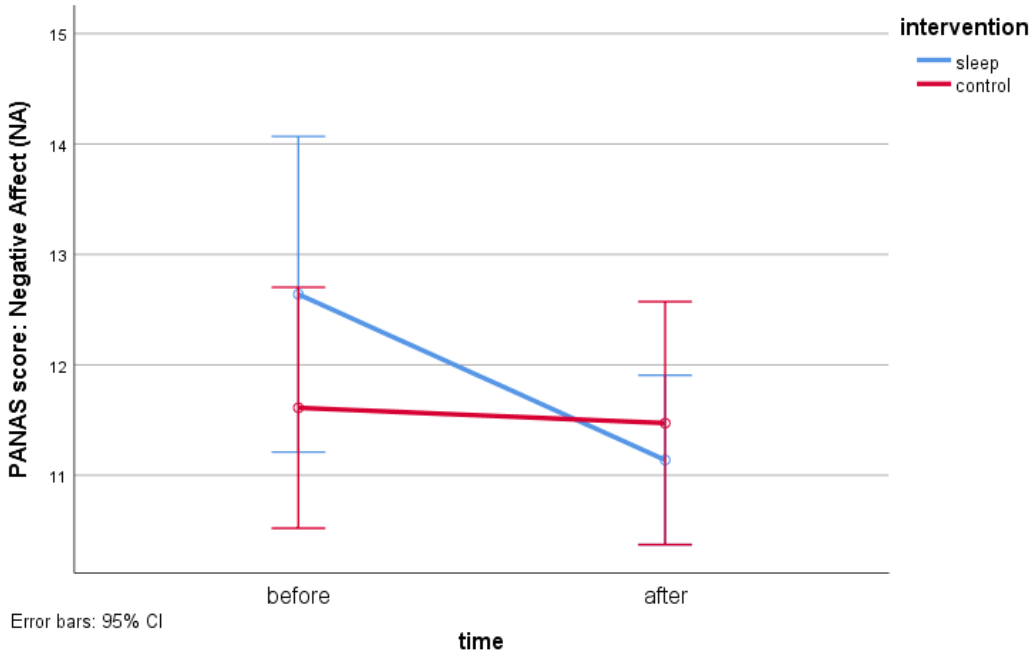

Figure 17 – PANAS score: Negative Affect (NA) ratings for the sleep and control day, before and after each intervention

Table 19 – Descriptive statistics: PANAS score: Negative Affect (NA) ratings, before and after the sleep vs. control intervention, pre-conditioning (1) vs. post-conditioning (2)

	N	Min	Max	*M*	*SD*
sleep: before (1)	18	10	22	12.44	3.634
sleep: before (2)	18	10	24	12.83	3.585
sleep: after (1)	18	10	16	11.17	1.948
sleep: after (2)	18	10	17	11.11	1.676
control: before (1)	18	10	15	11.17	1.543
control: before (2)	18	10	23	12.06	3.226
control: after (1)	18	10	19	11.00	2.142
control: after (2)	18	10	20	11.94	2.508

Table 20 – Tests of Within-Subject Effects: PANAS: Negative Affect (NA), repeated measures ANOVA

	df	F	p	η^2
intervention	1; 17	.751	.398	.042
time	1; 17	6.687	.019*	.282
conditioning	1; 17	2.392	.140	.123
intervention * time	1; 17	5.404	.033*	.241
intervention * conditioning	1; 17	1.303	.270	.071
time * conditioning	1; 17	.106	.749	.006
intervention * time * conditioning	1; 17	.136	.716	.008

3.4 Vigilance measures (Stanford Sleepiness, Vigilance test)

As can be seen in the Stanford Sleepiness scale results in Figure 18, Table 21 and Table 22, sleepiness decreased significantly after the 2-hour nap (-0.73) compared to the wakefulness day, where sleepiness increased (+0.22).

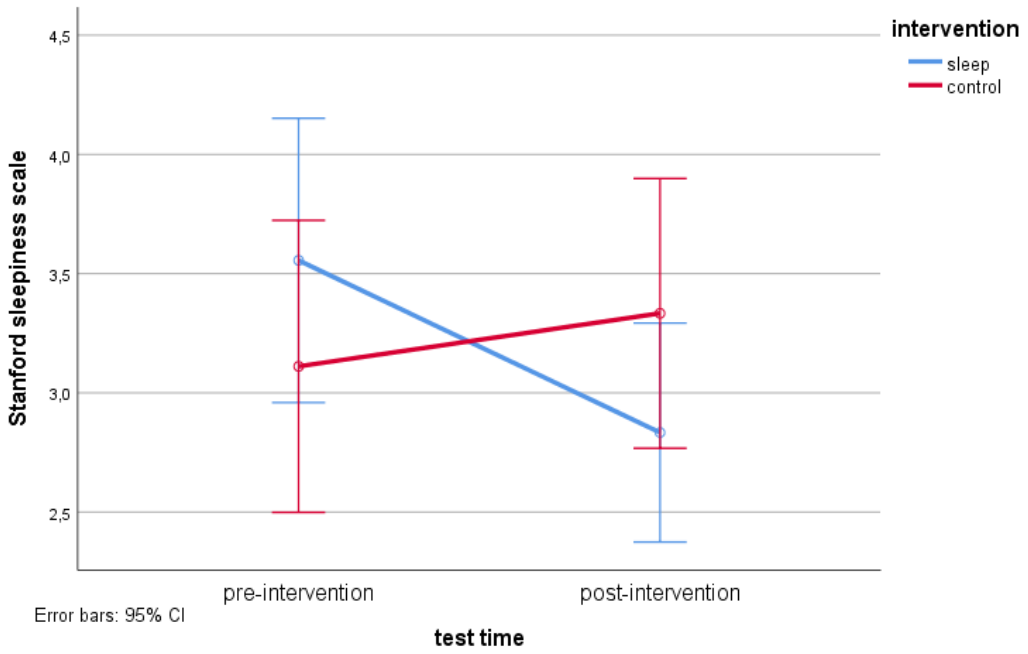

Figure 18 – Stanford sleepiness scale ratings for sleep and control day, before (test no. 3) vs. after (test no. 4) each intervention

Table 21 – Descriptive statistics: Stanford sleepiness scale ratings for sleep and control day, before (test no. 3) vs. after (test no. 4) each intervention

	N	Min	Max	*M*	*SD*
sleep (test 3)	18	2	6	3.56	1.199
sleep (test 4)	18	1	5	2.83	.924
control (test 3)	18	1	5	3.11	1.231
control (test 4)	18	2	5	3.33	1.138

Table 22 – Tests of Within-Subject Effects: Stanford sleepiness scale, repeated measures ANOVA

	df	F	p	η^2
intervention	1; 17	.013	.909	.001
time	1; 17	1.515	.235	.082
intervention * time	1; 17	4.968	.040*	.226

The vigilance test (Table 23, Table 24) showed similar changes: reaction times decreased slightly after sleep (-9.344 msec) but increased after a wakefulness interval (+17.873 msec) leading to a significant Intervention x Time interaction (see Figure 19).

