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Direktor: Prof. Martha Merrow

NEUROPHYSIOLOGICAL CORRELATES OF THE PLACEBO EFFECT IN NAUSEA

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Anja Eva-Maria Haile
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Berichterstatter:	Prof. Dr. med. habil. Karin Meißner
Mitberichterstatter:	Prof. Dr. Michael Ewers
	Priv. Doz. Dr. Ilonka Eisensehr
Mitbetreuung durch den promovierten Mitarbeiter:	
Dekan:	Prof. Dr. med. dent. Reinhard Hickel
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1. Introduction

1.1 The Placebo Effect: a Neurobiological Phenomenon

Mounting evidence from different methodological approaches indicate that the placebo effect is a ´true´ quantifiable neurobiological phenomenon (Benedetti, Mayberg, Wager, Stohler, & Zubieta, 2005; Meissner et al., 2011; Price, Finniss, & Benedetti, 2008). Specifically, it reflects a measurable health response that is in fact triggered by a sham therapy, which contains no pharmacological ingredient and is not inherently designed to give rise to any specific medical effects (Price et al., 2008). It is generally assumed that the placebo effect begins by a modulation of the mind (Benedetti, 2010; Benedetti, Carlino, & Pollo, 2011; Pollo, Carlino, & Benedetti, 2008), which in turn can activate a cascade of measurable neurobiological changes similar to those activated by real drugs.

For example, in placebo analgesia (i.e. pain reduction induced by placebos), it has been shown that the release of endogenous opiates was associated with the subjective placebo response (for a review on placebo analgesia, see Colloca, Klinger, Flor, & Bingel, 2013). Notably, it was not possible to create the same placebo response when the endogenous opiates were blocked by naloxone (Benedetti, 1996). In other words, the placebo effect on pain required its biological correlate – endogenous opiates released from the central nervous system (CNS) – to act. Another study by Hunter and colleagues revealed that the placebo effect plays a key role in the actual treatment of depression. In patients with severe depression, prefrontal cortex (PFC) activity was measured prior to the beginning of a treatment with antidepressants (i.e. during the placebo lead-in phase). Nineteen percent of the benefit (reduction on Hamilton depression scale) in these medication-treated subjects could be predicted by a decrease in PFC activity during the placebo-lead in phase (Hunter, Leuchter, Morgan, & Cook, 2006). The authors suggested that the altered PFC activity was induced through positive associations with the administration of the drug. Positive expectations triggered by clinical interventions are referred to as

the ‘influence of the psychosocial context of a treatment’ and are considered to be a crucial mechanism of the placebo effect (Benedetti, 2006). It has also been suggested that the placebo effect is modulated via reward-specific mechanisms (de la Fuente-Fernández, Lidstone, & Stoessl, 2006), such as the release of dopamine in the striatum in Parkinson´s disease (de la Fuente-Fernández, Ruth, Sossi, Schulzer, Calne, & Stoessl, 2001).

These examples elucidate that the placebo effect can play a significant role in health care and transform its previous label of ‘pure imagination’ into a more accurate label as a true quantifiable neurobiological phenomenon. On top of that, placebo research sheds new light on mind-body interactions by connecting subjective experience to neurobiological changes (Colloca & Benedetti, 2005). However, despite the growing evidence pointing to the validity of the placebo effect, more substantial knowledge is needed before applying the placebo effect to medicine (Price et al., 2008). For example, little is known about the mechanism of the placebo effect in nausea, even though it is a commonly occurring clinical condition known to be sensitive to placebo interventions (Quinn & Colagiuri, 2015). To fill this gap, the present study investigates the placebo effect in a nausea model, specifically its underlying cognitive mechanism and its linkage to expectation and stress.

1.2 Placebo Effect in Nausea

The placebo effect plays a crucial role in the treatment of acute nausea and has been described in chemotherapy patients (Enblom et al., 2012), pregnant women (Borrelli, Capasso, Aviello, Pittler, & Izzo, 2005), and healthy populations. In these populations, nausea has been shown to be alleviated by placebo interventions in comparison with a no-treatment control group, but with varying level of evidence (Quinn & Colagiuri, 2015). A competitive distinction between ‘verbal instructions’ and ‘conditioning’ has been proposed in the past (Peck & Coleman, 1991; Voudouris, Peck, & Coleman, 1990), however others (Quinn & Colagiuri, 2015; Stewart-Williams & Podd, 2004) argue that both processes can induce placebo effects and

should be integrated. Broadly defined, instruction based interventions include conscious manipulations, e.g. 'this pill was shown to positively affect your stomach and therefore will prevent you from experiencing nausea'. In contrast, the conditioned placebo effect more likely comprises the hidden actions (e.g. context factors such as the form of a drug, Wickramasekera, 1980) and has been shown to act even without conscious perception of the conditioned stimuli (e.g. in the domain of pain, Jensen et al., 2012). In fact, it seems that both interventions trigger expectancy towards a positive treatment outcome, independently of whether this happens consciously or unconsciously (Stewart-Williams & Podd, 2004).

In a study that integrated both concepts, a lemon flavored water drink, presumed to be an effective antiemetic (placebo group) or pure water without instruction (control group) was administered prior to a nauseating stimulus (Horing et al., 2013). The nauseating stimulus was performed on three consecutive days, and had in fact been surreptitiously decreased following the placebo treatment at day two relative to each participant's first session. This was important in order to induce the conditioning effect at session three, in which the nauseating stimulus was returned to the initial high level after another placebo treatment was applied. In this session, the placebo subjects showed twice the magnitude of symptom reduction compared to the control group. However, similar placebo effects have also been observed at day two, where only the placebo instruction was applied. Hence, the authors suggested that the placebo instruction alone was as effective as in combination with conditioning.

In another study by Quinn and colleagues (2015), 56 participants received either the instruction that a sham treatment – a peppermint essence vapor - would reduce nausea (instruction and conditioning) or no information (only conditioning). The conditioning was induced by pairing the sham treatment with a surreptitious decrease in the severity of nausea induction versus no prior pairing (control group). In a final session, peppermint essence vapor induced equal placebo effects in all three groups, i.e. those who only received instruction, those who only

received conditioning and those who received both. These results confirm the study by Horing and colleagues (2013) and further suggest that placebo effects in nausea can be induced to a similar degree through conditioning, instruction or their combination.

Based on these results, Quinn and colleagues (2015) proposed three key factors to ensure powerful placebo effects in nausea, namely: a) high initial expectations for experiencing nausea (Horing et al., 2013; Müller, Remus, Hoffmann, Tschöp, & Meissner, 2016), b) reasonable and convincing expectancy manipulations (i.e. verbal instructions of the placebo intervention), and c) verbal instructions should be followed by a placebo intervention (e.g. sham acupuncture: Müller et al., 2016 or placebo pills: Weimer et al., 2012).

Recently, a study by Müller and colleagues (2016) supported these claims by implementing the key factors and obtaining powerful placebo effects in nausea in female participants (*partial* $\eta 2$ = .71)*:* first, high initial expectations of experiencing nausea were induced by exposing participants to the nauseating stimulus prior to the main experiment and including only those who experienced moderate nausea. Second, the placebo intervention included a reasonable instruction in the form of a positive verbal suggestion that the intervention would improve nausea. Third, instructions were followed by a sham acupuncture point stimulation, which has been indicated as the most effective placebo intervention (Linde, Niemann, Schneider, & Meissner, 2010; Meissner et al., 2013).

In sum, these findings show that the placebo effect can be systematically induced and studied in nausea. Despite the fact that placebo interventions can powerfully reduce nausea, little is known about the exact underlying mechanisms. However, the bodily and cortical mechanisms of nausea have been studied more thoroughly, providing a reliable basis to investigate the placebo mechanisms in more detail.

1.3 Nausea

Nausea is a widespread and highly individual condition, which ranges from relatively harmless during motion in approximately 30% of the car drivers (Turner & Griffin, 1999) to intolerable in 25% of cancer patients receiving chemo-therapy (Morrow, Hickok, DuBeshter, & Lipshultz, 1999) and in 52% of pregnant women (Gadsby, Barnie-Adshead, & Jagger, 1993). Its signs and symptoms include physical (e.g. sweating or vomiting), emotional (e.g. stress), cortical (e.g. changes in brain activity) and gastrointestinal (e.g. stomach ache) afflictions (Levine, 2017).

1.3.1 Motion Sickness Induced Nausea

One appropriate way to experimentally study nausea is to induce nausea through motion. Motion sickness can be induced by means of a vection drum, which works through illusory self-motion (Hettinger, Schmidt-Daly, Jones, & Keshavarz, 2014). Vection drums usually consist of black and white stripes moving constantly from left to right (Reason & Brand, 1975). Moving dots have also been used to induce motion sickness (Klosterhalfen, Muth, Kellermann, Meissner, & Enck, 2008). These types of stimuli recreate sensory conflicts similar to those encountered while driving a car or piloting an airplane. As a result, sensory organs report conflicting spatial and moving information due to difference in expected and the sensed motion cues. This theory is called the sensory rearrangement theory (Reason & Brand, 1975), which was later refined to the subjective vertical conflict theory (Bles, Bos, de Graaf, Groen, & Wertheim, 1998; de Graaf, Bles, & Bos, 1998). The latter implies that motion sickness can only be induced when the sensory conflict is between the expected internal calculated vertical and the perceived vertical. It follows then that the vection drums usually work with vertical illusions. Both theories assume that such a sensory mismatch in susceptible subjects (which varies greatly) leads to neural mismatching (error signaling) in the brain-stem causing several symptoms, predominantly nausea (also headache or dizziness).

Bodily changes of nausea in the context of motion sickness are well studied and have been suggested to act in a bidirectional network composed of mechanisms within the autonomic nervous system (ANS), the central nervous system (CNS), and the endocrine system (Singh, Yoon, & Kuo, 2016). Recently, this was evidenced by an fMRI study (Farmer et al., 2015), in which nauseated subjects were shown to have gastric dysrhythmia, blunted levels of plasma ghrelin as well as increased activation in the inferior frontal and the anterior cingulate cortex (ACC). Nevertheless, little is known about the psychosocial influences on nausea in the context of motion sickness, such as stress.

1.3.2 Nausea and Stress

Notably, a wealth of research has shown that psychosocial aspects play a key role in nausea (Levine, 2017). For example, nausea is often accompanied by elevated stress levels. ANS response to stress shows robust similarities with the ANS pattern in nauseated patients in the context of motion sickness (Stern & Koch, 1996), chemotherapy (Gianaros, Stern, Morrow, & Hickok, 2001), and pregnancy (Koch et al., 1990; Levine, 2017). The ANS acts via two branches to direct bodily reactions, namely: (a) the parasympathetic nervous system (PNS) and (b) the sympathetic nervous system (SNS). The PNS regulates bodily activity at rest, such as digestion. In contrast, the SNS is important for the so called 'fight-or-flight' reaction (McCarty, 2007). At times of stress, the ANS is indexed by increased SNS and decreased PNS activity to mobilize energy resources (McEwen, 1998). The experience of nausea is indexed by similar ANS patterns (Stern & Koch, 1996). The order in which stress and nausea act together (whether nausea causes stress or vice versa) is not yet clear. It has however, been shown that acute stress can evoke abnormal gastric myoelectrical activity (Muth, Koch, Stern, & Thayer, 1999). It is possible that at times of stress, there is little energy left for digestion and the gastrointestinal system may be suppressed, which in turn may trigger nausea (Gianaros, Quigley, et al., 2001).

The interplay between SNS and PNS is mainly navigated via three brain regions, the hippocampus, amygdala, and PFC (taking into account that stress leads to far more intricate bio-behavioral changes; McEwen et al., 2015). Together, they regulate the release of stress related hormones via the hypothalamic-pituitary-adrenal (HPA) axis in the ANS, such as cortisol or amylase.

1.3.3 Nausea and Salivary Enzyme Alpha-Amylase

Though alpha-amylase has been indicated to play a crucial role in digestion, little is known about the link between alpha-amylase and nausea (Layer, Zinsmeister, & DiMagno, 1986). However, based on the fact that acute nausea is accompanied by altered stomach activity, thus altered digestive tract activity (Farmer, 2015), it follows that alpha-amylase may be directly involved in the development of acute nausea.

Additionally, alpha-amylase was shown to indicate increased stress levels (Nater et al., 2006). For example, one study measured changes in alpha-amylase, salivary cortisol and heart rate in 30 healthy men following Trier Social Stress Test (TSST). All three measures significantly increased. An investigation of the associations between the measures revealed that while sympathetic cardiovascular tone was positively correlated with alpha-amylase, changes in cortisol levels were not significantly associated with changes in alpha amylase ($r < 0.25$). Therefore, alpha-amylase was included in the current study as a potential stress-related mediator in addition to cortisol and to index altered SNS activity (Nater & Rohleder, 2009) underlying the placebo effect in nausea.

1.3.4 Nausea and Cortisol

Nausea in the context of motion sickness has been shown to be accompanied by elevated cortisol levels (Xu et al., 1993). On the other hand, high levels of cortisol measured prior to the induction of nausea in females were associated with decreased nausea responses (Meissner,

Enck, Muth, Kellermann, & Klosterhalfen, 2009). In general, the hormone cortisol plays a crucial role in the HPA-axis. Released from the adrenal cortex, it can redistribute energy via negative feedback loops at mineralocorticoid and glucocorticoid receptors, primarily found in the hypothalamus. For example, cortisol can nourish muscles with energy in form of increased glucose levels (Gonzalez-Bono, Rohleder, Hellhammer, Salvador, & Kirschbaum, 2002) or reduces gastro-intestinal activity (Bhatia & Tandon, 2005). It also closely interacts with certain brain areas, such as the PFC (Dedovic, D'Aguiar, & Pruessner, 2009). However, the exact relationship between PFC and cortisol has not yet been clarified and seems to differ between females and males (Wang et al., 2007). In summary, cortisol was shown to be relevant in nausea, though its exact function is still unclear. Changes in cortisol levels may index, among other components, the magnitude of the placebo effect in nausea.

1.3.5 Nausea and the Central Nervous System

Regarding the central nervous system (CNS), several studies revealed electroencephalography (EEG) frequencies of the fast Fourier transformed (FFT) spectrum to be altered during acute nausea. FFT transforms data from the time domain into the frequency domain and shows the extent to which each frequency band are represented within the EEG recordings (Cooley & Tukey, 1965). The frequency bands can be defined according to the spectrum of interest. In the investigation of nausea, frequency bands are usually defined between '0' and '30' hertz (Hz). For example, Hu and colleagues (1999) investigated theta ('0' – '4' Hz), delta ('4.1'-'8' Hz), alpha ('8.1'-'13' Hz), beta I (13.1-20), and beta II ('13.1' - '30' Hz) bands and showed that the percentage of delta power (compared to the whole spectrum of '0' to '30' Hz) in the central electrodes C3 and C4 was increased during acute nausea. Additionally, brain imaging programs such as exact low resolution brain electromagnetic tomography algorithm (eLORETA; Pascual-Marqui, 2002; Pascual-Marqui, 2007) have allowed researchers to localize these nausea related

EEG changes. For example, parietal, motor, and occipital areas were revealed to exhibit significant nausea related EEG changes (Ko, Wei, Jung, & Lin, 2011). Specifically, parietal and motor components exhibited alpha power suppression and occipital components elevated power of theta and delta bands, measured during acute nausea (Chelen, Kabrisky, & Rogers, 1993; Chen et al., 2010). Furthermore, in an fMRI study that was based on 28 female participants, nausea was accompanied by changes in the insular, ACC, orbitofrontal, somatosensory, and prefrontal cortices (Napadow et al., 2013). In particular, the authors observed a strong correlation between anterior insula (thought to be involved in emotion regulation) and ACC activation and suggested a closer linkage between these regions within the brain circuitry underlying nausea. These findings align perfectly with fMRI findings by Farmer and colleagues (2015) in which anterior cingulate as well as the frontal cortex were identified as key cortical components in nausea.

In summary, these studies clearly show that changes in the CNS are related to the subjective experience of nausea and that EEG reflects a potential measurement to investigate, in more detail, psychosocial top-down modulations, such as anticipatory nausea.

1.4 Expectation and the Treatment of Nausea

While some medical treatments of nausea lead to remission of symptoms (e.g. vomiting, Jordan, Sippel, & Schmoll, 2007), most medical approaches fail to successfully alleviate nausea (for a review, see Sanger & Andrews, 2006). The mechanisms underlying such failure in reducing symptoms are not fully understood, but may involve the missed attempt to explicitly trigger the psychosocial components of nausea, for example, to take away high initial expectation of nausea (Colagiuri & Zachariae, 2010).

Indeed, research has increasingly suggested that expectation plays a crucial role in the treatment of nausea, as it is a robust predictor of the actual experience of nausea. For example, in patients receiving chemotherapy, expectation of nausea measured prior to the beginning of the chemotherapy predicted the magnitude of chemotherapy induced nausea (Hickok, Roscoe,

& Morrow, 2001), more so than other personal variables, like age, gender or experience with nausea in the past (Montgomery & Bovbjerg, 2001). When making precise distinctions of expected nausea, Colagiuri and colleagues (2008) found that patients classified as highly expectant experienced more nausea than all other classifications (i.e. somewhat, slightly, no expectation). The authors concluded that patients with high initial expectations of nausea appear to be at particular risk of developing chemotherapy induced nausea and that it is important to find treatments which alleviate fearful expectations in these patients (Colagiuri & Zachariae, 2010). This was also shown to be true in cancer patients treated with radiotherapy: nausea could effectively be reduced following sham acupuncture but only in those subjects who really believed that the treatment would effectively prevent nausea (Enblom et al., 2012).

In general, expectation plays a crucial role in placebo interventions, and underlying PFC activity has been suggested to modulate expectation-specific mechanisms. For example in Alzheimer patients, a local anesthetic was less effective when the expectation component was absent (Benedetti et al., 2006). The expectation component could be studied in isolation in an open-hidden design, by administering the drug covertly (hidden condition) versus overtly (open condition). The analgesic placebo response in Alzheimer patients that also showed abnormal PFC activity was almost completely absent.

Overall, the investigation of cortical top-down mechanisms of the placebo effect in nausea, particularly expectation, plays a crucial role in understanding the underlying mechanisms of the placebo effect in nausea and thus may explain why nausea is relatively sensitive to placebo interventions.

