Psychophysiological correlates of treatment effects – in search of potential biomarkers for the course of major depressive disorder

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Danksagung

Contents

List of tables

List of figures

Abbreviations

ANS	Autonomic Nervous System
APA	American Psychiatric Association
AUC	Area Under the Curve
AUCi	Area Under the Curve increase
AUCg	Area Under the Curve ground
BDI	Beck Depression Inventory
BDI-II	Beck Depression Inventory - II
BDI 6WF	Beck Depression Inventory six week follow up
BDI 6MF	Beck Depression Inventory six month follow up
BMI	Body Mass Index
CAR	Cortisol Awakening Response
CAN	Cardiac-Autonomic-Network
CBT	Cognitive Behavioral Therapy
CVD	Cardiovascular Diseases
Df	Degrees of freedom
DSM	Diagnostic and Statistical Manual of Mental Disorders
ECG	Electrocardiogram
HF	High Frequency
HiTOP	Hierachical Taxonomy Of Psychopathology
HPA	Hypothalamic Pituitary Adrenal
HRSD	Hamilton Rating Scale of Depression
HRV	Heart Rate Variability
ICD	International Statistical Classification of Diseases and Related Health Problems
LF/HF Ratio	Low Frequency / High Frequency Ration
M	Mean
Max	Maximum
MDD	Major Depressive Disorder
Min	Minimum
N	Sample Size
PFC	Prefrontal Cortical
PTSD	Post-Traumatic Stress Disorder

RMSSD	Root Mean Sum of Squared Distanace
SD	Standard Deviation
SDNN	Standard Deviation of RR-Intervals
SSRI	Selective Serotonin Reuptake Inhibitors
tDCS	Transcranial direct current stimulation

Abstract

Major depressive disorder (MDD) is still one of the most common and highly debilitating mental disorders. Although there are several treatment possibilities, the relapse rate is still high and some patients even suffer from treatment-resistant depression. First research results suggest that the measurement of psychophysiological correlates ("biomarkers") of MDD might be promising as a means to identify high-risk subgroups for poor outcomes in respect of MDD treatment. However, findings about the association between these correlates and MDD are still inconsistent, especially in naturalistic settings.

To address this research gap, this dissertation focusses on assessing the explanatory value of psychophysiological correlates of depression to predict the course of MDD after inpatient treatment. This research employs two psychophysiological correlates of MDD that are typically used: Heart Rate Variability (HRV) and Cortisol Awakening Response (CAR). The overarching methodological goal that guided the studies within this dissertation is to use multiple measurement designs to increase the robustness of psychophysiological data in naturalistic settings.

Vagally-mediated HRV is seen as a psychophysiological marker for mental health and MDD. However, up to now, little has been known about the association between HRV and the severity of depression and whether this association mirrors treatment effects following inpatient psychotherapy. Therefore a multiple measurement study was conducted to assess the association between the severity of MDD symptoms and HRV before and after therapy. The sample consisted of 50 patients suffering from moderate to severe MDD. HRV was assessed three times at the beginning of, and three times at discharge from, inpatient psychotherapy. Depressive symptoms were assessed by self-reports (Beck Depression Inventory, BDI-II) and a third-party questionnaire (Hamilton rating scale for depression, HRSD) at the beginning of psychotherapy and at discharge. Results confirm an expected negative correlation between HRV and depressive symptoms at the beginning of the inpatient treatment. At discharge, results show a de-coupling between HRV and symptom severity: Depressive symptoms improve significantly (d=0.84) without corresponding changes in HRV as a psychophysiological indicator.

CAR reflects a dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis which is associated with MDD. Initial research results suggest that CAR resembles the accumulated effects of MDD and therefore might be a predictor for the course of depression after discharge. Therefore CAR was measured at intake to inpatient psychotherapy. The BDI was assessed at

four time-points (intake, discharge, 6 weeks after discharge and 6 months after discharge). The sample included 123 inpatients diagnosed with MDD. The results show that a blunted CAR at intake predicts mood deterioration six weeks and six months following discharge.