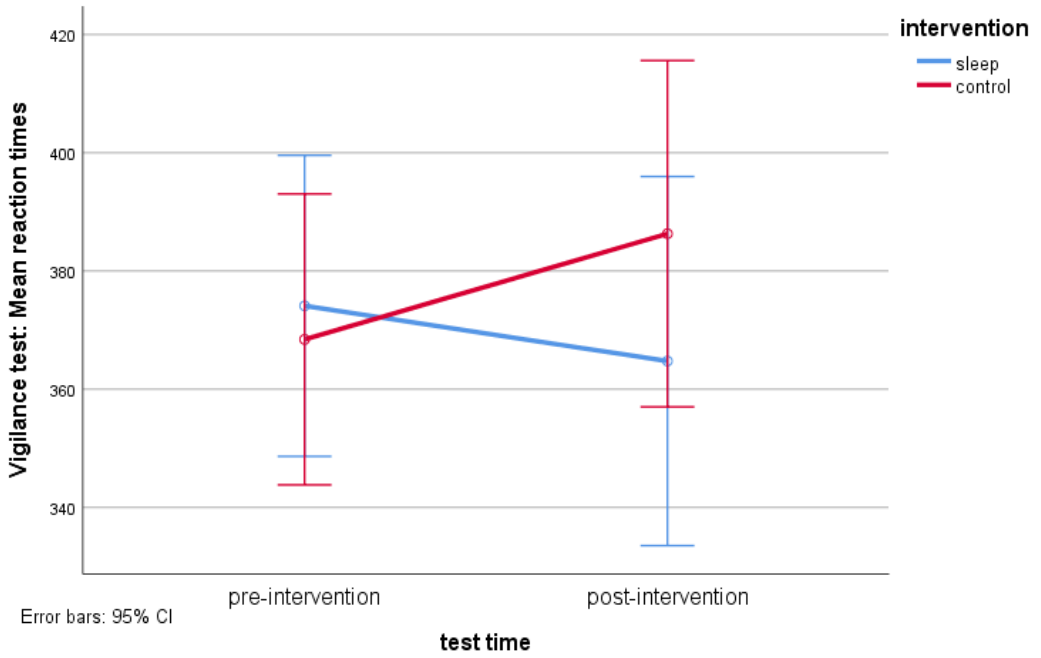

Figure 19 – Vigilance test / psychomotor vigilance task (PVT): mean reaction times for sleep and control day, before (test no. 2) vs. after (test no.3) each intervention, in msec

Table 23 – Descriptive statistics: Vigilance test / psychomotor vigilance task (PVT): mean reaction times for sleep and control day, before (test no. 2) vs. after (test no.3) each intervention, in msec

	N	Min	Max	*M*	*SD*
sleep (test 2)	18	297.775	496.725	374.104	51.203
sleep (test 3)	18	281.900	534.925	364.760	62.788
control (test 2)	18	316.175	487.000	368.424	49.473
control (test 3)	18	307.550	519.200	386.297	58.901

Table 24 – Tests of Within-Subject Effects: Vigilance test: mean reaction times, repeated measures ANOVA

	df	F	p	η^2
intervention	1; 17	2.601	.125	.133
time	1; 17	.575	.459	.033
intervention * time	1; 17	8.591	.009**	.336

3.5 Personality traits (STAI trait, SSS, NEO-FFI)

There was a significant positive correlation between neuroticism scores and STAI trait anxiety scores (*rho* = 0.56, *p* = 0.015), indicating internal consistency between measures of the same psychological construct. However, neither neuroticism scores nor trait anxiety correlated with physiological or behavioural scores.

Total scores of the SSS (M = 22.778; standard deviation (SD) = 4.558) were comparable with previous normative data (Beauducel et al., 2003). There was a significant correlation between the arousal after CS+ and SSS scores (*rho* = -0.55, *p* = 0.018). In other words, participants with higher SSS scores were less aroused by CS+ than participants with lower SSS scores.

3.6 Sleep measures (SF-A/R)

Mean sleep times are demonstrated in Figure 20. Participants' average sleep was 8.05 ± 0.73 hours. Average sleep the night before tests was 7.55 ± 0.82 hours. On the nights after the interventions, the average sleep time returned to normal values (8.14 ± 0.73 hours).

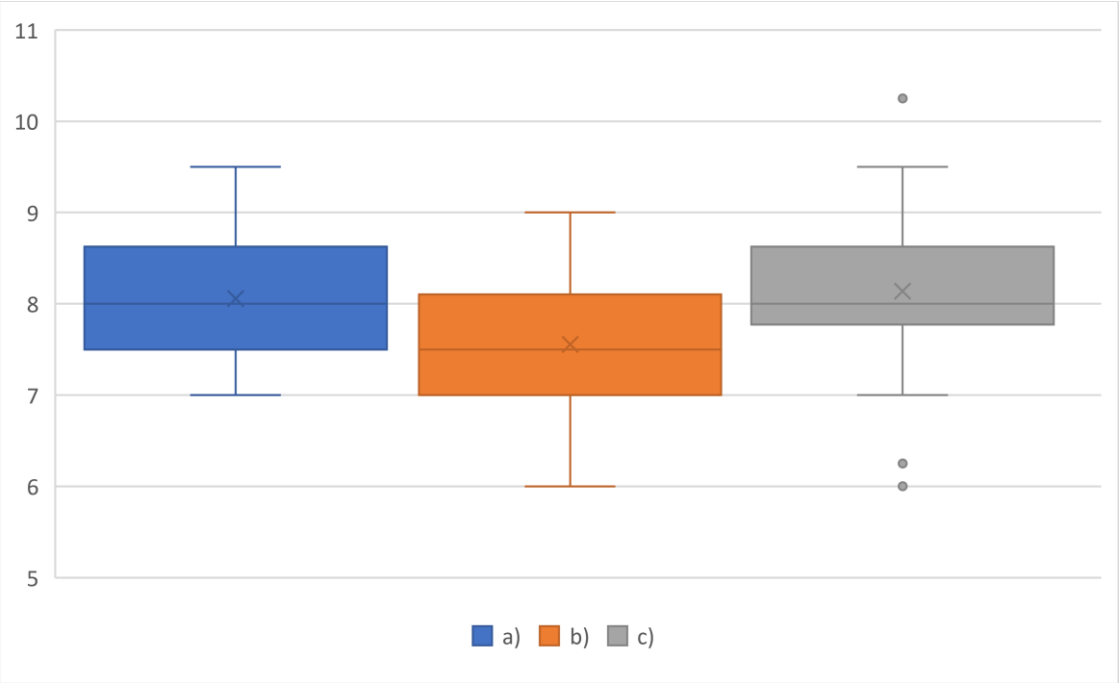

Figure 20 – a) Average sleep per night in hours b) Average sleep the night before the experiment in hours c) Average sleep the night after the experiment in hours