1.5 This Study and Its Hypothesis

The present study was designed to investigate whether individuals who receive a placebo-intervention (i.e. a sham acupuncture combined with a positive verbal suggestion) would show decreased nausea ratings compared to a group who received no treatment and if so, whether this

difference could be evidenced by changes in EEG outcomes, in terms of stress and expectancy. Based on previous literature, the following main hypothesis were formulated:

Placebo intervention would directly reduce the initial level of expected nausea, indexed by decreased NRSs and changes in the PFC and ACC during the anticipation phase in the placebo group compared to the control group (Amanzio, Benedetti, Porro, Palermo, & Cauda, 2013, Benedetti et al., 2006). The anticipation phase in the present study is the period following verbal placebo instruction - or the instruction that they would not receive any treatment in the control group - before the nausea induction has been commenced.

Placebo intervention would alleviate perceived nausea (i.e. placebo effect on nausea, Müller et al., 2016), indexed by decreased NRSs and changes in nausea related brain activities in the placebo group compared to the control group (e.g. decreased delta power, Hu et al., 1999; or changes in frontal or anterior cingulate activity, Farmer et al., 2015).

Placebo intervention would attenuate perceived stress during acute nausea in the placebo group compared to the control group, which would be indexed by decreased cortisol and amylase levels. Interestingly, changes in PFC activity have been linked to nausea related ANS changes (Toschi et al., 2017). Therefore, the placebo-induced stress reduction was also assumed to be accompanied by altered PFC activity in the placebo group compared to the control group.

Besides expectation and stress, also, reward-specific mechanism have been proposed to play a crucial role in the modulation of placebo responses (de la Fuente-Fernández, 2001, 2004), therefore self-reported positive aspects of motivation (Carver & White, 1994) were hypothesized to enhance nausea-related placebo responses. In contrast, negative aspects of motivation were hypothesized to attenuate nausea-related placebo responses.

2. Methods

The present study focuses on data collected at the Institute of Medical Psychology, Ludwig-Maximilians-University Munich, as part of a "Deutsche Forschungsgemeinschaft" (DFG) grant (ME3675/1-1) on "Effects of emotional context and tactile stimulation on the placebo response in a nausea model" in healthy individuals.

2.1 Participants

In total, 90 (45 females, 45 males) healthy participants were recruited for the study, between March, 2014 and April, 2015. They were recruited via flyers, advertisements on our institute homepage, an LMU email distribution list, and screening phone calls (to check for eligibility). The recruitment was addressed to people who tend to suffer from motion sickness.

To reduce potential confounding sources, participants were identified based on the following preliminary inclusion criteria: age between 18 and 50, right-handed (Chapman & Chapman, 1987), normal or corrected-to-normal vision and hearing, body mass index between 18 and 25. Exclusion criteria included: ongoing pregnancy, current drug use, alcohol or drug abuse, implant devices or metal implants, regular medication use (except hormonal contraceptives, thyroid medications, allergy medications), history of diseases of the inner ear, skin diseases, diabetes, cardiovascular disease, epilepsy, cancer, history of blood-clotting disorders or tendency for thromboembolic diseases, ongoing acute disease, surgery during the past 4 weeks, and any prior participation in a placebo or nocebo experiment.

After fulfilling preliminary inclusion criteria, participants were asked to complete the motion sickness susceptibility questionnaire (MSSQ). This questionnaire ascertains how vulnerable one feels to motion sickness and which kinds of motion are critical. Only subjects who rated significant susceptibility (≥ 80 on a 200 point scale) were asked to go through a pre-test session. The pre-test session aimed to test their sensibility to a nauseating stimulus. Participants were only included in the main experiment (control – and testing day) if moderate nausea was

reached. All participants provided written consent to a protocol approved by the LMU ethical review committee. Participants were compensated with 100 euros after completion of study.

2.2 Vection Drum

Nausea was induced in the context of motion sickness, which was simulated by means of a vection drum. The drum consisted of a curved shaped blank screen which was placed 30 cm distal to the participant's head. Black and white stripes were presented on the screen on a rotating basis from left to right at 60 degrees per second. The left-to-right horizontal translation induces a circular vection sensation wherein participants experience a false sensation of translating to the left (Napadow et al., 2013). This in turn produces illusory self-motion, which causes moderate nausea in subjects who are predisposed to motion sickness (Müller et al., 2016). The present method is similar to a 'rotating' optokinetic drum successfully applied in previous studies (Gianaros et al., 2001; Levine et al., 2006), but here the drum did not rotate, only the stripes were in regular motion. This allowed participants to sit still, which was important for EEG quality.

2.3 Placebo Device

Figure 1 illustrates the location of the sham- and the real acupuncture point. A placebo, 'sham acupuncture point stimulation' was used, which was applied with a transcutaneous electrical nerve stimulation (TENS) device (Digital EMS/TENS unit SEM 42, Sanitas, Uttenweiler, Germany). In contrast to real acupuncture needles, the TENS device works through electrical nerve stimulation acting via two electrodes that can be placed on the skin. It also includes a nonspecific massage program (somatosensory stimulation). Both types of stimulation cause a tingling sensation about the electrodes site, making the two difficult to distinguish.

In the TENS group, the two electrodes were placed at the 'P6' acupuncture point, which is located at the antebrachial region of the forearm, 2 cun (ca. 6,67 cm) distal to the anterior crease of the wrist (Arnberger et al., 2007). Stimulation was turned on at a frequency of 7 Hz

(hertz), with a current between 7 and 13 mA (milliampere; Pfab et al., 2011).

In the placebo condition, the two TENS electrodes were placed around a dummy point at the left and right forearm. The dummy point was evidenced to have no specific acupuncture effects (Witt et al., 2012). Its exact location is at the ulnar side of the forearm, a six centimeter distance to the elbow. The level of the tingling can be regulated and was adjusted based on participant's feedback to a low intensity level but clearly noticeable. During placebo instruction, participants were informed to either receive placebo or real intervention, though the experimenter always pretended to place the electrodes at real acupuncture points and to switch on nerve stimulation.

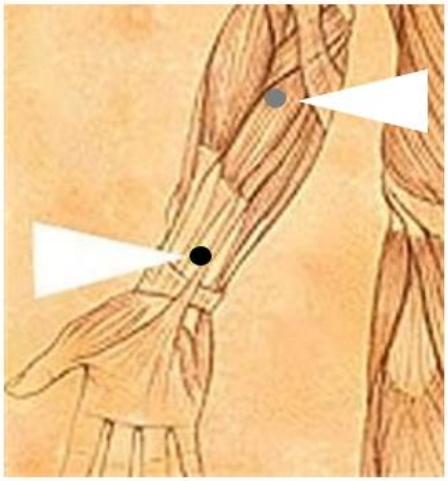

Figure 1. Diagram depicting the real acupuncture point (PC6, black dot) and the sham acupuncture point (grew dot) at the right forearm, on which the TENS (transcutaneous electrical nerve stimulation) electrodes were attached according to the assigned condition (real treatment: TENS at PC6, simple placebo: attaching TENS electrodes to the sham acupuncture point with the TENS device turned off, enhanced placebo: attaching TENS electrodes to the sham acupuncture point and turning on a massage program which induced somatosensory stimulation).

2.4 Verbal Instruction

After the baseline measures, the randomization envelope was opened by the experimenter. Subjects assigned to TENS as well as sham acupuncture point stimulation were instructed that they would receive a therapy for nausea and that the therapy could either be a placebo or a real

TENS treatment. Next, the experimenter articulated that nausea was found to be associated with gastric abnormalities and that the TENS therapy was shown to positively influence stomach activity (Pfab et al., 2011), thus prevent motion sickness induced nausea (Streitberger et al., 2006).

Additionally, subjects were advised that the treatment will start ten minutes prior to the nausea induction because then positive treatment outcome was shown to be most effective (Ezzo et al., 2006). In contrast, participants assigned to the no-treatment group were instructed not to receive any treatment in order to measure spontaneous responses to the nauseating stimulus. In order to avoid frustration in the no-treatment group, the importance of control groups in medical studies was also explained. The very same placebo instruction has been shown to powerfully induce placebo effects in females, in nausea related behavioral as well as bodily correlates (Müller et al., 2016).

2.5 Group Assignment

Table 1 presents an overview of the four study groups and their conditions on day 1 and day 2. The group assignment was randomized single-blind. To guarantee comparable baseline data across all subjects, the randomization envelopments were opened right after completion of the baseline measurement (t=-10). The participants were informed that they would either receive intervention, placebo, or no treatment. No information was given about the probability of the actual group assignment. Only 10% received real treatment, 45% placebo, and 45% no intervention, respectively. The data conducted in the real treatment group was not of interest to the present study. The reason for implementing the TENS group was to provide a meaningful and ‘relatively’ truthful expectancy manipulation, which also fulfilled the ethical standards not to deceive the participants.

Table 1. Design of the present study showing conditions for each group across the experiment

	Day 1	Day 2
Control Group	Nausea induction	Nausea induction; no intervention
Simple Placebo Group	Nausea induction	Nausea induction; sham acupuncture without somatosensory stimulation
Enhanced Placebo Group	Nausea induction	Nausea induction; sham acupuncture with somatosensory stimulation
TENS	Nausea induction	Nausea induction; real nerve stimulation

Note. Nausea was induced at both days across all groups. Only placebo group received intervention at day 2: Enhanced placebo group received sham acupuncture with somatosensory stimulation: TENS massage program at fake acupuncture point. Simple placebo group received sham acupuncture without somatosensory stimulation: TENS device stayed off but electrodes were placed at fake acupuncture point. Real acupuncture group received electrical nerve stimulation at real acupuncture point (PC6). TENS: transcutaneous electrical nerve stimulation.

2.6 Self-Report Measures

2.6.1 Expectation, Nausea and Stress

Ratings on numeric rating scales (NRS) for expected nausea (on a ‘10’-point NRS, with ‘0’ indicating no nausea and ‘10’ maximal tolerable nausea), perceived nausea (moderate nausea corresponds to ≥‘5’ on a ‘10’-point NRS, with ‘0’ indicating no nausea and ‘10’ maximal tolerable nausea), and perceived stress (‘10’-point NRS, with ‘0’ indicating no stress and ‘10’ maximal stress) were obtained.

2.6.2 Hospital Anxiety and Depression Scale

Placebo interventions have been shown to activate emotional components (Petrovic et al., 2005), therefore the contribution of mood disorders related to anxiety and depression was assessed using the Hospital Anxiety and Depression scale (HADS-A, HADS-D, Zigmond & Snaith, 1983). In total, the HADS consists of 14 items, seven of them are related to anxiety (i.e. I feel tense) and seven to depression (i.e. I feel as if I am slowed down). Each question is rated on a ‘0’ to ‘3’-point scale, which leads to a maximum score of ‘21’ for each scale. A sum score of greater than ‘7’, which also was the cut-off score of including participants in the present

study, was suggested to indicate mood disorders (Snaith, 2003).

2.6.3 State and Trait Anxiety

To control for emotional variations between groups and testing days, trait and state anxiety was ascertained using the State-Trait Anxiety Inventory (i.e. STAI-State and STAI-Trait; Spielberger, 2010). STAI-Trait was rated on the control day. STAI-State was conducted twice, at the control- and placebo day. Participants were requested to report on a '4'-point scale how much 20 statements apply to them 'at this moment' (i.e. state) and in general (i.e. trait). Items described both the absence of anxiety (e.g. 'I am calm') and the presence of anxiety (i.e. 'I am worried.'), with higher scores indicating greater levels of anxiety. The questionnaire was administered by paper-and-pencil.

2.6.4 Motivation

Motivational aspects have been shown to modify placebo responses (de la Fuente-Fernández et al., 2001). It is possible that the level of motivation also modulates the placebo effect in nausea. Therefore, aspects of motivation underlying behavior were obtained at baseline during day 1 using the behavioral inhibition - and behavioral approach system scale (BIS/BAS; Carver and White, 1994). Participants were required to state the extent to which the 24 statements applied to them. Items described both negative (BIS, e.g. 'Criticism or scolding hurts me quite a bit.') and positive (BAS drive, e.g. 'I go out of my way to get things I want') aspects of motivation. The questionnaire was administered by paper-and-pencil.

2.7 Cortisol and Amylase

To measure the humoral response of the hypothalamic-pituitary-adrenal (HPA)-axis and the sympathetic nervous system, respectively, saliva samples of cortisol and alpha-amylase were collected before and after baseline and at acute nausea (see Figure 3) by a cotton swab, on which participants chewed for at least sixty seconds before storing it back into a tube. After

each session, saliva concentrations were centrifuged at 2000 rpm at 4°C and 2x 300µl per sample stored at -20°C.

2.8 Electroencephalography Recordings

Figure 2 illustrates the exact positions coordinates of the 32-electrodes used in the present study. EEG was recorded using the ActiveTwo system (BioSemi) with thirty two-channels arrayed in a regular distribution based on a standard 10-20 system. Additionally, two external mastoid channels as well as horizontal and vertical electrooculography (EOG) were recorded. After placing the electrodes, the offset signal was controlled individually for each channel and kept below 20 mV. Sampling rate was 2,048 (2 KHz). Data was offline down sampled to 265 Hz and re-referenced to the mastoids after a 50-Hz high cut-off filter was applied.

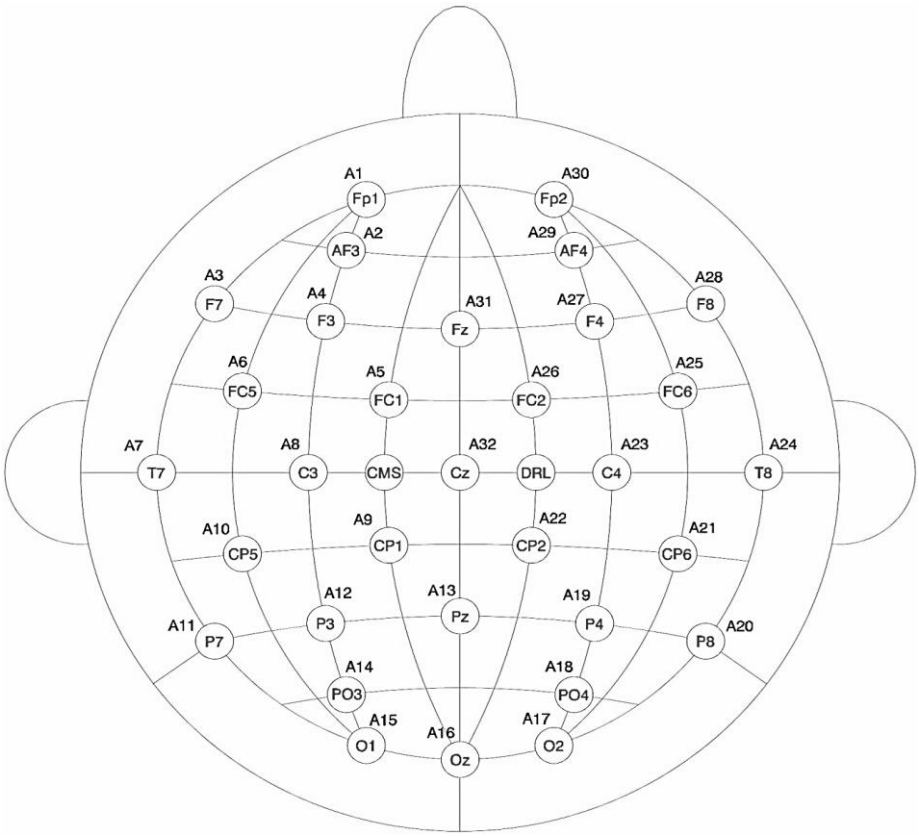

Figure 2. Position coordinates of the 32-electrodes for the BioSemi headcaps, which were used in the present study.

3. Procedure

The sessions included verbal ratings, electroencephalography (EEG), electrocardiography (ECK), electrogastrogram (EGG), saliva and blood collection, and several questionnaires. From this large dataset, this study focuses on: (1) the following questionnaires: motion sickness susceptibility questionnaire (MSSQ, Golding, 2006), German version of hospital depression scale (HADS-A and HADS-D, Zigmond & Snaith, 1983), and behavioral inhibition - and behavioral approach system scale (BIS/BAS, Carver & White, 1994), (2) verbal ratings on a numeric scale of expected nausea, perceived nausea, and perceived stress (Müller et al., 2016), (3) saliva samples of cortisol and amylase, and (4) 32- channel EEG recordings.

The study included two experimental sessions, referred to as day 1 (control condition) and day 2 (test condition). At each session and across all subjects, nausea was induced by means of the vection drum, however randomization to either placebo intervention or control was applied only at day 2.

Day 1 and day 2 were conducted on two consecutive days, at least 48 hours apart, constantly between 14:00 and 19:00 o'clock. This was important to avoid circadian influences, which were shown to affect the hormone level (Ca & Eb, 1999; Czeisler & Klerman, 1999). Participants were instructed not to eat or drink liquids other than water three hours prior to the experiment. After that, the EEG net was applied. Saliva probes were collected before baseline measurement (amylase and cortisol), after the nauseating stimulus was stopped (peak of induced nausea, amylase), and at the end of each session (cortisol). Furthermore, at baseline and during nausea exposure ratings on the NRS for nausea (moderate nausea corresponds to ≥'5' on a '10'-point NRS, with '0' indicating no nausea and '10' maximal tolerable nausea) and stress levels (on a '10'-point NRS) were obtained. Furthermore, before baseline and directly following the expectancy manipulation, participants rated the maximal level of expected nausea (on a '10'-point NRS) of the day´s session.

3.1 Nausea Exposure

Study day 1 involved three saliva probes, nausea induction, NRS of expected and perceived nausea, perceived stress, and perceived motion sickness as well as EEG recordings. It included a ten minute baseline followed by a ten minute rest measurement during which participants sat in front of a blank screen and were instructed to look straight ahead and keep still. Next, the nauseating stimulus was turned on for 20 minutes. The session was completed with a resting period of 15 minutes during which participants again sat in front of the blank screen, while looking straight ahead and keeping still.

3.2 Placebo Intervention

Figure 3 presents an overview of the study protocol on day 2. This session comprised three saliva probes, nausea induction, NRS of expected and perceived nausea, perceived stress, and perceived motion sickness as well as EEG recordings and sham acupuncture point stimulation for the placebo group. After the ten minutes baseline measurement, participants were randomized to receive either no treatment (control group) or sham acupuncture point stimulation (placebo group). Immediately following randomization, participants in the placebo group received a standardized instruction of positive verbal suggestion of nausea improvement (see above, Placebo Instruction) and the device for the sham acupuncture point stimulation was applied and switched on for twenty minutes. After ten minutes of intervention or rest, the nauseating stimulus was presented to the participants for twenty minutes. After the first ten minutes of nausea exposure the placebo intervention was stopped (device was turned off), so that the last ten minutes of nausea exposure were free of any uncontrolled actions through the device or the electrodes.