Due to a lack of comparative data, and to verify these initial results regarding the association of CAR and MDD, a replication study was conducted. The replication study used an improved methodology with stricter assessment protocols and monitoring. The sample included 122 inpatients diagnosed with moderate to severe MDD. CAR and self-ratings were assessed at the same measurement points as in the original study. Results could be replicated in terms of nearly identical effect sizes but do not reach statistical significance ($p=.054$). The replication of effect sizes with a concurrent lack of statistical significance may well inform research on psycho-endocrinological predictors for treatment success, but raises the question of practical relevance for CAR as a predictor for the further course of MDD.

Results are discussed for each study individually and on an aggregate level with respect to the overarching research question. Taken together, they show that the associations between biological and psychological aspects are very complex and are influenced by many factors. Robust measurement designs are required to improve scientific understanding. However, when measured strictly, additional information regarding the association between biomarkers and the course of depression can be gathered. However, the explanatory power of a single biomarker seems to be too small to have clinical impact. Therefore biomarkers can be viewed only as an additional source of information to predict treatment effects.

1. General Introduction

"Unter allen Leidenschaften der Seele bringt die Trauer am meisten Schaden für den Leib."
(Thomas von Aquin)

1.1 Major Depressive Disorder

Major Depressive Disorder (MDD) is one of the most common, and highly debilitating, mental disorders. It is typically a complex and long-term disorder with an overall significant reduction in quality of life, a high suicide rate and high economic burden costs (Murray et al., 2012). Compared to healthy people, the mortality rate for depressive patients is doubled (Ösby et al., 2001). The main criteria for depressive disorder in the Diagnostic and Statistical Manual of Mental Disorders (DSM 5) are low mood and a general disinterestedness for at least two weeks (American Psychiatric Association (APA), 2013). Other depressive symptoms are, for example: difficulty in concentrating, sleeping disorders and/or restlessness. Depression affects the body as well, which is also shown by MDD development and maintenance models, including additional biological aspects like genetic variables and hormones (Beck & Bredemeier, 2016; Hyde et al., 2008). Symptoms such as disturbance of sleep and eating patterns or changes in blood values are also mainly biological aspects of depression. Depression is not just about a pronounced sadness, but about disturbances of the entire organism with symptoms on the emotional, cognitive, physiological, motor, social-interactive and behavioral level (Wittchen & Hoyer, 2011).

Findings indicate an association between MDD and several physical illnesses (Kang et al., 2015; Kemp & Quintana, 2013). For example, MDD is strongly associated with cardiovascular diseases (CVD), the primary cause of mortality worldwide (Christopher & Murray, 2016). CVD include diseases like heart failure, stroke or peripheral arterial diseases (National Health Service, 2021). Studies show that CVD and MDD are bidirectionally associated (e.g. Nicholson et al., 2006): On the one hand MDD patients are more likely to die because of a myocardial infarct (Pratt et al., 1996; van der Kooy et al., 2007) or a stroke (Pan et al., 2011). On the other hand it is estimated that approximately one in five CVD patients suffers from at least one depressive episode (Carney et al., 1987). Patients suffering from both disorders have a poorer prognosis even though there are several treatment options (Goldston & Baillie, 2008).

The further development of treatment options aims to build on understanding causal relationships and the biological basis of the development and maintenance of depressive symptoms. Research into neurobiological and psychophysiological factors in MDD has thus become a field of research with increasing relevance in recent years (e.g. Enneking et al., 2020; Kemp et al., 2008; Lopresti et al., 2014). Since there is a comorbidity between physical illnesses and MDD (or the respective neurobiological correlates), one possible promising approach is to take the physical aspects more into account in order to improve and evaluate treatment approaches. In particular, changes in the hypothalamic-pituitary-adrenal (HPA) axis have received most attention due to the well-known role cortisol plays in stress reactions (Murphy, 1991). Therefore, for many years, numerous studies have dealt with the interaction between psychotherapy and cortisol release in depressed patients (e.g. Hammen et al., 1992; Jones et al., 2015; McKnight et al., 1992). These have shown that cortisol is associated with symptom severity of depression and might be a possible predictor for treatment outcomes.

