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Psychological and genetic predictor variables of nocebo responses after cyclosporine A and placebo intake

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Contents

1 Introduction

1.1 Psychoneuroimmunology

Psychoneuroimmunology (PNI) is the investigation of the interaction between neuroendocrine and neural functions, behavior, and immune processes, which has been established as an interdisciplinary field in the late 1970s. Over the past 30 years it has become evident that these systems influence each other reciprocally (Ader, 2000). Neuroendocrine mediators released by the pituitary gland (Kelley et al., 1985) as well as the sympathetic division of the autonomic nervous system affect immune cell activity. Contrarily, products of the immune system such as hormones and cytokines, influence both neuroendocrine and neural activities. Evidence for this has been provided as early as 1975, where plasma concentration of glucocorticoids rose during a specific immune response, indicating that components of the immune system are able to influence the nervous- and hormonal system (Besedovsky et al., 1975). In addition, PNI research also elucidated the effects of behavioral processes on the neuroendocrine and immune system and vice versa. Stressful life events (Glaser and Kiecolt-Glaser, 2005) have been shown to affect cellular immune und humoral responses, as well as disease development, while immune responses demonstrated an influence on behavior and affective state (Maier and Watkins, 1998; Miller et al., 2009) (Figure 1).

This multi-directional communication is possible because cells of the immune system express receptors for hormones and neurotransmitters (Blalock and Smith, 2007) and hormones in turn can influence the regulation of an immune response and affect processes of the central nervous system (CNS) (Yirmiya and Goshen, 2011). Furthermore, receptors for cytokines and prostaglandins have been found on glial and neuron cells (Blalock and Smith, 2007) and the CNS can influence immunity through the release of cortisol from the hypothalamus-pituitary-adrenal axis (HPA-axis), which functions as a humoral pathway of the CNS (Viveros-Paredes et al., 2006) (Figure 1).

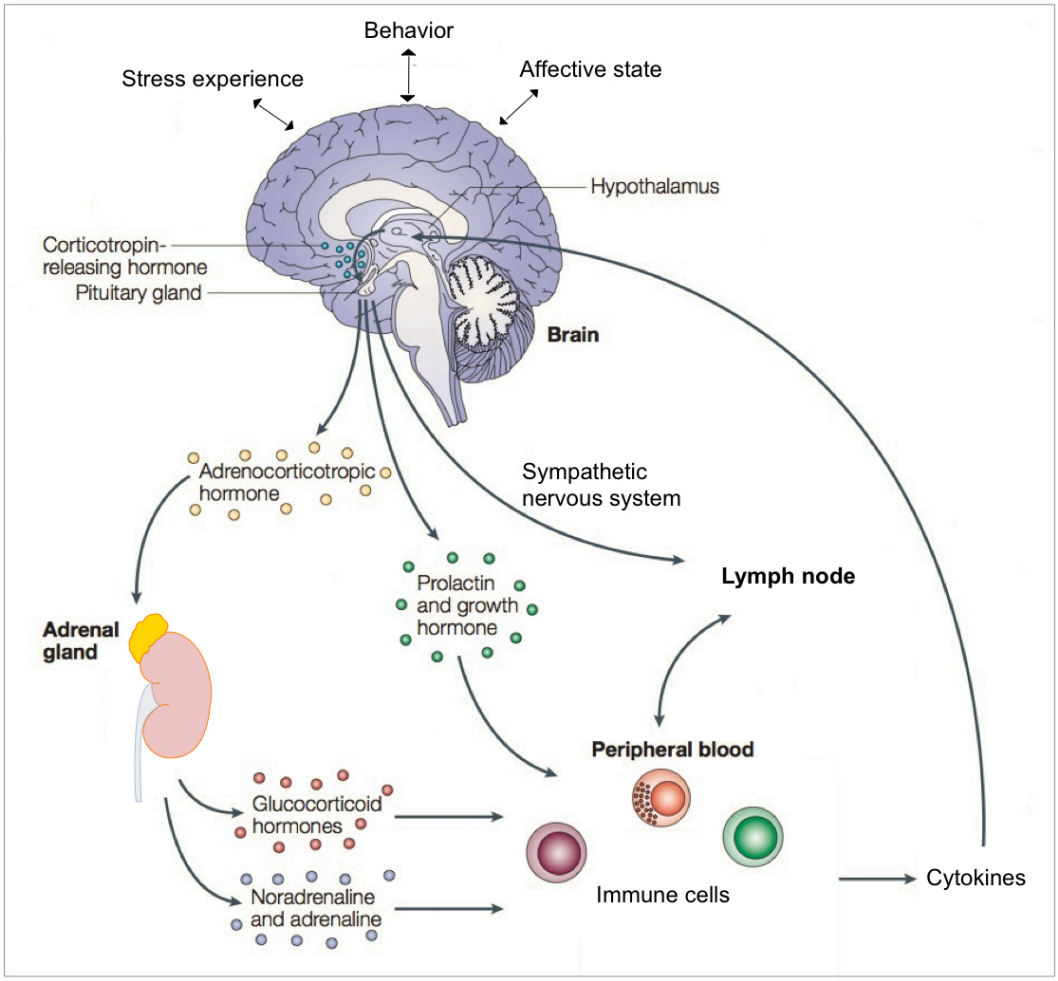

Fig. 1: Bidirectional communication between the CNS, the neuroendocrine, the peripheral immune system and behavioral processes

Neuroendocrine agents and autonomic processes of the nervous system can influence immune activity, whereas components of the immune system are able to influence the nervous- and hormonal system. Stress experience can affect cellular immune und humoral responses and immune responses in turn can influence behavior and affective state. The CNS and the immune system are connected through afferent and efferent pathways. Sensory fibers of the vagus nerve serve as an afferent and efferent neural route of communication. In addition, messengers such as cytokines operate on the humoral afferent route and can reach the brain through circumventricluar organs. Another major efferent route comprises the sympathetic nervous system, which is directly connected with primary and secondary lymphoid organs. In addition, the HPA-axis also functions as a connection pathway between these systems. Modified from (Glaser and Kiecolt-Glaser, 2005).

Unambiguous evidence of the functional interactions between the CNS, behavior, the neuroendocrine and immune systems is derived from immune conditioning (Schedlowski and Pacheco-López, 2010). Here, the mechanisms of classical conditioning of immune functions offer an elegant way to unravel the communication network between these systems.

1.1.1 Classical conditioning of immune functions

Classical conditioning can be defined as learning between causal and temporal relations of internal and external stimuli. This enables the organism to access and choose the appropriate response set before significant biological events occur. Ivan Pavlov initially described this paradigm, eventually leading to studies of learned placebo responses, in which immunobiological processes are modified through behavioral conditioning (Pavlov, 1927). In short, during a behavioral conditioning paradigm, there are two distinct processes involved, the first of which is a learning phase (acquisition). Here, a novel stimulus, such as an inert pill (placebo), an odor, or a flavor (conditioned stimulus, CS) is given paired with a substance, which induces physiological changes (unconditioned stimulus, US). Following repeated pairings, is a memory phase (evocation), in which the CS is represented alone in order to elicit a behavioral and/or a physiological response (conditioned response, CR), which mimics the effect of the US. As a result, the characteristics of a biologically important stimulus (US) are transmitted to another stimulus without these characteristics (CS).

Based on the pioneering studies of classical conditioning of the immune system (Ader and Cohen, 1993), a conditioned taste aversion (CTA) paradigm within rats has been developed. Here, saccharin, a novel tasting stimulus is utilized as a CS while the potent immunosuppressive drug, cyclosporine A (CsA) acts as an US during the acquisition phase (Exton et al., 2002; Wirth et al., 2011). CsA is widely used in organ transplantation to prevent rejection and within treatment of chronic inflammatory autoimmune diseases (Kapturczak et al., 2004), when a suppression of immune functions, particularly T cell activity, is needed (see section 2.2). Subsequently, during the evocation phase, the CS is represented alone and induces behavioral (taste aversion) and immunological changes, which mimic the effects of CsA (US). Further animal studies provided impressive evidence of successful implementation of this conditioning paradigm. In the context of supportive therapies within organ transplantation and chronic inflammatory autoimmune diseases, both mortality rates and disease symptoms were reduced (Schedlowski and Pacheco-López, 2010). Eventually, this taste-immune conditioning paradigm was established within humans and has been shown to induce a learned immunosuppression (Goebel et al., 2002). Here, a novel tasting drink (CS) is repeatedly paired

with CsA (US) during the acquisition phase. When the CS is represented alone during the evocation phase, it elicits a significant suppression of T cell activity, thereby resembling the effects of CsA (Goebel et al., 2002).

Conditioning procedures with pharmacological agents can create memory traces of the learned responses and consequently be recalled in the future (Amanzio and Benedetti, 1999), which offers the possibility of implementing these paradigms within a clinical context as a supportive therapy. Studies within animals and humans have shown that the higher the number of learning trials during the acquisition phase, the stronger the placebo response during evocation (Albring et al., 2012; Niemi et al., 2007). The learned immunosuppression described above can also be elicited after a second sole presentation of the CS (Wirth et al., 2011), where psychological and biological variables, such as state anxiety and plasma noradrenaline concentrations, predicted the learned immunosuppression before the CS re-exposition during evocation (Ober et al., 2012). Furthermore, just like any other learning process, the learned immunosuppression is subject to extinction over time, where the learned behavior continuously recedes. In order to integrate learned immune responses as a supportive therapy within a clinical context, it is essential that this receding process is inhibited. This has already been successfully done within experimental animals (Exton et al., 1999) and recently, the extinction process was counteracted in a study with healthy males, which received a subtherapeutic dose of CsA paired with the CS during the evocation phase (Albring et al., 2014).

Besides the potential therapeutic relevance, the paradigm of behavioral conditioned immunosuppression can also be utilized to elucidate afferent, efferent and central mechanisms steering the learned immune response.

1.1.2 Mechanisms and pathways of the behavioral conditioned immune response

During acquisition, the brain must be able to detect and associate the sensory characteristics of the CS, as well as the alterations within the immune system induced through the US. Additionally, the immune system needs to sense these alterations and be able to communicate its peripheral response through a shared

chemical language to the nervous system (Blalock and Smith, 2007). This is only possible by means of a link between the brain and immune system via afferent and efferent pathways.

To date, the afferent mechanisms through which the brain detects immunosuppressants, such as CsA, are poorly understood, although first evidence indicates that CsA may exert its influences on the brain through a direct mechanism (Pacheco-López et al., 2012). The afferent immune-to-brain signaling comprises humoral and neuronal pathways. The systemic or humoral pathway, through which messengers such as cytokines and prostaglandins reach the brain via cerebral vasculature or circumventricluar organs (reviewed in Quan and Banks, 2007) and the neuronal or hardwired signaling pathway, which transforms peripheral immunological changes directly into neurological activity. This has been demonstrated through alterations in EEG-activity, increased noradrenaline levels, cytokine messenger ribonucleic acid (mRNA) expression and through induction of the neuronal activity marker c-fos (Engler et al., 2011). The vagus nerve serves as a neural route of communication between the immune system and the CNS (Goehler et al., 2000) while the effects of immunomodulators, which can induce autonomic reflexes and behavioral responses can be inhibited or even blunted through vagotomy (Fleshner et al., 1998). However, within the paradigm of behavioral conditioning of immune responses, utilizing CsA as an US, the vagus nerve did not seem to be involved within the afferent pathway, as its deafferentation did not prevent the conditioning process (Pacheco-López and Bermúdez-Rattoni, 2011).

One major efferent neural pathway linking the brain with the immune system is the sympathetic nervous system (Nance and Sanders, 2007). It is directly connected with primary and secondary lymphoid organs (Bermúdez-Rattoni, 2004) through sympathetic nerve fibers that have been shown to regulate the immune system through the neurotransmitter noradrenaline (Sanders and Straub, 2002). Within the CTA paradigm, where CsA is utilized as an US, it was repeatedly demonstrated that conditioned suppression of T_H1 cytokine production and lymphocyte proliferation was mediated through the splenic nerve, noradrenaline and adrenoceptor-dependent mechanisms (Exton et al., 2002). Nonetheless, the splenic nerve appears to be one of many efferent neural routes which are mobilized during learned immunosuppression (Exton et al., 2000). Recently, utilizing the CsA-saccharine

conditioning paradigm within rodents, the enzymatic activity of calcineurin (CaN) has been reduced through the immunosuppressive conditioning procedure via a β-receptor dependent mechanism (Pacheco-López et al., 2009). This finding is noteworthy, as the CR appears to transform the same sub-cellular components of CaN as the pharmacologic effects of CsA, but through a different route.

A humoral efferent pathway, comprising the HPA-axis and elevated glucocorticoid levels has also been proposed as being responsible for behaviorally conditioned immunosuppression. This appears to be unlikely, as studies reported no difference in corticosterone (cortisol in humans, respectively) levels in either animal or humans in both control and experimental groups (Niemi et al., 2007; Wirth et al., 2011). In conclusion, studying behaviorally conditioned immunosuppression paradigms is an elegant way to elucidate neuro-immune communication by only employing a single model and current research is dedicated to unravel the underlying mechanisms and signaling pathways, as well as utilizing these findings for therapeutical purposes. Here, placebo and nocebo effects have been shown to affect behavioral processes, the neurochemistry of the CNS and subsequently the periphery of the body and medical treatment outcomes.

1.2 The placebo and nocebo effect

Placebo effects are positive physiological responses following sham treatments, which cannot only be elicited through drugs, containing inactive substances, but also through a wide range of interventions (Pacheco-López et al., 2006). The term placebo originates from the 13th century and used to be considered a fraud. It was not until the middle of the 20th century that the relevance of the placebo was recognized as a positive influence in the treatment of various diseases (Beecher, 1955). However, to this date the definition of the placebo effect remains controversial and from an evolutionary perspective it remains unclear, why placebo effects have developed at all (Sullivan et al., 2010).

The concept of the nocebo effect evolved, because negative side effects were observed in the context of placebo therapies (Enck et al., 2008). Placebo groups of randomized studies reported side effects, which were according to the known side effects of the drug (Colagiuri et al., 2012; Rief et al., 2009) and which may as a

consequence lead to high drop out rates (Mitsikostas et al., 2011). These symptom developments do not only occur after the intake of inert substances paired with negative expectations, or within simulated medical interventions, but as with the placebo effect, also in the context of any treatment when subjects or patients expect to experience adverse reactions (Bingel et al., 2011). This in turn can have serious effects, as treatment improvements may be abolished and adverse symptoms can develop, interfering with drug and treatment effects (Enck et al., 2008). For this reason, in contrast to the meaning of placebo (“I shall please”), the definition of the nocebo as meaning “I shall harm” was developed (Hahn, 1997). In comparison to the placebo effect, the nocebo effect has been investigated to a much lower degree. Although, lately the nocebo effect gained more scientific attention and its influence within the clinical context is increasingly considered.

1.3 Mechanisms mediating the placebo and nocebo effect

Today, the mechanisms and effects of the placebo and nocebo phenomenon have been increasingly investigated but many questions still prevail. However, research over the last years revealed that two mechanisms steer the placebo and nocebo response: Associative learning processes such as Pavlovian or behavioral conditioning and expectation (Enck et al., 2008). Depending on the physiological response system, one or both of these mechanisms have been shown to steer the placebo response. Behavioral conditioning of placebo effects has been demonstrated for the immune and neuroendocrine system (reviewed in Wendt et al., 2014), which were modulated only through behavioral conditioning (Benedetti et al., 2003; Goebel et al., 2002) and not by induced expectations (Albring et al., 2012; Benedetti et al., 2003). Both associative learning and expectation mechanisms have been shown to steer placebo effects within numerous physiological systems, such as the respiratory, cardiovascular and gastrointestinal system, as well as diseases, amongst others depression, multiple sclerosis and Parkinson’s disease (reviewed in Enck et al., 2013). Their affects on the nocebo response have only been identified in the context of pain and experienced side effects. This is due to the ethical agreement of nonmaleficence considering the infliction of pain, anxiety and stressful experiences on individuals during clinical or experimental study setup in order to gain more insight about its psychological and physical effects.