In contrast, participants in the control group were informed that they would not receive any treatment. Consistent with day 1, the experiment was completed with a 15 minute resting period during which participants were asked to look at the blank screen.

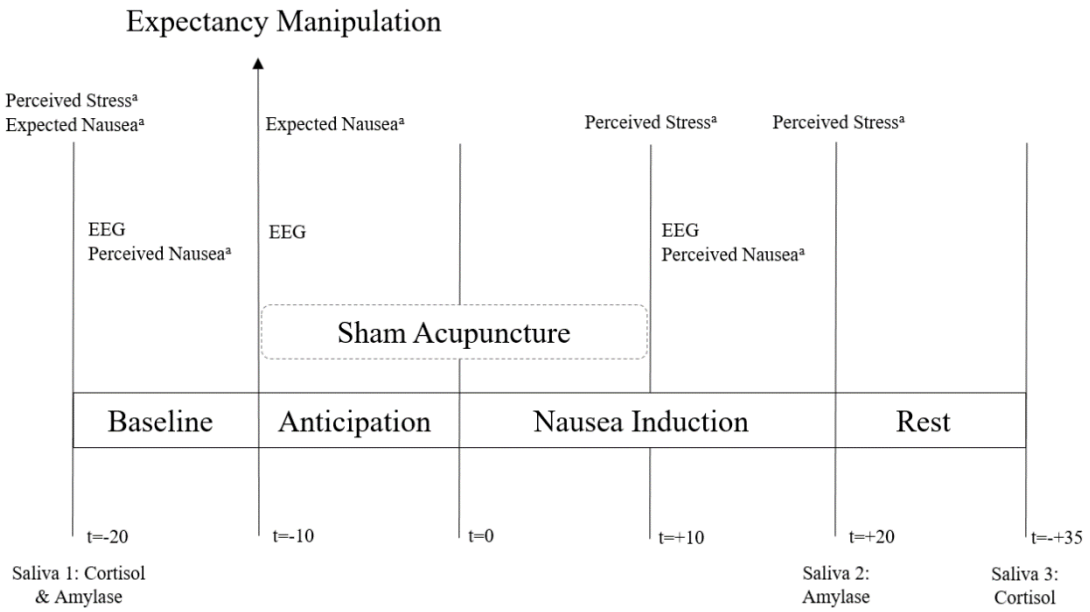

Figure 3. Overview of the study protocol on day 2. Expectancy manipulation and sham acupuncture was only applied in the placebo group. The control group received no intervention. Abbreviations: EEG: Electroencephalography. [a]Numeric rating scale (0-10).

4. Data Analysis

4.1 Behavioral

In total, the behavioral measures consisted of the following NRS: (1) expected nausea, (2) perceived nausea, and (3) perceived stress. At day 1 and day 2, the primary analyses focused on measures during baseline and nausea exposure. Expected nausea was directly rated after expectancy manipulation. Thus, the following means of ratings during these time points (note: time points referred to as t) were computed:

(1) Expected nausea at day1 and day 2, computed as mean NRS scores after baseline (t=-10, which was shortly after the expectancy manipulation at day 2) minus expected nausea rated at the beginning of the session (t=-20), with lower scores reflecting decreased levels of expected nausea.

(2) Perceived nausea (at day 1 and day 2: rated every minute during baseline from t=-20 to t=-10, and acute nausea from t=+10 to t=+20), operationalized as mean NRS scores during acute nausea minus nausea rated during baseline, higher scores indicating increased magnitude of nausea induction.

(3) Perceived stress at day 1 and day 2, computed as NRS scores rated at acute nausea (t=+10) minus at rest (t=-20), with higher scores reflecting increased stress levels.

(4) Placebo effects (spontaneous changes from day 1 to day 2 in the control group, respectively) on expected nausea, perceived nausea, and stress were defined as baseline corrected mean NRS scores [mean NRS scores during nausea minus mean NRS scores during baseline] at day 1 minus at day 2, whereby higher scores indicate greater placebo effects.

4.2 Amylase and Cortisol

Salivary amylase and cortisol levels were ascertained via ‘cortisol saliva assay’ and ‘alpha-amylase saliva assay’ kit from IBL International GMBH (catalogue number of cortisol kit: RE52611 and amylase: RE80111). All saliva samples were analyzed in duplicate and performed

following the manufacturer's protocol. Finally, the values of amylase and cortisol levels at acute nausea were logarithmized to a natural factor and corrected for the levels measured at baseline by computing (for amylase: level at acute nausea minus level at baseline, for cortisol: level at the end of the session minus level at baseline). Final analyses were conducted in SPSS.

4.3 Electroencephalography

The EEG data was processed offline using Brain Vision Analyzer 2.0 (Brain Products GmbH, Gilching, Germany). First, 'Sinc-Interpolation' was used to down sample the EEG data to a rate of 256-Hz as well as a low cut-off of 0.5 Hz and a high cut-off of 50 Hz with a notch filter of 50-Hz which was applied. Further, gross artifacts were removed and EEG data was re-referenced to the mastoids. Next, ocular ICA was run to remove blink artifacts. Bad individual channels were spatially weighted and linear interpolated. To exclude any remaining artifacts, a final semi-automatic artifact rejection was applied.

Additionally, voltage levels at the 32 electrodes were replaced by valid head coordinates through the current source density (CSD; order of splines: 4, max. degree of Legendre polynomials: 10). Then, each ten minute period was segmented into one second segments and fast Fourier transformed (FFT), transforming EEG data from the time into the frequency domain. Finally, mean values of the following frequency bands were exported and analyzed in SPSS: δ, 0.5-4 Hz, θ, 4.1-8 Hz; α, 8.1-13 Hz; β I, 13.1-20 Hz; β II, 20.1-30 Hz; and total EEG (0.5-30 Hz) for both the baseline period as well as during nausea exposure (frequency bands adapted by Hu, 1999).

4.3.1 Delta Power

Delta power has been shown to be associated with nausea as well as stress (Hu, 1999; Hall et al., 2007). Therefore, delta frequency band was extracted from electrodes C3 and C4. Specifically, the mean delta values of spectral power were extracted from EEG recordings dur-

ing baseline and acute nausea on both day. Next, the delta percentage of total power was computed by dividing delta spectral power by total spectral power (total EEG power, 0.5-30 Hz). Finally, to investigate the placebo effect on nausea related changes in Delta percentage of total power, an additional variable was computed indicating nausea related increase in Delta percentage of total power (computed as: Delta percentage of total power during nausea minus Delta percentage of total power during baseline).

4.3.2 Exact Low Resolution Electromagnetic Tomography

To localize nausea and placebo related brain activation, exact low resolution brain electromagnetic tomography algorithm (eLORETA), invented by Pascual-Marqui (Pascual-Marqui, 2002; Pascual-Marqui, 2007) and freely available at the LORETA webpage (http://www.uzh.ch/keyinst/loreta.htm), was applied. Out of many, it is one of the most commonly used algorithms to estimate the location of EEG sources (Grech et al., 2008). By now, eLORETA has been evidenced as an accurate solution to map the origin of EEG data recorded at the scalp, shown by studies comparing LORETA to other source localization methods, for example to fMRI (Mulert, 2004; Vitacco, 2002), positron emission tomography (PET; Pizzagalli, 2004) or implanted electrodes in epilepsy patients (Zumsteg, 2006).

eLORETA estimates the exact current density of different frequencies at 6239 voxels at 5mm spatial resolution in the grey matter based on the Montreal Neurological Institute (MNI)-reference brain. eLORETA analyses were based on recordings of 32-channel EEG, measured during baseline, during anticipation period (in the placebo group, this is the period following verbal instruction before the nauseating stimulus was turned on; in the control group this is the period following instruction that they would receive no treatment), and during nausea exposure (each period consist of 10 minutes of EEG recording). The raw EEG data was preprocessed offline in BVA (see above), segmented into 1 minute sequences, and exported as .dat files. In LORETA the 1 minute sequences were merged into SLOR files on which the following analysis

was run separately for each participant:

(1) To indicate regions presenting brain activity related to nausea, voxel-wise paired t-tests were performed by comparing participants at rest and during nausea exposure at day 1 (control condition).

(2) To identify regions showing placebo-induced changes in nausea-related activity, voxel-wise unpaired t-tests were performed, comparing the magnitude of nausea at day 1 to the magnitude of nausea at day 2, separately for the control and the placebo group.

(3) To reveal regions reflecting changes in activity due to the expectation manipulation, voxel-wise paired t-tests were conducted, comparing the EEG recordings on day 2 during baseline to the recordings during anticipation period, separately for the control and the placebo group.

Study groups were stratified by gender. Significant nausea or placebo related changes in the different frequency oscillations (δ, 0.5-4 Hz, θ, 4.1-8 Hz; α, 8.1-13 Hz; β I, 13.1-20 Hz; β II, 20.1-30 Hz) were defined at $p \leq 0.05$.

4.4 Statistical Analysis

To examine the magnitude of nausea induction and the placebo effect on nausea, which was defined as the decrease in the magnitude of nausea sensation from day 1 to day 2 in the placebo group compared to the control group (for the behavioral as well as for the EEG data) 'mixed' 2x2x2 analyses of variance (ANOVAs) was conducted. The between subject factors were defined as *group* (no treatment vs sham acupuncture point stimulation) and *sex* (male vs female), and the within subject factor as *day* (control condition versus placebo condition). Present ANOVA results were Greenhouse-Geisser corrected. Post hoc t-tests and univariate ANOVAs were conducted following significant ANOVA findings ($p \leq 0.05$). To evaluate the effects of expectation and stress on nausea and delta power as well as potential relationships between those variables, the degrees of Spearman´s rho correlations between baseline corrected mean NRS scores of perceived stress, perceived nausea, and the percentage of delta power in C3 and

C4 were assessed. To evaluate potential mechanism of the placebo effect, the degrees of Spearman´s rho correlations between the placebo effect on expected and perceived nausea, perceived stress, and delta power in C3 and C4 were conducted.

Significant nausea or placebo related changes were defined at $p \leq 0.05$. Finally, due to frequently occurring sex differences in placebo studies (Meissner et al., 2016), the study groups were stratified by gender.

5. Results

5.1 Demographics and Self-Reported Data

Out of 494 individuals who responded to initial recruitment, 384 (203 females and 181 males) scored at least 80 in the MSSQ and were invited to participate in the pre-test session. In total, 245 individuals completed the pre-test session among which 121 (63 females and 58 males) tested positive (in the nausea rating) and were invited to the experimental days. After day 1, eleven females and eight males dropped out because they either were no longer interested in completing the study, had trouble with taking blood samples (fear, circulation problems, or hidden veins), or the level of nausea was less than moderate.

In total, 100 subjects completed the study. To fulfill ethical standards, ten participants received real treatment, though this experimental condition was of no interest to the present study. Thus, the present data focuses on data of 90 participants, split into two groups (30 control and 60 placebo participants). The mismatched group numbers (30 relative to 60) were a consequence of the initial hypothesis that a sham acupuncture condition with somatosensory stimulation (‘enhanced placebo group’, including 30 subjects) would induce more powerful placebo effects on nausea compared to a sham acupuncture condition without stimulation (‘simple placebo group’, including 30 subjects). However, this did not turn out to be the case. Instead, both placebo groups displayed similar levels of decreased nausea (these results will be reported in detail in a separate dissertation). Preliminary analyses of the psychophysiological outcomes also did not show any difference between the two placebo groups. Consequently, the two groups were pooled for the present analysis to increase power. Sex was equally distributed across the two groups. Table 2 summarizes the demographic data and questionnaires at baseline and illustrates that no significant differences existed in baseline characteristics between the control and the placebo group (i.e. age, MSSQ, BMI, HADS-D, education; all $p > 0.05$).

Table 2. *Demographic sample description separately for control and placebo group at baseline on day 1*

	Control Group (n=30)		Placebo Group (n=60)		
	Mean	SD	Mean	SD	p-value
Age	23.50	2.70	23.45	3.42	0.94
MSSQ	141.55	42.92	135.57	36.48	0.51
BMI	22.31	2.75	21.61	2.10	0.19
Education	16.16	3.30	16.67	2.34	0.40
HADS-D	1.41	1.57	1.76	1.64	0.34
HADS-A	3.96	2.65	4.00	2.19	0.95
BAS	3.20	0.23	3.18	0.30	0.82
BIS	2.88	0.37	2.79	0.42	0.42
STAI-trait	38.75	6.39	37.78	6.49	0.51
STAI-state day 1	34.70	4.87	35.79	8.33	0.51
STAI-state day 2	35.51	8.96	34.88	8.14	0.74

Note. Entries show mean and standard deviation (SD), p-values (One-Way ANOVA´s), motion sickness susceptibility questionnaire (MSSQ), hospital depression scale (HADS-D), hospital anxiety scale (HADS-A), body mass index (BMI), education (sum of total number of years of school and university), behavioral inhibition and approach system scale (BIS/ BAS), state-trait anxiety inventory (STAI).

5.2 Behavioral Data

Table 3 summarizes the behavioral and humoral data as well as delta percentage of total power conducted at day 1, and shows that no significant differences existed between the control and the placebo group at the control condition (i.e. expected and perceived nausea, perceived stress, perceived motion sickness, cortisol, amylase, delta power, all $p > 0.05$). In contrast and in line with our main hypothesis, one-way ANOVA´s displayed significant group differences in expected- and perceive nausea as well as perceived stress at day 2 (all $p \leq 0.01$, see Table 4).

Table 3. *Behavioral, EEG, and humoral sample description separately for placebo and control group at times of acute nausea (average over t=+10 to +20) at day 1*

	Control Group (n=30)		Placebo Group (n=60)		
	Mean	SD	Mean	SD	p-value
Ctrl Expected Nausea$^{NRS(0-10),\ bc,\ t(-10)}$	5.59	2.52	5.72	2.25	0.81
Ctrl Perceived Nausea$^{NRS(0-10),\ bc,\ avg\ t(+10\ to\ +20)}$	5.60	1.45	5.60	1.83	0.99
Ctrl Perceived Stress$^{NRS(0-10),\ bc,\ t(+10)}$	2.88	1.78	2.83	1.79	0.91
Ctrl Perceived Stress$^{NRS(0-10),\ bc,\ t(+20)}$	4.04	2.12	3.62	2.44	0.47
Ctrl Delta Power C3$^{\%\ of\ total\ FFT\ power,\ bc,\ t(+20)}$	0.04	0.11	0.03	0.12	0.62
Ctrl Delta Power C4$^{\%\ of\ total\ FFT\ power,\ bc,\ t(+20)}$	0.05	0.12	0.04	0.12	0.70
Ctrl Amylase$^{ln,\ bc,\ t(+20)}$	-0.10	0.50	-0.16	0.41	0.58
Ctrl Cortisol$^{ln,\ bc,\ t(+35)}$	0.31	0.85	0.07	0.70	0.17

Note. Entries show mean, standard deviation (SD), and p-values (One-Way ANOVA´s, * indicates $p \leq .05$). Day 1 (control condition, ctrl), numeric rating scale (NRS), logarithmized (ln), all values were baseline corrected (minus baseline recordings, bc), average (avg).

Table 4. *Behavioral, EEG, and humoral sample description separately for placebo and control group at times of acute nausea (average over t=+10 to +20) at day 2*

	Control Group (n=30)		Placebo Group (n=60)		
	Mean	SD	Mean	SD	p-value
Test Expected Nausea$^{NRS(0-10),\ bc,\ t(-10)}$	5.61	1.57	4.16	1.99	0.00**
Test Perceived Nausea$^{NRS(0-10),\ avg\ t(+10\ to\ +20)}$	4.82	1.77	2.37	1.98	0.00**
Test Perceived Stress$^{NRS(0-10),\ bc,\ t(+10)}$	2.29	2.21	1.07	1.93	0.01*
Test Perceived Stress$^{NRS(0-10),\ bc,\ t(+20)}$	2.98	2.52	1.44	2.17	0.01*
Test Delta Power C3$^{\%\ of\ total\ FFT\ power,\ bc,\ t(+20)}$	-0.01	0.11	0.01	0.10	0.52
Test Delta Power C4$^{\%\ of\ total\ FFT\ power,\ bc,\ t(+20)}$	0.02	0.13	0.01	0.10	0.70
Test Amylase$^{ln,\ bc,\ t(+20)}$	-0.21	0.31	-0.13	0.35	0.35
Test Cortisol$^{ln,\ bc,\ t(+35)}$	-0.02	0.60	-0.05	0.78	0.86

Note. Entries show mean, standard deviation (SD), and p-values (One-Way ANOVA´s, * indicates $p \leq .05$). Day 2 (placebo condition versus no treatment condition, test), numeric rating scale (NRS), logarithmized (ln), all values were baseline corrected (minus baseline recordings, bc), average (avg).

5.2.1 Expected Nausea

A main effect of *day* (control versus intervention day) emerged for expected nausea ($F(1, 80) = 6.13$, $p = 0.02$) as well as a two-way interaction between *day* x *group* ($F(1, 80)=5.87$, p=.02). As hypothesized, the interaction was driven by lower levels of expected nausea ($M = 4.16$, $SD = 0.29$) in the placebo group compared to the control group ($M = 5.60$, $SD = 0.29$) at day 2 ($t(56) = 4.19$, $p = 0.00$; Figure 4). This indicates that our expectancy manipulation elicited the intended effect on a behavioral level, i.e. positive believe led to lower perceived nausea through the intervention. Neither a two-way interaction of day x *sex* nor a three-way interaction between *day* x *group* x *sex* emerged ($p \geq 0.05$).

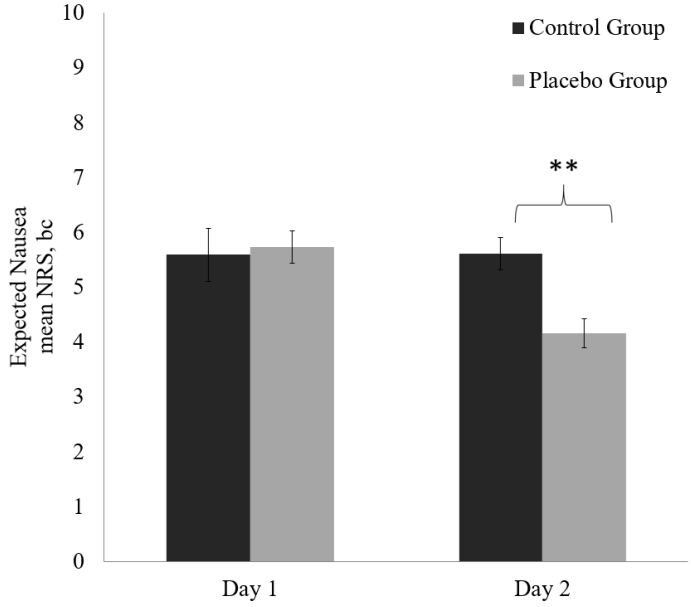

Figure 4. Baseline corrected (bc) means of expected nausea for the control (n=30) and the placebo group (n=60), rated at t = -10, separately for day 1 and day 2. NRS: '10'-point Numeric rating scale, with '0' indicating no expected nausea and '10' maximal level of expected nausea. **indicates $p \leq 0.001$. Error bars indicate standard error.