In the SF-A/R, total sleep time (GSD) was slightly higher (mean sleep time in hours: sleep day = 7.79 ± 0.94, control day = 8.5 ± 0.87) in the night following the control day compared to the sleep day ($F(1;12) = 5.648$, $p = 0.035$). There was no measurable effect of the variables on sleep quality (Table 25).

Table 25 – Tests of Within-Subject Effects: SF-A/R: sleep quality (SQ), repeated measures ANOVA

	df	F	p	η^2
intervention	1; 12	.268	.614	.022
time	1; 12	1.937	.189	.139
intervention * time	1; 12	1.405	.259	.105

Sleep quality (SQ) during the nap was calculated as described in the manual (see 2.8), resulting in a mean SQ of 3.008 (1 = low SQ / 5 = high SQ), which was the lowest overall SQ. SQ on the nights before each experimental day was 4.128 and 4.137, respectively. SQ on the nights after each experimental day was 3.861 and 3.961, respectively. SQ during the nap varied between a minimum of 1.625 and 4.125 (Table 26).

Table 26 – Descriptive statistics: Sleep quality (SF-A/R) during nap and on the nights before and after each experimental day

	N	Min	Max	*M*	*SD*
SQ (nap)	16	1.625	4.125	3.008	.815
SQ (before sleep)	18	3.208	4.750	4.128	.410
SQ (after sleep)	14	2.583	4.917	3.861	.713
SQ (before control)	18	3.417	4.750	4.137	.329
SQ (after control)	15	2.500	4.750	3.961	.721

4 Discussion

4.1 Cardiovascular measures

In all three cardiovascular measures, the type of intervention had a significant effect on the participants' physiological responses. Before the intervention, there was no major difference between sleep and control day measures. After the intervention, the cardiovascular measures changed more on the control day than on the sleep day. Specifically, after the period of wakefulness, HR decreased, and HRV increased, which was not observed after sleep. This was the opposite of what we expected from most previous literature. We regarded the control day as a kind of "sleep deprivation" compared to a 2-hour nap. Sleep deprivation has been found to decrease the parasympathetic nervous system and increase the sympathetic nervous system (Hynynen et al., 2006) and to cause an autonomic imbalance (Takase et al., 2004). The sympathetic activation, e.g. through sleep deprivation, would decrease HRV, whereas vagal activity would increase it (Gruber & Cassoff, 2014; Malliani A et al., 1991; Pagani M et al., 1986).

Leary et al. (2002) describe a morning surge in heart rate influenced by physical activity after waking. In our study, this could be the reason for the comparatively higher heart rate measures in the conditioning experiment after the 2-hour nap. According to Boudreau et al. (2012), this is due to a sympathetic activation after sleep with higher arousal levels and lower HRV compared to night times, whilst during the nap, the parasympathetic nervous system predominates (Takahashi et al., 1998). Importantly, though, we did not start the post-sleep experiment immediately after awakening but gave participants half an hour to recover and to fill in several questionnaires, so it is questionable whether there was still a sympathetic surge during the second testing.

Most probably, a decrease of HR and increase of HRV in the wake condition during the post-intervention experiments could be attributed to the circadian rhythm of cardiovascular measures. Cardiovascular measurements of participants during a normal sleep-wake cycle show maximum HR and minimum HRV between 11:00-12:00 h (Krauchi & Wirz-Justice, 1994). Minimum HR and maximum HRV are between 00:00-01:00 h. Typical graphs of hourly HR and HRV demonstrate that after reaching their peaks around midday, heart rate decreases gradually during in the afternoon and

evening, reaching its lowest point around midnight; HRV increases at the same time during a wake phase (Massin, 2000; Sapoznikov et al., 1992; Stein, 2007). This is consistent with our cardiovascular results in the wake condition. The effect of the nap can, therefore, be primarily regarded as an interruption of this cycle.

If this hypothesis is correct, the error in our a priori expectations was that we transferred the results of night sleep experiments to afternoon sleep. This analogy is, however, incorrect because circadian factors are quite different in these two kinds of sleep.

It should be said, however, that the question whether the pattern of HR and HRV depends on the actual time of day (implying that the body has a "physiological clock") or, rather, on the time of rest, is still a matter of debate. Krauchi & Wirz-Justice measured heart rate during a 34-h period in which participants rested, stayed awake during the whole time and were unaware of the time of day. Participants still demonstrated a circadian rhythm of HR with the usual peaks, indicating that there must be an endogenous circadian component for certain physiological measures (Krauchi & Wirz-Justice, 1994). Ewing et al measured HRV for participants with normal sleep-wake cycles, for a sleep-deprived group and for a group working nightshifts. During sleep-deprivation, HRV was similar to the wake periods. The night shift group showed higher HRV when sleeping during the day. In total, this study indicated that actual sleeping or waking periods influence HRV results stronger than a fixed circadian rhythm (Ewing et al., 1991).

Pace-Schott et al, 2011, analysed the effect of a daytime nap on inter-session habituation to aversive visual stimuli, including the analysis of heart rate deceleration (HRD), as mentioned in chapters 1.7 and 1.8. Although this study is not a classical conditioning experiment, the setup is very similar to the setup of our study and the effect of aversive stimuli on the cardiovascular measure HRD can be analysed. HRD to a negative stimulus is common and is seen as a part of the orienting reflex as discussed in chapter 1.3.1 (Bradley, 2009; Hare, 1972). In Pace-Schott et al.'s (2011) study HRD responses habituated in the wake group, leading to a less pronounced HRD in the second session. In contrast, there was no habituation in the sleep group. These findings are quite similar to the findings of our study with a greater change in the post-intervention wake group. The authors suspect that a rise in parasympathetic outflow

may augment the capacity of a stimulus to mediate a HRD response. In our study this would mean that the parasympathetic activation due to the nap, still present during post-intervention conditioning, additionally modifies cardiovascular measures, counteracting the effect of the intervention itself. In contrast to the "sympathetic surge" as mentioned at the beginning of this chapter, in this case ongoing parasympathetic activation is held responsible for the effect. In a 2014 study, the same authors investigated the effect of night sleep compared with a 12 hour wake period and obtained exactly the opposite result: HRD responses decreased after sleep, but increased after wakefulness (Pace-Schott et al., 2014). This is another indication that the time of the day has a strong influence on cardiovascular responses towards aversive stimuli.