1.3.1 Associative learning processes steering the nocebo effect

Associative learning processes such as Pavlovian or behavioral conditioning have given the most insight into the origin of placebo and nocebo effects. In general, stimuli that have been associated with an adverse reaction or symptom development in the past are able to induce unwanted adverse reactions upon re-exposure (Figure 2). This has been documented with anticipated nausea in cancer patients during chemotherapy (Bovbjerg et al., 1992). Cytotoxic chemotherapy agents (US) commonly cause adverse side effects such as nausea and vomiting. During drug infusion, neutral stimuli such as the hospital atmosphere, clinical personnel or distinct odors are present which can, when encountered alone in the future, elicit vomiting and nausea (CR) (Bovbjerg et al., 1992). Visual cues alone can recall this learning process as allergy patients who were presented a picture of a hayfield showed allergic symptoms (reviewed in Ader and Cohen, 1993) and the presentation of a sealed glass vessel filled with dust caused an asthmatic attack within asthma patients (Dekker and Groen, 1956).

However, the mechanisms of associative learning and expectation cannot be viewed dichotomously, as they are interrelated and influence each other. Through prior experience, an individual can learn to associate the procedure of taking medication with the development of side effects, which can be viewed as behavioral conditioning, but this also influences the expectation of experiencing symptoms in future drug intakes. Furthermore, expectations induced through verbal suggestions can also function as a CS as they may restore earlier acquired stimuli associations.

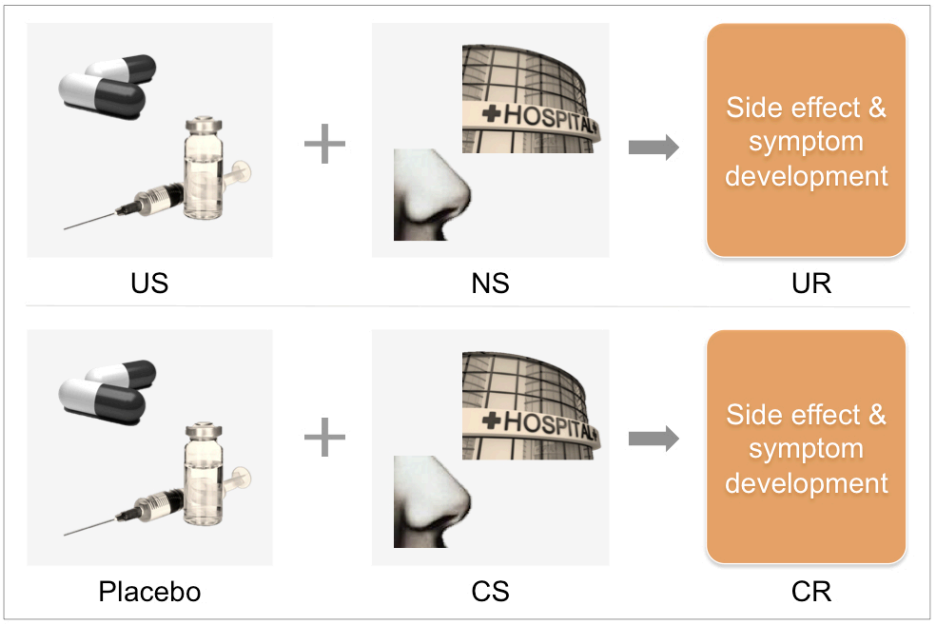

Fig. 2: Associative learning processes and their influence on the nocebo response

An associative learning process, such as classical conditioning, is an important mechanism steering the nocebo response. Previous encounters with a drug or treatment (US), which induce adverse responses (UR), such as an increase of side effects or symptom development are accompanied with neutral visual or olfactory stimuli (NS). Later on when a placebo or sham treatment occurs in the presence of the same formerly neutral visual and olfactory stimuli (CS), these may induce similar adverse effects (CR). US= unconditioned stimulus, NS= neutral stimulus, UR= unconditioned response, CS= conditioned stimulus, CR= conditioned response

1.3.2 Expectation processes steering the nocebo effect

The expectation of either symptom improvement (placebo) or experienced side effects or pain (nocebo), play an essential role when patients or study participants receive a treatment. Interestingly, as outlined in section 1.4, these expectations of experiencing side effects or pain activate similar brain areas as the actual experience of side effects or pain themselves, which may in turn intensify these perceptions (Bingel et al., 2011; Koyama et al., 2005).

Expectations are typically induced through verbal suggestions and social observational learning. Verbal suggestions of harm within experimental pain studies, overruled the effects of previous conditioning with a pain reliever (Benedetti et al., 2003) and abolished the effect of a potent opioid (Bingel et al., 2011). In another experiment, subjects were told about the side effects of the immunosuppressive drug CsA, which they expected to take. They reported the experience of four typical side effects of CsA, although they received placebos throughout the entire study (Wendt et al., forthcoming).

Within the medical treatment context, verbal suggestions have been shown to induce pain and anxiety. The experienced pain during local anesthetic injections was higher in patients who were told to expect a slight discomfort (nocebo information) than in those who were told to expect to feel comfortable throughout the procedure (placebo information) (Varelmann et al., 2010). A further example is a group of patients, who were warned about undesirable experiences of noxious stimuli during interventional radiological procedures and reported higher levels of pain and anxiety than those without negative information (Lang et al., 2005).

Observational learning of other individuals, who show adverse symptoms after a placebo treatment, can increase pain sensitivity and induce symptom development as well as the experience of side effects within the observer (Mazzoni et al., 2010; Vögtle et al., 2013). This may also induce associative learning mechanisms, as the observation of negative events experienced by the demonstrator could operate as an US. In addition, further aspects influence the nocebo effect through implicit and explicit mechanisms, which can induce associative learning and expectations (Figure 3).

1.3.3 Further factors steering the mechanisms of the nocebo effect

The mechanisms of the nocebo response, associative learning and expectations, are further influenced through factors such as information sources, the doctor-patient relationship, the personal medical history as well as psychological and biological factors.

Information sources can induce strong nocebo effects through negatively worded informed consents and patient information leaflets (Wendt et al., forthcoming) while increased information given about potential side effects can lead to their subsequent rise (Wise et al., 2009). Media information about the amount of side effects induced through antidepressants has influenced nocebo responses within placebo groups taking two different antidepressants. The placebo group of the tricyclic antidepressant (TCA) trial reported more side effects than the placebo group of the selective serotonin reuptake inhibitors (SSRIs) trial. SSRIs were introduced in the late 1980s and are known to cause fewer side effects than TCAs (Rief et al., 2009). Today, it has been speculated that the increase in lactose intolerance

(Vernia et al., 2010) and in side effects of oral contraceptives (Grimes and Schulz, 2011) may be due to negative information sources.

Moreover, a negative doctor-patient relationship influences the occurrence and strength of the nocebo response (Colloca and Finniss, 2012) and the personal medical history can also influence present treatment outcomes. The development of symptoms after treatments in the past are strong predictors for developing adverse symptoms in the present (Petrie et al., 2005) and within the treatment context, visual and olfactory cues of a former treatment may induce nocebo responses upon re-exposure (Bovbjerg et al., 1992). Lastly, biological and psychological variables have shown to influence nocebo responses. Females have reported significantly more nocebo effects within various experiments (Lorber et al., 2007; Ströhle, 2000). However, these effects may be partly due to the fact that males report less pain to female experimenters (Flaten et al., 2006). Psychological factors such as anticipatory anxiety (Elsenbruch et al., 2012), beliefs about medicines (Nestoriuc et al., 2010) and somatosensory amplification (Davis et al., 1995) have been associated with nocebo responses as well (Figure 3).

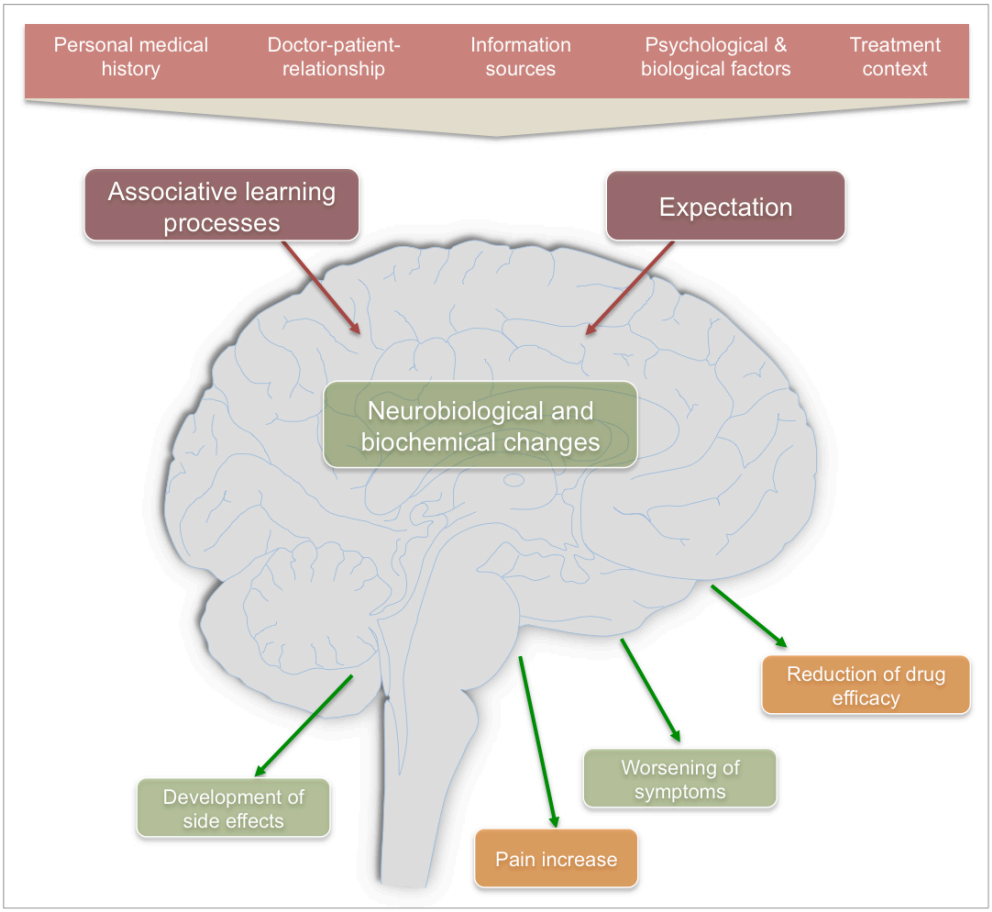

Fig. 3: Mechanisms of the nocebo response and their adverse effects

The nocebo effect is based on two different mechanisms. It can be induced through associative learning processes, such as classical conditioning and expectation, which can be induced through verbal suggestions or observational learning. Current research shows that these mechanisms can induce neurobiological and biochemical changes, which in turn may cause the development of side effects, reduction of drug efficacy, pain increase and worsening of symptoms. Further factors have been shown to steer and modulate associative learning processes and expectation, such as the personal medical history, the doctor-patient relationship, information sources, psychological and biological factors, as well as the general treatment context.

In conclusion, the mechanisms of nocebo and placebo responses have been shown to influence various treatment outcomes, in both negative and beneficial ways. These mechanisms need to be investigated in more detail. Insights into their effects have been provided by neuroimaging and pharmacological investigations, where they have been shown to produce neurobiological changes, which in turn interact with biochemical signaling paths of pharmacological drugs.

1.4 Neurobiological and biochemical actions of the placebo and nocebo effect

Current research revealed that placebo and nocebo effects cannot be explained by a single model utilizing neurobiological or biochemical processes. Depending

on the physical system studied or the experimental setup, different mechanisms take effect. In opposition to the placebo effect, which occurs in a positive psychosocial environment, the nocebo phenomenon is mainly investigated during pain experiments and therefore embedded in a negative context. As a consequence, the acquired neurobiological and biochemical measurements reflect those negative effects on the physical and psychological state of an individual.

1.4.1 Neurobiological actions of the placebo and nocebo effect

Neuroimaging methods have provided fascinating insights of neurobiological processes during the investigation of expectancy induced nocebo effects (Bingel et al., 2011; Kong et al., 2008; Koyama et al., 2005). The nocebo effect seems to be based on a cognitive and affective pain path, as a magnetic resonance tomography (MRT) study revealed activity within neurobiological structures involved in these pathways during a nocebo response (Kong et al., 2008).

Opposing effects of placebo and nocebo responses and their neurobiological correlates have been demonstrated in an elegant study in which the opioid analgesic remifentanil was administered (Bingel et al., 2011). Participants received painful thermal stimuli, which they had to rate on a pain intensity scale from 0 to 100. Subsequently, remifentanil was administered intravenously without the subjects' knowledge (hidden application) and the pain intensity decreased from an average of 65 to 55. However, when they were informed about the pain reducing properties of remifentanil, pain sensation decreased to 39, although the intravenous concentration remained unchanged. In contrast, when the nocebo information was given that no more analgesic would be given and the sensation could get worse, pain ratings rose to 64, corresponding to the initial value, where no opiods were given. Here, the anticipatory anxiety of experiencing pain overruled the effects of the opioid analgesic. Functional magnetic resonance imaging (fMRI) revealed that during placebo and nocebo conditions endogenous pain modulatory control systems were engaged, but in antagonistic ways. When subjects expected an analgesic effect during the placebo condition, the dorsolateral prefrontal cortex, the rostral anterior cinculate cortex and the periaquedutal grey were active, which are involved in inhibiting pain and which enhanced the effects of the drug. Whereas dur-

ing the nocebo condition, increased activity was measured in the hippocampus (Bingel et al., 2011).

Further opposing effects have been shown in an fMRI study where the expectancy of increased pain (nocebo) was associated with increased activity in the insular cortex (Ic), prefrontal cortex, anterior cingulate cortex (ACC), thalamus and further brain areas. Whereas, when the expectation of decreased pain was induced, the activity of pain-related brain areas (e.g. ACC, amygdala (Am) and primary somatosensory cortex) was reduced along with the subjective perception of pain (Koyama et al., 2005). In summary, negative expectations may not only shape neural processes of painful stimuli, but may also enhance and alter the unpleasantness of painful stimuli on a psychophysical level.

1.4.2 Biochemical actions of the placebo and nocebo effect

Changes of brain activity during the nocebo effect are associated with neurochemical alterations and pharmacological investigations have increased the understanding of biochemical actions of the nocebo effect (Benedetti et al., 2006). Here, similar to neuroimaging pain studies, negative and positive expectations are induced within study participants regarding the intensity of pain experience. During the administration of saline solution (placebo), the activation of the endogenous opioid and dopaminergic neurotransmitter systems were measured through positron emission tomography (PET). The placebo effect induced an analgesic effect along with the activity of opioid neurotransmission in the ACC, Am, nucleus accumbens, Ic, oribitofrontal cortex and periaqueductal grey matter. The nocebo response caused a hyperalgesic effect and was associated with a deactivation of opioid release. In addition, the placebo response was reflected in dopaminergic activity in the ventral basal ganglia and the nucleus accumbens, whereas the nocebo response decreased the dopaminergic activity in those areas (Benedetti et al., 2006). Therefore, nocebo and placebo responses seem to induce opposing effects in brain circuits associated with reward and motivational behavior.

Further evidence for these opposing effects has been demonstrated in a model investigating the antagonistic action of cholecystokinin (CCK) on opiodergic systems (Benedetti et al., 2007). The hormone CCK is involved in the development of

anxiety and panic and appearing to play a modulating role during pain within nocebo and placebo responses. A verbally induced expectation of declining pain (placebo) resulted in decreased pain and mobilized the endogenous μ-opioid neurotransmission, but this process can be blocked by CCK. Its antagonist proglumide binds to CCK-A and CCK-B receptors, which has been shown to inhibit a hyperalgesic nocebo effect and an enhancement of the placebo effect (Benedetti et al., 2007).

Furthermore, anxiety seems to play an important part during the nocebo effect (Benedetti et al., 2006). This has been shown in an experiment, where the intake of a placebo pill was combined with the induction of experiencing pain hyperalgesia. Participants reported an intensification of pain, paralleled by increased blood concentrations of the hormones adrenocorticotropin and cortisol, indicating a stress-induced activation of the HPA-axis. Further test groups additionally received either the benzodiazepine diazepam, which reduces tension and anxiety; or proglumide before the experimental procedure. The hyperalgesic nocebo effect was reduced through both diazepam and proglumide with the difference being that diazepam also reduced the stress-induced activation of the HPA-axis. Proglumide did not seem to reduce anxiety, as the increased release of stress hormones was not blocked. In summary, there seem to be two different biochemical means of transmission during the nocebo response; the pain enhancing effect of CCK and the activation of the HPA-axis respectively (Figure 4).