5.2.2 Perceived Nausea

A main effect of *day* ($F(1, 86) = 128.08$, $p = 0.00$) as well as an interaction between *day*

x *group* ($F(1, 86) = 47.66$, $p = 0.00$) were observed. Post-hoc tests showed that this effect resulted from a significant decrease in perceived nausea in the placebo group compared to the control group at day 2 ($F(1, 79) = 7.86$, $p = 0.01$; day 2: control group: $M = 4.82$, $SD = 0.32$, placebo group: $M = 2.37$, $SD = 0.27$). By contrast, at day 1 both groups perceived similar levels of nausea ($p \geq 0.05$; day 1: control group: $M = 5.60$, $SD = 0.27$, placebo group: $M = 5.59$, $SD = 0.24$; Figure 5). These findings indicate that the placebo intervention successfully induced a placebo effect on nausea on the NRS. No *sex* effects emerged on perceived nausea ($p \geq 0.05$).

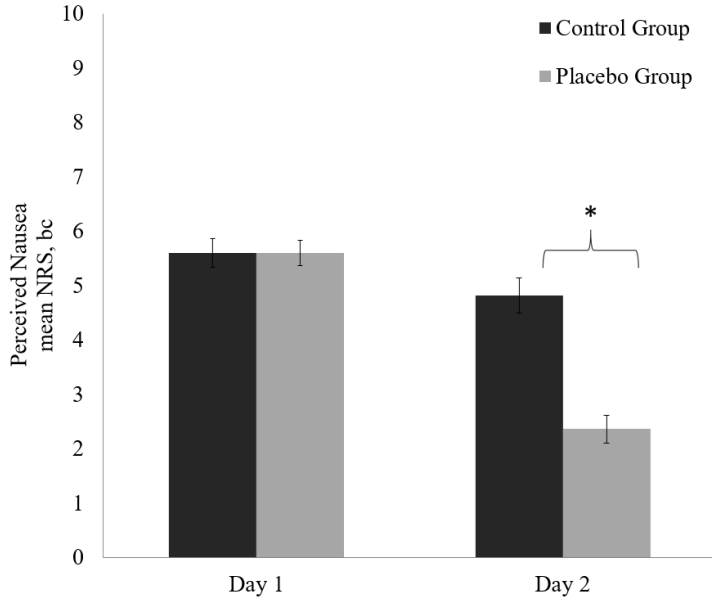

Figure 5. Baseline corrected (bc) mean NRS scores of perceived nausea for the control (n=30) and the placebo group (n=60), average over t = +10 to +20, separately for day 1 and day 2. NRS: Numeric rating scale. * indicates $p \leq 0.01$. Error bars indicate standard error.

5.2.3 Perceived Stress

The ANOVA based on stress ratings conducted at time point +20 minutes at each session indicated no placebo effect on stress, only a main effect of *dayt* emerged. This was due to a reduction in stress from day 1 to day 2 ($t(77) = 6.52$, $p = 0.00$; day 1: $M = 3.76$, $SD = 2.34$; day 2: $M = 1.96$, $SD = 2.40$).

Instead, the ANOVA based on stress ratings obtained at time point +10 at day 1 and day

2 revealed a main effect of *day* (F (1, 74) = 30.71, p = 0.00), an interaction between *day* x *group* (F (1, 74) = 4.88, p = 0.03), and a three-way interaction between *day* x gro*up* x *sex* (F(1, 74) = 6.10, p = 0.02). This was due to blunted stress levels in the females' placebo group compared to the control group at day 2 (F(1, 44) = 9.80, p = 0.00; females: day 1: control group: M = 2.83, SD = 0.55, placebo group: M = 2.97, SD = 0.33; day 2: control group: M = 2.9, SD = 0.46, placebo group: M = 0.95, SD = 0.38; Figure 6: A), which was not true in females at day 1. Neither was there a significant group difference in males at day 1 or day 2 ($p \geq 0.05$; day 1: control group = 2.9±0.38, placebo group=2.65±0.37; day 2: control group = 2.65±0.37, placebo group = 1.22±0.37). In contrast, the males showed a stress reduction above both groups from day 1 to day 2 (t(32) = 4.60, p = 0.00; Figure 6: B).

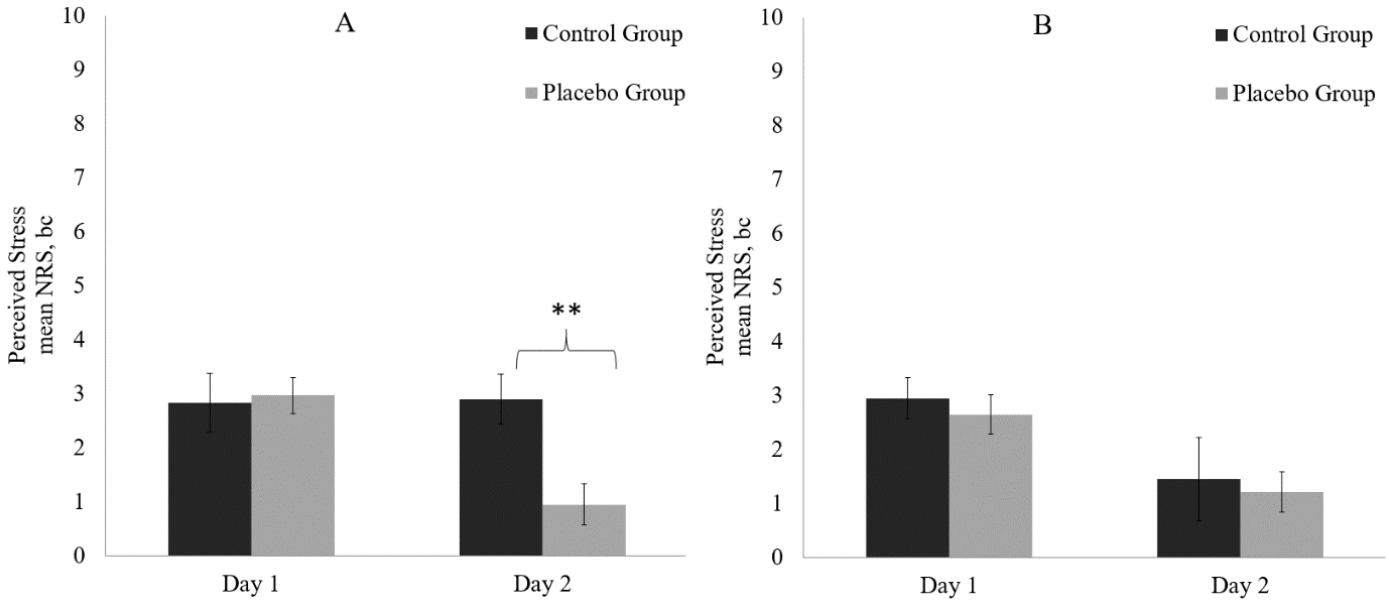

Figure 6. Baseline corrected (bc) NRS scores of perceived stress rated at t = +10 for the control (n=30) and the placebo group (n=60) at day 1 and day 2 for (A) females and (B) males. NRS: Numeric rating scale. ** indicates $p \leq 0.001$. Error bars indicate standard error.

5.3 Humoral Stress Parameter

5.3.1 Cortisol

For cortisol, the ANOVA resulted in a main effect of *day* (F(1, 85) = 6.13, p = 0.02) and

a trend towards an interaction between *day* and *sex* (F(1, 85)=3.79, p=0.06). This was driven by reduced stress levels in females at day 2 compared to day 1 ($t(44) = 3.17$, $p = 0.00$; female cortisol level day 1: $M = 0.33$, $SD = 0.79$, female cortisol level day 2: $M = -0.06$, $SD = 0.67$, Figure 7), which was not the case in males ($p \geq 0.05$; male cortisol level day 1: $M = -0.03$, $SD = 0.68$, male cortisol level day 2: $M = -0.03$, $SD = 0.78$; Figure 7). No main or interaction effects of group emerged ($p \leq 0.05$).

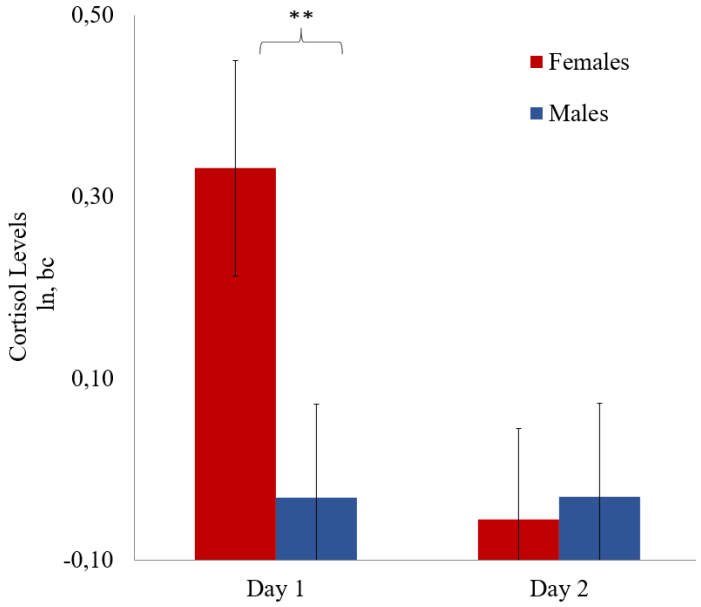

Figure 7. Mean levels of cortisol, logarithmized (ln) and baseline corrected (bc), separately for females and males at day 1 and day 2. Error bars indicate standard error.

5.3.2 Amylase

There were no main effects on amylase neither for *day* nor for *group* or *sex,* or interactional effects (all p's ≥ 0.05; amylase level in the control group at day 1: $M = 4.75$, $SD = 0.58$, and day 2: $M = 4.65$, $SD = 0.59$; amylase level in the placebo group at day 1: $M = 4.67$, $SD = .80$, day 2: $M = 4.64$, $SD = .65$; Figure 8).

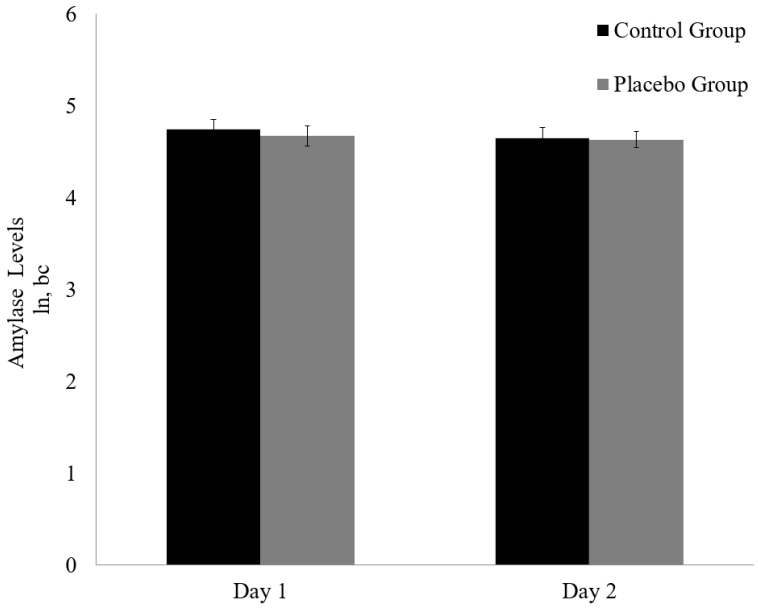

Figure 8. Mean levels of amylase conducted at t = +20, logarithmized (ln) and baseline corrected (bc), separately for the placebo and the control group at day 1 and day 2. Error bars indicate standard error.

5.4 Electroencephalography: Fast Fourier Transformation

5.4.1 Percentage of Delta Power in C4

A repeated measures ANOVA comparing *day*, *groups* and *sex* in percentage of delta power (0.5 – 4 Hz) in the C4 electrode revealed no significant effect of day or *day*by *group* ($p \geq 0.05$), though there was a significant three-way interaction between *day*, *group*, and *sex* ($F(1, 76) = 4.67$, $p = 0.03$). A univariate ANCOVA, separately conducted for sex, with *group* as the between subject factor and *the increase of delta power* on day 2 (difference between delta power at acute nausea minus at baseline) as the dependent variable, controlled for the *increase of delta power* at day 1 as a covariate revealed a significant group effect on the percentage of delta frequency during nausea at day 2 in females only (females: $F(1, 38) = 4.02$, $p = 0.05$; female control group increase delta power in C4 day 1: $M = 0.05$, $SD = 0.07$, day 2: $M = 0.06$, $SD = 0.14$; female placebo group increase delta power in C4 day 1: $M = 0.04$, $SD = 0.12$, day 2: $M = -0.01$, $SD = 0.10$; Figure 9: A; males: $p \geq 0.05$; male control group increase delta power in C4 day 1: $M =$

0.05, *SD* =0.15, day 2: *M* = -0.02, *SD* =0.11; male placebo group increase delta power in C4 day 1: *M* = 0.03, *SD* =0.13, day 2: *M* = 0.03, *SD* =0.11, Figure 9: B). This suggests a placebo effect on the percentage of delta power above central areas in females but not in males.

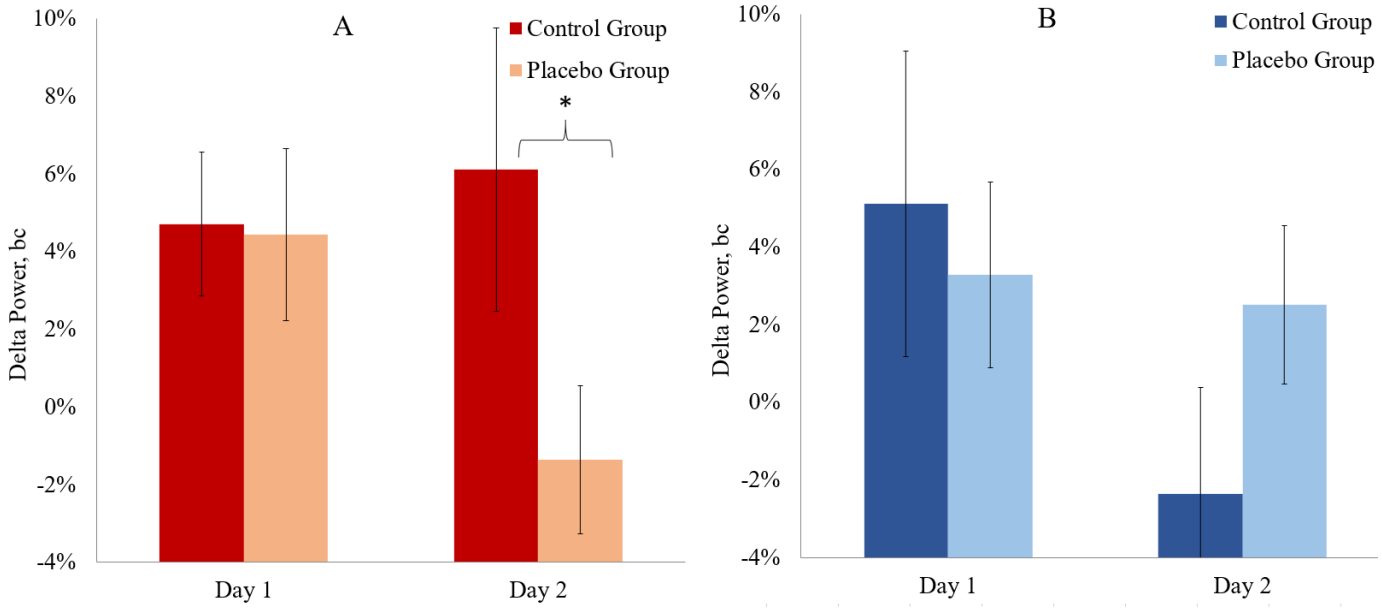

Figure 9. Percentage of delta power of the total FFT (fast Fourier transformed) spectrum at acute nausea [averaged over t=+10 to +20] in (A) females and (B) males at day 1 and day 2, separately for the placebo (n = 30) and the control participants (n = 60), baseline corrected (bc).

5.4.2 Percentage of Delta Power in C3

There was a main effect of *day* ($F(1, 76) = 3.97$, $p = .05$), though post-hoc t-test across groups indicated no significant differences between the nausea related percentage of delta power on day 1 versus day 2 ($p \geq 0.05$; increase delta power in C3 at day 1: *M* = 0.03, *SD* = 0.11, day 2: *M* = 0.00, *SD* =0.10). No other significant main effects or interactions were found ($p \geq 0.05$).

5.5 Gender and the Placebo Effect

In order to understand the influence of gender, *table 5* (day 1) and 6 (day 2) list stress related parameters (i.e. perceived behavioral stress at t = +10 and +20, increase in cortisol, amylase, and delta power) across control and placebo groups, separately for females and males (for more detail see section 5.6.3). A placebo effect was found only in the female group on delta power in C4 as well as on perceived stress at t=+10 minutes (for more detail see sections 5.2.3 and 5.4.1).