PWA, on the other hand, did react as we had expected. This is interesting since PWA is a rarely used tool for studying cardiovascular responses and we are not aware of any studies examining PWA during aversive conditioning. It is known that PWA is on a higher level during sleep but decreases in response to external or internal stress. As mentioned in chapter 1.8, PWA drops in response to auditory disturbances (Catcheside et al., 2002), during obstructive apnoea events (Haba-Rubio et al., 2005; Ramirez et al., 2013), but also during undisturbed sleep marking a change in cortical activity (Delessert et al., 2010). If less sleep increases the SNS activity, it makes sense that PWA should correlate negatively with wakefulness (Lavie et al., 2000). In previous studies analysing cardiovascular responses towards a sympathetic activation, both PWA and HRV decreased (Colombo et al., 2015). In our study these two measures had an inverse relationship. A possible explanation could be the missing circadian variation of PWA. As mentioned above, during the hours of our experiment, HR decrease and HRV increase is part of the expected circadian variance, which may have concealed our expected cardiovascular responses. This effect may not be present for PWA, but the evidence is weak. Up to date there are no studies analysing the circadian dynamics of PWA.

As can be seen in Figure 8, there was a larger difference between the PWA of block 1 and 2 than between block 2 and 3. In the first block, no aversive stimulus was presented. In the second block, participants must have instantaneously developed a response to the aversive stimulus. In the third block, there was no additional learning effect. Thus, the response to CS+ was learnt immediately during partial reinforcement and was not

substantially influenced by the following total reinforcement. This is further confirmed by SAM measurement data, which will be discussed below in chapter 4.2.

Notably, Heathers and Goodwin, 2017, mention a number of papers which criticize the use of HRV as a measure for sympathovagal balance. In particular since the 1996 Task Force publication of standards for HRV analysis, thousands of scientists have used this method (Camm et al., 1996). It is comparatively cheap, non-invasive and easy to assess. One must be careful to simplify the results though: HRV and the LF/HF-ratio are dependent on many factors such as respiratory rate, heart rate, cardiac function, or the current level of activity. Respiratory rate can particularly affect LF HRV and is rarely controlled in cardiovascular studies (Brown et al., 1993), including our own. HRV is directly influenced by the heart rate – lower HR automatically causes higher oscillations (Sacha & Pluta, 2008). It is too simple to state that high HRV indicates parasympathetic activation and vice versa (Berntson et al., 1997). For example, facial emersion in cold water increased sympathetic activity but caused bradycardia (Eckberg et al., 1984). Sympathetic-parasympathetic interactions are not always linear, the stimulation of one can actually increase the action of the other (Billman, 2013; Levy Matthew N., 1971).

For this reason, we decided to analyse HR and PWA as additional cardiovascular measures. HR may have advantages to HRV, e.g. it is a better indicator of cardiac health compared to HRV (Grant et al., 2013). As expected and mentioned in the references above, HR and HRV showed an inverse relationship (Sacha, 2014). PWA on the other hand showed a similar reaction than HR. Interestingly, as mentioned above, there was a significant effect of the reinforcement rate on PWA, which was not the case for HR and HRV. A partial explanation might be that the HRV response includes both phases of accelerations and decelerations (Sevenster et al., 2015), which could lead to the effect being averaged out. PWA seems to be a good marker of general stress. Like skin conductance responses, PWA may be a more direct measure of sympathetic activity than HRV (Bach, 2014) and is thought to be a more reliable, although more complex, measure of autonomic balance (Heathers & Goodwin, 2017).

4.2 Self-assessment measures (arousal, valence)

Mean arousal level after the aversive sound indicated medium to high initial arousal upon first hearing of the sound. Valence was clearly negative with means of 6.94/9

respectively 7.72/9. Although valence scores ranged from 5 until the maximum value of 9, indicating strong interindividual differences, all participants felt the US to be unpleasant. The aversiveness of our US was comparable to other aversive US used in previous studies. E.g., in a study using electric shocks as aversive US participants had a mean arousal of 5.15/9, SD = 2.07 and a mean valence of 4.87/9, SD = 1.76 in trials with a shock (Fayolle et al., 2015). In another study using an aversive odour as US, participants scored 4.58, SD = 0.67 (patients) and 4.33, SD = 0.49 (controls) on a 5-point scale SAM for emotional valence (Schneider et al., 2000). Overall, our aversive US seems to have been of reasonable strength in comparison to other types of stimuli.

Since the main effect of condition (CS+ vs. CS-) on the valence scores was highly significant, clearly the participants learned to react differently to each stimulus. Emotional reaction to the CS+ was conditioned through the combination with the aversive stimulus US+ and created higher (i.e., more negative) valence scores than reaction to the CS-, which was combined with the neutral (slightly positive) US-. As can be seen in Figure 9, the differential response was formed in the 50% reinforcement block. The third block did not have an additional effect on the learning process; here the scores did not differ significantly between blocks 2 and 3. Conditioning with the aversive stimulus therefore resulted in a very fast learning effect with no additional benefit of a higher reinforcement rate. The same effect could be seen in the PWA reactions (see 3.1).

This rapid conditioning may be due to the "preparedness" of the body for fear-relevant stimuli, resulting in faster CR acquisition, stronger CRs and greater resistance to extinction compared to non-aversive stimuli (Öhman & Dimberg, 1978; Öhman & Soares, 1993). In a study comparing conditioning of fear-relevant (facial expression of fear) vs. non-fear-relevant (happy expression) stimuli (CS+) paired with an electrical shock US, fear-relevant stimuli as CS+ acquired the ability to evoke physiological and subjective arousal as well as enhanced negative emotions. Non-fear-relevant stimuli did not have such a strong ability to elicit conditioned fear, although a stronger association may have been achieved if there had been more acquisition trials (Williams & Rhudy, 2007).