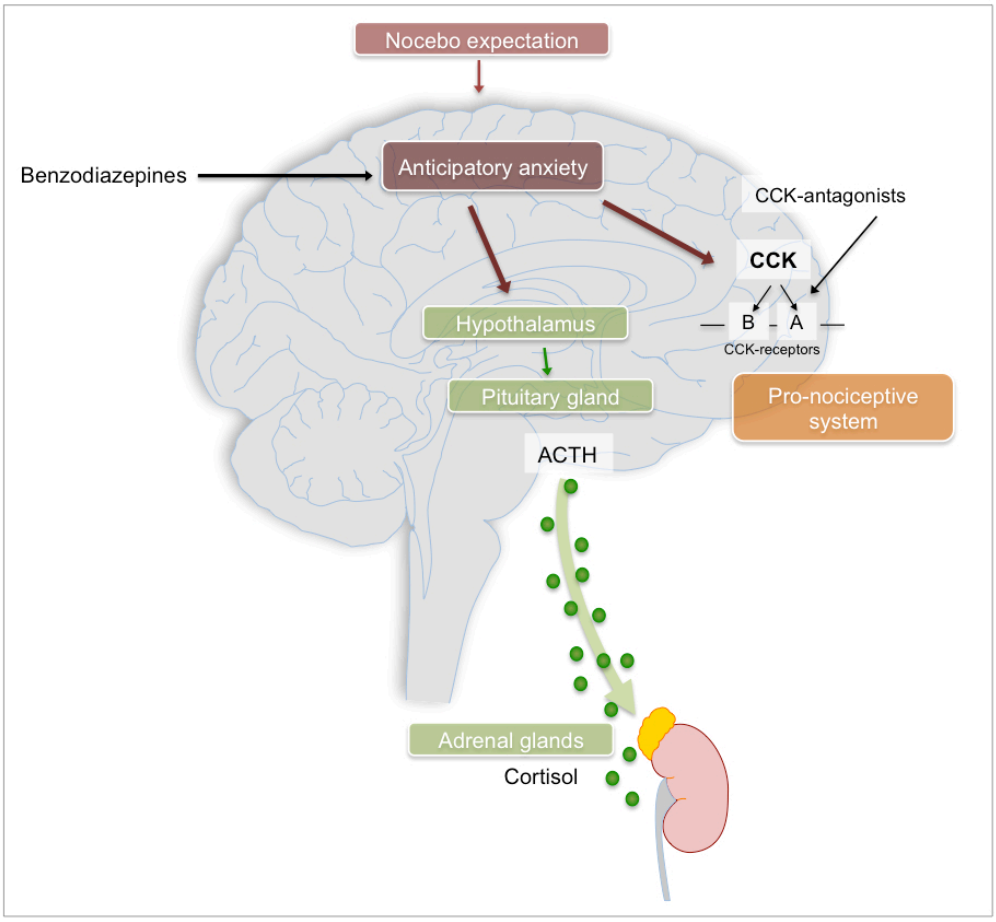

Fig. 4: Biochemical actions of the nocebo response

The expectation of receiving a painful stimulus (nocebo) leads to anticipatory anxiety, which activates two different biochemical means of transmission: the pain enhancing effect of the CCK-ergic pronociceptive system and the stress-induced activation of the HPA-axis. Only the pronociceptive system is modulated through CCK-antagonists such as proglumide, where the hyperalgesic effects of the nocebo response is blocked, while the increased release of stress hormones through activation of the HPA-axis is not affected. The intake of benzodiazepines however, causes a reduction of anxiety and blocks both biochemical paths. Note: Anatomical accuracy was subjected in order to illustrate the biochemical actions of the nocebo response. Adapted from (Enck et al., 2008).

In conclusion, neurobiological and biochemical actions behind nocebo responses are still poorly understood to this date. Current pharmacological studies however, show that the induction of a placebo and nocebo response leads to a complex involvement of various neurotransmitters such as CCK, opioids and dopamine. Furthermore, the same neurobiological and biochemical systems seem to mediate both placebo and nocebo responses.

1.5 The search for predictors of nocebo responses

Nocebo-induced side effects can result in nonadherence behavior and medication discontinuation, unnecessary additional physician visits and added drug intake to

counteract occurring side effects. Moreover, as they form confounds within the evaluation of clinical drug trials, they may contribute to an overestimation of side effects. Consequently, this leads to wasted drug intake and higher health care costs. The identification of predictors of nocebo responses can be utilized to improve the efficacy of drug effects as well as the overall physical constitution of patients. Several studies have identified psychological variables that increase and induce nocebo responses. Up to this date, there are no data available indicating whether or not and to what extent biological factors, such as gene polymorphisms contribute to the nocebo response. Although a few studies have reported stronger nocebo effects within females (Lorber et al., 2007; Ströhle, 2000), these effects may be partly due to the fact that males report less adverse effects to female experimenters (Flaten et al., 2006). Therefore, within this thesis, the psychological variables, somatosensory amplification and beliefs about medicines, as well as the gene polymorphism, catechol-O-methyltransferase (COMT) were tested considering them to be possible nocebo predictor variables.

1.5.1 Somatosensory amplification and beliefs about medicines

Various psychological factors predicting nocebo responses have been identified, such as anticipatory anxiety for the experience of visceral pain (Elsenbruch et al., 2012), worries about experiencing symptoms (Petrie et al., 2005), the trait pessimism for inducing unpleasant feelings after pill intake (Geers et al., 2005), neuroticism (Davis et al., 1995) and a higher somatosensory awareness and amplification (Barsky et al., 1999; Davis et al., 1995).

Somatosensory amplification is a disposition to identify naturally occurring somatic and visceral sensations as being very strong, unpleasant and harmful and has recently been associated with hypochondria (Barsky et al., 1990). Patients and study participants with increased sensitivity of bodily processes, reported more symptoms after placebo intake (Barsky et al., 1999); and healthy subjects with higher scores on the somatosensory amplification acale (SSAS), measuring somatosensory amplification, experienced more side effects when taking an antidepressant or a placebo (Davis et al., 1995). This may be due to the fact that many individuals who experience symptoms, examine their bodies mentally to detect physical symptom changes (Rief and Broadbent, 2007). As shown in section 1.4, expecta-

tions of developing symptoms induce similar neurobiological and biochemical pathways, as pharmacological drugs or pain stimuli do. This in turn may promote the perception of symptoms, and contributes to nocebo responses. Individuals with a medical history of somatic symptoms, have shown more likely to experience a nocebo response in the future (Papakostas et al., 2004). Therefore, individuals with higher scores in the assessment of somatosensory amplification seem to be more likely to experience more side effects.

Furthermore, nocebo effects may develop because of previous negative experiences with drugs and medical treatments, resulting in classical conditioning processes, where individuals associate side effects with such regimens (Figure 2) and shape negative expectations regarding side effect development. Acquired beliefs about medicines seem to play a pivotal role within nocebo effects and treatment efficacy, as negative beliefs about medicines have predicted increased side effect reporting during treatment with arthritis medication (Nestoriuc et al., 2010); and numerous studies have associated more negative beliefs of medicines with lower medical treatment adherence (reviewed in Nestoriuc et al., 2010).

1.5.2 Gene polymorphisms

Biological and/or genetic variables predicting nocebo responses are lacking, whereas clinical and experimental data on biological and genetic variables predicting placebo responses are rare; however, plasma noradrenaline concentration (Ober et al., 2012) and more recently, gene polymorphisms (Furmark et al., 2008; Hall et al., 2012; Leuchter et al., 2009; Peciña et al., 2014) have been identified. The major degrading enzyme of endocannabinoids FAAH has been found to induce higher placebo analgesia for the FAAH Pro129/Pro129 homozygote (Peciña et al., 2014) and within major depressive disorder, the placebo response was linked to gene polymorphisms, which modulate monoaminergic tone (Leuchter et al., 2009). A serotonin-related gene polymorphism has been linked to placebo-induced anxiety relief (Furmark et al., 2008) and genetic polymorphisms in the COMT gene ($Val^{158}Met$) predicted placebo responses in patients with irritable bowel syndrome (Hall et al., 2012).

The neurological pathway of the placebo response has been intensively investigated and points to the involvement of a dopamine-reward circuitry (Pacheco-López et al., 2006). The enzyme catechol-O-methyltransferase (COMT), degrades dopamine and plays a critical role in the regulation of prefrontal and mid-brain dopamine signaling (Yavich et al., 2007); its coding gene occurs in allelic variants. The COMT Val^{158}Met polymorphism has been investigated most extensively and has been linked to memory function, cognition, as well as emotional and pain processing (reviewed in Hall et al., 2012). In this particular functional single-nucleotide polymorphism in the COMT gene, is a base G to base A transition, which leads to a valine to methionine substitution at position 158 (Val^{158}Met) rs4680 (Lotta et al., 1995). The valine form catabolizes dopamine three to four-times more than the methionine form (Lachman et al., 1996). The less efficient catabolization in Met/Met homozygote carriers leads to a higher amount of dopamine in the brain compared to Val/Val homozygote carriers and individuals with the Val/Met variant.

This seems to influence the perception of well-being, as individuals with the Met variant have been shown to have a higher ability to experience reward compared to Val/Val homozygous carriers (Lancaster et al., 2012). Complementing these results, Met/Met homozygous carriers showed the strongest placebo response in a recent study, especially when examined by a caring practitioner, compared to individuals of the other genotypes (Hall et al., 2012).

In the experiment of this thesis, the nocebo effect was investigated after the intake of the drug CsA and placebo administration. Animal studies, utilizing CsA as an US, have shown that conditioned immunosuppressive effects are regulated by the sympathetic nervous system with noradrenaline being the main transmitter (Exton et al., 2002). Noradrenaline and adrenaline are regulated by the enzyme COMT (Molinoff and Axelrod, 1971) and plasma noradrenaline levels have been shown to predict the individual placebo response in humans within behavioral conditioned immunosuppressive effects with CsA (Ober et al., 2012). It seems therefore likely to find potential biomarkers of nocebo responses within the COMT Val^{158}Met polymorphism.

1.6 Thesis objectives and hypotheses

The aim of the present study was to identify possible psychological and biological predictors of nocebo responses after drug and placebo intake. Nocebo-induced side effects are not only of relevance for clinical trials, but also play a major role in drug discontinuation in clinical practice, thereby negatively affecting treatment efficacy as well as patient adherence and compliance (Enck et al., 2013; Rief et al., 2011). Since experimental and clinical data document a large interindividual variability in nocebo responses, one of the major challenges in this research area is to identify such predictors (Barsky et al., 1999; Davis et al., 1995; Elsenbruch et al., 2012; Geers et al., 2005) in order to minimize nocebo effects and increase medical treatment benefits for patients.

A number of psychological predictors of the nocebo response have been identified, such as anxiety, beliefs about medicines and somatosensory amplification (see section 1.5.1). However, to this date, there are no biological predictors for the nocebo response, although recently, polymorphisms in the COMT gene (Val^{158}Met) have been linked to placebo responses. As a result, this research project investigated whether the trait somatosensory amplification, beliefs about medicines and the COMT functional Val^{158}Met polymorphism could be also possible predictors of nocebo effects.

In order to assess nocebo responses after medication and placebo intake, a study program on learned immunosuppressive placebo responses (Albring et al., 2014) with 62 healthy male subjects, was employed. The unique advantage of this conditioning model is the ability to analyze intra-individual nocebo responses, measured through CsA-specific and general side effects, after the intake of CsA during the acquisition phase as well as after placebo intake during the evocation phase. Psychological, immunological, cardiovascular and neuroendocrine parameters were analyzed before and after medication or placebo intake in order to test their influence on specific and general side effect development. Drug specific and general side effects were assessed throughout the whole study and analyzed to determine if they were associated with somatosensory amplification, beliefs about medicines and the COMT functional Val^{158}Met polymorphism; therefore being possible nocebo predictors.

2 Materials and Methods

2.1 Study participants

This study consisted of 62 healthy males of Caucasian descent (age range: 18-40 years, mean age: 25.5 ± 0.5 years), recruited through public advertisement in the surrounding community. Before the first study day, all participants received a written form as well as a personal briefing about the details of the experimental setup and possible risks of cyclosporine A (CsA) intake. However, they received no information about the study hypothesis or the conditioning paradigm. Participants gave written informed consent and were informed that they were able to discontinue participation at any point in time. The study was approved by the local ethics committee for human investigations of the University Hospital Essen, and participants received 500 Euros as compensation; they were also insured through the University Hospital Essen.

Subsequently, subjects underwent an extensive physical and psychological assessment (self-reported questionnaires, general anamnesis and medical history). An electrocardiogram and ultrasonography of the kidneys were performed and evaluated by the physicians of the Department of Nephrology. Subjects were excluded if one of the following criteria was identified: daily intake of medication, blood donations >200 ml within the last two months, intolerance for substances (e.g. lactose) used in the study, previous participation in pharmacological studies or other medical exclusion criteria (e.g. disorders of immune or neuroendocrine system, previous or persistent psychiatric disorders, addiction, allergies, signs of cardiovascular, hematologic or nephrologic disorders, respiratory problems or diabetes mellitus).

2.2 Cyclosporine A

In this thesis, the drug CsA was utilized for assessing the nocebo effect, in the form of reported side effects after medication and placebo intake. CsA is a potent immunosuppressant widely used in organ transplantation to prevent rejection, as well as in cases of psoriasis, atopic dermatitis and occasionally in rheumatoid arthritis (Kapturczak et al., 2004). CsA inhibits the activity of the Ca^{2+}/calmodulin-

dependent-activated serine / threonine phosphatase calcineurin (CaN), which operates as a phosphatase, amongst other cells, within immunocompetent T-cells, where it promotes the transcription of interleukin (IL)-2, interferon (IFN)-γ and related genes through dephosphorylating the transcription factor nuclear factor of activated T-cells (NF-AT). When Ca^{2+} enters the cell, the displacement of an autoinhibitory domain by the Ca^{2+}-calmodulin complex activates the catalytic subunit of CaN (Steinbach et al., 2007). A DNA-binding complex is formed through hypophosphorylated NF-AT entering the nucleus, which leads to enhanced IL-2 and IFN-γ production.

Therefore, CaN modulates a key transcription factor involved in immune feedback and is therewith directly responsible for the activation of T cells. CsA is a calcineurin inhibitor, which employs its cellular effects by binding to a family of proteins called immunophilins and targets CaN. The immunophilin cyclophilin A predominantly binds CsA, which in turn enhances the immunophilin's affinity to CaN. (reviewed in Kapturczak et al., 2004). An inhibitory complex with CaN is subsequently formed, leading to the inhibition of CaN activity. Conclusively, CsA prevents the dephosphorylation of NF-AT through calcineurin and as a result its immunosuppressive effect is manifested through the inhibition of the IL-2 and IFN-γ production (Batiuk and Halloran, 1997) (Figure 5).

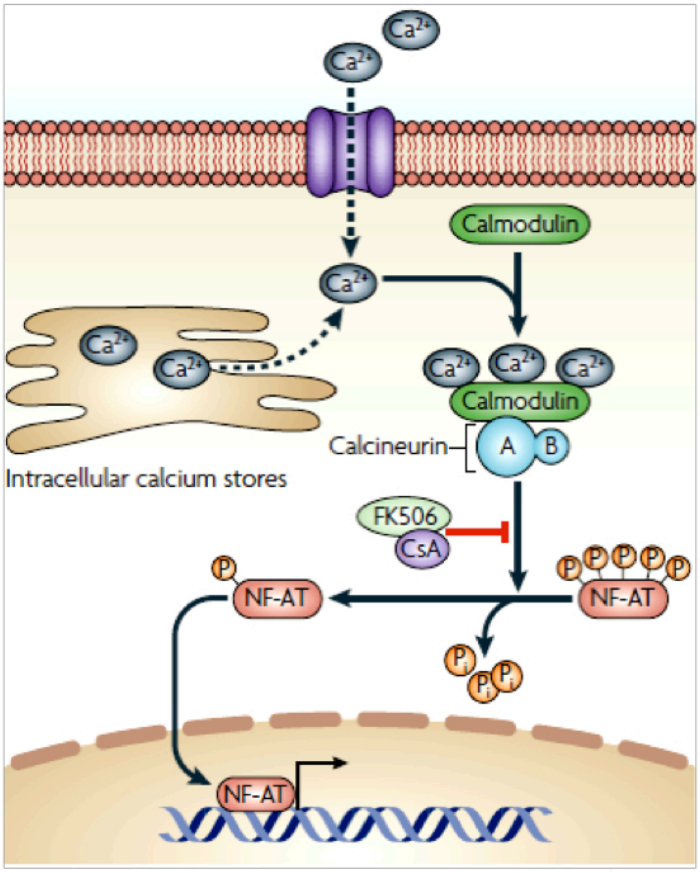

Fig. 5: The calcineurin signaling pathway and the action mechanism of CsA

An increase of intracellular Ca^{2+} activates the development of a Ca^{2+}–calmodulin complex. This in turn, activates the enzyme calcineurin (CaN), which acts as a serine/ threonine phosphatase. Subsequently it dephosphorylates the transcription factor NF-AT (nuclear factor of activated T-cells), which then passes through into the nucleus, where it initiates the transcription of NF-AT regulated genes. CsA inhibits the enzymatic activity of CaN and consequently inhibits the expression of NF-AT regulated genes. Modified from (Steinbach et al., 2007).