1.1.1 Epidemiology of MDD

In 2020, an estimated 264 million people were affected by depression worldwide (World Health Organization, 2020). MDD is the second most common disorder (after CVD), with great economic and social consequences (Murray & Lopez, 1996). In Germany, 8.2 % of the total population between 18 and 79 years (i.e. 5.3 million adult people) developed unipolar or persistent depressive disorder within one year (Jacobi et al., 2014). The lifetime prevalence of depression is estimated at 16-20% (Bijl et al., 1998; Ebmeier et al., 2006; Jacobi et al., 2004; Kessler et al., 2012). However, it should be noted that the prevalence figures vary greatly, depending on how the diagnosis was carried out. The prevalence numbers of MDD collected on the basis of clinical diagnoses by general practitioners were significantly lower. In the medical care system and in physically ill people, an even higher prevalence of depressive disorders can be assumed (Moussavi et al., 2007).

Depression is a disorder that affects women twice as often as men (approximate lifetime prevalence: Women = 25%; Men = 12.3%; see Härter et al., 2017; Jacobi et al., 2004). The risk of developing MDD is rather low in childhood to mid-adolescence and then increases steadily into advanced adulthood (Jacobi et al., 2004).

The course of the depression disorder shows great inter-individual variability (Härter et al., 2017). Typically, MDD is characterized by an episodic course: the episodes of symptoms

are limited in time and often remit completely or partially, sometimes even without antidepressant treatment (Ustun et al., 2004). On average, one episode lasts 6-8 months without medication (Berger & van Calker, 2004). Different antidepressant treatments are able to reduce the episode length and symptom severity (Härter et al., 2017). Within the entire life of depressed patients, at least half of them suffer from at least one further depressive episode after an initial depressive episode (Murray & Lopez, 1996). Recurrent depressive disorders have an average cycle length of five years. Fifteen percent of depressed patients suffer from a chronic depressive disorder, i.e. depressive symptoms without remission or partial remission for more than two years (Eaton et al., 2008; Spijker et al., 2002).

Analyzes at annual intervals show that, depending on the type of treatment, the risk of relapse is 30-40% within the first year (Belsher & Costello, 1988). After a two-year interval the risk of relapse increases to 40-50 % (Hautzinger & Jong-Meyer, 1996). Previous depressive episodes or only partial remission also increase the likelihood of further episodes and of a poor prognosis for the disorder (Judd et al., 1998; Kupfer, 1991).

1.1.2 Diagnosis and Diagnostic Challenges

The diagnosis of mental illnesses such as MDD is based mainly on self-assessments and clinical judgements. The consequence of this is a high susceptibility to subjectivity and variability (Young et al., 2016). Therefore, in order to diagnose MDD, at least one structured interview is required (APA, 2013). This interview takes into account the diagnostic criteria of depressive symptoms and exclusion criteria (e.g. exclusion of dysthymia, depressive symptoms as a result of somatic diseases, or drugs / toxins) as well as temporal patterns (episodic, chronic, recurrence interval, relapse rates etc.). The main criteria for the diagnosis MDD are low mood, and a general disinterestedness (DSM 5). The DSM 5 prescribes for a MDD diagnosis that at least five of the following depressive symptoms must have been present for at least two weeks: (1) depressed mood, (2) a general disinterestedness, (3) loss/more appetite, (4) sleeping disorder, (5) psychomotor retardation/restlessness, (6) fatigue or loss of energy, (7) feelings of worthlessness or guilt, (8) difficulties to think or concentrate, or (9) recurrent thought of death. Either symptom 1 or 2 are mandatory (APA, 2013).

In addition to a structured interview there are various self-assessment questionnaires or third-party questionnaires available to assess the severity of the depression, e.g. Beck-Depres-

sion-Inventory (BDI; Beck et al., 1996a), Hamilton Rating Scale of Depression (HRSD; Hamilton, 1960) or General Depression Scale (German: Allgemeine Depressions Skala; Stein & Luppa, 2012).