The intervention (i.e., sleep versus wakefulness) did not influence behavioural responses. As mentioned in chapter 1.6, this is consistent with the findings of the meta-analysis by Lipinska et al., 2019, who did not find an overall effect of sleep-dependent consolidation for emotional material. Although most studies showed a better consolidation of emotional material compared to neutral material, sleep only had an additional effect in certain testing conditions. For example, sleep had a strong effect on consolidation in studies using free recall outcome measures but not in studies using recognition measures. Some data indicate that free recall tasks are more dependent on hippocampal activity, which may be more strongly influenced by sleep (Girardeau et al., 2017), whereas recognition-based retrieval relies more on frontal-subcortical structures (Squire & Dede, 2015). Since our study did not include free recall tasks, this may be a reason for a missing influence of the intervention.

Ratings of pre- vs. post-intervention measurement also did not differ significantly. This indicates a rapid extinction of aversive conditioning because the data from block 1 (with no reinforcement) did not differ between pre- and post-intervention experiments. This is interesting since aversive conditioning is usually more resistant to extinction than non-aversive conditioning. This effect is particularly strong in phobias, which are highly resistant to extinction (Seligman, 1971). In more recent studies, conditioned fears actually have proven easy to extinguish, but a relapse is not uncommon. The extinction is easy to “learn” but difficult to “remember” (Vervliet et al., 2013). In our study, there were 60 non-reinforced CS presentations in the first block of the post-intervention experiment, which seem to have allowed a successful extinction.

In contrast to cardiovascular measures, behavioural measurements were not influenced by the type of intervention. Chapter 1.3.1 provided a short introduction of the research concerning the connection of the heart and the brain. HRV is said to be an objective measure of regulated emotional responding (Appelhans & Luecken, 2006). In the past, a neurovisceral integration model has been described, suggesting a connection of cardiovascular measures such as HRV with the ability to regulate emotion on a central neural level (Thayer & Lane, 2000, 2009). Imaging of the brain has identified a central autonomic network (CAN) including structures such as the anterior cingulate, insular, ventromedial prefrontal cortices and the amygdala, which can regulate HRV (Benarroch, 1993; Lane et al., 2001). This connection has also been shown in HRV

biofeedback training, its repeated application being associated with a reduction of stress and anxiety according to a recent meta-analysis (Goessl et al., 2017). In our study, however, the cardiovascular effect of the intervention did not modify the effect on behavioural measures, thus providing an indication that the CAN is more complex than the previous literature suggests. It may be speculated, for example, that circadian factors affect autonomic responses much stronger than the corresponding emotional responses. Also, critics of the HRV measure, as mentioned in chapter 4.1, could argue that the cardiovascular responses are not simple measures of the autonomic balance as many authors suggest.

4.3 Emotional state measures (STAI state, PANAS)

State anxiety increased during each conditioning experiment. We observed that the aversive stimuli had a negative effect on the participants' emotional state. Interestingly, anxiety levels increased more strongly in the pre-intervention experiments. After the intervention, anxiety levels did not increase as much. Participants had probably learnt to deal with the aversive stimuli and did not have such a negative reaction to the discomforting sounds.

PA increased and NA decreased following the 2-hour nap compared to scores following the 2-hour wakefulness. This provides more evidence that a 2-hour nap had an emotionally revitalizing effect on the participants, although even during wakefulness negative affect was reduced compared to the first post-conditioning levels.

Positive affect was also higher before each conditioning experiment and declined after each experiment. The experiment itself therefore had a significantly unpleasant effect on the participants' emotional status. The decline was particularly large following the first conditioning and less during the second, similar to the dynamics of state anxiety.

The fact that anxiety levels and affect levels did not change so much after the post-intervention experiment compared to the pre-intervention experiment could also be the consequence of UR diminution. When a US is repeated in presence of a newly learned CS+, the UR amplitude is attenuated (Kimmel & Pennypacker, 1962). The body adapts to the US over time, which could also be shown in a recent fMRI study, which showed an inverse relationship between US expectancy and UR amplitude within the amygdala, anterior cingulate and dorsolateral prefrontal cortex (Dunsmoor et al., 2008). The same

effect was found for skin conductance responses, with reduced amplitudes of URs when the US was preceded by a CS+ (Marcos & Redondo, 1999). On the other hand, one would expect an increase in CR at the same time the UR decreases. Our study design did not allow us to discriminate between UR and CR amplitudes since emotional state measures were only taken at the end of each conditioning experiment, therefore it remains unclear whether the results can be attributed to UR diminution.

Since the test was not performed between blocks, we do not know whether the fear extinction during block 1 of the post-intervention experiment was accomplished for emotional state measures similar to the results of the behavioural measures. The less pronounced increase of anxiety levels and smaller decline of positive affect when comparing the pre- and post-intervention reactions to the experiment indicate that there was still a trace of the CR directly before the post-intervention experiment, indicating that the reaction had been consolidated during the first experiment and not completely extinguished during the 2-hour intervention interval.

4.4 Vigilance measures (Stanford Sleepiness, Vigilance test)

After a 2-hour nap, reaction times significantly decreased, whereas the reaction time of the control group increased further, supporting the hypothesis that sleep had a refreshing effect on vigilance. The wakefulness period led to a decrease in vigilance. Sleepiness also decreased after the nap compared to wakefulness. This effect is consistent with the results of other studies, which show that lunch-time naps decrease sleepiness and improve performances for mental tasks afterwards (Takahashi et al., 1998). Sleepiness on the other hand reduces peoples' attention in the PVT (Lim & Dinges, 2008) and increases their distractibility during the test (Anderson & Horne, 2006).

4.5 Personality traits (STAI trait, SSS, NEO-FFI)

Participants with higher SSS scores had a significantly weaker arousal reaction towards the CS+. This reaction was to be expected as the creators of this test also found a significant negative correlation between SSS and anxiety (Zuckerman et al., 1964). People with higher levels of sensation-seeking are likely to show a less intense reaction to an aversive stimulus, whereas people with low sensation seeking scores show a greater fear-potentiated startle in response to threatening stimuli (Lissek, Baas, et al., 2005).

The correlation between neuroticism scores and STAI trait anxiety scores was to be expected, as both scores measure a stable, quite similar, personality trait. A high anxiety/neuroticism individual is described as follows: “He is overly emotional, reacting too strongly to all sorts of stimuli, and finds it difficult to get back on an even keel after each emotionally arousing experience” (Eysenck & Eysenck, 1975; Jorm, 1989). Both scores therefore indicate an anxious personality which is more likely to react to fear in a similar way. Surprisingly, neither scores correlated with physiological or behavioural data, even though previous studies have demonstrated that people with higher trait anxiety scores have a facilitated detection and processing of threats (Byrne & Eysenck, 1995).