During the treatment with CsA, unwanted side effects such as paresthesia, nephrotoxicity, high blood pressure, hirsutism and a higher vulnerability towards infections and malignant tumors may occur (Mihatsch et al., 1989); however, subjects of this study only took four dosages of CsA during three days. For this reason, only mild side effects could have occurred, such as heat sensation in the hands and head, nausea and discomfort in the intestine and stomach, fatigue and a tingling sensation in the hands.

2.3 Study design

2.3.1 The behavioral conditioning paradigm

In a well-established design of a study program on behavioral conditioning of immune functions (Albring et al., 2014), subjects were randomly allocated to three different double-blind placebo-controlled groups, in order to identify possible biological and psychological predictors of nocebo responses after treatment with the immunosuppressive drug CsA. Within this program, CsA (unconditioned stimulus/US) was paired with a gustatory stimulus (conditioned stimulus/CS) during acquisition. Mere re-exposition to the CS during evocation is mimicking the immunopharmacological properties of CsA, reflected by impaired Th1 cytokine production and decreased T cell proliferation (Goebel et al., 2002; Wirth et al., 2011). The unique advantage of the design described here, is the ability to analyze intra-individual nocebo responses, after intake of CsA during the acquisition phase as well as after placebo intake during the evocation phase.

On experimental days 1 (6 pm), 2 (8 am and 6 pm) and 3 (8 am) during the first week of medication intake, all subjects received four oral doses of 2.5mg/kg body weight of the immunosuppressive drug CsA (Sandimmun optoral®, Novartis) in capsule form. This quantity was chosen based on previous studies successfully utilizing this dosage (Albring et al., 2014; Goebel et al., 2002; Wirth et al., 2011). The CsA capsules were manufactured by the pharmacy of the University Hospital Essen to be indistinguishable from the placebo capsules in taste and smell. To achieve this, the CsA capsules were coated with a white film of gelatine and the interstitial were filled with lactose powder. The first (*Csa_placebo_drink,* n=24) and second (*Csa_10%CsA_drink,* n=26) groups received a green-colored novel-tasting drink (150 ml strawberry milk aromatized with lavender oil; CS) with each capsule intake during the whole study, which has been previously established and utilized (Albring et al., 2014; Goebel et al., 2002; Wirth et al., 2011) (Figure 6).

Fig. 6: The conditioned and unconditioned stimuli.

The conditioned stimulus is made out of a green-colored novel-tasting drink (150 ml strawberry milk aromatized with lavender oil) and taken together with the unconditioned stimulus; the immunosuppressive drug CsA (Sandimmun optoral®, Novartis) in capsule form.

A pause of five days followed to allow drug wash out. During the following eight days, subjects of group 1 (*Csa_placebo_drink*) received identical looking capsules containing a placebo (lactose powder), whereas subjects of group 2 (*Csa_10%CsA_drink,* n=26) and group 3 (*Csa_10%CsA,* n=12) took a subtherapeutic dose of CsA (0.25 mg/kg) fourteen times; twice a day (8 am and 6 pm respectively) (Figure 7). In order to increase the sample size of this study, all three groups were included, although only group 1 received placebo capsules during the "*Placebo*" phase and only groups 1 and 2 received the CS during the whole study. However, these different treatments had no effect on the investigated parameters of interest of this thesis. Since CsA can cause noticeable side effects, immunological, cardiovascular and neuroendocrine parameters were closely monitored during *Medication* and "*Placebo*" intake (Figure 7; *Pre I - Post II*).

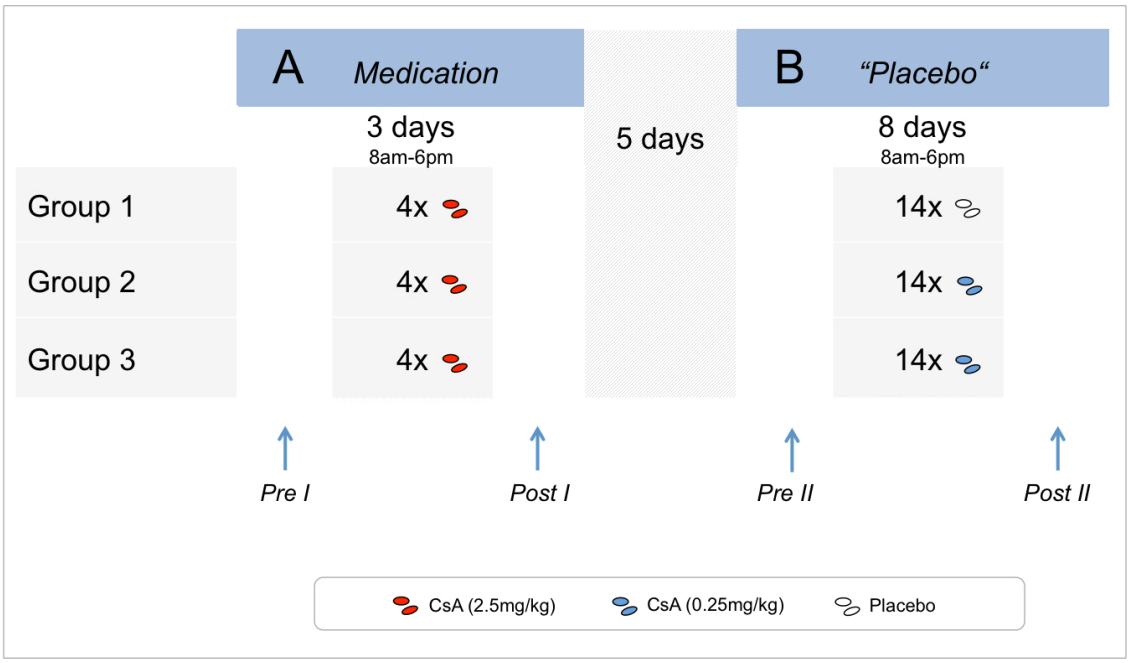

Fig. 7: Experimental design

On experimental days 1 (6 pm), 2 (8 am and 6 pm) and 3 (8 am) during medication intake, all subjects in each of the three treatment groups received four oral doses of 2.5mg/kg body weight of the immunosuppressive drug CsA (Sandimmun optoral®, Novartis) in capsule form. In addition, subjects in groups 1 (n=24) and 2 (n=26) received the CS (drink) with each capsule (CsA) intake, whereas subjects in group 3 (n=12) were not exposed to the CS (**A**). After five days wash out time, subjects either received identical looking capsules containing a placebo (lactose powder) or a subtherapeutic dose of CsA (0.25 mg/kg) fourteen times, twice a day (8 am and 6 pm respectively) with (groups 1 and 2) or without (group 3) the CS (**B**). To analyze possible nocebo effects, twelve hours after each capsule intake, participants were asked to report the amount and intensity of any drug (CsA) specific side effect. Before the start of the study, before *(Pre II)* as well as after *"Placebo" (Post II)* intake, the GASE (Generic Assessment of Side Effects) questionnaire was additionally filled out to analyze general treatment side effects. Blood was drawn on the first day for baseline measurement (*Pre I*), on day 3 (*Post I*) to analyze the pharmacological effect of CsA, on day 8 (*Pre II*) and 15 (*Post II*) to determine both possible residual effects of the drug and effects on physiological parameters after treatment with *"Placebo"* (subtherapeutical doses of CsA).

2.3.2 CsA dose response: definition of the subtherapeutic dose

The utilized subtherapeutic dose of 0.25 mg/kg CsA, was determined in a previous pilot study (Albring et al., 2014). Participants received pills containing 0.25 mg/kg CsA (10% of the dose used as an US) four times during three consecutive days delivered at the same time points as during the acquisition phase of the conditioning process. Blood was drawn each time before and after CsA intake and analyzed for CsA levels as well as functional immune parameters. A subtherapeutic dose of CsA was barely detectable in whole blood and did not significantly affect IL-2 secretion of anti-CD3 stimulated peripheral blood mononuclear cells (PBMCs).

2.4 Behavioral parameters

2.4.1 Measurement of side effects

Before the start of the study, subjects completed the Generic Assessment of Side Effects questionnaire (GASE) (Rief et al., 2011), assessing psychological and medical indispositions of the last seven days (Figure 7A; *Pre I*). The GASE inquires the most frequent side effects in clinical trials according to FDA statistics and it also allows the assessment of the attribution of symptoms to a specific drug. Before the first intake of placebo capsules, the GASE was completed again, assessing those side effects that occurred during the medication intake (Figure 7A; *Pre II*). In addition, subjects filled out the GASE after fourteen intakes of capsules containing either no pharmacological agents (placebo) or a subtherapeutic dose of CsA (0.25 mg/kg) respectively, in order to analyze possible unwanted side effects, which were ascribed to the "*Placebo*" treatment (Figure 7B; *Post II*). During those two measurement points, only symptoms that were attributed to the alleged drug were counted. Additionally, subjects were asked at each visit during *Medication* and "*Placebo*" intake, to rate CsA-specific side effects on a five-point Lickert scale (heat sensation in the hands and head, nausea and discomfort in the intestine and stomach, fatigue, tingling sensation in the hands, (0/"not at all''; 4/''very intense'') (Figure 7). To measure the expectation of experiencing side effects during study participation, subjects were asked before the first pill intake how high they would estimate the chance of experiencing adverse effects on a percentage scale from 0 to 100 (Fig. 7A; *Pre I*).

2.4.2 Psychological trait variables

Questionnaires assessing psychological trait variables were filled out before the first study day (Figure 7A; *Pre I*). A general anamnesis; traits such as optimism and pessimism (revised Life Orientation Test /LOT-R) (Herzberg et al., 2006); somatosensory amplification (somatosensory amplification scale/SSAS) measuring a disposition to identify naturally occurring somatic and visceral sensations as being very strong, unpleasant and harmful (Barsky et al., 1990); beliefs about medicines (beliefs about medicines questionnaire_extended version/BMQ)(Horne et al., 1999), assessing patients' expectations and perceptions regarding the effects of

medicines, through judging the personal need of drugs relative to concerns about their risk factors, such as side effect development. The BMQ is comprised of six subscales: “BMQ_general harm,” the belief that medication will do harm in general; “BMQ_general overuse,” the perception of how doctors use medicine and place too much emphasis in them; “BMQ_general benefit,” the belief that a beneficial treatment can be achieved through medicines; “BMQ_sensitive soma,” the sensitivity towards drug effects; “BMQ_specific necessity,” the dependence on medication; “BMQ_specific concerns,” being concerned about drug side effects and feeling uneasy when taking drugs. In addition, the trait version of the State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1983), as well as physical activity (Freiburger Fragebogen zur körperlichen Aktivität; FFkA) (Frey et al., 1999) were analyzed. In order to exclude any participants with high depression scores, the Hospital Anxiety and Depression Scale (HADS-D) (Bjelland et al., 2002) was also utilized.

2.5 Measurement of CsA blood concentrations and cardiovascular parameters

CsA concentrations in whole blood were assessed by the central laboratory of the University Hospital of Essen, using Siemens Dimension Flex reagent cartridge (Erlangen, Germany), according to the manufacturer’s instructions. The blood pressure was measured before every blood withdrawal using a sphygmomanometer and in addition the heart rate was recorded.

2.6 Immunological analyses

2.6.1 Cell isolation

In order to determine the effects of CsA, PBMCs were isolated by density gradient centrifugation (Ficoll-Paque™ Plus, GE Healthcare, Munich, Germany) from heparinized whole blood. To achieve this, blood was diluted in the ratio of 1:1 with PBS (Phosphate Buffered Saline, Life Technologies, Darmstadt, Germany) and subsequently layered on Ficoll. Erythrocytes and granulocytes were then centrifugalized through the Ficoll due to their higher density, whereas PBMCs and thrombocytes sediment between Ficoll and plasma. This layer was carefully collected with a pi-

pette and in order to remove the thrombocytes, as well as the remains of plasma and Ficoll, cells were washed twice with Hanks' Balanced Salt Solution (HBSS, Life Technologies, Darmstadt, Germany). The cell pellet was subsequently resuspended in 2ml of cell culture medium (Roswell Park Memorial Institute (RPMI) 1640 supplemented with GlutaMAX I, 25 mMHepes, 10% fetal bovine serum, 50 µg/ml gentamicin; Life Technologies, Darmstadt, Germany) and counted with an automated hematology analyser (KX-21 N, Sysmex Deutschland GmbH, Norderstedt, Germany) and in the last step adjusted to 2.5×10^6 cells/ml in RPMI.

2.6.2 Determination of IL-2 production

PBMC suspensions (2.5×10^6 cells/ml) were transferred to 96-well flat bottom tissue culture plates and were stimulated with 20 ng/ml of soluble mouse anti-human CD3 monoclonal antibody (clone: HIT3a; BD Pharmingen, San Diego, USA) for 24h (37°C, 5% CO_2). Concentration of IL-2 in culture supernatants was quantified using a commercial enzyme linked immunosorbent assay (ELISA) (Biolegend, San Diego, USA) according to the manufacturer´s instructions with the usage of Fluostar OPTIMA Microplate Readers (BMG Labtech, Offenbach, Germany). The samples were diluted for the subsequent measurement with Assay Diluent in the ratio of 1:5.

2.6.3 IL-2 mRNA expression analysis

PBMCs (2.5×10^6 cells/ml) were stimulated with 40 ng/ml of soluble mouse anti-human CD3 monoclonal antibody (clone: HIT3a, BD Pharmingen) for 4 h (37°C, 5% CO_2). Total ribonucleic acid (RNA) was extracted using the RNeasy Micro Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. The concentration of RNA was spectrometrically determined in the wavelength of 260 nm with the BioPhotometer (Eppendorf, Hamburg, Germany). Single-stranded complementary deoxyribonucleic acid (cDNA) was synthesized using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Darmstadt, Germany). Real-time quantitative polymerase chain reaction (PCR) was performed on a 7500 Fast Real-Time PCR system (Applied Biosystems, Darmstadt, Germany) using Fast qPCRMasterMix Plus Low Rox (Eurogentec, Cologne, Germany) and the following cycling conditions: 5 min at 95°C followed by 40 cycles of 3 sec at

95°C, 20 sec at 60°C and 26 sec at 72°C. Primers (forward: 5'-CCAGGATGCTCACATTTAAGTTTTAC-3'; reverse: 5'-GAGGTTTGAGTTCTTCTTCTAGAC-3' ACTGA) and probes (5'-6-FAM-TGCCCAAGAAGGCCACAGAACTGAA-BHQ1-3') were purchased from Microsynth (Balgach, Switzerland). For quantification of IL-2 mRNA expression, serially diluted cDNA samples generated from purified specific PCR products (High Pure PCR Product Purification Kit, Roche Diagnostics, Mannheim, Germany) were used as external standards in each run.

2.6.4 Determination of proliferation rate of $CD4^+$ T cells

The proliferation rate of $CD4^+$ T cells was measured by flow cytometry using the Click-iT® EdU cell proliferation assay (Invitrogen, Darmstadt, Germany) according to the manufacturer's instructions. Briefly, PBMCs (1.25 x 10^6 cells/ml) were stimulated in 96-well round bottom tissue culture plates with 2.5 µg/ml of soluble mouse anti-human CD3 monoclonal antibody (clone: HIT3a, BD Pharmingen,) for 72 h (37°C, 5% CO_2). The thymidine analogue 5-ethynyl-2´-deoxyuridine (EdU), which is incorporated during DNA synthesis, was added to the cells at a concentration of 10 µM for the last 48 h of culture. After incubation, cells were washed and stained with APC conjugated anti-human CD4 (clone RPA-T4, BD Pharmingen) antibody. Cells were fixed with 4% paraformaldehyde and permeabilized using a saponin-based permeabilization reagent. Afterwards, cells were incubated with the Click-iT® reaction cocktail. The percentage of proliferating $CD4^+$ T cells was analyzed on a FACS (fluorescence-activated cell sorting) Canto II flow cytometer using FACS Diva software (BD Immunocytometry Systems, Heidelberg, Germany).