Until now, symptoms were the most common way to describe MDD, but without any psychophysiological correlates being required inside the definition or diagnostic system (APA, 2013). This must be viewed critically, since many patients suffer from physical symptoms (e.g. deviations in serotonin and dopamine levels or an overactivity of the HPA axis (Borsboom et al., 2019, Murphy, 1991). In Germany, most patients first consult general practitioners about various unspecific physical symptoms (Paykel & Priest, 1992; Tylee & Gandhi, 2005). A study from Wittchen and Pittrow (2002) shows that 11% of German general practitioners' patients fulfilled the aforementioned MDD criteria, but only half of the patients were correctly diagnosed as depressed. This shows that many depressed patients are not recognized as such and therefore do not have access to appropriate treatment or medication (Bramesfeld & Stoppe, 2006; Hach et al., 2003; Rost et al., 1998; Wittchen & Pittrow, 2002; Wittchen et al., 2010). International results show similar recognition rates (30-60% depending on depression test instrument) by general practitioners (Gilbody et al., 2003; Gilbody et al., 2005). The large number of misassigned or undiagnosed patients shows the difficulties of making a correct diagnosis for depressed patients. One explanation for this high number of undiagnosed or wrongly diagnosed patients seems to be that most patients primarily have a somatic disorder understanding and therefore first describe the physical symptoms of depression (e.g. loss of appetite and energy, or sleeping disorders) to their general practitioner without mentioning psychological symptoms (Becker & Abholz, 2005; Wittchen et al., 2000). In addition, there is still a great fear of stigmatization (Nature Editorial, 2013). By describing physical symptoms, the disease becomes tangible/real and requires medication. Apart from that, the high number of psychological comorbidities, e.g. 60% of MDD patients suffer from a comorbid anxiety disorder (Kessler et al., 2005) and physical comorbidities e.g. CVD or cancer (Kang et al., 2015) make correct diagnoses even more difficult.

Another difficulty in correctly diagnosing depression is that it is a very heterogeneous diagnosis with many different manifestations and groups of symptoms (Goldberg, 2011). There are around 227 ways to meet the diagnostic criteria of MDD (Zimmermann et al., 2015). When looking at the diagnostic criteria, it is also noticeable that patients with completely opposed symptoms of MDD (e.g. loss of or more appetite, restlessness or psychomotor retardation) receive the same diagnosis (DSM 5). In his review, Goldberg (2011) therefore describes various

approaches to subgroup formation, e.g. depending on somatic syndromes, panic attacks, obsessional traits, depression accompanying physical illnesses or pseudodementia depression in older patients. All of these exemplary subgroups will be classified as depressed and therefore get similar drugs and psychotherapy treatments even though the symptom clusters are very different.

1.1.3 Development and Maintenance Model of MDD

Until the present, different MDD disorder models have represented different aspects of depression and its treatment options: e.g. cognitive triad (Beck, 1967), cognitive model (Clark & Beck, 1999), the cognitive-behavioral disorder concept (Hautzinger, 2003) or the diathese-stress-model (Hammen, 2005)). The development and maintenance of MDD seems to be complex and has not yet been fully clarified. Therefore, a multi-causal disorder development and a multifactorial bio-psychosocial explanatory and disorder model are currently assumed, like the diathese-stress-model (Hammen, 2005) or the unified model of depression (Beck & Bredemeier, 2016). The latter integrates psychological and biological aspects in the development and maintenance of depressive disorders and describes the biological and psychological relationships which are analyzed in this dissertation. It integrates advances in neurobiology, genetic research and evolutionary perspectives within a framework (Clack & Ward, 2019). The unified model of depression is based on the cognitive triad model (Beck, 1967; Clark & Beck, 1999) but includes actual biological research results and biological-psychological interactions (Beck & Bredemeier, 2016). According to their model, the predisposition for MDD is attributed to four risk factors:

1. **Genetic risk:** Results from family and twin studies show that vulnerability for MDD seems to be inheritable (e.g. see Sullivan et al., 2000). In addition Caspi and colleagues (2010) pointed out that there seems to be a moderating effect from genetic polymorphism of the 5-HTT gene on the link between stress and depression.
2. **Early traumatic experiences**: A recent meta-analysis shows that early experiences especially like neglect or emotional abuse are associated with depression in adulthood (Mandelli et al., 2015). It could be assumed that an early trauma sensitized an individual for negative effects of subsequent stressors, which increases the risk for MDD (Heim & Binder, 2012; Kendler et al., 2004)
3. **Information processing biases**: Recent research results suggest that attention, memory and attributional bias are a result of a weakness of executive control mediated by an

impairment of the prefrontal cortex (Levin et al., 2007). Negative events and experiences are over-interpreted and in turn lead to negative views and expectations over time (Joormann & Gotlib, 2006), which can be seen as a vulnerability factor for MDD (Gotlib & Krasnoperova, 1998). Simultaneously information processing biases are common in depressed patients e.g. in the sense of selective attention to negative information and lower attention to positive information (Gençöz et al., 2001).

4. **Biological stress reactivity**: Among others, Pariante and Lightman (2008) point out that a dysregulation of the HPA axis increases the risk of MDD episodes via changes of cognitive functions (Keller et al., 2017; Pariante and Lightman, 2008). Apart from that, early traumatic experiences seem to be associated with changes in inflammatory processes during the Trier Social Stress Test (Pace et al., 2006). Thompson and colleagues (2011) suggest that biological reactivity to environmental stress seems to foster affective instability.

All factors influence each other via feedback loops. For example, it has been shown that an earlier trauma is associated with a reduced hippocampus volume (Rao et al., 2010) and a dysregulation of the HPA axis which represents a reduced biological stress reactivity (Tyrka et al., 2008). The way in which these predisposing factors are connected or interact is complex and not yet fully understood. However, all of the above-mentioned predisposing factors seem to promote the emergence of depressogenic beliefs about the self, the world and the future over time (comparable to the cognitive triad; Beck, 1967). Depressogenic beliefs simultaneously influence information processing and biological stress responses via feedback loops. Beck and Bredemeier (2016) emphasize that on the one hand single occurrence of one predisposition factor does not to lead to MDD, but rather that the interactions and presence over time lead to a manifestation of clinically relevant depressogenic beliefs. On the other hand the absence of one factor (for example genetic risk) does not protect against falling ill with depression.