Table 5. *Behavioral, EEG, and humoral parameters across control and placebo group, separately for females and males at times of acute nausea at day 1*

	Females					Males				
	Control Group (n=15)		Placebo Group (n=30)			Control Group (n=15)		Placebo Group (n=30)		
Variables at Day 1	Mean	SD	Mean	SD	p-value	Mean	SD	Mean	SD	p-value
Ctrl Expected Nausea$^{NRS(0\text{-}10),\ bc,\ t(+20)}$	5.80	2.34	5.93	2.46	0.86	5.33	2.81	5.50	2.01	0.83
Ctrl Perceived Nausea$^{NRS(0\text{-}10),\ bc,\ avg\ t(+10\ to\ +20)}$	5.47	1.73	5.78	1.96	0.61	5.73	1.17	5.41	1.70	0.52
Ctrl Perceived Stress$^{NRS(0\text{-}10),\ bc,\ t(+10)}$	2.83	2.12	2.97	1.81	0.83	2.95	1.21	2.65	1.79	0.64
Ctrl Perceived Stress$^{NRS(0\text{-}10),\ bc,\ t(+20)}$	3.97	2.42	3.83	2.54	0.87	4.15	1.67	3.35	2.34	0.34
Ctrl Delta Power C3$^{\%\ of\ total\ FFT\ power,\ bc,\ avg\ t(+10\ to\ +20)}$	0.02	0.09	0.03	0.07	0.67	0.07	0.12	0.03	0.15	0.42
Ctrl Delta Power C4$^{\%\ of\ total\ FFT\ power,\ bc,\ avg\ t(+10\ to\ +20)}$	0.05	0.07	0.04	0.12	0.93	0.05	0.15	0.03	0.12	0.68
Ctrl Amylase$^{ln,\ bc,\ t(+20)}$	0.00	0.47	-0.15	0.47	0.34	-0.21	0.53	-0.16	0.33	0.77
Ctrl Cortisol$^{ln,\ bc,\ t(+35)}$	0.41	0.90	0.29	0.75	0.65	0.20	0.81	-0.15	0.58	0.10

Note. Entries show mean, standard deviation (SD), and p-values (One-Way ANOVA´s, * indicates $p \leq .05$, ** $p \leq .001$), *day 1* (control condition, ctrl), all values were baseline corrected (i.e. minus baseline recordings, bc) humoral parameters were logarithmized (ln).

Table 6. *Behavioral, EEG, and humoral parameters across control and placebo group, separately for females and males at times of acute nausea at day 2*

	Females					Males				
	Control Group (n=15)		Placebo Group (n=30)			Control Group (n=15)		Placebo Group (n=30)		
Variables at Day 2	Mean	SD	Mean	SD	p-value	Mean	SD	Mean	SD	p-value
Test Expected Nausea$^{NRS(0\text{-}10),\ bc,\ t(+20)}$	5.93	1.44	4.34	2.38	0.02*	5.23	1.69	3.96	1.50	0.02*
Test Perceived Nausea$^{NRS(0\text{-}10),\ bc,\ avg\ t(+10\ to\ +20)}$	5.15	1.75	2.68	2.20	0.00**	4.49	1.78	2.05	1.72	0.00**
Test Perceived Stress$^{NRS(0\text{-}10),\ bc,\ t(+10)}$	2.90	1.78	0.95	2.06	0.00**	1.45	2.54	1.22	1.80	0.76
Test Perceived Stress$^{NRS(0\text{-}10),\ bc,\ t(+20)}$	3.70	2.24	1.52	2.38	0.01*	2.00	2.65	1.35	1.91	0.42
Test Delta Power C3$^{\%\ of\ total\ FFT\ power,\ bc,\ avg\ t(+10\ to\ +20)}$	0.03	0.04	0.01	0.09	0.42	-0.04	9.14	0.00	0.08	0.16
Test Delta Power C4$^{\%\ of\ total\ FFT\ power,\ bc,\ avg\ t(+10\ to\ +20)}$	0.06	0.14	-0.01	0.10	0.05*	-0.02	0.11	0.03	0.11	0.17
Test Amylase$^{ln,\ bc,\ t(+20)}$	-0.17	0.30	-0.19	0.38	0.87	-0.25	0.33	-0.07	0.32	0.12
Test Cortisol$^{ln,\ bc,\ t(+35)}$	-0.05	0.62	-0.06	0.70	0.96	0.00	0.60	-0.05	0.86	0.85

Note. Entries show mean, standard deviation (SD), and p-values (One-Way ANOVA´s, * indicates $p \leq 0.05$, ** $p \leq 0.001$), *day 2* (condition including sham intervention in the placebo group versus no treatment in the control group, test), all values were baseline corrected (i.e. minus baseline recordings, bc) humoral parameters were log-arithmized (ln).

5.6 Correlations

5.6.1 Acute Nausea, Stress and Delta Power

Table 7 shows the results of the correlation analysis (Spearman´s rho) across all subjects for expected and perceived nausea, perceived stress, cortisol and amylase as well as percentage of delta power in C3 and C4 at day 1. Perceived nausea was positively associated with expected nausea (r_s (78) = 0.23, p = 0.04,), indicating that expected nausea may be a predictor of actual experience levels of nausea. Additionally, perceived nausea positively correlated with perceived stress (r_s (78) = 0.60, p = 0.00, Figure 10: A), and an increase in cortisol (r_s (89) =0.25, p = 0.02; Figure 10: C). Furthermore, stress was associated with the rise in cortisol (r_s (86) =0.26, p = 0.02; Figure 10: B) and there was a trend towards a positive relation between the increase of delta power in C3 and rise in cortisol (r_s (85) =0.20, p = 0.07; Figure 10: D*).* This trend seems to be meaningful since the raw percentage of delta power in C3 was also significantly associated with the rise in cortisol *(*r_s (86) =0.23, p = 0.03). Finally, in line with previous literature, there was no correlation between cortisol and amylase ($p \geq 0.05$), despite the fact that they have both robustly been shown to indicate stress levels.

Table 7. *Correlations including all reported variables across all subjects at day 1*

	A			B			C			D			E			F			G		
Variables at Day 1	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p
A. Expected Nausea																					
B. Perceived Nausea	85	.29	.01*																		
C. Perceived Stress $^{t(+10)}$	78	.10	.37	78	.43	.00**															
D. Perceived Stress $^{t(+20)}$	78	.23	.04*	78	.60	.00**	78	.83	.00**												
E. Cortisol	84	.10	.36	89	.25	.02*	78	.21	.06 [a]	78	.26	.02*									
F. Amylase	74	-.16	.16	78	-.02	.86	73	.08	.49	73	-.01	.90	78	-.16	.15						
G. Delta Power C3	80	-.18	.11	85	.10	.36	74	.09	.44	74	.08	.50	85	.20	.07 [a]	75	.00	.98			
H. Delta Power C4	80	.04	.75	85	.00	1.00	74	.13	.26	74	.11	.36	85	.18	.10	75	-.04	.72	85	.49	.00**

Note. A: expected nausea rated at t=+10, B: perceived nausea computed as the average of nausea rated at the NRS every minute from t=+10 to t=+20, C: perceived stress rated at t=+10, D: perceived stress rated at t=+20, E: logarithmized cortisol levels assessed at t=+35, F: logarithmized amylase levels assessed at t=+20, G: percentage of delta power of the total FFT (fast Fourier transformed) spectral power in electrode C3, H: percentage of delta power of the total FFT spectral power in electrode C4. All values are corrected for baseline [mean values during nausea – mean values during baseline]. [a] indicates trend towards significant correlation $p \leq 0.08$, * indicates $p \leq 0.05$, ** indicates $p \leq 0.001$.

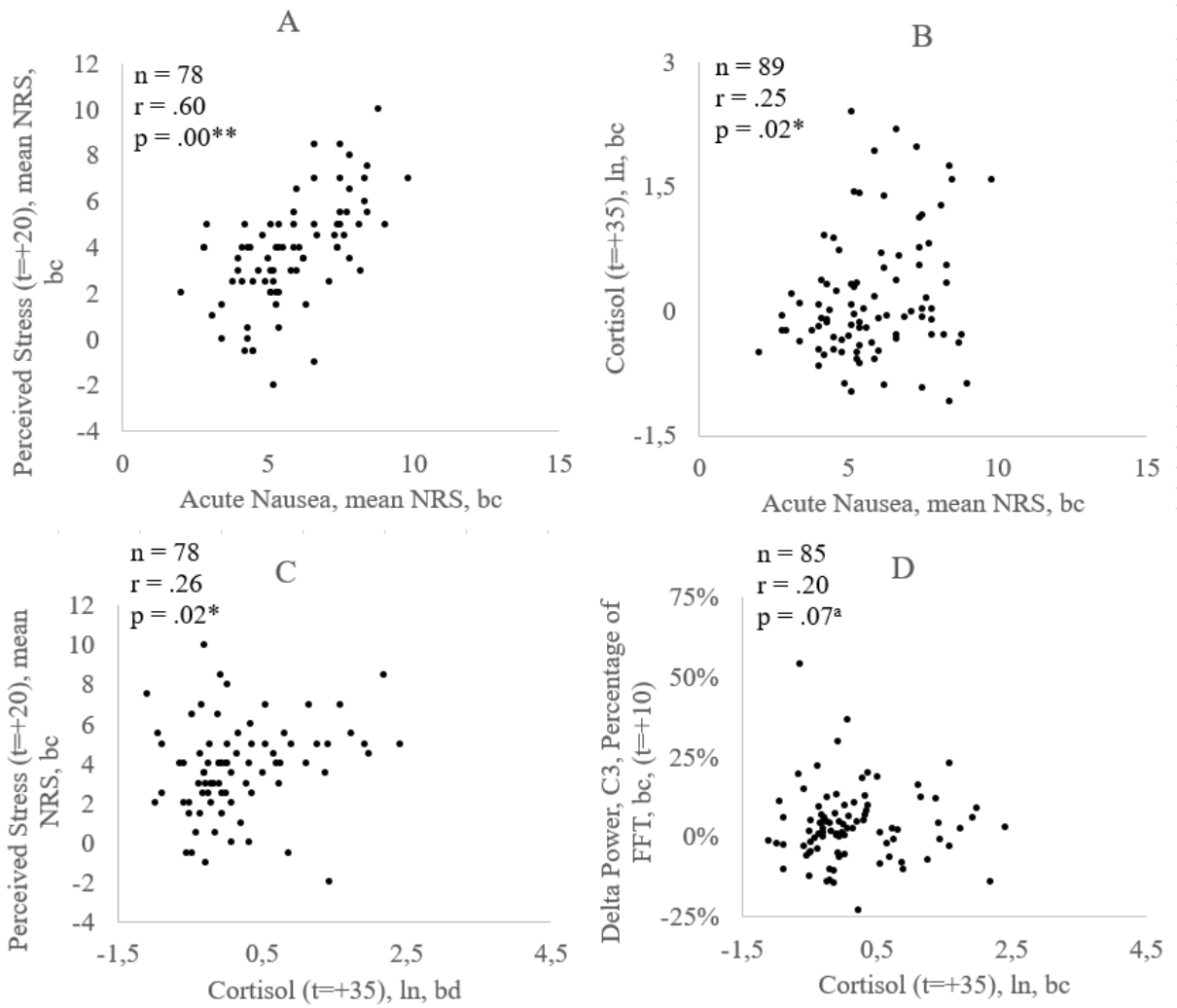

Figure 10. Scatterplots A-D. Positive correlations at day 1 between: (A) perceived stress (t = +20) and perceived nausea (average over t = +10 to +20); (B) perceived stress (t = +20) and logarithmized (ln) rise in cortisol (t = +35 minus t = -20); (C) perceived nausea (t = +10) and rise in cortisol; (D) percentage of delta power (average over t = +10 to +20) and ln rise in cortisol, all mean values are baseline corrected (bc) and presented across all subjects. [a] indicates trend towards significant correlation $p \leq 0.08$, * indicates $p \leq 0.05$, ** indicates $p \leq 0.001$.

5.6.2 Placebo Effect on Nausea, Stress and Delta Power

Table 8 shows correlations across no-treatment subjects for the spontaneous changes in all reported variables. Table 9 shows correlations across placebo participants for the placebo effect on all reported variables. No significant correlations for any spontaneous changes from day 1 to day 2 in the control group emerged (all $p > 0.05$). In contrast to our main hypothesis

that the placebo effect on nausea would be regulated via expectation and stress modulation, no significant correlation between the placebo effect on nausea and stress, expectation, cortisol, or delta power emerged (all $p > 0.05$). However, there was a trend between the placebo effect on delta power in C3 and nausea ($r_s(51) = 0.26$, $p = 0.06$).

Table 8. *Correlations across control group for spontaneous changes in all reported variables from day 1 to day 2*

	A			B			C			D			E			F			G		
Spontaneous changes in:	n	p	r	n	p	r	n	p	r	n	p	r	n	p	r	n	p	r	n	p	r
A. Expected Nausea																					
B. Perceived Nausea	27	.62	.10																		
C. Perceived Stress$^{t(+10)}$	25	.70	-.08	25	.19	-.27															
D. Perceived Stress$^{t(+20)}$	25	.21	-.26	25	.24	-.25	25	.00	.69**												
E. Cortisol	27	.24	.20	30	.98	-.00	25	.64	-.10	25	.51	.14									
F. Amylase	25	.54	-.13	27	.35	.20	24	.95	-.01	25	.24	-.25	30	.98	-.00						
G. Delta Power C3	26	.30	.21	29	.40	.16	24	.63	-.10	24	.85	.04	29	.33	.20	26	.25	-.24			
H. Delta Power C4	26	.77	.06	29	.67	.08	24	.44	-.16	24	.30	-.22	29	.52	-.13	26	.23	-.24	29	.02	.42*

Note. Spontaneous changes from day 1 to day 2 in A - H were defined as the baseline corrected mean values at day 1 minus the baseline corrected mean values at day 2. A: Expected nausea rated at t = +10, B: Perceived nausea computed as the average of nausea from t = +10 to +20, C: Perceived stress rated at t = +10, D: Perceived stress rated at t = +20, E: Logarithmized cortisol levels assessed at t = +35, F: Logarithmized amylase levels assessed at t=+20, G: Percentage of delta power of the total fast Fourier transformed (FFT) spectral power in electrode C3, H: Percentage of delta power of the total FFT spectral power in electrode C4. [a] indicates trend towards significant correlation $p \leq 0.08$, * indicates $p \leq .05$, ** indicates $p \leq 0.001$.

Table 9. Correlations across placebo group for the placebo effect on all reported variables

	A			B			C			D			E			F			G		
Placebo effect on:	n	p	r	n	p	r	n	p	r	n	p	r	n	p	r	n	p	r	n	p	r
A. Expected Nausea																					
B. Perceived Nausea	57	.43	.12																		
C. Perceived Stress$^{t(+10)}$	52	.90	.02	53	.71	-.05															
D. Perceived Stress$^{t(+20)}$	52	.42	.11	53	.11	-.23	53	.00	.54**												
E. Cortisol	56	.23	.16	59	.54	.08	53	.64	-.07	53	.70	.05									
F. Amylase	48	.87	-.03	51	.14	-.21	49	.83	.03	49	.50	-.10	48	.06	-.27[a]						
G. Delta Power C3	49	.52	.10	51	.06	.26[a]	45	.88	.02	45	.80	.04	51	.25	-.16	45	.73	-.05			
H. Delta Power C4	49	.23	-.20	51	.83	.03	45	.18	-.02	45	.32	.-.20	51	.70	-.06	45	.91	-.02	51	.00	.46**

Note. Placebo effects on the variables A – H were defined as the baseline corrected mean values at day 1 minus the baseline corrected mean values at day 2. A: Expected nausea rated at t = +10, B: Perceived nausea computed as the average of nausea t = +10 to +20, C: Perceived stress rated at t = +10, D: Perceived stress rated at t = +20, E: Logarithmized cortisol levels assessed at t = +35, F: Logarithmized amylase levels assessed at t = +20, G: percentage of delta power of the total fast Fourier transformed (FFT) spectral power in electrode C3, H: Percentage of delta power of the total FFT spectral power in electrode C4. [a] indicates trend towards significant correlation p ≤ 0.08, * indicates $p \leq .05$, ** indicates $p \leq 0.001$.

5.6.3 Gender and the Placebo Effect in Nausea, Stress and Delta Power

Since a placebo effect on delta power in C4 and stress at t = +10 was observed only in females, a correlation analysis stratified by gender which included placebo effects (spontaneous changes in the placebo group) was run on expected and perceived nausea and all stress relevant parameters. Due to the fact that some of the stress related parameters showed no reduction from day 1 to day 2, mean amylase and cortisol levels measured at day 2 (i.e. not subtracted by the data conducted at day 1) were also included in the correlation analysis. Table 10 – 13 shows correlations of stress relevant parameters and placebo effects separately for gender and group.

Table 10. *Correlations including spontaneous changes in nausea, delta power in C4 and stress, and stress-relevant parameters at day 2 for the female control group*

	A			B			C			D			E			F		
Variables	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p
A. Spontaneous Changes in Nausea																		
B. Spontaneous Changes in Delta Power C4	14	-.16	.59															
C. Spontaneous Changes in Stress$^{t(+10)}$	15	.-10	.73	14	-.06	.84												
D. Test Perceived Stress$^{t(+10)}$	15	-.03	.86	14	-.05	.86	15	-.07	.80									
E. Test Perceived Stress$^{t(+20)}$	15	-.02	.18	14	-.38	.18	15	.28	.32	15	.48	.07						
F. Test Amylase	14	.31	.14	13	-.43	.14	14	-.38	.18	14	-.03	.93	14	-.11	.71			
G. Test Cortisol	15	.23	.29	14	-.31	.29	15	.11	.68	15	.18	.52	15	.30	.28	14	.43	.13

Note. Spontaneous changes from day 1 to day 2 in A - C were defined as the baseline corrected mean values at day 1 minus the baseline corrected mean values at day 2. A: Perceived nausea computed as the average of nausea from t = +10 to +20, B: Percentage of delta power of the total FFT spectral power in electrode C4, C: Perceived stress rated at t = +10. Variables D – G represent baseline corrected parameters conducted at day 2 (test). D: Perceived stress rated at t = +10, E: Perceived stress rated at t = + 20, F: Logarithmized amylase levels assessed at t=+20, G: Logarithmized cortisol levels assessed at t = +35. [a] indicates trend towards significant correlation $p \leq 0.08$, * indicates $p \leq 0.05$, ** indicates $p \leq 0.001$.