2.6.5 Determination of $CD3^+$, $CD3^+CD4^+$ and $CD3^+CD8^+$ cells

In order to determine $CD3^+$, $CD3^+CD4^+$ and $CD3^+CD8^+$ cells, PBMC suspensions (2.5 × 10^6 cells/ml) were incubated with the following fluorochrome-conjugated monoclonal antibodies: anti-human CD3 (clone SK7, BD Pharmingen, Heidelberg, Germany), anti-human CD4 (clone RPA-T4, AbD Serotec, Düsseldorf, Germany) and anti-human CD8 (clone SK1, BD Pharmingen, Heidelberg, Germany). Antibody labeling was performed by a standard lyse-wash procedure using FACS™ lysing solution (BD Pharmingen, Heidelberg, Germany) and supplemented PBS

(Dulbecco's PBS without calcium and magnesium, 2% FBS, 0.1% NaN_3). Lymphocytes were identified by forward and sideward scatter characteristics. Ten thousand lymphocytes from each sample were analyzed on a FACS Canto II flow cytometer using FACS Diva 6.01 software (BD Immunocytometry Systems, Heidelberg, Germany). T cells were identified by CD3 staining, T helper cells by CD3/CD4 double-staining and cytotoxic T cells by CD3/CD8 double-staining. Total cell counts were obtained with an automated cell counter (KX-21N, Sysmex Deutschland GmbH, Norderstedt, Germany).

2.7 Neuroendocrine parameters

2.7.1 Measurement of cortisol concentration in plasma

Plasma cortisol levels were measured using a commercial enzyme-linked immunosorbent assay (Cortisol ELISA, IBL International, Hamburg, Germany) according to the test protocol of the manufacturer and were analyzed on a Fluostar OPTIMA Microplate Reader (BMG Labtech, Offenbach, Germany). The samples were diluted for the subsequent measurement with PBS in the ratio of 1:50. Intra- and interassay variances were 5.6% and 6.9%, respectively. The detection limit for cortisol was 0.3 ng/ml.

2.7.2 Measurement of catecholamine concentration in plasma

Catecholamines were isolated from plasma through selective adsorption on aluminium oxide. Noradrenaline and adrenaline concentrations were determined at the Department of Nephrology, University Hospital Essen, through high-performance liquid chromatography (HPLC) with electrochemical detection (ChromSystems, Instruments and Chemicals GmbH, Munich, Germany) according to the manufacturer's instructions.

2.8 Genotyping

Genomic DNA was extracted from whole blood using peqGOLD Blood DNA Mini Kit (Erlangen, Germany) according to the manufacturer's protocol. DNA concentration was determined through the Eppendorf BioPhotometer® plus. Genotyping was performed on a 7500 Fast Real-Time PCR System using the TaqMan SNP Geno-

typing assay for rs4680 (C_25746809_50) and the TaqMan genotyping master mix (Applied Biosystems, Darmstadt, Germany) following the manufacturer's instructions. Each individual DNA sample was amplified in a total volume of 25 µl, consisting of 11.25 µl DNA, containing 10ng of DNA, 12.5 µl master mix and 1.25 µl SNP assay. In addition, negative and positive controls were run through the cycle and determination was performed with duplicates. Cycling conditions were as follows: 10 min at 95°C HOLD, 15 sec at 92°C DENATURE, 1 min at 60°C ANNEAL/EXTEND. Allelic discrimination analysis was performed with the SDS version 1.4 software (Applied Biosystems, Foster City, USA).

2.9 Statistical analysis

Neuroendocrine, immunological, cardiovascular parameters, as well as CsA levels in whole blood were analyzed using multivariate analysis of variance (ANOVAs). Psychological characteristics as well as behavioral parameters were compared with univariate analysis of variances (ANOVA) followed by Bonferroni post hoc tests. Pearson correlations were additionally, where applicable, measured. Calculations were performed with PASW statistics version 18 (SPSS, Chicago, IL, USA). If not stated otherwise, all p-values are asymptotic, two-sided and corrected for multiple testing. The significance-level was set at $p<0.05$. Results are displayed as mean ± standard error of the mean (SEM).

3 Results

3.1 Analysis of the three experimental groups

Biological and psychological predictors of nocebo responses after short term treatment with the immunosuppressive drug CsA were analyzed after three days of medication intake, as well as during eight days of receiving either placebo or a subtherapeutic dose of CsA (0.25 mg/kg) respectively.

In the first step, possible differences between treatment groups 1 to 3 in all variables were analyzed. Subjects in these groups did not differ in any behavioral parameters, such as sociodemographic and psychological trait variables (Table 1).

Treatment group	Group 1 (n=24)	Group 2 (n=26)	Group 3 (n=12)
SSAS	24.8 ± 1.1	25.9 ± 1.1	27.1 ± 1.4
BMQ_general harm	9.8 ± 0.6	9.2 ± 0.7	10.5 ± 0.7
BMQ_general overuse	13.7 ± 0.5	12.9 ± 0.6	13,7 ± 0.7
BMQ_general benefit	15.7 ± 0.5	16.4 ± 0.5	15.6 ± 0.7
BMQ_sensitive soma	7.7 ± 0.6	8.3 ± 0.7	9.6 ± 1.0
BMQ_specific necessity	7.6 ± 0.4	7.8 ± 0.5	8.1 ± 0.8
BMQ_specific concerns	9.6 ± 0.7	9.2 ± 0.9	11.3 ± 1.1
Age (years)	25.0 ± 0.7	25.9 ± 0.9	25.5 ± 0.9
Body mass index (kg/m²)	22.5 ± 1.1	22.3 ± 1.4	24.2 ± 1.0
Physical activity (FFkA)	42.3 ± 6.8	37.2 ± 5.3	51.5 ± 9.3
Trait anxiety (STAI)	33.1 ± 1.3	37.3 ± 1.8	36.3 ± 1.8
LOT(R) Pessimism	4.0 ± 0.5	4.9 ± 0.5	4.8 ± 0.7
LOT(R) Optimism	9.4 ± 0.5	9.0 ± 0.4	8.8 ± 0.5

Tab. 1: Sociodemographic and psychological characteristics of the three experimental groups

SSAS, BMQ_general harm, BMQ_general overuse, BMQ_general benefit, BMQ_sensitive soma, BMQ_specific necessity and BMQ_specific, age, body mass index, physical activity (FFkA), trait anxiety (STAI) scores, LOT(R) pessimism and optimism were compared between all three treatment groups using univariate ANOVA. Groups did not significantly differ in any of the variables listed. Data are shown as mean ± SEM; all $p>0.05$

The analysis of immunological and cardiovascular parameters also revealed no differences between treatment groups 1 to 3 during *Medication* and "*Placebo*" intake (Table 2). In all groups, the CsA concentration in whole blood rose significantly after four intakes, whereas the IL-2 concentration in culture supernatant decreased significantly. Post hoc tests revealed significant time effects for the CsA levels from *Pre I* to *Post I* (Group 1: $t=-11.4$; $p<0.001$; Group 2: $t=-30.6$; $p<0.001$;

Group 3: t=-8.8; p<0.001), as well as significant time effects for the IL-2 protein concentrations from *Pre I* to *Post I* for all three groups (Group 1: t=5.0; p<0.001; Group 2: t=3.9; p<0.001; Group 3: t=6.6; p<0.001) (Table 2). The only difference between the three treatment groups was measured after fourteen subtherapeutical dose intakes of CsA in groups 2 and 3, where marginal values were measured compared to group 1. Post hoc tests revealed significant time effects from *Pre II* to *Post II* for the groups 2 and 3 (Group 2: t=-6.5; p<0.001; Group 3: t=-8.8; p<0.001); however, this treatment difference had no influence on IL-2 production and cardiovascular parameters for all groups (Table 2).

	Group	***Medication***		***"Placebo"***	
		Pre I	*Post I*	*Pre II*	*Post II*
CsA levels in whole blood *(ng/ml)*	Group 1	n.d.	1285.8 ± 41.3 t*	n.d.	n.d.
	Group 2	n.d.	1096.1 ± 94.0 t*	n.d.	60.4 ± 5.5 t*
	Group 3	n.d.	1482.3 ± 77.6 t*	n.d.	81.9 ± 6.5 t*
IL-2 in culture supernatant *(pg/ml)*	Group 1	488.4 ± 76.4	128.3 ± 18.3 t*	452.2 ± 68.8	432.5 ± 75.8
	Group 2	350.5 ± 78.2	115.7 ± 22.2 t*	341.6 ± 60.9	303.0 ± 54.5
	Group 3	278.4 ± 43.9	164.4 ± 31.6 t*	378.6 ± 60.8	492.8 ± 83.5
Heart rate *(beats/min)*	Group 1	71.9 ± 2.4	69.1 ± 1.8	64.8 ± 1.9	65.6 ± 2.2
	Group 2	73.4 ± 1.8	68.1 ± 1.9	70.9 ± 2.1	65.7 ± 2.0
	Group 3	71.3 ± 2.1	69.7 ± 2.3	72.0 ± 3.2	71.4 ± 5.2
Systolic BP *(mmHG)*	Group 1	122.8 ± 2.1	123.1 ± 2.4	122.7 ± 1.7	121.6 ± 2.2
	Group 2	127.5 ± 2.0	127.1 ± 1.8	125.7 ± 2.2	123.9 ± 1.7
	Group 3	125.4 ± 3.6	126.9 ± 2.3	126.7 ± 3.6	119.6 ± 3.9
Diastolic BP *(mmHG)*	Group 1	83.5 ± 1.5	85.1 ± 2.0	82.1 ± 1.4	81.5 ± 1.6
	Group 2	85.0 ± 1.5	87.7 ± 1.5	81.9 ± 1.4	82.8 ± 1.1
	Group 3	84.6 ± 2.3	88.3 ± 1.3	82.5 ± 1.7	83.3 ± 1.7

Tab. 2: CsA levels, IL-2 concentrations and cardiovascular parameters of the three experimental groups during *Medication* and "*Placebo*" intake

CsA treatment during *Medication* significantly increased CsA serum levels in all groups significantly and suppressed IL-2 protein concentrations after anti-CD3 stimulation, however, it did not affect cardiovascular parameters in all groups. During the "*Placebo*" condition, treatment with subtherapeutical doses of CsA slightly increased CsA levels (groups 2 and 3), however, effected neither IL-2 production nor cardiovascular parameters in these groups. CsA levels, immune and cardiovascular parameters were analyzed with multivariate ANOVAs (t= time effect; n.d= not detectable, <25 ng/ml). In case of significant F tests, these were followed by Bonferroni post hoc tests. Data are shown as mean ± SEM; *p<0.001

Subjects of all three groups did not significantly differ in their perceived treatment side effects documented with the GASE before study entry (Baseline; F=0.1; n.s.), as well as in their general (F=1.0; n.s.) and CsA-specific side effects (F=1.4; n.s.) during *Medication* intake (Figure 7, *Post I*). Moreover, during *"Placebo"* treatment, no significant group differences were observed either in reported general (GASE; *Post II* (F=0.5; n.s.) or in CsA-specific side effects (F=0.4; n.s.)(Table 3).

	Medication		*"Placebo"*	
Group	CsA-specific side effects	General side effects (GASE)	CsA-specific side effects	General side effects (GASE)
Group 1	7.1 ± 1.3	6.3 ± 1.1	13.0 ± 3.5	1.0 ± 0.4
Group 2	8.0 ±1.6	5.9 ± 1.7	18.7 ± 5.4	1.2 ± 0.4
Group 3	12.1 ± 3.9	9.9 ± 3.7	19.5 ± 11.5	2.3 ± 2.1

Tab. 3: CsA-specific and general side effects of the three experimental groups during *Medication* and *"Placebo"* intake

Treatment groups did not significantly differ in reported CsA-specific side effects and general side effects analyzed with the GASE, either during the Medication or during the "Placebo" condition. All measured side effects were compared between all three treatment groups using univariate ANOVA. Data are shown as mean ± SEM; all $p>0.05$

3.2 Genotyping and subsequent analysis

After confirming that treatment groups showed no significant differences in reported side effects, or psychological and physical parameters, volunteers were then put into one group and subsequently compared according to the respective three genotype groups: homozygotes for the Val^{158}allele (Val/Val), heterozygotes (Val/Met), and homozygotes for the Met^{158}allele (Met/Met). Genotyping revealed allele frequencies of 30.7% (Val/Val; n=19), 54.8% (Val/Met; n=34) and 14.5% (Met/Met; n=9) for the COMT Val^{158}Met polymorphism.

3.2.1 General and CsA-specific side effects

Subsequent analyses revealed significant differences between the three genotype groups and their experienced side effects. When analyzing general psychological and medical indispositions with the GASE questionnaire before study entry, Val/Val homozygote carriers reported significantly more general psychological and medical indispositions (7.8 ± 1.8) compared to the Met/Met (1.3 ± 0.7) and Val/Met groups (3.9 ± 0.8) ($F=4.6$; $p<0.01$). Post hoc analysis showed a significant difference between Val/Val and Met/Met ($p<0.05$). Furthermore, Val/Val homozygote carriers also reported significantly more general psychological and medical indispositions (GASE) after *Medication* intake ($F=5.9$; $p<0.01$). Post hoc analysis showed a significant difference between Val/Val and Val/Met; $p<0.01$, as well as between Val/Val and Met/Met; $p<0.05$ (Figure 8a). Interestingly, also after *"Placebo"* treatment, this group reported more experienced general side effects recurrently ($F=4.7$; $p<0.05$) (Val/Val vs. Val/Met $p<0.05$) (Figure 8b).

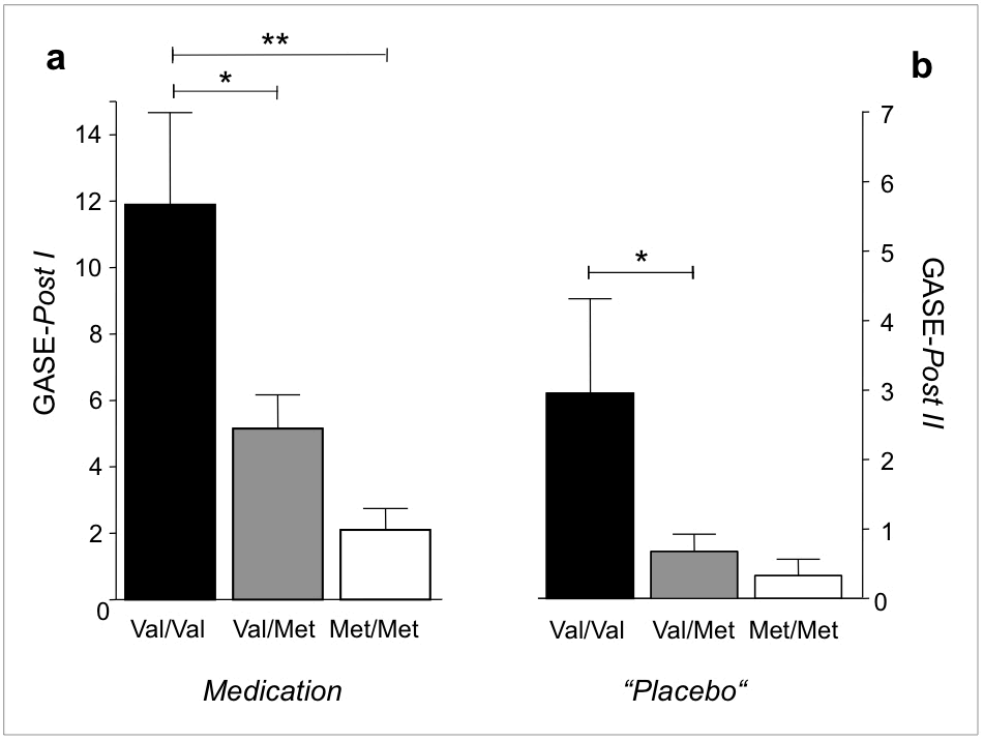

Fig. 8: General side effects after *Medication* and "*Placebo*" intake

General psychological and medical indispositions were analyzed with the GASE after *Medication* **(a)** and *"Placebo"* intake, respectively **(b)**. Val/Val homozygote carriers experienced significantly more general psychological and medical indispositions after four medication intakes **(a)** and also showed the strongest nocebo response, measured by most reported side effects after *"Placebo"* intake **(b)**. Data were analyzed with univariate ANOVAs. In case of significant F tests, these were followed by Bonferroni post hoc tests. Bars represent mean ± SEM; *p<0.05, **p<0.01

In parallel with the findings of increased perceived general side effects in Val/Val homozygote carriers, ANOVAs showed significant differences for CsA-specific side effects ($F=11.9$; $p<0.001$) with significantly more side effects reported in Val/Val and Met/Met groups than compared with the Val/Met group after *Medication* intake. Post hoc analysis showed a significant difference between Val/Val and Val/Met, $p<0.001$, as well as Val/Val and Met/Met, $p<0.05$ (Figure 9a). Even more noticeable differences in reported CsA-specific side effects between individuals of the three genotype groups were observed during *"Placebo"* treatment ($F=13.1$; $p<0.001$) with the most pronounced side effects reported from the Val/Val homozygote carriers compared to the other two groups. Post hoc analysis showed a significant difference between Val/Val and Val/Met ($p<0.001$) as well as Val/Val and Met/Met ($p<0.01$). Remarkably, after fourteen intakes of *"Placebo"* capsules, the reported CsA-specific side effects were twice as high for the Val/Val homozygote carriers when compared to four intakes of CsA during *Medication* (Figure 9b).