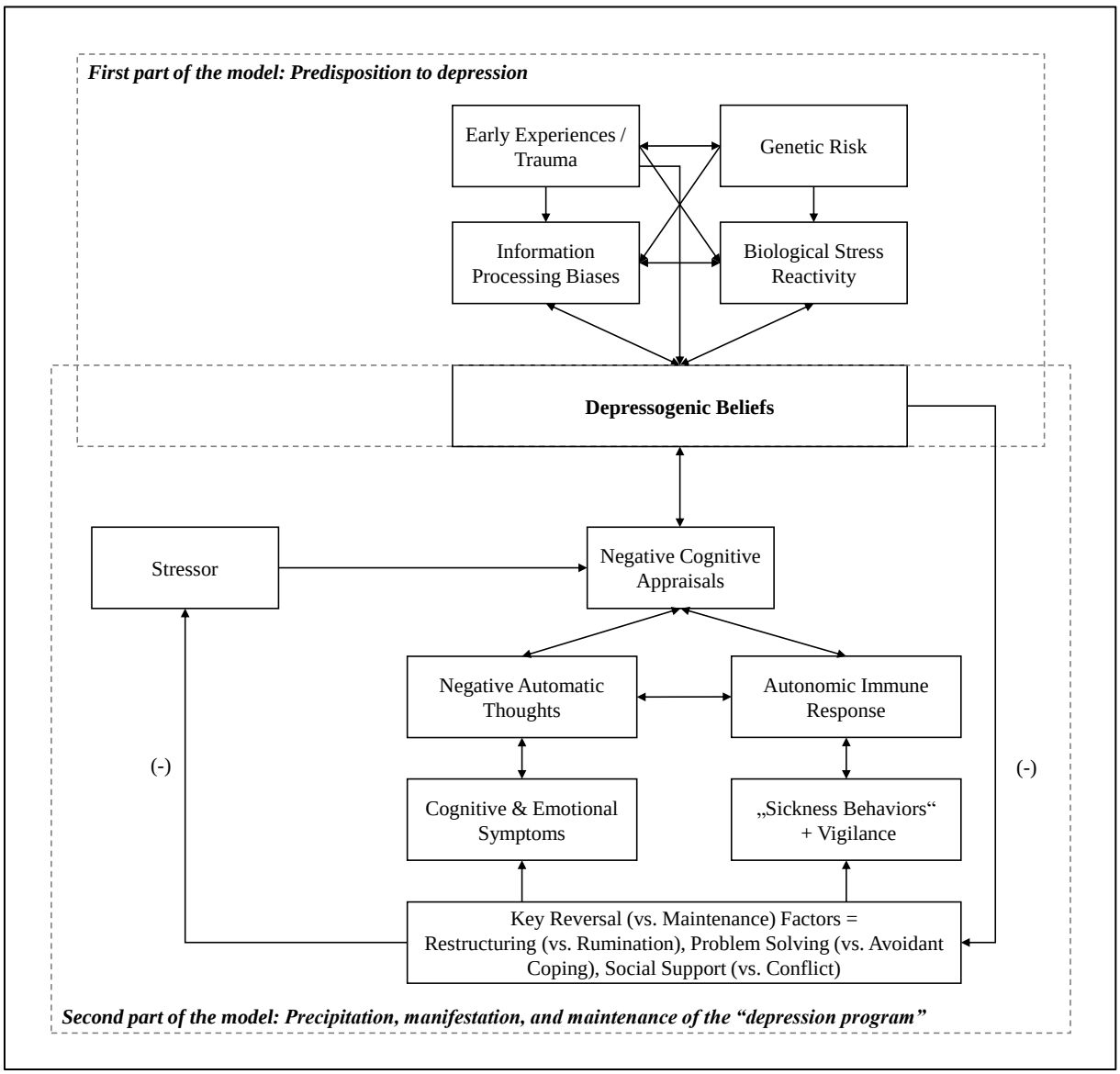
The second part of the unified model of depression describes the depressive program (Beck & Bredemeier, 2016). Depressogenic beliefs and external or internal stressors generate negative cognitive appraisals. These negative cognitive appraisals become clinically relevant when they are perceived as a strong “loss of an investment in a vital resource” (Beck & Bredemeier, 2016, p. 608), e.g. a person invests heavily in an important relationship and would be vulnerable to MDD if this relationship ends, because this end is perceived as a loss of important vital investment. The authors propose that both negative thoughts and biological aspects

are heavily influenced by negative cognitive appraisals. From a biological perspective, autonomic/immune responses are the key aspect in this part of the model. Several interactions with negative thoughts, cognitive/emotional aspects and sickness behavior lead to a manifestation and maintenance of typical MDD symptoms. Lastly, key reversal or maintenance factors influence both cognitive / emotional symptoms and sickness behavior (like social conflicts = maintenance vs. social support = key reversal). Due to the many mutual relationships, there are possible feedback loops from key reversal / maintenance factors to the aforementioned biological aspects in both parts of the model.

Figure 1.1 summarizes the complex relationship of factors and their mutual influence in the Unified Model of Depression (for details see Beck & Bredemeier, 2016):

Figure 1.1.