Table 11. *Correlations including spontaneous changes in nausea, delta power in C4 and stress, and stress-relevant parameters at day 2 for the male control group*

	A			B			C			D			E			F		
Variables	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p
A. Spontaneous Changes in Nausea																		
B. Spontaneous Changes in Delta Power C4	15	.19	.49															
C. Spontaneous Changes in Stress$^{t(+10)}$	10	-.03	.93	10	-.12	.73												
D. Test Perceived Stress$^{t(+10)}$	11	.06	.87	11	.05	.88	10	.79*	.01									
E. Test Perceived Stress$^{t(+20)}$	11	-.11	.74	11	.07	.83	10	.61	.06	11	.69*	.02						
F. Test Amylase	13	.27	.37	13	-.47	.11	10	.32	.37	11	.32	.34	11	.22	.53			
G. Test Cortisol	15	.51	.05	15	.35	.20	10	.34	.33	11	.62*	.04	11	.39	.24	13	-.07	.83

Note. Spontaneous changes from day 1 to day 2 in A - C were defined as the baseline corrected mean values at day 1 minus the baseline corrected mean values at day 2. A: Perceived nausea computed as the average of nausea from t = +10 to +20, B: Percentage of delta power of the total FFT spectral power in electrode C4, C: Perceived stress rated at t = +10. Variables D – G represent baseline corrected parameters conducted at day 2 (test). D: Perceived stress rated at t = +10, E: Perceived stress rated at t = + 20, F: Logarithmized amylase levels assessed at t = +20, G: Logarithmized cortisol levels assessed at t = +35. [a] indicates trend towards significant correlation $p \leq 0.08$, * indicates $p \leq 0.05$, ** indicates $p \leq 0$ .001.

Table 12. *Correlations including placebo effect on nausea, delta power in C4, and stress, and stress-related parameters at day 2 for the female placebo group*

	A			B			C			D			E			F		
Variables	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p
A. Placebo Effect on Nausea																		
B. Placebo Effect on Delta Power C4	24	-.08	.72															
C. Placebo Effect on Stress $^{t(+10)}$	30	-.12	.53	24	-.22	.30												
D. Test Perceived Stress$^{t(+10)}$	30	-.23	.23	24	-.41	.05*	30	.61	.00**									
E. Test Perceived Stress$^{t(+20)}$	30	-.34	.07 [a]	24	-.35	.10	30	.39	.03*	30	.85	.00**						
F. Test Amylase	27	-.45	.02*	23	-.40	.06[a]	27	.02	.92	27	.17	.38	27	.33	.10			
G. Test Cortisol	30	-.21	.27	24	.08	.71	30	-.09	.65	30	-.23	.21	30	-.18	.35	27	.00	.99

Note. Placebo effects in A - C were defined as the baseline corrected mean values at day 1 minus the baseline corrected mean values at day 2. A: Perceived nausea computed as the average of nausea from t = +10 to +20, B: Percentage of delta power of the total FFT spectral power in electrode C4, C: Perceived stress rated at t = +10. Variables D – G represent baseline corrected parameters conducted at day 2 (test). D: Perceived stress rated at t = +10, E: Perceived stress rated at t = + 20, F: Logarithmized amylase levels assessed at t = +20, G: Logarithmized cortisol levels assessed at t = +35. [a] indicates trend towards significant correlation $p \leq 0.08$, * indicates $p \leq 0$ .05, ** indicates $p \leq 0.001$.

Table 13. *Correlations including placebo effect on nausea, delta power in C4 and stress, and stress-relevant parameters at day 2 for the male placebo group*

	A			B			C			D			E			F		
Variables	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p
A. Placebo Effect on Nausea																		
B. Placebo Effect on Delta Power C4	27	.23	.26															
C. Placebo Effect on Stress $^{t(+10)}$	23	-.04	.87	21	-.12	.61												
D. Test Perceived Stress$^{t(+10)}$	23	.26	.23	21	.04	.88	23	.56	.01*									
E. Test Perceived Stress$^{t(+20)}$	23	.11	.63	21	-.11	.64	23	.42	.05*	23	.82	.00**						
F. Test Amylase	24	-.15	.48	22	-.20	.38	22	.37	.09	22	.25	.27	22	.34	.12			
G. Test Cortisol	29	-.22	.25	27	.07	.75	23	.22	.31	23	-.02	.93	23	-.02	.94	23	.18	.40

Note. Placebo effects in A - C were defined as the baseline corrected mean values at day 1 minus the baseline corrected mean values at day 2. A: Perceived nausea computed as the average of nausea from t = +10 to +20, B: Percentage of delta power of the total FFT spectral power in electrode C4, C: Perceived stress rated at t = +10. Variables D – G represent baseline corrected parameters conducted at day 2 (test). D: Perceived stress rated at t = +10, E: Perceived stress rated at t = + 20, F: Logarithmized amylase levels assessed at t = +20, G: Logarithmized cortisol levels assessed at t = +35. [a] indicates trend towards significant correlation $p \leq 0.08$, * indicates $p \leq 0.05$, ** indicates $p \leq 0.001$.

Among females only, the placebo effect on nausea was negatively associated with the amylase level at acute nausea at t = +35 at day 2 ($r_s(30) = -0.46$, $p \leq 0.02$; Figure 11: E). Additionally, the placebo effect on delta power in C4 negatively correlated with baseline corrected stress levels at t = +10 at day 2 ($r_s(30) = -0.41$, $p \leq 0.05$; Figure 11: F) and amylase level at day 2 (trend, $r_s(23) = -0.40$, p=0.06; Figure 11: G). No correlations were found for other stress related parameters, neither in females nor in males ($p > 0.05$).

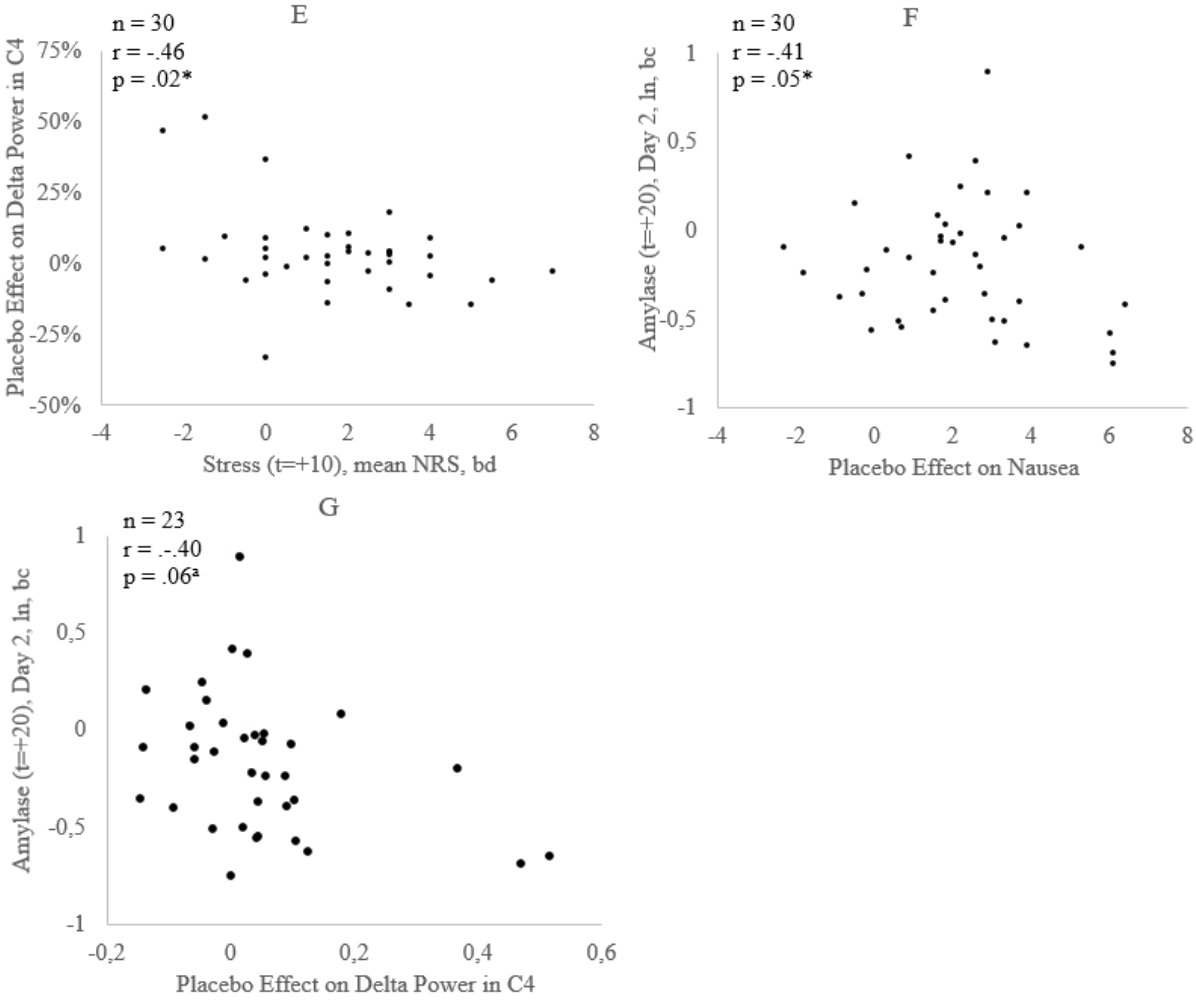

Figure 11. Scatterplots E - G. Negative correlations across female placebo subjects between (E) the placebo effect on delta power in C4 and baseline corrected mean scores of perceived stress (t=+10) at day 2; and (F) the placebo effect on nausea and baseline corrected amylase level (t=+20) at day 2; and (G) the placebo effect on delta power in C4 and baseline corrected amylase level (t=+20) at day 2. [a]indicates $p \leq 0.08$, * indicates $p \leq 0.05$, ** indicates $p \leq 0.001$.

5.6.4 Motivation and the Placebo Effect in Nausea

The BIS/BAS was included in the present study to investigate negative (BIS) as well as positive (BAS) aspects of motivation on the placebo effect. Therefore, sum of BIS and BAS scores were correlated with the placebo effect (spontanous changes from day 1 to day 2 in the control group, respectively) on expected and perceived nausea, perceived motion sickness, perceived stress at t = +10 and +20, cortisol, amylase, and delta power, spereately for the control and the placebo subjects (Tables 14 and 15).

Table 14. Correlations across control subjects separately for the BIS and the BAS with spontaneous changes on expected and perceived nausea, perceived stress, cortisol, amylase, and delta power

	BAS			BIS		
Spontaneous Changes in:	n	p	r	n	p	r
Expected Nausea	18	.89	-.04	18	.28	-.27
Perceived Nausea	20	.60	-.13	20	.43	.19
Perceived Stress $^{t(+10)}$	16	.01	.61*	16	.14	-.38
Perceived Stress $^{t(+20)}$	16	.11	.42	16	.86	-.05
Cortisol	20	.62	.12	20	.90	.03
Amylase	18	.76	-.08	18	.49	.18
Delta Power C3	19	.28	-.26	19	.85	.05
Delta Power C4	19	.04	-.48*	19	.51	.16

Note. Spontaneous changes were defined as the baseline corrected mean values at day 1 minus the baseline corrected mean values at day 2 in the listed variables: Expected nausea at t=+10, perceived nausea as the average over t = +10 to t = +20, perceived stress at t = +10 and t = +20, logarithmized (ln) cortisol levels at t = +35, ln amylase levels at t=+20, percentage of delta power of the total FFT (fast Fourier transformed) spectrum in electrode C3, percentage of delta power of the total FFT spectrum in electrode C4. BAS: Behavioral approach system scale. BIS: behavioral inhibition scale. * indicates $p \leq 0.05$.

Table 15. *Correlations across placebo subjects separately for the BIS and the BAS with placebo effects on expected and perceived nausea, perceived MS, perceived stress, cortisol, amylase, and delta power*

	BAS			BIS		
Placebo Effect on:	n	p	r	n	p	r
Expected Nausea	46	.06	-.28	46	.35	-.14
Perceived Nausea	49	.36	.13	49	.95	-.01
Perceived Stress $^{t(+10)}$	42	.06	-.29[a]	42	.34	-.15
Perceived Stress $^{t(+20)}$	42	.02	-.36**	42	.37	-.14
Cortisol	48	.46	-.11	48	.94	-.01
Amylase	41	.68	.07	41	.10	.26
Delta Power C3	42	.00	.46**	42	.31	.16
Delta Power C4	42	.00	.46**	42	.61	.28

Note. Placebo effects were defined as the baseline corrected mean values at day 1 minus the baseline corrected mean values at day 2 in the listed variables: Expected nausea at t=+10, perceived nausea as the average over t = +10 to t = +20, perceived stress at t = +10 and t = +20, logarithmized (ln) cortisol levels at t = +35, ln amylase levels at t=+20, percentage of delta power of the total FFT (fast Fourier transformed) spectrum in electrode C3, percentage of delta power of the total FFT spectrum in electrode C4. BAS: Behavioural approach system scale. BIS: behavioural inhibition scale. [a] indicates trend towards significant correlation $p \leq .08$, * indicates $p \leq .05$, ** indicates $p \leq .001$.

In the control group, mean BAS values were positively associated with the magnitude of spontaneous decrease from day 1 to day 2 in: (a) perceived stress at t = +10 ($r_s(16) = 0.61$, p=0.01), and (b) delta power in C4 ($r_s(19) = -0.48$, p=0.04; Figure 12: H).

In the placebo group, BAS scores were negatively associated with the placebo effect on: (a) perceived stress at t = +20 ($r_s(42) = -0.36$, p=0.02), and (b) delta power in C3 and C4 (C3: $r_s(42) = 0.46$, p=0.00; C4: $r_s(42) = 0.46$, p=0.00; Figure 12: I).

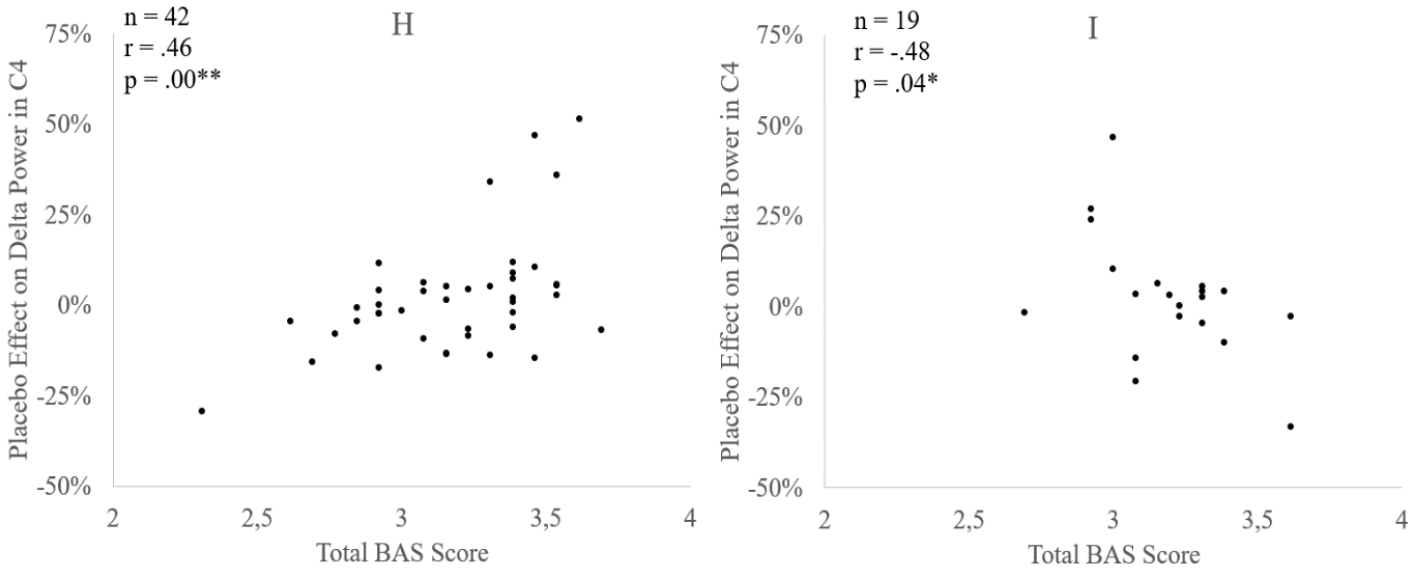

Figure 12. Scatterplots H: Positive correlation in the placebo group between the total BAS scores and the placebo effect on G) delta power in C4. Scatterplot I: Negative correlation in the control group between the total BAS scores and the spontaneous changes in delta power in C4. * indicates $p \leq .05$, ** indicates $p \leq .001$.

5.7 Exact Low Resolution Brain Electromagnetic Tomography

5.7.1 Acute Nausea

eLORETA analysis revealed no significant group differences in baseline recordings, neither on day 1 nor on day 2 (all $p \geq 0.05$). This was important to ensure meaningful comparison of nausea and placebo-related changes between groups. During acute nausea at day 1, nausea-related changes compared to the baseline recordings were shown in the following areas (extreme $p = 0.00$, $t = 7.49$, Table 16, Figure 13): the limbic, parietal, occipital, temporal, frontal lobe, and the insula.

Table 16. *Coordinates for brain areas showing significant differences between acute nausea and baseline at day 1 in MNI current density across all participants*

Frequencies	Lobe, Structure, Brodmann area	Peak voxel (X, Y, Z)	t-value (two-tailed)
Alpha, beta I, beta II	Limbic lobe, posterior cingulate, 30, 31	-10, -70, 15	7.49
Alpha, beta I	Parietal lobe, precuneus, 7, 31	-15, -70, 20	7.48
Alpha, beta I	Occipital lobe, cuneus, 18, 31	-15, -70, 15	7.46
Alpha, beta I	Temporal lobe, middle temporal gyrus, 22, 39	-35, -75, 25	7.06
Alpha, beta I	Frontal lobe, paracentral lobule, 4, 5, 31, 32	0, -35, 45	6.17
Alpha	Sub-lobar, insula, 13	-40, -45, 20	6.09

Note. Current density during acute nausea was computed from EEG (electroencephalography) recordings over t = +10 to +20, baseline current density from EEG over t = -20 to -10 at day 1. MNI: Montreal Neurological Institute. All t-values ≥ 3.52 indicate p-values ≤ 0.05.

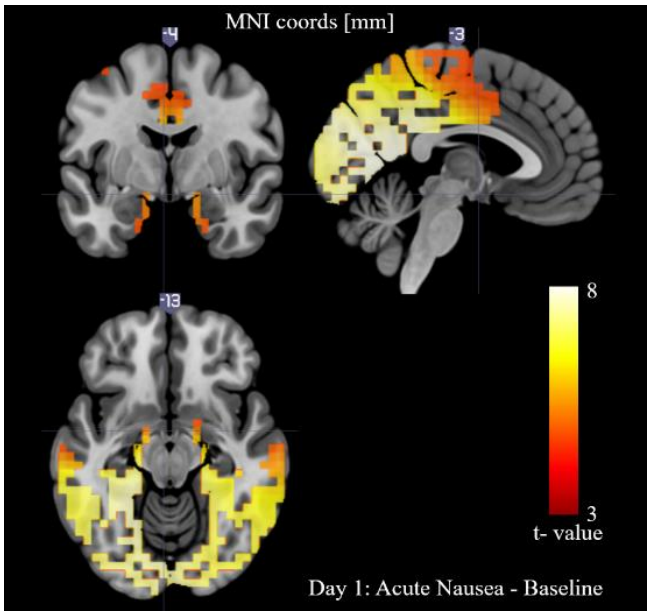

Figure 13. Comparison of acute nausea (mean over t = +10 to t = +20) and baseline (mean over t = -20 to t = -10) at day 1 across both sexes. The color bar represents t-statistic with t ≥ 3.52 (two-tailed) indicating p ≤ 0.05. Source: Montreal Neurological Institute coordinate system (MNI).