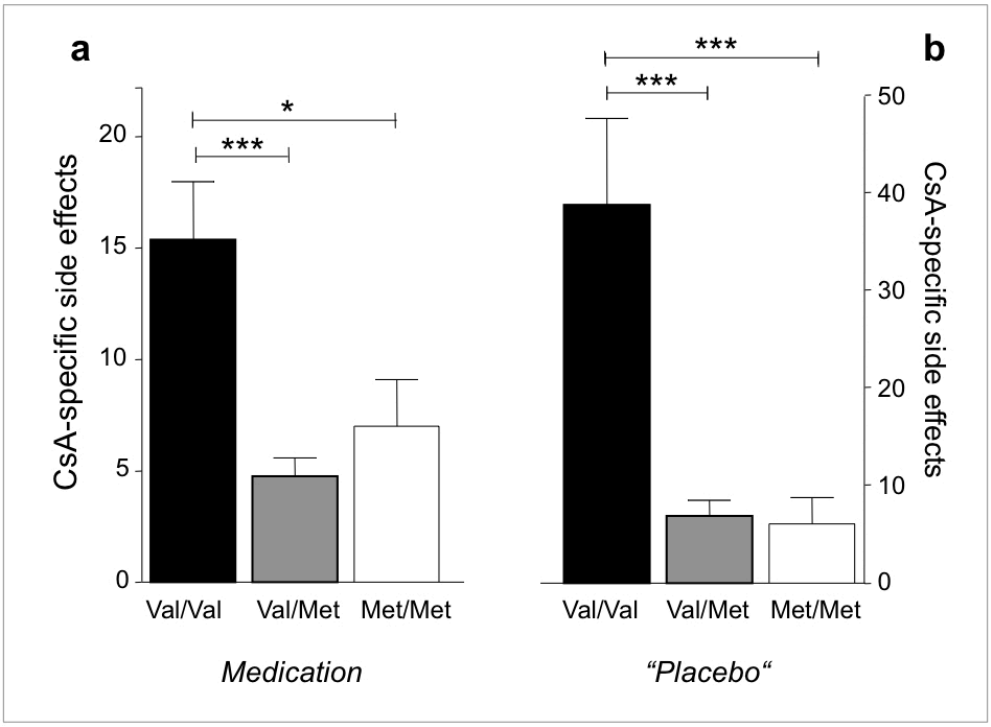

Fig. 9: CsA-specific side effects after *Medication* and *"Placebo"* intake

Reported CsA-specific side effects after *Medication* **(a)** and *"Placebo"* intake, respectively **(b)**. After *Medication* intake, significantly higher CsA-specific side effects were reported by Val/Val homozygote carriers **(a)**. This difference was even more pronounced after fourteen *"Placebo"* intakes **(b)**. Data were analyzed using univariate ANOVA. In case of significant F tests, these were followed by Bonferroni post hoc tests. Bars represent mean ± SEM; *p<0.05, ***p<0.01

3.2.2 Psychological trait and screening parameters

Analysis of sociodemographic and psychological trait and screening variables with univariate ANOVAs showed no differences between all three genotypes for age, body mass index, physical activity, trait anxiety, as well as for the traits pessimism and optimism (Table 4).

Genotype	Val/Val (n=19)	Val/Met (n=34)	Met/Met (n=9)
Age (years)	25.7 ± 1.1	25.3 ± 0.6	25.6 ± 1.3
Body mass index (kg/m²)	21.5 ± 1.9	23.7 ± 0.5	22.0 ± 2.9
Physical activity (FFkA)	46.6 ± 8.7	38.2 ± 4.2	46.0 ± 11.2
Trait anxiety (STAI)	38.5 ± 1.8	34.4 ± 1.3	33.3 ± 2.6
LOT(R) Pessimism	5.3 ± 2.8	4.2 ± 0.4	4.0 ± 0.7
LOT(R) Optimism	8.9 ± 0.5	8.9 ± 0.4	10.1 ± 0.5

Tab. 4: Sociodemographic and psychological characteristics of the COMT genotype groups

COMT genotype groups did not significantly differ in age, body mass index, physical activity, trait anxiety (STAI) scores, as well as within the traits pessimism and optimism LOT(R). Data were analyzed using univariate ANOVA and are shown as mean ± SEM; all $p>0.05$

However, significant differences between allele carriers of the COMT Val^{158}Met polymorphism and four of six subscales of the beliefs about medicines questionnaire (BMQ) were found, in which prescribed medication for personal use (BMQ_specific), as well as beliefs about medicines in general (BMQ_general) are rated. Individuals with the Val/Val genotype reported more negative beliefs about medicines, judging the intake of medicines as a potential danger, having concerns about side effects, as well as accumulated long-term effects. They were significantly more convinced that medication harms them in general (BMQ_general harm; $F=4.2$; $p<0.05$; Post hoc analysis showed a significant difference between Val/Val and Val/Met ($p<0.05$)) and they react more sensitive towards their effects (BMQ_sensitive soma; $F=13.1$; $p<0.001$; Post hoc analysis showed a significant difference between Val/Val and Val/Met ($p<0.001$), as well as Val/Val and Met/Met ($p<0.001$)). They also view themselves as significantly more dependent on medication (BMQ_specific necessity; $F=5.6$; $p<0.01$; Post hoc analysis showed a significant difference between Val/Val and Val/Met ($p<0.05$), as well as Val/Val and Met/Met ($p<0.05$)) and at the same time were more concerned about their side effects and feeling uneasy when taking them (BMQ_specific concerns; $F=6.3$; $p<0.01$; Post hoc analysis showed a significant difference between Val/Val and Val/Met ($p<0.05$), as well as Val/Val and Met/Met ($p<0.01$)). No differences were found in the scales BMQ_general overuse, which assess the perception that doctors prescribe medicines too much and within the BMQ_general benefit, which measures the belief that a beneficial treatment can be achieved through medication.

In addition, the Val/Val homozygote carriers obtained significantly higher scores compared to the Val/Met and Met/Met carriers in the SSAS, measuring a disposition to identify natural occurring somatic and visceral sensations as being very strong, unpleasant and harmful ($F=8.8$, $p<0.001$). Post hoc analysis showed a significant difference between participants of the genotypes Val/Val and Val/Met ($p<0.001$), as well as Val/Val and Met/Met ($p<0.01$) (Table 5).

Genotype	Val/Val (n=19)	Val/Met (n=34)	Met/Met (n=9)
SSAS	29.4 ± 1.3 **	24.3 ± 0.8	22.9 ± 1.3
BMQ_general harm	11.0 ± 0.7 *	8.8 ± 0.5	10.6 ± 0.7
BMQ_general overuse	14.1 ± 0.7	12.9 ± 0.5	13.7 ± 0.8
BMQ_general benefit	15.7 ± 0.6	16.0 ± 0.4	16.0 ± 0.7
BMQ_sensitive soma	11.0 ± 0.8 ***	7.4 ± 0.4	6.2 ± 0.5
BMQ_specific necessity	9.2 ± 0.6 *	7.2 ± 0.4	6.8 ± 0.5
BMQ_specific concerns	12.1 ± 1.1 **	9.1 ± 0.6	7.4 ± 0.7

Tab. 5: Differences in the somatosensory amplification scale (SSAS) and four subscales of the beliefs about medicines questionnaire (BMQ) depending on the COMT genotype group

SSAS, BMQ_general harm, BMQ_general overuse, BMQ_general benefit, BMQ_sensitive soma, BMQ_specific necessity and BMQ_specific concerns were compared between all COMT genotype groups. The significantly highest scores in the SSAS and four of the BMQ subscales (BMQ_general harm, BMQ_sensitive soma, BMQ_specific necessity and BMQ_specific concerns) were reported by carriers of the Val/Val homozygote, indicating a significantly higher belief that medication will harm in general and a higher sensitivity to bodily sensations, as well as more concerns about prescribed medication based on beliefs about the danger of dependence and long-term toxicity and the disruptive effects of medication. However, the three genotype groups did not differ in the two subscales BMQ_ general overuse and BMQ_ general benefit. Data were analyzed using univariate ANOVA. In case of significant F tests, these were followed by Bonferroni post hoc tests. Data are shown as mean ± SEM; *p<0.05, **p<0.01, ***p<0.001

The scores of the depression scale of the Hospital Anxiety and Depression Scale (HADS-D) revealed values within normal range for all participants (3.5 ± 0.3).

3.2.3 Genetic and psychological variables and their influence on general and CsA-specific side effects

The increased sensitivity of perceived general and CsA-specific side effects after *Medication* as well as *"Placebo"* treatment in Val/Val homozygote carriers is paralleled by significantly higher values within the SSAS and the four subscales of the BMQ (Table 5). In a subsequent step, the association of general as well as CsA-specific side effects with a higher somatization tendency was tested. Pearson correlations between the trait somatosensory amplification (SSAS) and the specific and general side effects were calculated. These correlations were significant for

somatosensory amplification and the specific side effects after *Medication* (r=.36; p<0.01) and *"Placebo"* intake (r=.41; p<0.001), as well as for the three time points, when the GASE was measured (Baseline: r=.47, p<0.001; after *Medication* intake: r=.54; p<0.001; after *"Placebo"* intake: r=.27; p<0.05).

After showing a significant association between specific and general side effects and a higher somatization tendency, Pearson correlations between the trait somatosensory amplification (SSAS) of the three respective genotypes and the specific and general side effects were analyzed (Table 6). No correlations were observed between SSAS scores of the Met/Met genotype and CsA-specific and general side effects. In contrast, SSAS scores were positively correlated with CsA-specific and general side effects in the Val/Val genotype during *Medication* intake but not during *"Placebo"* treatment. In the Val/Met group, somatosensory amplification and CsA-specific side effects were also significantly correlated during *Medication*.

	Medication		***"Placebo"***	
SSAS scores of Genotype	CsA-specific side effects	General side effects (GASE)	CsA-specific side effects	General side effects (GASE)
Val/Val (n=19)	r=.52, p<0.05 *	r=.67, p<0.01 **	r=.31, n.s.	r=.16, n.s.
Val/Met (n=34)	r=-.38, p<0.05 *	r=.16, n.s.	r=.01, n.s.	r=.18, n.s.
Met/Met (n=9)	r=.08, n.s.	r=-.09, n.s.	r=.13, n.s.	r=.30, n.s.

Tab. 6: Pearson correlations between SSAS of the three COMT genotype groups and CsA-specific and general side effects during the *Medication* and *"Placebo"* phase

SSAS scores of the Met/Met genotype and CsA-specific and general side effects did not correlate. One significant correlation was measured for the Val/Met genotype between SSAS and CsA-specific side effects after *Medication* intake and two significant correlations for the Val/Val genotype between SSAS and CsA-specific as well as general side effects after *Medication* intake. Data are shown as Pearson correlations; *p<0.05, **p<0.01

3.2.4 Manipulation of expectation

Participants of all three groups estimated that after the first week, 62% of capsules contained CsA; after the first five capsules of placebo pills that estimation decreased to 38% and after the following five placebo intakes, lower to 34%. After the last four placebo intakes the estimation was 33% and the only observed group difference was that the Val/Val group estimated the amount of capsules containing CsA at 48%, whereas the Met/Met group thought only 26% and the Val/Met group 27% of the pills contained the drug (F=3.6; p<0.05; Post hoc analysis showed a significant difference between Val/Val and Val/Met (p<0.05)) (Figure 10). Individu-

als of the Val/Val homozygote group estimated on the first day before taking any capsules that the chance of experiencing side effects would be 28%, whereas the Val/Met group thought it would be 20% and the Met/Met individuals rated the chance to be 11%. Although the Val/Val group had on average the highest expectation to experience side effects, this estimation was not significantly higher compared to the ratings of the other two groups (F=1.9; n.s.).

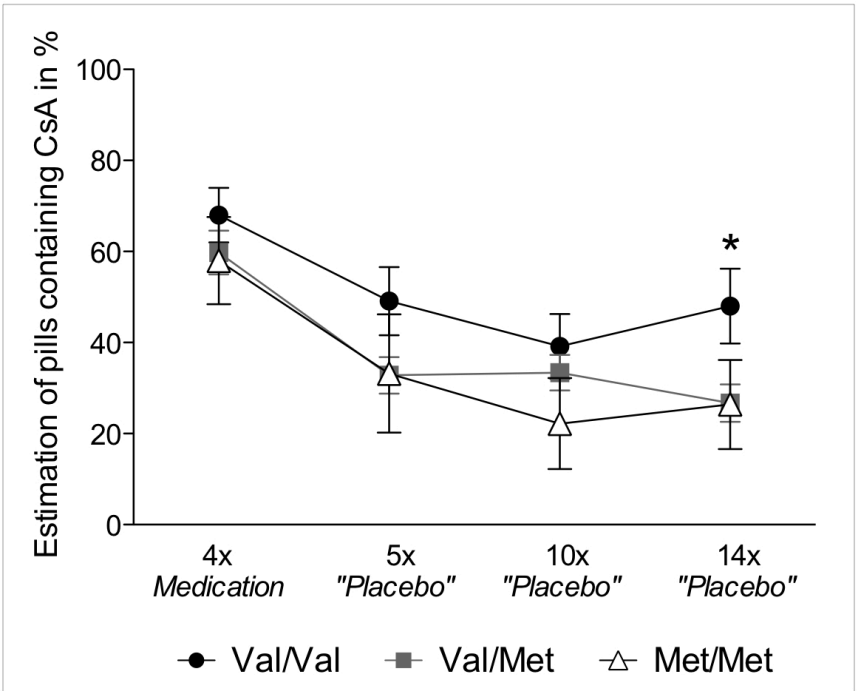

Fig. 10: Estimation of percentage of ingested pills that contained CsA

Subjects were told before each pill intake, that the chance of receiving CsA was always 50%. They were given visual analogue scales from 0 to 100 percent, in order to estimate the percentage of ingested pills that contained CsA after every four to five pill intakes. Individuals with the Val/Val genotype always rated the amount of ingested CsA capsules higher than the other two genotype groups. This difference in rating was only significant after the last four *"Placebo"* intakes. Data were analyzed with multivariate ANOVAs. In case of significant F tests, these were followed by Bonferroni post hoc tests. Line charts represent mean ± SEM; *p<0.05

3.2.5 CsA levels, immunological and neuroendocrine parameters

CsA levels were determined two hours after the last of four CsA intakes (10 am; peak level). After the *Medication* phase, CsA concentrations were significantly increased in all three genotype groups without significant differences between groups. Post hoc tests revealed significant time effects from *Pre I* to *Post I* for all three genotype groups (Val/Val: t=-21.0, p<0.000; Met/Met: t=-11.9, p<0.000; Val/Met: t=-18.0, p<0.000) (Table 7). After treatment with subtherapeutic CsA doses, marginal CsA concentrations could be detected in peripheral blood with no differences between groups. Post hoc tests revealed significant time effects for all

three genotype groups when the CsA levels of *Pre II* were compared to *Post II* (Val/Val: t=-4.9, p<0.001; Met/Met: t=-4.1, p<0.001; Val/Met: t=-4.4, p<0.001) (Table 7).