Unified Model of Depression (see Beck & Bredemeier, 2016)



1.1.4 Treatment of MDD

Not only the diagnosis (as mentioned above) but also an effective and sustained treatment of depressive disorders is currently still in need of improvement. Even if patients are correctly diagnosed as depressive, only 38% of them get an adequate antidepressant medication from general practitioners (Wittchen et al., 2000). And even in this case, most of them do not get any additional psychological or psychiatric treatment, even though there are many different treatment opportunities available (e.g. pharmacological/somatic or psychological therapy). The APA showed that antidepressant pharmacotherapy and cognitive behavioral therapy (CBT) are evidence-based effective treatments for MDD as a monotherapy or combination therapy (APA, 2010). In Germany, treatment guidelines specify which treatment approaches or treatment combinations should be used for which degree of severity and the course of the disorder (Härter et al., 2017), but without correct diagnosis this approach is difficult to implement. This could be one reason that, although there are several scientifically proven treatment options, for a high proportion of patients (up to 50%) these treatments fail to achieve full remission of MDD (Fava, 2003; Rush et al., 2006; Warden et al., 2007). Another explanation for this contradiction could be that evaluation studies of drugs or treatment protocols often include only patients with unipolar depression without somatic problems or comorbidities (Zimmerman et al., 2002). However, such a sample selection does not represent patients in the clinical setting who are characterized by complex comorbidities, somatic disorders or chronic disorder course. In clinical practice, too, there is an increasing number of antidepressants prescribed, but the evidence for the effectiveness of antidepressant drugs is weaker than is commonly assumed (Moncrieff, 2001). Apart from that, it is still unclear which patient will benefit from what kind of antidepressant medication (Thase, 2014) As a result, the the symptoms often worsen during this time of inadequate antidepressant treatment (Mago et al., 2018).

In addition, MDD is associated with a high relapse rate and recurrent depressive episodes, even after remission (Pintor et al., 2003). Therefore, the current psychotherapy and psychiatry research tries to find more predictors that may help to understand the course of depression and the respective influencing factors to improve treatment of MDD. A distinction is made between direct outcome measures, such as mood, anxiety, worries or satisfaction with treatment and biological measures, such as blood values, electrocardiogram data or body weight (Califf, 2018). Above all, the direct measurements that are assessed by the patient themselves are not entirely uncritical, as they can be influenced by many different factors like recent life changes, or a history of chronic and disabling physical or emotional problems (Garrity et al., 1978). For

these reasons, psychotherapy and psychiatry research in recent years have increasingly looked for physiological correlates of mental disorders – "biological markers" that can be measured objectively in order to get valid data to describe treatment response (Young et al., 2016). In addition, recent research suggests that such biomarkers might be able to identify subgroups at high risk of treatment-resistant depression and that would make psychological processes objectively measurable (Enneking et al., 2020, Strawbridge et al., 2017). Clinically useful biomarkers have to be specific for the disorder and sensitive to change. They might significantly improve an objective (differential-)diagnosis and treatment of psychiatric disorders like MDD (Brand et al., 2015; Young et al., 2016).

1.2 Biomarkers in psychiatry and psychotherapy research

Biomarkers are defined as "A characteristic that can be objectively measured and considered as an indicator of a normal biological process, a pathological process, or a response of an individual to a therapeutic intervention" (Biomarkers Definitions Working Group, 2001: p.91). Califf (2018) distinguishes between the following subtypes of biomarkers:

- **Diagnostic** biomarkers: Detection or confirmation of a disorder, subtype of disorder or condition of interest.
- **Monitoring** biomarkers: Serial measurements to assess the status of disorders.
- **Response / pharmacodynamic** biomarkers: Biomarker level changes in response to exposure to a drug, treatment or environmental agent. These changes in a physiological measure (e.g. change in heart rate) can provide early indications that a treatment may have an effect on a clinical endpoint or whether a medication is effective.
- **Predictive** biomarkers: Identification of people who are more likely than others without this type of biomarker to have a beneficial or adverse effect from exposure to the drug, treatment, or environmental factor. These physiological measures can provide early information that a treatment has a favorable effect on the course of disorder. Typically, these biomarkers are collected at the beginning or before an intervention and provide additional objective and reliable information that can be used to improve making appropriate treatment decisions (Enneking et al., 2020).
- **Prognostic** biomarkers: Identification of groups of people at high risk for a disorder. These biomarkers become commonly used to set eligibility and exclusion criteria for studies.