5.7.1.1 Gender Effects on Acute Nausea

In order to reveal sex specific cortical mechanism underlying nausea, acute nausea was compared to baseline at day 1, separately for male and female. The following significant nausea-related changes emerged in the female group (extreme $p = 0.00$, $t = 5.24$, Table 17): alpha and beta I activity in the limbic, parietal, occipital, tempo lobe, and insula. Similar nausea-related changes emerged in the male group (extreme $p = 0.00$, $t = 6.44$, Table 18): alpha and beta activity changes in the limbic, parietal, occipital, temporal, frontal lobe, and the insula. This may indicate that during acute nausea, similar brain regions are active in males and females.

Table 17. *Coordinates for brain areas showing significant differences between acute nausea and baseline at day 1 in MNI current density across female subjects*

Frequencies	Lobe, Structure, Brodmann area	Peak voxel (X, Y, Z)	t-value (two-tailed)
Alpha, beta I	Limbic lobe, posterior cingulate, 30	-10, -70, 10	5.26
Alpha, beta I	Parietal lobe, precuneus, 31	-15, -75, 20	5.18
Alpha, beta I	Occipital lobe, cuneus, 18	-10, -76, 0	5.23
Alpha,	Temporal lobe, fusiform gyrus, 37	-30, -45, -20	4.87
Alpha	Right frontal lobe, paracentral lobule, 5	20, -45, 50	4.36
Alpha	Sub-lobar, insula, 13	-30, -40, 20	4.13

Note. Current density during acute nausea was computed from EEG (electroencephalography) recordings over t = +10 to +20, baseline current density from EEG over t = -20 to -10 at day 1. MNI: Montreal Neurological Institute. All t-values ≥ 3,58 indicate p-values ≤ .05.

Table 18. *Coordinates for brain areas showing significant differences between acute nausea and baseline at day 1 in MNI current density across male subjects*

Frequencies	Lobe, Structure, Brodmann area	Peak voxel (X, Y, Z)	t-value (two-tailed)
Alpha, beta I	Limbic lobe, posterior cingulate, 35	-30, -25, -25	6.44
Alpha, beta I	Parietal lobe, precuneus, 7	20, -65, 30	6.09
Alpha, beta I	Occipital lobe, precuneus, 31	15, -60, 25	6.14
Alpha, beta I, beta II	Temporal lobe, middle temporal gyrus, 39	35, -65, 25	6.18
Alpha	Frontal lobe, paracentral lobule, 31	0, -30, 45	4.50
Alpha, beta I	Sub-lobar, insula, 13	-35, -25, 5	5.81

Note. Current density during acute nausea was computed from EEG (electroencephalography) recordings over t = +10 to +20, baseline current density from EEG over t = -20 to -10 at day 1. MNI: Montreal Neurological Institute. All t-values ≥ 3.59 indicate p-values ≤ 0.05.

5.7.2 Expectancy

In line with the main hypothesis on the source localization analysis, a placebo related decrease was shown at day 2 (anticipatory period, measured during t = -10 to 0, the time period following expectancy manipulation) in delta (0.5 - 4 Hz) activation arising from peak voxels in the frontal and anterior cingulate cortex (extreme $p \leq 0.001$, $t \geq 3.63$, Table 19, Figure 14), as well as decreased theta (4.1 - 8) oscillations arising from peak voxels in the frontal lobe. Contrastingly, in the control group, no significant differences between the recordings during baseline and after the randomization at day 2 emerged ($p \geq 0.05$). When stratifying by gender, no significant effects in the placebo groups during the anticipation phase emerged ($p \geq 0.05$).

Table 19. *Coordinates for brain areas showing significant differences following expectancy manipulation compared to baseline at day 2 in the placebo group*

Frequency	Lobe, Structure, Brodmann area	Peak voxel (X, Y, Z)	t-value (two-tailed)
Delta, theta	Frontal lobe, 6, 9, 10	55, 0, 30	4,30
Delta	Anterior cingulate, 32	10, 40, 15	3.83

Note. Current density during anticipation phase (following expectancy manipulation) was computed from EEG (electroencephalography) recordings over t = -10 to 0, baseline current density from EEG over t = -20 to -10 at day 2. MNI: Montreal Neurological Institute. All t-values ≥ 3.63 indicate p-values ≤ 0.05.

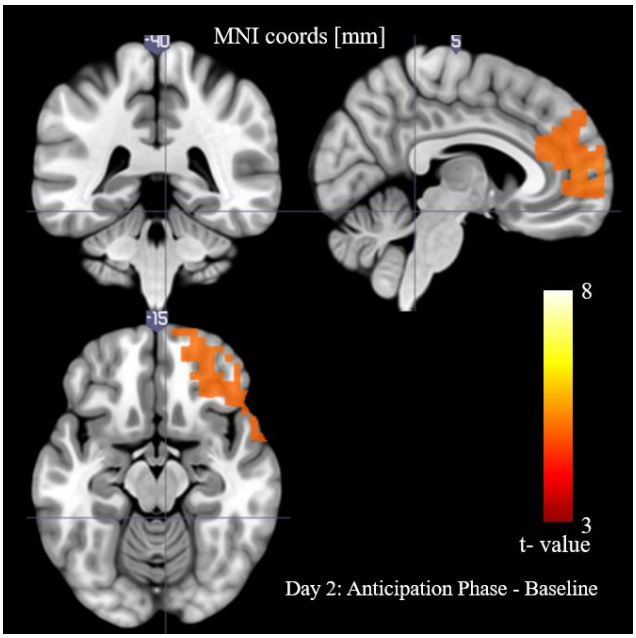

Figure 14. Comparison of the anticipation phase following expectancy manipulation (mean over t = -10 to t = 0) and baseline (mean over t = -20 to t = -10) at day 2 for the placebo group including both sexes. The color bar represents t statistic with t ≥ 3.63 (two-tailed) indicating p ≤ 0.05. Source: Montreal Neurological Institute coordinate system (MNI).

5.7.3 Placebo Effect on Nausea

To reveal placebo-related effects following sham acupuncture, acute nausea at day 2 was compared to acute nausea at day 1, separately for the placebo and the control group. No significant effects emerged in the control group (all $p \geq 0.05$), not even if stratified by gender (in males: all $p \geq 0.05$, in females: all $p \geq 0.05$).

Although, no placebo-related changes were found in the placebo group across both sexes, when stratifying by gender, placebo related activation in females (extreme $p \leq 0.02$, $t = 4.04$, Table 20, Figure 15) and males (extreme $p \leq 0.03$, $t = -3.78$, Table 21, Figure 16) emerged. Specifically, the female placebo group showed changes in alpha and beta activation from peak voxels in parietal, temporal, and frontal lobe. In the male placebo group, there was a change in alpha activation in the limbic lobe and the insula.

Table 20. *Coordinates for brain areas showing significant differences in MNI current density during acute nausea following placebo intervention at day 2two compared to acute nausea at day 1 in the female placebo group*

Frequency	Lobe, Structure, Brodmann area	Peak voxel (X, Y, Z)	t-value (two-tailed)
Alpha	Parietal lobe, postcentral gyrus, 1, 3	-65, -20 35	4,04
Alpha	Frontal lobe, precentral gyrus, 4	10, 40, 15	4,04
Beta I	Temporal lobe, middle temporal gyrus, 39	60, -60, 10	3,98

Note. Current density during acute nausea was computed from EEG (electroencephalography) recordings over t = +10 to +20 at day 1 and day 2. MNI: Montreal Neurological Institute. All t-values ≥ 3.98 indicate p-values ≤ .05.

Table 21. Coordinates for brain areas showing significant differences in MNI current density during acute nausea following placebo intervention at day 2 compared to acute nausea at day 2 in the male placebo group.

Frequency	Lobe, Structure, Brodmann area	Peak voxel (X, Y, Z)	t-value (one-tailed)
Alpha	Limbic lobe, parahippocampal gyrus, 28, 35	25, -20, -10	-3,78
Alpha	Sub-lobar, insula, 13	35, -15, 20	-3,54

Note. Current density during acute nausea was computed from EEG (electroencephalography) recordings over t = +10 to +20 at day 1 and day 2. MNI: Montreal Neurological Institute. All t-values ≥ -3.54 indicate p-values ≤ 0.05.

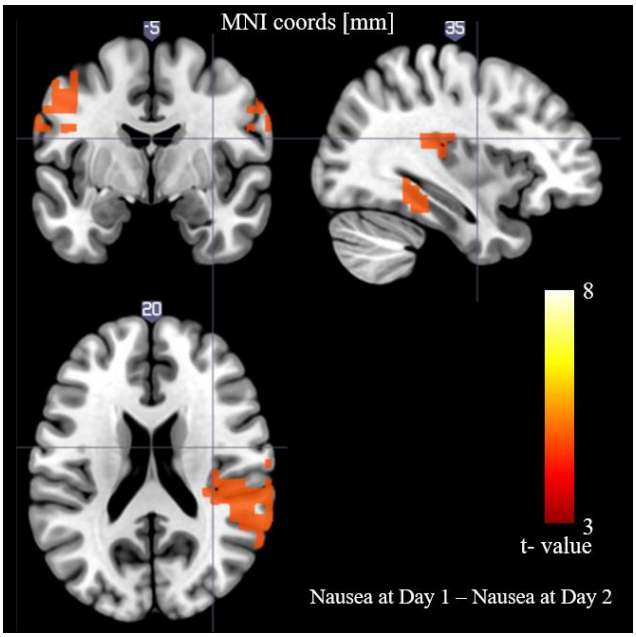

Figure 15. Comparison of acute nausea at day 1 (mean over t = +10 to +20) and acute nausea at day 2 (mean over t=+10 to +20) in the female placebo group. The color bar represents t-statistic with $t \geq 3.98$ (two-tailed) indicating $p \leq 0.05$. Source: Montreal Neurological Institute coordinate system (MNI).

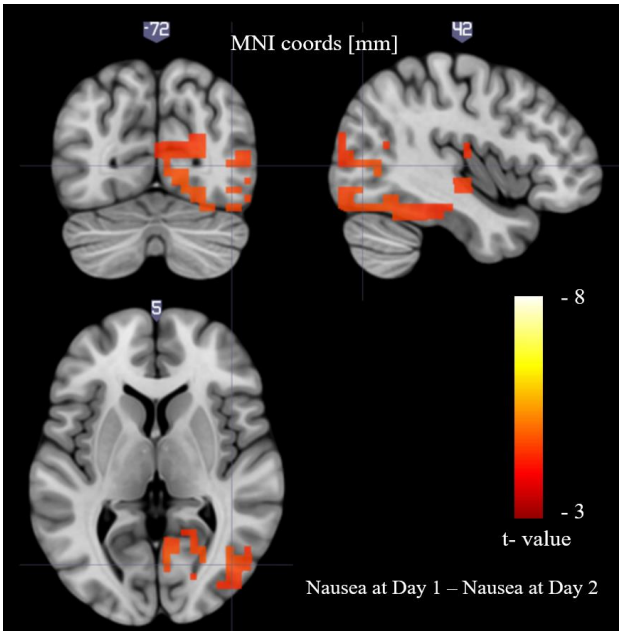

Figure 16. Comparison of acute nausea at day 1 (mean over t = +10 to +20) and acute nausea at day 2 (mean over t = +10 to +20) in the male placebo group. The color bar represents t-statistic with $t \geq -3.57$ (one-tailed) indicating $p \leq 0.05$. Source: Montreal Neurological Institute coordinate system (MNI).

6. Discussion

Nausea is a debilitating side effect that can seriously impair daily functioning in numerous populations (e.g. chemotherapy patients, pregnant women, car drivers/passengers etc.). Unfortunately, most treatments fail to alleviate nausea in these populations. However, nausea has been shown to be sensitive to placebo interventions. The present study was designed to achieve a better understanding of the placebo effect (psychosocial factors) in nausea and its underlying neurobiological mechanisms. To this end, placebo effects in nausea in relation to stress hormones and altered brain activity were studied. This investigation was crucial because it is assumed that neurobiological mechanisms underlying nausea may explain why nausea is sensitive to placebo interventions and therefore yield powerful implications to the treatment of nausea.

6.1 Main Findings

The research results emphasize the robustness of sham acupuncture in combination with verbal instruction to elicit placebo effects in nausea, as it clearly reduced expected and perceived nausea in both sexes, on a behavioral, cortical, and hormonal level. Specifically, the following three main findings in respect of the study hypothesis and sex differences emerged:

The verbal placebo instruction successfully reduced expected nausea on the NRS compared to the no-treatment group. On a cortical level, this effect was accompanied by changes in PFC and ACC activity in both sexes.

The placebo intervention significantly alleviated acute nausea on the NRS compared to the no-treatment group. The cortical correlates of the placebo effect on nausea differed for males and females: in males, the placebo effect on nausea displayed changes in the limbic, sub-lobar, and occipital lobe. In females, the placebo effect was accompanied by reduced delta power in the FFT spectrum and changes in parietal, frontal, and temporal lobe.

The placebo intervention elicited the intended effect in reducing stress exclusively in the female placebo group, which was indexed on a cortical and a hormonal level: females who

experienced a lower level of stress also showed greater placebo effects on nausea and delta power in C4 relative to subjects who stayed relatively stressed during acute nausea. Furthermore, the placebo-induced stress reduction was indexed by reduced amylase levels (which indicate a decrease in SNS activity) and amylase in turn was associated with the placebo effect on perceived nausea and delta power. Hence, stress, delta power, and amylase seem to modulate the placebo effect in nausea in females. In contrast, the stress levels in males remained unaffected by the placebo intervention, that is to say, there was a spontaneous stress reduction from day 1 to day 2 in both male groups.

In summary, these data indicate the effectiveness of sham acupuncture in nausea and shows that cortical mechanisms are involved in females and males. In particular, the positive expectation of nausea improvement following verbal instruction seems to be similarly modulated in females and males, specifically via PFC and ACC. On the other hand, the placebo effect on nausea itself may be differentially modified in females and males. Different cortical areas were found to be active during the placebo effect in nausea for females and males and placebo-induced reductions in stress, delta power, and amylase were only observed in females.

6.2 Critical Evaluation

Certain limitations of the present study design and its measurements should be acknowledged. Firstly, the present analyses focuses on 90 participants, which was split into two groups (30 control and 60 placebo participants). The mismatch between group sizes was due to an initial hypothesis that somatosensory stimulation (tingling in 30 enhanced placebo subjects) compared to no somatosensory stimulation (in 30 simple placebo subjects) at the sham acupuncture points would enhance the placebo effect on nausea. As preliminary analysis indicated no significant differences between simple placebo and enhanced placebo, both placebo groups were combined to increase the efficiency of all present results. In other words, the sample size in the control group was smaller compared to the placebo group, which increases the chances

of type II errors for the findings in the control group. Furthermore, even though there were no significant differences on a behavioral and bodily level between the two placebo groups; it is possible that the somatosensory stimulation in the enhanced placebo group could have influenced cortical processes not detected by the present study.

Secondly, there was an overall stress reduction in men from day 1 to day 2, which may have hindered the detection of differences in placebo-induced stress reductions between the male placebo and control groups. This should be taken into account when discussing the finding that stress was exclusively reduced during acute nausea at day 2 in the female placebo group.

Thirdly, the spacial resolution of EEG is less reliable compared to fMRI recordings and may have failed to reveal nausea or placebo-relevant brain structures, most prominently the absence of ACC activity during acute nausea at day 1 (see section 6.3.1). Also, one should take into account that more activation in the EEG does not automatically mean more activation in certain areas or vice versa. An increase in current density (LORETA) or spectral power (FFT) can also reflect electron inhibition.

6.3 Discussion of the Main Findings

6.3.1 Nausea Induction

The implemented nausea induction across all subjects at day 1 allowed to control behavioral, cortical, and hormonal correlates - naturally triggered by the vection drum. The following results are important in order to understand placebo-related changes. The subjective nausea response at day 1 was accompanied by elevated stress, accompanied by increased cortisol levels and delta power (0.5-4 Hz). This replicates previous findings that increased delta power above central cortical electrodes (Hu et al., 1999) and stress are involved in acute nausea (Levine, 2017). Additionally, the present study allowed for a deeper investigation into the relation between delta power and nausea due to the addition of stress relevant parameters, important parameters which have been absent in previous studies.

Furthermore, correlation analysis revealed that the increase in delta power did not directly correlate with the elevated nausea experience as reported in previous studies (Hu et al., 1999). In contrast, nausea positively correlated with an increase in stress and cortisol. In turn, cortisol positively correlated with the magnitude of stress and the increase in delta power. Therefore, the increase in delta power may reflect changes in stress-relevant brain areas underlying nausea rather than the direct nausea-related brain activity.

Furthermore, source-level analyses revealed nausea-related changes in the limbic, parietal, occipital, and PFC as well as in the insula. All these areas were previously found to be involved in nausea in the context of motion sickness (Chelen et al., 1993; Chen et al., 2010; Farmer et al., 2015; Hu et al., 1999; Ko et al., 2011; Napadow et al., 2013). On the other hand, some areas that have also been shown to be involved in nausea were not detected in the present study: the motor (Ko et al., 2011), orbitofrontal, somatosensory area and the ACC (Napadow et al., 2013). Specifically, the absence of the ACC is in contrast to Farmer (2015) and Napadow (2013) who suggested that the ACC strongly interacts with the PFC and together act as key components in the development of nausea. One explanation for this discrepancy could be that the induction of nausea was implemented differently and the source localization was based on fMRI recordings instead of EEG. Farmer (2015) and Napadow (2013) induced nausea within the fMRI scanner via special goggles and Farmer used a different video to induce illusory motion, with real pictures instead of alternating black and white stripes. Regarding the measurement, fMRI detects the level of oxygen in the blood and allows a higher level of local resolution compared to EEG, which records the average of voltage (electrical potentials) at the scalp. Electrical potentials have a higher temporal resolution than fMRI signals but in order to define local brain activity, they have to be traced back to their source within the brain, which is based on estimation analysis. Since no other EEG study has revealed the ACC while contrastingly, all fMRI studies on nausea did detect the ACC, it is possible that the ACC was involved in acute

nausea in the present study but was not significant because of the limited local resolution of EEG compared to fMRI.