These results are in accordance with the immune parameters, which were assessed to measure the influence of the CsA effect and to ensure no differences between the allele carriers of the COMT $Val^{158}Met$ polymorphism. The IL-2 protein concentrations, IL-2 mRNA expression and the percentage of proliferating $CD4^+$ T cells did not differ between the three groups during *Medication* and *"Placebo"* intake. However, after four CsA intakes, all these immune parameters were significantly suppressed. Post hoc tests revealed significant time effects from *Pre I* to *Post I* for the IL-2 protein concentrations (Val/Val: t=4.2, p<0.001; Met/Met: t=5.1; p<0.001; Val/Met: t=5.6; p<0.001), the IL-2 mRNA expression (Val/Val: t=5.2; p<0.001; Met/Met: t=5.0; p<0.01; Val/Met: t=7.4; p<0.001) and the percentage of proliferating $CD4^+$ T cells (Val/Val: t=8.5; p<0.001; Met/Met: t=4.0; p<0.01; Val/Met: t=14.1; p<0.001) (Table 7). Additionally, the amount of circulating $CD3^+$, $CD3^+CD4^+$ and $CD3^+CD8^+$ cells were determined, using multivariate ANOVAs. There were no differences detected in all three groups (Table 7).

	Group	*Medication*		*"Placebo"*	
		Pre I	*Post I*	*Pre II*	*Post II*
CsA levels in whole blood *(ng/ml)*	Val/Val	n.d.	1315.4 ± 61.4 t***	n.d.	62.1 ± 7.6 t***
	Val/Met	n.d.	1209.9 ± 65.9 t***	n.d.	48.5 ± 5.3 t***
	Met/Met	n.d.	1340.2 ± 110.3 t***	n.d.	49.9 ± 9.4 t***
IL-2 in culture supernatant *(pg/ml)*	Val/Val	375.4 ± 61.0	160.4 ± 23.9 t***	395.4 ± 61.0	393.5 ± 60.3
	Val/Met	393.4 ± 63.6	127.4 ± 19.9 t***	375.9 ± 54.0	402.8 ± 59.6
	Met/Met	333.4 ± 68.2	86.8 ± 16.3 t***	398.6 ± 115.3	300.0 ± 103.8
IL-2 mRNA *(fg/µg total RNA)*	Val/Val	183.1 ± 31.4	45.3 ± 9.7 t***	194.7 ± 23.6	167.5 ± 26.5
	Val/Met	222.2 ± 24.7	60.1 ± 8.4 t***	224.1 ± 21.5	201.2 ± 19.3
	Met/Met	160.4 ± 31.0	46.6 ± 12.8 t**	170.9 ± 28.6	147.1 ± 16.3
Proliferation *(% proliferating $CD4^+$ T cells)*	Val/Val	26.1 ± 1.9	12.6 ± 1.2 t***	25.7 ± 2.3	23.6 ± 2.3
	Val/Met	26.3 ± 1.2	12.7 ± 0.8 t***	27.8 ± 1.8	25.6 ± 1.7
	Met/Met	32.0 ± 3.7	16.9 ± 2.3 t**	31.7 ± 3.2	32.1 ± 3.3
$CD3^+$ **cells** *(cells/µl)*	Val/Val	3023.7 ± 96.4	3037.9 ± 107.4	2960.8 ± 125.3	2978.1 ± 96.5
	Val/Met	3131.1 ± 77.6	3249.1 ± 79.5	2940.1 ± 101.3	3009.6 ± 72.2
	Met/Met	3053.7 ± 155.6	3185.2 ± 144.4	2965.7 ± 172.0	3127.8 ± 174.4
$CD3^+CD4^+$ **cells** *(cells/µl)*	Val/Val	1728.5 ± 78.4	1772.0 ± 86.7	1662.3 ± 78.7	1724.1 ± 58.9
	Val/Met	1870.0 ± 77.4	2014.0 ± 76.5	1756.0 ± 79.5	1808.7 ± 60.0
	Met/Met	1808.9 ± 91.8	2027.0 ± 128.3	1778.0 ± 113.7	1877.0 ± 135.5
$CD3^+CD8^+$ **cells** *(cells/µl)*	Val/Val	1295.2 ± 96.1	902.3 ± 31.9	1298.4 ± 109.0	2003.5 ± 200.4
	Val/Met	1227.8 ± 79.4	965.0 ± 23.6	1180.1 ± 63.5	1954.1 ± 163.4
	Met/Met	1244.7 ± 150.5	946.0 ± 42.9	1003.3 ± 152.2	1869.7 ± 351.5

Tab. 7: CsA levels and immunological parameters during *Medication* and *"Placebo"* intake

CsA treatment during *Medication* significantly increased CsA serum levels and significantly suppressed IL-2 protein concentrations, IL-2 mRNA expression and the percentage of proliferating $CD4^+$ T cells in all COMT genotype groups. However, CsA treatment did not affect $CD3^+$, $CD3^+CD4^+$ and $CD3^+CD8^+$cells in all groups. During the "*Placebo*" condition, treatment with subtherapeutical doses of CsA slightly increased CsA levels in Val/Val, Val/Met as well as Met/Met allele carriers; however it did not affect immune parameters or the circulating $CD3^+$, $CD3^+CD4^+$ and $CD3^+CD8^+$cells in these groups. CsA levels and immune parameters were analyzed with multivariate ANOVAs (t= time effect; n.d= not detectable, <25 ng/ml). In case of significant F tests, these were followed by Bonferroni post hoc tests. Data are shown as mean ± SEM; **p<0.01 ***p<0.001

In order to exclude any effects due to the activation of the HPA-axis or any increased physical activation that could have influenced the perception of side effects, catecholamines (adrenaline, noradrenaline) and cortisol were measured before and after *Medication* and *"Placebo"* intake, using multivariate ANOVAs. There were no differences detected in all three groups (Figure 11, 12).

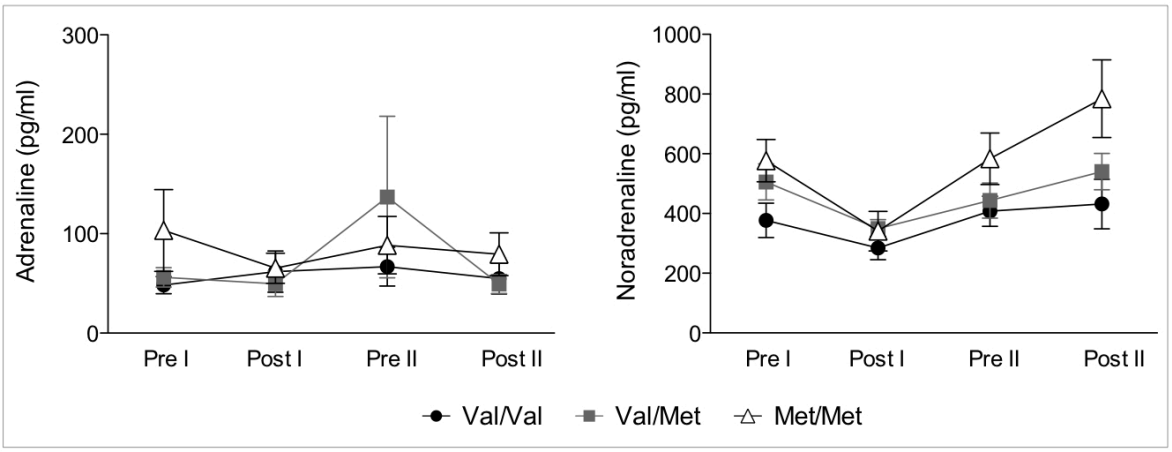

Fig. 11: Adrenaline and noradrenaline concentration in plasma before and after *Medication* and "*Placebo*" intake

Participants of all three genotype groups did not differ in either adrenaline or noradrenaline concentration in plasma at all measured time points. Data were analyzed with multivariate ANOVAs. Line charts represent mean ± SEM; all p>0.05

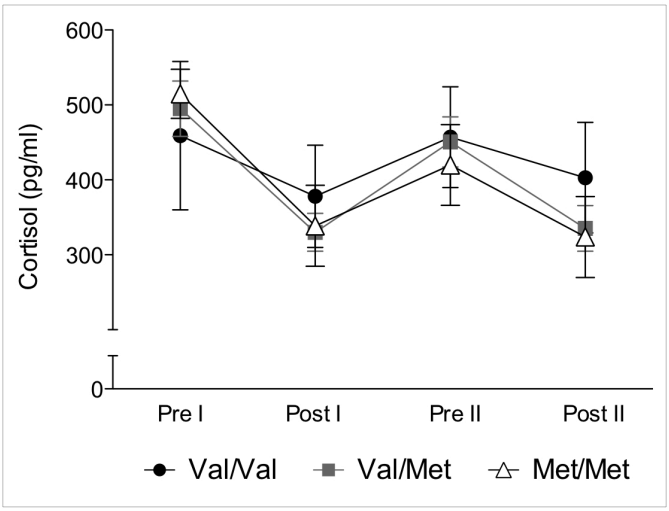

Fig. 12: Cortisol concentration in plasma before and after *Medication* and "*Placebo*" intake

Individuals of all three genotype groups did not differ in cortisol concentration in plasma at all measured time points. Data were analyzed with multivariate ANOVAs. Line charts represent mean ± SEM; all p>0.05

Lastly, cardiovascular parameters (systolic and diastolic blood pressure, heart rate) were measured before and after *Medication* and *"Placebo"* intake, in order to identify any side effects caused by CsA intake (*Post I*) or any physical arousal during study participation (*Pre I – Post II*), using multivariate ANOVAs. There were no differences detected in any of the three groups (Figure 13, 14).

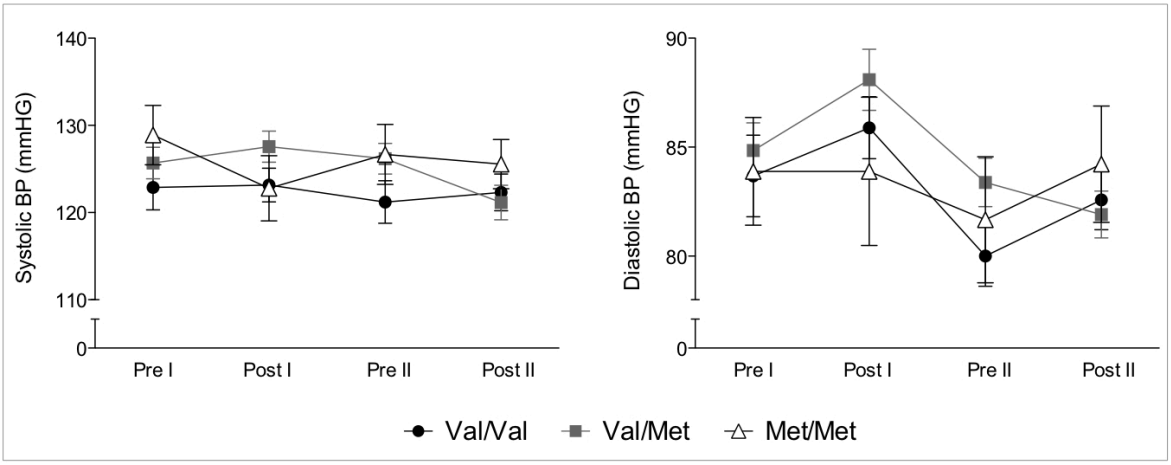

Fig. 13: Systolic and diastolic blood pressure before and after *Medication* and "*Placebo*" intake

Participants of all three genotype groups did not differ in systolic and diastolic blood pressure at all measured time points. Data were analyzed with multivariate ANOVAs. Line charts represent mean ± SEM; all $p>0.05$

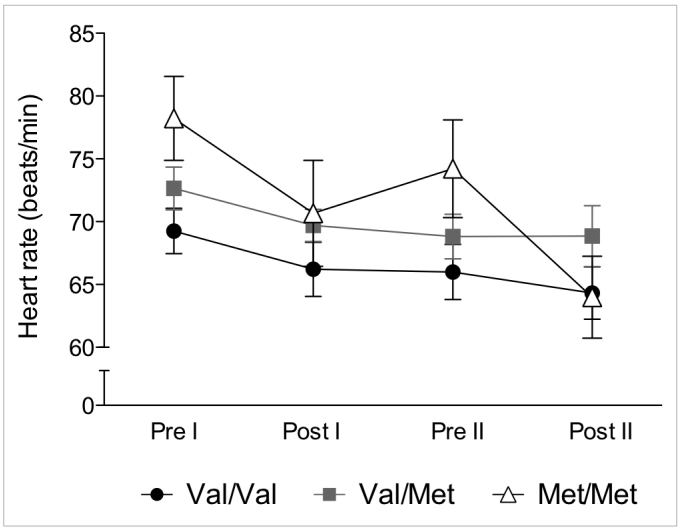

Fig. 14: Heart rate before and after *Medication* and "*Placebo*" intake

Participants of all three genotype groups did not differ in heart rate at all measured time points. Data were analyzed with multivariate ANOVAs. Line charts represent mean ± SEM; all $p>0.05$

Thus, the significantly highest reported specific and general treatment side effects in Val/Val allele carriers during *Medication* and "*Placebo*" intake is most likely not due to increased or decreased physiological responses to the treatments. Together, these data suggest that individuals of the Val/Val genotype have a higher somatosensory sensibility, more negative beliefs about medicines and experience more specific and general side effects after *Medication* and "*Placebo*" intake.

4 Discussion

4.1 Somatosensory amplification, beliefs about medicines and the COMT Val^{158}Met polymorphism as predictors of nocebo responses

Finding psychological and biological variables that can predict nocebo responding within medical treatment contexts is of high value, as nocebo effects have been shown to contribute to decreases of drug efficacy and increases of side effects. Furthermore, the recognition of placebo and nocebo responders within drug testing trials will be invaluable for estimating the real drug effects, as placebo responders may contribute to an underestimation of drug effects, whereas nocebo responders may lead to an overestimation of adverse medical side effects.

Only few psychological predictor variables have been identified, such as somatosensory amplification and the beliefs about medicines (Davis et al., 1995; Nestoriuc et al., 2010), whereas biological and/or genetic predictor variables are to this date lacking. However, gene polymorphisms, for example in the COMT gene, have recently been linked to placebo responding, in which individuals with the Met/Met genotype of the COMT Val^{158}Met polymorphism have shown the strongest placebo response (Hall et al., 2012). Therefore, the experiment underlying this thesis sought to identify psychological and genetic predictor variables of nocebo responses, with the aim being to minimize nocebo effects and to increase medical treatment benefits for patients.

In order to identify nocebo predictor variables, an established design of a study program on behavioral conditioning of immune functions was utilized (Albring et al., 2014). The unique advantage of this design is the ability to analyze intra-individual nocebo responses, after the intake of the immunosuppressive drug CsA during the acquisition phase as well as after placebo intake during the evocation phase. Reported CsA-specific and general side effects during *Medication* and "*Placebo*" intake were analyzed in relation to the respective homocygotic (Val/Val; Met/Met) or heterocygotic (Val/Met) genotype of the COMT Val^{158}Met polymorphism, as well as the degree of somatosensory amplification and negative beliefs about medicines.

This experiment showed for the first time, that significantly more CsA-specific as well as general side effects were experienced by individuals with the Val variant during medication and "*Placebo*" intake. In addition, homocygotic Val/Val carriers showed a significantly higher disposition to identify naturally occurring somatic and visceral sensations as being harmful, unpleasant, and very strong, which was measured with the SSAS. They also reported more negative beliefs about medicines, judging the intake of medicines as a potential danger, having concerns about side effects, as well as accumulated long-term effects, which were measured with the BMQ. Numerous studies have associated more negative beliefs about medicines with lower medical treatment adherence (reviewed in Nestoriuc et al., 2010). No differences between the three genotype groups were found in the scales BMQ_general overuse, which assess the perception that doctors prescribe medicines too much and within the BMQ_general benefit, which measures the belief that a beneficial treatment can be achieved through medicines. These subscales assess the behavior of doctors and positive attitudes towards medicines and are not related to the higher negative belief about medicines reported from individuals with the Val variant.