4.6 Sleep measures (SF-A/R)

In the night following the sleep day, participants needed less sleep than in the night following the control day. This could be due to the additional stress of undergoing the conditioning experiment without a break, which as a result led to a higher sleep deficit. It could also merely be due to the fact that participants had slept during the experimental day and their bodies therefore needed less sleep in the following night to be well-rested. Another possible explanation is that sleep may be necessary to consolidate fear memory, as aversive conditioning experiments modify the way we sleep (Graves et al., 2003; Menz et al., 2013). Therefore, the 2-hour afternoon nap might have given participants the additional time they needed to process the aversive conditioning experiments, whereas in the nights following control days, more sleep was needed to process the new information.

Quality of sleep was not affected by the conditioning experiment. It is known that increased agitation, e.g. in patients with anxiety disorders (Papadimitriou & Linkowski, 2005) or with PTSD (Mellman et al., 1995), reduces sleep quality. In our study it seems like the conditioning experiment was not present in the following night in a way that it affected the participants’ sleep.

Sleep quality during the nap was 3.008 on a scale of 1 (low quality) to 5 (high quality). It was measured as a combination of several categories of the ST-A/R test, including ESS (difficulties falling asleep), DSS (difficulties during sleep), VZA (waking up to early) and ASC (general sleep characteristics) and therefore provides the most accurate

estimate of participants' sleep during the nap. All participants did sleep during the nap, but there was a large SD of 0.815, indicating a high interindividual variance of the sleep quality. On the nights spent at home, SQ was considerably higher, especially on the nights before experimental days with a SQ of > 4. These results indicate that sleeping conditions were not ideal for the participants, even though they had all undergone the adaptation day to get used to the new environment. On the other hand, we did not compare the data of our 2-hour nap with a 2-hour nap in familiar settings. Perhaps a "regular" night's sleep will always cause higher SQ compared to a short nap. This effect cannot be completely ruled out.

4.7 Study limitations and future direction

An obvious limitation of our study is the small sample size. 18 participants took part in the experiment. Due to incorrect or incomplete measuring, only 14 (for PWA) or 15 (for HR and HRV) samples were included in the analysis. Thus, the effect may have been stronger with a larger sample size. With an average age of 24.7 years, our participants were very young. It would have been interesting to have a larger variation of age, since HRV and emotional reactions change during a lifetime. There is a decline in cardiovascular control for elderly people (Colosimo et al., 1997). This has also been demonstrated in longitudinal studies (Sinnreich et al., 1998). Activation of brain regions during emotional reactions also changes with age: the lateral prefrontal cortex activation declines, as does the connectivity with the amygdala (Sakaki et al., 2016). Another factor we did not take into account, which may influence emotional reactions to aversive conditioning, is the menstrual cycle of female participants, as lower oestrogen levels are associated with inhibition of fear and extinction deficits (Glover et al., 2012, 2013).

In our study we chose an aversive auditory stimulus presented through pneumatic earphones. Simple harmonic tones have been shown to elicit stable ERP effects for healthy participants (Tervaniemi et al., 2000) and neurological patients (Kotchoubey et al., 2003). Tactile aversive stimuli have a large interindividual variation and require individual calibration of the stimulation level (Lipp, 2006). Interestingly, the reactions to auditory stimuli were not stable in our study. Several participants found the stimuli almost unbearable; others did not show a great reaction to the stimuli. Factors determining this variability should be examined in future studies.

Photoplethysmography is more sensitive to environmental effects. To analyse HR and HRV, ECG recording is probably a more sensitive method with less artefacts during measurement. PPG allowed us to measure the PWA, which is not possible to measure with ECG, and therefore gain additional cardiovascular information. With longer interstimulus intervals (ISI), it would have been possible to measure direct PPG responses to stimuli, which may permit the researcher to distinguish accelerators and decelerators (Sevenster et al., 2015) or to detect a possible UR decrement to the aversive stimulus (Grings & Schell, 1971). These responses could have been compared with behavioural responses. It would have also been interesting to measure continuous PPG or ECG during the sleep or wake intervention. This might have helped to find clues why the cardiovascular measures differed for the post-intervention experiments and differentiate whether the effect depended on the intervention or perhaps on other modifying factors.

An obviously important factor is the strength of the aversive US, because if the US+ is weak, all responses can be different compared with conditioning with a very strong, painful US+. As far as we can see from the assessment of arousal and valence (e.g., Section 3.2), our US+ did not substantially differ in strength in comparison with typical fear conditioning studies. In future experiments, additional measures of stimulus strength may be used.

We hypothesized in chapter 4.1 that there is a strong circadian effect on cardiovascular measures. Around midday HR reaches a maximum and HRV a minimum. The effect of HR and HRV in the wake group could therefore be due to the circadian rhythm of the participants' cardiovascular system, rather than due to the lack of sleep. Although fewer people sleep in the morning than in the afternoon, it would be interesting to collect such a sample and to compare the effects of morning versus afternoon nap; on the morning, the trend of HR and HRV would be the opposite of the afternoon trend.

Finally, sleep quality during the 2-hour nap was most likely not as good as sleep quality on the nights spent at home. Participants spent a variable amount of time awake. Therefore, the refreshing effect of a sleep phase will have been diminished compared to a nap with better sleep quality and will have possibly diminished differential effects on the cardiovascular and behavioural measures.

5 Conclusion

In this work, we analysed the effect of a 2-hour period of sleep or wakefulness on behavioural and cardiovascular responses during aversive conditioning. Specifically, we examined reactions of heart rate, heart rate variability and pulse wave amplitude, measured through photoplethysmography. We also examined the change in behavioural measures in response to each stimulus as well as emotional state and vigilance before and after each test. Additionally, we calculated correlations between the conditioned responses and stable personality traits and tested whether sleep quality was affected by the conditioning experiment.

After the period of wakefulness, as compared with the pre-intervention session, HR decreased, whereas HRV increased. These changes were much less pronounced after the 2-hour nap, resulting in a strong and significant interaction between Time (pre- versus post-) and the type of intervention. This might be due to the circadian dynamics of cardiovascular parameters in the control condition, whereby sleep is supposed to break this circadian trend. Another, less plausible, interpretation might be a general sympathetic surge of the sleep group directly after waking up. Other studies hold an ongoing parasympathetic activation responsible for less pronounced cardiovascular responses following sleep. PWA decreased after the period of wakefulness. In contrast to HR and HRV, possibly its response was not concealed by circadian variance. All three cardiovascular measures significantly responded to aversive stimulation, but PWA, in contrast to HR and HRV, also depended on the rate of the aversive stimulation indicating that PWA provided additional information beyond that of HR and HRV.