Taken together, this analysis on nausea, cortisol, stress, and delta power at day 1 showed that participants who are relatively stressed also showed increased cortisol levels and increased nausea. Additionally, higher stress levels were accompanied by increased delta power in C3 and several nausea-related brain areas were revealed, such as the PFC. Again, the PFC is crucial in stress regulation and the increase in delta power directly correlated with stress. Hence, the present data may indicate cortisol and delta power as potential physiological stress modulators in nausea and strengthens the assumption that nausea-related brain areas overlap with cortical stress components (Levine, 2017; Toschi et al., 2017).

6.3.2 Expectancy Manipulation

Both sexes showed reduced expectation of nausea following verbal placebo instruction and sham acupuncture on the NRS. This was accompanied by changes in PFC and ACC, which may index the placebo-related modulation of the mind towards a positive treatment outcome. This assumption is reasonable, especially for the PFC activity, in light of the many previous placebo studies which have shown that the PFC is key area during placebo instruction effects (Benedetti, 2010; Hunter et al., 2006). In placebo anxiolysis, which reflects the reduction of fear and anxiety following placebo treatment, changes in frontal activity and ACC were also detected, and positive expectation towards the placebo treatment and ACC activity directly correlated with the subjective placebo effect (Meyer et al., 2015; Petrovic et al., 2005). The authors suggest that subjects recruit frontally based cognitive control functions during an expectancy manipulation that diminishes feelings of unpleasantness. The present findings are in line with these assumptions, even though anxiety and fear were not explicitly controlled, though they are closely linked to stress, which was reduced during acute nausea in the female placebo group.

6.3.3 Placebo Effect on Nausea

The placebo intervention elicited the intended effect of reducing nausea on a behavioral, cortical, and hormonal level (though the latter was only true in females). This is in line with the pilot study by Müller and colleagues (2016). Additionally, in this study the placebo intervention was also successfully tested in males (even without somatosensory stimulation at the electrodes site of the placebo TENS device) and found no sex differences on the placebo effect in nausea on a behavioral level. In contrast, the bodily correlates of the placebo response in nausea differed between males and females: in males, the placebo effect on nausea displayed changes in the limbic, sub-lobar, and occipital lobe. In females, on the other hand, the placebo effect was accompanied by reduced delta power in parietal, frontal, and temporal regions.

This pattern fits with placebo research in other domains, where gender does not appear to have a significant influence on the placebo response on the behavioral level (in placebo analgesia: Averbuch & Katzper, 2001; Weimer, Colloca, & Enck, 2015; in depression: Casper, Tollefson, & Nilsson, 2001), but instead plays a larger modulatory role on the cortical (Aslaksen, Bystad, Vambheim, & Flaten, 2011) and hormonal level (Colloca, Pine, Ernst, Miller, & Grillon, 2016). Specifically, vasopressin agonists enhanced the placebo response in women but not in men (Colloca et al., 2016). In contrast, in the study by Aslaksen and colleagues (2011) only men displayed a placebo response in N2/P2 ERP. Such discrepancies show the importance of controlling for gender effects, especially when investigating bodily correlates.

One possible explanation for the gender effects in the present study may be that the placebo response in females was more strongly modulated through a down-regulation of stress. This assumption is plausible, based on the finding that there was a placebo-induced stress reduction during acute nausea on the NRS only in females. The present study cannot clarify to which extent the placebo intervention reduced stress because stress itself is a correlate of nausea

(Levine, 2017) and no regression analysis was run, but the placebo effect in females was accompanied by changes in delta power, PFC activity, and amylase levels. All these correlates have been linked to stress in previous studies (Hall et al., 2007; Nater et al., 2006; McEwen et al., 2015). Specifically, delta power was found to index nausea-induced stress at day 1 in the present study. Additionally, PFC activity could be associated with nausea related changes in the ANS (Napadow et al., 2013; Toschi et al., 2017) and the ANS response of stress and nausea showed similar pattern (Levine, 2017). In summary, the present data supports the idea that not only the ANS response but also the cortical circuitry underlying nausea is closely linked to stress and is modifiable via placebo interventions in females.

Furthermore, it was hypothesized that the placebo-induced reduction in stress would be indexed by decreased cortisol levels; this turned out not to be the case. Cortisol was linked to stress, delta power, and nausea at day 1 but not to the behavioral placebo response, neither in females nor in males. At first glance, this seems surprising because females did show a placebo-induced stress reduction during acute nausea and cortisol robustly indexes stress (Lovallo & Thomas, 2000) yet, on the other hand, a previous study detected the very same pattern of cortisol in placebo analgesia (Johansen, Brox, & Flaten, 2003). They investigated cortisol in three groups: a no-treatment group, a placebo group, and a nocebo group. Pain was significantly reduced in the placebo group following sham intervention compared to the other groups but changes in cortisol did not correlate with placebo analgesia (increased cortisol levels were observed in all three groups, with the most significant rise in the nocebo group). In summary, cortisol might play a critical role in nausea-related stress but does not modulate the actual placebo response in nausea. Since amylase levels were associated with the placebo response on nausea in females, future studies may include amylase to reveal hidden placebo-induced stress mechanisms.

6.3.4 Motivation

It was hypothesized that positive aspects of motivation (BAS) were related to the behavioral placebo response in nausea. Although BAS scores did not correlate with the placebo effect on NRS nausea, BAS scores positively correlated with the magnitude of the placebo effect on delta power in both electrodes (C3 and C4). In the control group it was the other way around, here BAS scores correlated negatively with the spontaneous changes in delta power from day 1 to day 2. In placebo analgesia, reward-related brain activity predicted the placebo analgesic response, specifically within the ACC and the PFC (Schweinhardt, Seminowicz, Jaeger, Duncan, & Bushnell, 2009). Notably, the present study revealed placebo-related activity in the PFC and the ACC during the anticipation phase following expectancy manipulation, which emerged within the delta spectrum. Hence, the present data suggests that delta power in nausea is not only linked to nausea-related stress but also to higher cortical functions such as motivation (or in this case, to the anticipation of reward which was the positive treatment outcome - nausea reduction).

Furthermore, the BAS scores correlated negatively with the placebo effect on stress and positively with the spontaneous changes in stress from day 1 to day 2 in the control group. Even though no specific hypotheses were formulated for motivation in relation to stress, it is surprising that the BAS correlated negatively with the placebo effect on stress. We would rather have expected to find the opposite: that positive aspects of motivation would increase placebo-induced stress reductions (de la Fuente-Fernández, Schulzer, & Stoessl, 2004). On the other hand, a previous study that investigated the BIS/BAS in relation to the placebo effect on stress displayed the very same inverse pattern (Darragh, Booth, & Consedine, 2014). Lower BAS scores predicted higher placebo effects on stress and the opposite was found in the control group. The authors suggested that different contextual contingencies activate different behavior so that similar motivated subjects can show inverse behavior when facing varying demands. The present

findings support this view as the randomization of subjects into control and placebo groups imposed different requirements on the subjects, such that highly motivated subjects may have habituated more quickly to the nausea induction and therefore experienced less overall stress at day 2. In contrast, high motivated subjects in the placebo group may have experienced higher levels of arousal (stress) at day 2 compared to day 1 because the sham acupuncture was novel and, as a result, benefitted more from the placebo intervention.

6.4 Summary and Conclusions

By now, it is clear that the psychosocial context of administering medication can influence neurobiological mechanism. Researchers have increasingly suggested that the placebo effect is mainly modulated via positive expectations towards a treatment, mediated by PFC and ACC activity, which seem to be additionally linked to motivational aspects (reward-based mechanism). Once positive expectations are induced, the 'inner doctor' acts specifically on the bodily correlates, depending on the relevant condition. The present study was successful in showing evidence for both assumptions in a nausea model. Firstly, the placebo intervention significantly reduced expected nausea in the placebo group compared to the control group during the anticipation phase and this was accompanied by changes in PFC and ACC activity within the delta spectrum. Secondly, the actual placebo response during nausea induction was accompanied by blunted stress levels (in females only), changes in amylase (in females only), decrease in delta power (in females only), and changes in nausea-related brain areas (this also was sex specific).

The German Medical Association claims that the placebo effect should be implemented in clinical practice but that domain specific evidence is needed for each to facilitate its application (Kupferschmidt, 2011). The present study was able to replicate that the placebo effect plays a significant role in nausea, in both men and women, and provided empirical evidence that it interrelates with underlying neurobiological mechanisms.

The placebo effect in the present study was induced by means of a positive and convincing

verbal instruction towards the placebo treatment and therefore reduced ‘fearful’ expectations of developing nausea. This strengthens the suggestion that treatments of nausea should more seriously focus on psychological aspects to reduce anticipatory nausea, for example reducing high initial expectations of nausea in cancer patients before receiving chemotherapy (Colagiuri & Zachariae, 2010).

In a way, the present study also contributed to the mind-body interaction debate as it clearly showed that the subjective experience of nausea was accompanied by neurobiological changes. Specifically, it was shown that during the anticipation phase - in which nothing else was manipulated but the mind - subjective expectation changed in the placebo participants, indexed by altered PFC and ACC activity. Hence, body and mind should never been treated in separation, but instead should be integrated and seen as important elements in health care.

6.5 Outlook

Future investigations may control more thoroughly for the impact of psychosocial factors (e.g. fear, unpleasant feelings, and tension) in nausea and the placebo effect for a better course in the treatment of nausea. For example, Quinn and colleagues (2015) just recently induced a method that allows for the study of nocebo effects on nausea. The method is called galvanic vestibular stimulation (GVS; Quinn, MacDougall, & Colagiuri, 2015; Quinn & Colagiuri, 2016). GVS works with electrical stimulation and also includes a sham stimulation program that induces a nocebo effect on nausea. One advantage of this method is that the sham GVS and the active GVS are difficult to distinguish because they both induce tingling at the electrodes site. One idea would be to study the sham GVS in comparison with the active GVS within the fMRI. This would allow to distinguish the psychosocial components (nocebo nausea induced by the sham GVS) from the pure bodily components (active GVS minus sham GVS).

Finally, cortisol was not affected by the placebo intervention but was indeed involved in acute nausea. Since the present study did not control for conditioning effects and a study by

Benedetti and colleagues (2003) detected placebo-induced hormonal changes only in response to conditioning not to expectancy manipulation, it is possible that cortisol is only involved in conditioning induced placebo effects in nausea. Hence, it will be of interest to study in more detail the role of cortisol in the placebo effect in nausea by conditioning.

In conclusion, more detailed insights about the exact psychosocial factors that modulate the development of nausea should be addressed. In particular, the exact influence of stress in comparison to fearful expectations. This will yield important implications to the treatment of nausea, especially because physicians can easily be trained to trigger the relevant psychosocial components.

7. Abstract

Background. Evidence indicates that nausea can be modulated by placebo interventions, however, little is known about the underlying cognitive mechanisms. Therefore, the present study examined behavioral and electroencephalography (EEG) correlates of the placebo effect on nausea.

Methods. On two consecutive days, 90 healthy subjects (45 females) were exposed to a nauseating visual stimulus for 20 minutes. Nausea was continuously rated on a numeric rating scale (a '10'-point NRS, with '0' indicating no nausea and '10' maximal tolerable nausea). Day 1 served as a control condition. Placebo intervention occurred during day 2. Subjects were split into two groups: the placebo group received sham acupuncture (coupled with expectancy manipulation of nausea improvement), whereas subjects in the control group did not receive any treatment. Thirty-two channels of EEG were recorded during baseline and nausea exposure on both days as well as during the placebo intervention on day 2. Fast Fourier transformation (FFT) at central electrodes C3 and C4 and exact low resolution electromagnetic tomography (eLORETA) were used to examine EEG correlates of the placebo effect in nausea.

Results. On the first day, both sexes showed severe nausea (NRS $\geq$ 5) in response to the nauseating stimulus. The nausea response was associated with elevated delta power (0.5-4 Hz) in the FFT spectrum. Additionally, source-level analyses revealed nausea related changes in the limbic, parietal, occipital, and frontal lobe, as well as in the insula. On day 2, both sexes showed reduced expectation of nausea following expectancy manipulation and sham acupuncture point stimulation on the NRS, indexed by changes in frontal lobe and anterior cingulate activity. In line with the main hypothesis in this study, both sexes showed a placebo effect on nausea, indexed by reduced nausea on the NRS. Among females, the placebo effect was accompanied by reduced delta power in the FFT spectrum, decreased placebo related activation in parietal, frontal, and temporal lobe as well as reduced stress levels. In contrast, the placebo effect on

nausea in males displayed increased activation in the limbic, sub-lobar, and occipital lobe.

Conclusion. Results emphasize the effectiveness of sham acupuncture in nausea and allow first insights into underlying central mechanisms in females and males. In particular, the positive expectation of nausea improvement following expectancy manipulation and sham acupuncture seems to be similarly modulated in females and males, specifically via frontal structures. On the other hand, the placebo effect on nausea alone may be differentially modified in females and males in the central nervous system.

Keywords: placebo effect, nausea, stress, motivation, electroencephalography

8. Zusammenfassung

Hintergrund. Bisher konnten zahlreiche Studien Placeboeffekte im Bereich Übelkeit nachweisen, zugrundeliegende neurokognitive Mechanismen sind jedoch weitgehend unklar. Im Rahmen dieses Projektes wurden verhaltensbezogene und elektroenzephalografische (EEG) Korrelate der Placeboantwort in einem Übelkeitsparadigma untersucht.

Methodik. An zwei aufeinanderfolgenden Tagen (Tag 1 und Tag 2) wurde Übelkeit in 90 gesunde Probanden (45 Frauen) über 20 Minuten lang mithilfe eines visuellen Stimulus erzeugt. Tag 1 diente als Kontrolltag. An Tag 2 wurden die Probanden in die Kontrollgruppe beziehungsweise in die Placebo Gruppe eingeteilt. Die Placebo Gruppe erhielt Scheinakupunktur in Kombination mit einer positiven Erwartungsmanipulation. In der Kontrollgruppe wurde keine Intervention angewandt. Verhaltensbezogene Übelkeit wurde auf einer Numerischen Rating Skala (‚10'-Punkte NRS, ‚0' entsprach keiner Übelkeit und ‚10' maximal tolerierbarer Übelkeit) und EEG mit 32 Elektroden kontinuierlich an beiden Tagen gemessen. Fast-Fourier Transformation (FFT) in den Elektroden C3 und C4 und exact Low Resolution Electromagnetic Tomography (eLORETA) wurden verwendet, um neurokognitiven Korrelate der Placeboantwort auf Übelkeit aufzuzeigen.

Ergebnisse. An Tag 1 zeigten beide Geschlechter signifikante Übelkeit (NRS $\geq$ 5) als Reaktion auf den visuellen Übelkeitsstimulus. Behaviorale Übelkeit wurde begleitet von erhöhter Delta Aktivität (0.5-4 Hz) im FFT Spektrum sowie veränderter Aktivität in limbischen, parietalen, okzipitalen und frontalen Hirnregionen und der Inselrinde. An Tag 2 zeigten beide Geschlechter in der Placebo Gruppe eine Reduzierung der erwarteten Übelkeit auf der NRS und Veränderungen im frontalen und anterioren cingulären Cortex im Vergleich zur Kontrollgruppe. Ebenso zeigten beide Geschlechter in der Placebogruppe eine signifikante Reduzierung der Übelkeit im Anschluss an die Placebo Intervention im Vergleich zur Kontrollgruppe. Bei Frauen

war die Placeboantwort begleitet von einer reduzierten Delta Aktivität im FFT Spektrum, reduzierter Aktivität in parietalen, frontalen und temporalen Hirnregionen sowie reduziertem subjektiven Stress. Im Gegensatz dazu ging die Placeboantwort auf Übelkeit bei Männern mit erhöhter Aktivität in limbischen und okzipitalen Hirnregionen einher.

Schlussfolgerung. Die vorliegenden Ergebnisse zeigen, dass Scheinakupunktur in Kombination mit einer positiven Erwartungsmanipulation erfolgreich eine Placeboantwort im Bereich Übelkeit bei beiden Geschlechtern induzieren konnte und erlauben erste Einblicke in die zugrundeliegenden neurobiologischen Korrelate. Speziell die positive Erwartungsmanipulation ging gleichermaßen für Männer und Frauen mit neurobiologischen Veränderungen im frontalen und anterioren cingulären Kortex einher. Im Gegensatz dazu, vielen die neurobiologischen Korrelate der Placeboantwort auf Übelkeit für Männer und Frauen unterschiedlich aus.

Schlüsselwörter: Placebo-Effekt, Übelkeit, Stress, Motivation, Electroenzephalografie

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11. Appendix

I. List of Tables

II. List of Figures

III. List of Abbreviations

ACC	Anterior cingula cortex
ANOVA	Analysis of variance
ANS	Autonomic nervous system
Avg	Average
BA	Brodmann area
BAS	Behavioral approach system scale
bc	baseline corrected
BIS	Behavioral inhibition approach system scale
CNS	Central nervous system
cun	Chinese unit of length, 1 cun = 3,333 cm
ECK	Electrocardiography
EEG	Electroencephalography
EGG	Electrogastrogram
eLORETA	exact Low Resolution Electromagnetic Tomography
EOG	Electrooculography
FFT	Fast Fourier transformation
GVS	Galvanic vestibular stimulation
HADS-A	Hospital anxiety scale
HADS-D	Hospital depression scale
HPA axis	Hypothalamic-pituitary-adrenal axis
Hz	hertz
ICA	Independent component analysis
KHz	Kilohertz
ln	logarithmized
mA	milliampere
MNI coords	Montreal Neurological Institute coordinates
MSSQ	Motion sickness susceptibility questionnaire
mV	millivolt
NRS	Numeric rating scale
PC 6	Pericardium 6
PFC	Prefrontal cortex
PNS	Parasympathetic nervous system
SD	Standard Deviation
SLOR	Standardized low resolution
SNS	Sympathetic nervous system
STAI	State-trait anxiety inventory
t	time
TENS	Transcutaneous electrical nerve stimulation
TSST	Trier social stress test

IV. Eidesstattliche Versicherung

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation mit dem Thema:

„Neurophysiological Correlates of the Placebo Effect in Nausea“

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