The differences in nocebo responses were not due to a range of possible interfering variables, as participants did not differ in sociodemographic and psychological trait variables, such as pessimism, trait anxiety and physical characteristics (physical activity, body mass index), which could have affected an increased experience of side effects. Differences in cardiovascular or neuroendocrine parameters (adrenaline, noradrenaline, cortisol plasma levels) were also not responsible, which could have been indicators of an increased stress reaction to the experimental procedure or medication intake. Moreover, CsA levels in whole blood were continuously surveyed and no increase in blood pressure, a side effect of CsA treatment (Mihatsch et al., 1989), was observed. The CsA levels were washed out after the five-day break and were therefore not responsible for continuously reported side effects when "*Placebos*" were taken. As an indicator of the immunosuppressive effects of CsA, immunological parameters (IL-2 production, IL-2 mRNA expression, proliferation rate of $CD4^+$ T cells, $CD3^+$, $CD3^+CD4^+$ and $CD3^+CD8^+$ cells) were monitored with no difference noted between the three genotype groups, which could have explained CsA-specific and general side effects.

Together, these data suggest that COMT Val^{158}Met, specifically the Val/Val genotype, higher degrees of somatosensory amplification and more negative beliefs about medicines are potential psychological and genetic predictor variables for nocebo responses. This is primarily true for the specific model employed here, in which healthy male subjects received a short-term treatment during the *Medication* period with an immunosuppressive drug; the calcineurin inhibitor CsA. Whether and to what extent this is a generalizable phenomenon and transferable to other drugs and/or patient populations (Furmark et al., 2008; Hall et al., 2012; Leuchter et al., 2009), needs to be investigated. Nevertheless, somatosensory amplification was associated with increased side effect reporting after the intake of placebos and antidepressants (Davis et al., 1995) and negative beliefs about arthritis medication predicted side effect occurrence within patients after taking them (Nestoriuc et al., 2010). The COMT polymorphism predicted placebo responses in patients with irritable bowel syndrome and depression (Hall et al., 2012; Leuchter et al., 2009). These observations argue for a role of COMT, somatosensory amplification and beliefs about medicines in nocebo responses; also for other physiological systems and diseases.

4.2 CsA-specific and general side effects

The reason for more reported CsA-specific and general side effects from homocygotic Val/Val allele carriers in this study remains unclear. The Val form catabolizes dopamine three to four-times more effective than the Met form (Lachman et al., 1996), consequently leading to significantly lower concentrations of prefrontal dopamine in Val/Val carriers compared to Met/Met carriers. This different availability of prefrontal dopamine seems to affect processes associated with the nocebo effect, such as pain and memory function (reviewed in Hall et al., 2012) in general, but also led to a more pronounced placebo response in Met/Met allele carrying patients with irritable bowel syndrome (Hall et al., 2012). A possible relationship between a smaller amount of available dopamine in the prefrontal cortex (Val/Val individuals) and a more pronounced nocebo response needs to be investigated in future studies.

Study participants with the Val variant reported 50% more CsA-specific side effects during the “*Placebo*” treatment compared to the intake of the drug CsA dur-

ing *Medication*, which were solely explained by the COMT polymorphism as a covariate. This increase may be related to the longer time frame during the "*Placebo*" phase, where participants were continuously reminded every twelve hours, by the investigators, of the four CsA-specific side effects. Studies showed that the more information is given about potential side effects, the more they develop (Wise et al., 2009). An increase of side effects from the *Medication to the* "*Placebo*" phase was not found within general side effects. This may be due to more general psychological and physical indispositions measured with the GASE, such as dejectedness, back pain, palpitation, irritability and sexual problems. Furthermore, the GASE was only filled out after every seven days and investigators did not verbally inform individuals about these general side effects.

Val/Val individuals had significantly more general symptoms measured with the GASE one week before study entry (GASE_Baseline) and patients with baseline somatic symptoms, have been shown to experience more adverse effects after medical treatments (Papakostas et al., 2004). However, more general side effects at Baseline did not predict general side effects at *Pre II* and *Post II*. Here, the somatosensory amplification scale had a significant effect on the experience of general side effects as a covariate. The increased somatosensory awareness, within the Val/Val group may have led to an attribution of already existing symptoms to the taken medication.

The number of valine alleles in COMT Val^{158}Met was linearly related to the amount of reported general side effects at Baseline, *Pre II* and *Post II*, where individuals with the Val variant reported most side effects; the Val/Met variant intermediate and the Met variant reported the fewest. This additive effect of the COMT genotype was not shown for the CsA-specific side effects at *Post I* and *Post II*, where the Val/Met group reported more CsA-specific side effects than the Met/Met group, although this was not statistically significant. This may be due to the small sample size and to the differently measured side effects and requires further investigation.

4.3 Expectations and the prevalence of side effects

Participants of this study equally believed the cover story of this experiment, that the probability of receiving CsA was always 50% during three of four (after four

CsA and after five and ten placebo intakes) estimation ratings of pills containing CsA. There was a significant difference between Val/Val and Val/Met individuals only after the last four placebo intakes, where Val/Val individuals thought that 48% of these capsules contained CsA, whereas Val/Met individuals estimated that 27% contained the drug. Remarkably, participants had the highest statistically significant estimation of 62% of pills containing CsA after the *Medication* phase compared to the other three time points, during the "*Placebo*" phase, where the estimation decreased from 38% to 34% and lastly to 33% (section 3.2.4). Nevertheless, the reported CsA-specific side effects increased by 50% for carriers of the Val/Val homozygote during "*Placebo*". It appears likely that more negative beliefs about medicines and a higher somatosensory amplification may have led to the increased experience of side effects, instead of the expected amount of taken medication.

In this study a control group (n=15) was included, which only received placebos throughout the whole study. This group serves as a control to rule out that the reported side effects during the "*Placebo*" phase are based on the previous intake of CsA, which could have induced expectation and classical conditioning of side effects. Subjects of the control group also reported CsA-specific and general side effects during the *Medication* phase, but were not included for further genotype analysis, as these were significantly less compared to the other three experimental groups (groups 1-3), which received CsA. However, after 14 placebo intakes there was no difference in reported CsA-specific and general side effects. Therefore, the encounter with the drug CsA during the *Medication* phase did not appear to influence the reported side effects during the "*Placebo*" phase. These are rather due to the identified psychological and genetic variables within this experiment. This is in line with a recent finding, where the expectation of receiving CsA, although placebo capsules were taken, caused the experience of CsA-specific side effects (Wendt et al., forthcoming).

4.4 Limitations of this study

There are a few limitations within this study. Firstly, the findings are limited due to the small number of young and healthy male study subjects and have to be thus interpreted with caution. In the future, female participants should be included, as

they have been shown to report more nocebo effects within various experiments (Lorber et al., 2007; Ströhle, 2000). However, these effects may be partly due to the fact that males report less adverse effects to female experimenters (Flaten et al., 2006) and within this study, 80% of the investigators were females. The validity of the identified predictor variables need to be further tested within a patient population that had frequent encounters with medical treatments and who may also display higher values of somatosensory amplification and have more negative beliefs about medicines.

Additionally, the information material and systematic procedure of requesting side effects could have led to an increased reported amount. Participants received information leaflets about CsA-specific side effects before study entry and were informed every twelve hours about common CsA-specific side effects. Numerous studies measured increased reported side effects within informed individuals compared to uninformed individuals (Lang et al., 2005; Varelmann et al., 2010; Wise et al., 2009). In addition, they received questionnaires for CsA-specific and general side effects (GASE), which may have contributed to increased side effect reporting as well. This was shown in studies which measured more reported side effects from individuals which were systematically asked or filled out standardized questionnaires about side effects compared to those which freely reported them (Rief et al., 2006).

Another limitation to consider is that two of the measured CsA-specific side effects can be defined as unspecific, such as fatigue and discomfort in the intestine and stomach and may have occurred independently from the experiment. Such symptoms also develop in individuals, which do not take any medication (Reidenberg and Lowenthal, 1968). Therefore, in future studies a “no treatment control group” should be included which only takes placebo capsules throughout the whole study in an open label procedure, in order to test if those symptoms occur independently from the experiment.

Lastly, this study could not confirm previous identified psychological nocebo predictor variables, such as anxiety and pessimism (Elsenbruch et al., 2012; Geers et al., 2005). This is probably due to the large diversity within methods, experimental setup and physical systems studied of research projects analyzing the nocebo re-

sponse. Furthermore, as outlined in section 1.3, multiple factors influence the nocebo response, which is why it is also not likely, that a single gene polymorphism alone can explain a complex behavioral response mechanism, such as the nocebo effect. Rather, further factors are involved, as was shown in a study, where the placebo response of individuals with the Met variant was enhanced through a positive doctor-patient relationship (Hall et al., 2012). Future investigations should nevertheless include identified predictor variables throughout different experiments and within the clinical context to test their reliability. However, if the mechanisms steering the nocebo response are carefully considered before a medical treatment is initiated and individuals with an increased risk of developing nocebo effects, identified through psychological and biological variables, receive a personalized medical treatment, the nocebo response can be reduced.

4.5 Utilizing associative learning processes and expectations to decrease nocebo responses

The placebo effect has been shown to positively influence the outcome of medical treatments through associative learning processes and expectations (reviewed in Enck et al., 2013). Through associative learning models, it has been possible to reduce clinical medication dosages, while at the same time maintaining their effects on the physical system. This was achieved through partial reinforcement within behavioral conditioning paradigms, in which the full dose of medication is given during the acquisition phase paired with a CS. Subsequently, during the evocation phase the CS is given again, but the medication dosage is decreased by intermittently replacing it with a placebo (Doering and Rief, 2012). Similar effects can be achieved through reconsolidation models of behaviorally conditioned pharmacological responses, in which subtherapeutic medical dosages are given during the evocation phase (Albring et al., 2014). In addition, positive expectations of treatment outcomes have influenced invasive medical interventions in beneficial ways for patients undergoing cardiac and orthopaedic surgery and for patients with Parkinson's disease receiving deep brain stimulation (reviewed in Enck et al., 2013).

Associative learning processes and expectations have demonstrated opposing effects for placebo and nocebo responses (Bingel et al., 2011; Varelmann et al.,

2010), which can be utilized to prevent the development of nocebo responses before a medical treatment is initiated. Numerous studies have shown that through positive verbal information and observational learning, a placebo response can be elicited instead of a nocebo response (Bingel et al., 2011; Colloca et al., 2008; Varelmann et al., 2010). Additional factors inducing nocebo responses (section 1.3) can also be preventatively modified, as psychological predictors of placebo and nocebo responses are often elicited and modified through environmental factors (Geers et al., 2005; Petrie et al., 2005). Symptoms and side effects may be the somatic manifestation of a negative affective state, stress or anxiety (Ferguson, 1993). These can be modified through a positive doctor-patient relationship, as this may activate neural mechanisms underlying the placebo response. This has been shown in patients with irritable bowel syndrome, when the doctor switched from a technical and short to a warm and empathetic interaction, resulting in a placebo response increase from 42% to 82% (Kaptchuk et al., 2008). Informed consents and patient information leaflets usually include numerous possible side effects, which are given to the patient before the onset of a medical treatment (Colagiuri et al., 2012), although this has been shown to induce nocebo effects (Lang et al., 2005; Varelmann et al., 2010; Wise et al., 2009). They should rather include beneficial treatment information, so that positive expectations are developed.

Besides minimizing factors that may induce nocebo responses, another approach is the identification of individuals with higher risk factors, to develop nocebo responses and offer them a personalized medical treatment.

4.6 Future outlook: Personalized medical treatments

Individuals with higher risks of developing nocebo responses could be identified conveniently and inexpensively through brief questionnaires, such as the SSAS and BMQ and/or the determination of the respective COMT genotype. These individuals may in turn receive a “personalized treatment”, instead of a common medical regimen. This can be achieved through the usage of a “contextualized informed consent”, education about the nocebo effect and re-attribution techniques of physical symptoms.

The usage of a “contextualized informed consent” offers a compromise between the judicial need of informed consent and the prevention of nocebo effects at the same time. The information given to the patient is adjusted so that neither the transparency of the procedure nor the principle of nonmaleficence, are compromised. Unspecific and harmless side effects are not mentioned; in order to avoid nocebo effects, whereas specific adverse effects are carefully explained so that the patient can decide if they want to take the drug (Wells and Kaptchuk, 2012).

Nocebo education in the health care system has been neglected, as three quarters of health care professionals are unaware of the nocebo effect (Berthelot et al., 2001). This can have negative implications, as the occurrence of side effects within individuals participating in drug trials have shown to represent the expectations of the investigators in both drug and placebo groups (Amanzio et al., 2009). Patients unaware of the nocebo effect, receive information about it before a treatment is initiated and with their consent, information limited to possible mild and transient side effects is given (Miller and Colloca, 2011).

The nocebo response may be partly due to the re-evaluation of already existing physical symptoms, common among healthy individuals. Various symptoms occurring within the last three days of being questioned, have been reported by 73% of 236 healthy individuals, who did not take any medication (Khosla et al., 1992). Therefore, the potential to attribute adverse effects to drugs or medical treatments, by misattributing already existing physical symptoms is large. Individuals with high scores within the SSAS, could receive re-attribution techniques, to learn to view somatic symptoms as naturally occurring, as well as techniques to reduce stressful reactions towards these symptoms.

In conclusion, through minimizing factors that may induce nocebo responses, as well as through implementing personalized medical treatment regimens for individuals with higher risk factors in developing nocebo responses, medical treatment efficacy can be increased and the burden caused by side effects minimized for the patient’s benefit.

Summary

Nocebo-induced side effects play a significant role in drug discontinuation in clinical practice, thereby negatively affecting treatment efficacy as well as patient adherence and compliance. Due to the large interindividual variability in nocebo responses, the goal of this thesis was to identify nocebo predictors in order to minimize nocebo effects and increase medical treatment benefits for patients. Psychological predictors, such as beliefs about medicines and somatosensory amplification have previously been linked to nocebo responses; however, to this date there are no known biological predictors. Recently, genetic polymorphisms in the catechol-O-methyltransferase gene (*COMT*) $Val^{158}Met$ have been identified as potential biomarkers of placebo responses.

Utilizing the unique model of behaviorally conditioned immunosuppressive effects, intra-individual nocebo responses of 62 healthy male subjects were analyzed after the intake of an immunosuppressive medication (CsA) and "*Placebos*". Psychological, immunological and neuroendocrine parameters were analyzed and CsA-specific and general side effects were assessed before and after medication or "*Placebo*" intake. The three *COMT* genotypes were analyzed with respect to their experienced side effects. Significantly more CsA-specific as well as general side effects were reported from Val/Val carriers during medication and "*Placebo*" treatment compared to the other genotype groups; and they had significantly higher scores in the somatosensory amplification scale (SSAS) and the BMQ (beliefs about medicine questionnaire).

Together these data demonstrate potential psychological and genetic nocebo predictor variables. They may be utilized in decreasing adverse nocebo effects within medical contexts by implementing personal treatment regimens for individuals with a heightened risk of developing nocebo responses.

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Figures

Tables

List of abbreviations

ACC	anterior cingulate cortex
Am	amygdala
BMQ	beliefs about medicines questionnaire
CaN	calcineurin
CCK	cholecystokinin
cDNA	complementary deoxyribonucleic acid
CNS	central nervous system
COMT	catechol-O-methyltransferase
CR	conditioned response
CS	conditioned stimulus
CsA	cyclosporine A
CTA	conditioned taste aversion
DNA	deoxyribonucleic acid
EdU	5-ethynyl-2´-deoxyuridine
ELISA	enzyme linked immunosorbent assay
FACS	fluorescence-activated cell sorting
FFkA	Freiburger Fragebogen zur körperlichen Aktivität
fMRI	functional magnetic resonance imaging
GASE	Generic Assessment of Side Effects
HADS-D	Hospital Anxiety and Depression Scale- Depression
HBSS	Hanks' Balanced Salt Solution
HPA-axis	hypothalamus-pituitary-adrenal axis
HPLC	high performance liquid chromatography
IC	insular cortex
IFN-	interferon-
IL-	interleukin-
LOT(R)	revised Life Orientation Test
mRNA	messenger ribonucleic acid
MRT	magnetic resonance tomography
NF-AT	nuclear factor of activated T-cells
NS	neutral stimuli
PBMCs	peripheral blood mononuclear cells
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PET	positron emission tomography
PNI	psychoneuroimmunology
RNA	ribonucleic acid
RPMI	Roswell Park Memorial Institute
SEM	standard error of the mean
SSAS	somatosensory amplification scale
STAI	State-Trait Anxiety Inventory
US	unconditioned stimulus

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Curriculum Vitae

Der Lebenslauf ist in der Online-Version aus Gründen des Datenschutzes nicht enthalten.

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