In line with the previous studies, subjective emotional responses exhibited fast learning, but also fast extinction. The type of intervention (i.e., sleep or wakefulness) did not have an effect on these measures. This is contrary to the cardiovascular measures that were affected by the type of intervention (see above), indicating the lack of a simple relationship between autonomic and subjective parameters.

Anxiety levels and negative affect increased with conditioning, and the increase was stronger before than after interventions, suggesting a preserved trace of memory after the 2-h interval. UR diminution may have also attributed to less pronounced post-intervention anxiety increase.

The nap had refreshing effect on participants, manifested in an increase of positive affect, decrease of subjectively experienced sleepiness, and faster reaction times in a vigilance task after the nap compared to wakefulness.

As expected, participants with a higher sensation seeking scores were less aroused by the CS+ than low sensation seeking individuals. No personality scores showed any correlation with cardiovascular measures.

Quality of sleep was not affected by the experiment, but participants needed less sleep after the sleep day than after the control day.

For future studies it would be interesting to analyse the effect of the circadian rhythm on cardiovascular measures, for example by performing a similar experiment with a morning intervention.

Additionally, with longer ISI it would be possible to measure cardiovascular reactions towards each individual stimulus. These could be correlated with the emotional reactions towards each stimulus. A continuous measurement of cardiovascular information during the interventions could also help to find an explanation for the significant difference of cardiovascular reactions in the post-intervention wake group compared to the sleep group.

6 English summary

In this work, we analysed the effect of a 2-hour period of sleep or wakefulness on behavioural and cardiovascular responses during aversive conditioning. Specifically, we examined reactions of heart rate (HR), heart rate variability (HRV) and pulse wave amplitude (PWA), measured through photoplethysmography. We also examined the change in behavioural measures in response to each stimulus as well as emotional state and vigilance before and after each test. Additionally, we calculated correlations between the conditioned responses and stable personality traits and tested whether sleep quality was affected by the conditioning experiment.

A total of 18 participants took part in four identical series of tests on two afternoons. One series of tests was performed before (pre-intervention) and one after (post-intervention) a 2-hour period of sleep or wakefulness. Participants were asked to sit still and attentively listen to 5 different tones, presented via headphones. Aversive conditioning was performed by using a very loud aversive tone (unconditioned stimulus, US+). This US+ was paired with an initially emotionally neutral tone (conditioned stimulus, CS+). Furthermore, there was a neutral standard tone as well as two other neutral tones (US- and CS-) which were also paired with each other. Each series of tests consisted of three blocks. In each of these blocks, 280 standard tones, 60 CS+ and 60 CS- were presented. In between blocks there was a short break and emotional responses towards each stimulus were assessed. In the first block the aversive tone was not introduced, thus the CS+ was unpaired (0% reinforcement rate). In the second block every second CS+ was followed by a US+ (50% reinforcement rate). In the third block each CS+ was followed by a US+ (100% reinforcement rate). During all tests finger plethysmography was measured continuously. All other results were acquired through questionnaires.

After the period of wakefulness, as compared with the pre-intervention session, HR decreased, whereas HRV increased. These changes were much less pronounced after the 2-hour nap, resulting in a strong and significant interaction between time (pre-intervention versus post-intervention) and the type of intervention (sleep versus wakefulness). This might be due to the circadian dynamics of cardiovascular parameters in the control condition, whereby sleep is supposed to break this circadian trend. Another, less plausible, interpretation might be a general sympathetic surge of the sleep

group directly after waking up. Other studies hold an ongoing parasympathetic activation responsible for less pronounced cardiovascular responses following sleep. PWA decreased after the period of wakefulness. In contrast to HR and HRV, possibly its response was not concealed by circadian variance. All three cardiovascular measures significantly responded to aversive stimulation, but PWA, in contrast to HR and HRV, also depended on the rate of the aversive stimulation indicating that PWA provided additional information beyond that of HR and HRV.

Self-assessment manikins (SAM) were used to evaluate emotional responses during the conditioning experiment. Valence and arousal scores in reaction to each stimulus were collected in between test blocks. In line with the previous studies, subjective emotional responses exhibited fast learning, but also fast extinction. The type of intervention (i.e., sleep or wakefulness) did not have an effect on these measures. This is contrary to the cardiovascular measures that were affected by the type of intervention (see above), indicating the lack of a simple relationship between autonomic and subjective parameters.

The State-Trait Anxiety Inventory (STAI) questionnaire and the Positive and Negative Affect Schedule (PANAS) questionnaire were used to measure emotional state measures before and after each of the four test series. Anxiety levels and negative affect increased with conditioning, and the increase was stronger before than after interventions, suggesting a preserved trace of memory after the 2-h interval.

The nap had refreshing effect on participants, manifested in an increase of positive affect, decrease of subjectively experienced sleepiness, and faster reaction times in a vigilance task after the nap compared to wakefulness.

As expected, participants with a higher sensation seeking scores were less aroused by the CS+ than low sensation seeking individuals. No personality scores showed any correlation with cardiovascular measures.

Quality of sleep was not affected by the experiment, but participants needed less sleep after the sleep day than after the control day.

For future studies it would be interesting to analyse the effect of the circadian rhythm on cardiovascular measures, for example by performing a similar experiment with a morning intervention.

Additionally, with longer interstimulus intervals it would be possible to measure cardiovascular reactions towards each individual stimulus. These could be correlated with the emotional reactions towards each stimulus. A continuous measurement of cardiovascular information during the interventions could also help to find an explanation for the significant difference of cardiovascular reactions in the post-intervention wake group compared to the sleep group.

7 Deutsche Zusammenfassung

In dieser Studie untersuchten wir in einem Konditionierungsexperiment den Effekt einer zweistündigen Schlafphase verglichen mit einer zweistündigen Wachphase auf kardiovaskuläre sowie emotionale Reaktionen während einer aversiven Konditionierung. Im Einzelnen untersuchten wir Herzfrequenz, Herzfrequenzvariabilität (HRV), Pulswellenamplitude (PWA) sowie emotionale Verhaltensänderungen infolge jedes Stimulus und den emotionalen Zustand sowie die Vigilanz vor und nach jeder Testreihe. Schließlich berechneten wir Korrelationen zwischen den konditionierten Reaktionen und unveränderlichen Persönlichkeitsmerkmalen und untersuchten, ob die Schlafqualität durch das Konditionierungsexperiment beeinflusst wurde.

Insgesamt 18 Probanden absolvierten jeweils vier identisch aufgebaute Testreihen an zwei Nachmittagen. Eine Testreihe wurde vor (präinterventionell) und eine Reihe nach (postinterventionell) einer zweistündigen Schlaf- bzw. Wachphase durchgeführt. Über Kopfhörer wurden insgesamt 5 verschiedene Töne präsentiert, während die Probanden still und aufmerksam auf einem Stuhl saßen. Hierbei wurde eine aversive Konditionierung mithilfe eines sehr lauten aversiven Tones (unkonditionierter Stimulus, US+) durchgeführt. Dieser wurde gepaart mit einem primär emotional neutralen Ton (konditionierter Stimulus, CS+). Des Weiteren gab es einen neutralen Standardton sowie zwei weitere primär neutrale Töne (US- und CS-), die ebenfalls miteinander gepaart wurden. Jede Testreihe bestand aus jeweils drei Abschnitten, in der jeweils 280 Standard-Töne sowie 60 CS+ bzw. CS- präsentiert wurden. Zwischen den Abschnitten erfolgte eine kurze Pause mit Bewertung der emotionalen Reaktionen auf die einzelnen Stimuli. Im ersten Teil wurde der aversive Ton noch nicht eingeführt, der CS+ wurde also ungepaart präsentiert (Verstärkungsrate 0%). Im zweiten Teil folgte jedem zweiten CS+ ein US+ (Verstärkungsrate 50%). Im dritten Teil betrug die Verstärkungsrate 100% (jedem CS+ folgte ein US+). Während aller Testreihen wurde eine kontinuierliche Aufzeichnung der kardiovaskulären Daten über Photoplethysmographie des Fingers erreicht. Die übrigen Tests wurden anhand von Fragebögen durchgeführt.

Im Vergleich zur präinterventionellen Testung sank die Herzfrequenz nach einer zweistündigen Wachphase, die HRV hingegen stieg an. Diese Veränderungen waren nach einer zweistündigen Schlafphase deutlich weniger ausgeprägt. Dies ergab eine starke und signifikante Interaktion zwischen der Testzeit (prä- vs. postinterventionell)

und der Interventionsart (Schlaf- vs. Wachphase). Am ehesten könnte das an der zirkadianen Dynamik kardiovaskulärer Parameter hängen, wobei Schlaf hier für eine Durchbrechung des üblichen Trends sorgt. Der Einfluss von sympathischer sowie parasympathischer Erregung spielt hierbei ebenfalls eine wichtige Rolle. PWA hingegen sank nach der Wachphase. Möglicherweise wurde ihre Messung nicht von zirkadianen Einflussfaktoren überdeckt. Alle drei kardiovaskulären Parameter zeigten eine signifikante Reaktion auf den aversiven Stimulus. Die PWA bot aber zusätzliche Informationen, da sie zusätzlich von der Verstärkungsrate der aversiven Stimulation abhing.

Emotionale Reaktionen wurden mithilfe von Selbstbewertungs-Figuren (SAM) erhoben. Es wurde die Wertigkeit (Valenz) und die Erregung der einzelnen Stimuli bewertet. Beide unterlagen einer raschen Akquisition sowie Extinktion. Die Interventionsart beeinflusste – im Gegensatz zu den kardiovaskulären – die emotionalen Reaktionen nicht. Es lag kein Zusammenhang zwischen diesen Parametern vor.

Der emotionale Zustand wurde mithilfe zweier Fragebögen zur Erfassung der Angst (STAI) sowie der positiven bzw. negativen Affekte jeweils vor und nach den vier Testreihen erhoben. Angst sowie negative Gefühle nahmen während der Konditionierung zu. Dieser Effekt war präinterventionell stärker. Postinterventionell war zu Beginn der Testung vermutlich das Gelernte noch teilweise präsent.

Schlaf hatte eine revitalisierende Wirkung auf die Probanden: Sie zeigten einen gesteigerten positiven Affekt, weniger subjektiv empfundene Schläfrigkeit sowie schnellere Reaktionszeiten im nach der Intervention durchgeführten Vigilanztest.

Probanden mit einer höheren Sensationslust (gemessen mit dem Sensation Seeking Scale) zeigten weniger emotionale Erregung infolge des konditionierten Stimulus. Die übrigen Persönlichkeitsmerkmale zeigten keine Korrelationen mit kardiovaskulären Parametern.

Die allgemeine Schlafqualität wurde durch die Experimente nicht beeinträchtigt, jedoch benötigten die Probanden in der auf den Schlaf-Interventionstag folgenden Nacht weniger Schlaf.

Für zukünftige Studien wäre es interessant, den Einfluss der zirkadianen Dynamik zu untersuchen. Hierzu wäre ein Experiment analog zu unserem denkbar, bei dem die Intervention morgens durchgeführt wird.

Zudem könnten zur genaueren Messung der kardiovaskulären Reaktionen auf die einzelnen Stimuli die Interstimulus-Intervalle (Zeitfenster zwischen Stimulus-Präsentationen) größer gewählt werden. Diese Reaktionen könnten mit den emotionalen Reaktionen auf die einzelnen Stimuli korreliert werden. Außerdem würde eine fortlaufende Messung der kardiovaskulären Daten während der jeweiligen Intervention zusätzliche Hinweise über den signifikanten Unterschied der kardiovaskulären Daten der postinterventionellen Wachgruppe im Vergleich zu Schlafgruppe liefern.

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9 Erklärungen zum Eigenanteil der Dissertationsschrift

Die Arbeit wurde am Institut für Medizinische Psychologie und Verhaltensneurobiologie der Eberhard Karls Universität Tübingen unter Betreuung von Prof. Dr. Boris Kotchoubey durchgeführt.

Die Konzeption der Studie erfolgte durch Prof. Dr. Boris Kotchoubey sowie Dr. Yuri Pavlov, postdoctoral researcher am selbigen Institut.

Die Versuche wurden von mir in Zusammenarbeit mit Dr. Yuri Pavlov durchgeführt. An den Versuchstagen erfolgte eine Reihe von Experimenten. Nur ein Teil dieser Experimente und Ergebnisse wurde in dieser Dissertationsschrift ausgewertet und beschrieben. Der andere Teil diente einer wissenschaftlichen Arbeit von Dr. Yuri Pavlov. Die in dieser Arbeit präsentierten Ergebnisse sind nicht Teil einer anderen wissenschaftlichen Arbeit oder Publikation.

Die statistische Auswertung erfolgte mit Unterstützung durch Dr. Yuri Pavlov durch mich.

Ich versichere, das Manuskript selbständig verfasst zu haben und keine weiteren als die von mir angegebenen Quellen verwendet zu haben.

_____ _____

Ort, Datum Unterschrift (Martin King)

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