In the following studies the focus will be on response/pharmacodynamic and predictive biomarkers for MDD with the aim of consolidating and enhancing recent knowledge in a naturalistic setting.

Another perspective suggests dividing biomarkers into state, trait and endophenotypic markers (Hacimusalar & Esel, 2018). The authors suggest that trait markers are persistent, exist before the onset of a disorder and do not change after remission. These markers may help to classify people with a higher risk for a disorder and are therefore relevant in prevention programs (Le-Niculescu et al., 2021). Endophenotypic markers can be classified as a subgroup of trait markers (Hacimusalar & Esel, 2018). The authors suggest that these markers are based on the association between genes and specific phenotypes. These biomarkers are also persistent and found to be higher in family members of sick people than in healthy people (Gururajan et al., 2016). In contrast to trait markers, state markers are situation-dependent, related to clinical conditions and transient. The authors suspect that biomarkers in this group change with the onset of the disorder and return to a normal level after remission.

In the following studies, the focus will be on the discussion whether state and trait markers can be distinguished at all in a naturalistic setting.

1.2.1 Biomarkers of MDD

There are currently efforts in mental health research to analyze biological profiles. One of the main purposes is to summarize heterogeneous complex diagnoses like MDD into robust homogeneous subgroups (within and across categorical diagnoses) and thus to improve treatment guidelines (Strawbridge et al., 2017). Kapur and colleagues (2012) pointed out that biomarkers are the most promising candidates from which these subgroups could be formed. Hofman and colleagues (2010) show, for example, that heart rate variability (HRV) is able to distinguish between patients suffering from generalized anxiety disorder with and without a comorbid MDD. Currently, there are many possible candidates for biomarkers for MDD of research interest, e.g. proteomic markers like growth factors or inflammatory markers, endocrine markers like cortisol, structural markers like brain volume or functional markers like heart rate (for an overview see Hacimusalar & Esel, 2018; Strawbridge et al., 2017).

This dissertation focuses on two biomarkers which might be promising candidates in the context of MDD treatment (see the following chapters for details):

1. HRV as a well-established biomarker for autonomic control that might be useful to show MDD treatment response (Kircanski et al., 2019).

2. Cortisol Awakening Response (CAR) as a predictive marker for HPA axis functioning (Chida & Steptoe, 2009; Stalder et al., 2016). Recent research shows that CAR could be a useful predictive biomarker for the course of depression (Beck et al., 2015; Hardeveld et al., 2014).

In addition, the distinctive classification between state and trait biomarkers regarding both biomarkers in the scope of this dissertation will be discussed critically.

1.2.2 Response biomarker: HRV

Another relevant indicator for psychopathological abnormalities in MDD patients is the balance of the autonomic nervous system (ANS) (Beauchaine & Thayer, 2015). The ANS consists of an interaction between the activating sympathetic and the inhibiting parasympathetic systems. A good balance between the two systems is an essential feature for healthy organisms (McCorry, 2007). A low adaptivity of the ANS is an indicator for a sympathetic dominance and restricted parasympathetic tone. It has been associated with all-cause mortality (Kluttig et al., 2010; Thayer & Lane, 2009; Thayer et al., 2010), increased susceptibility to stress, emotional instability (Koval et al., 2013) and increased risk of cardiovascular and mental disorders, especially depression (Karavidas et al., 2007; Thayer & Lane, 2009). In addition, a model from Carney and Freedland (2017) shows that CVD and depression are also linked to each other. The authors describe two possible mechanisms to describe the association between depression and coronary heart disease: Biological mechanisms (such as altered autonomic nervous system activity, elevated catecholamine levels or elevated inflammatory activity) and behavioral mechanisms (such as medication use, physical inactivity or smoking cessation). The mechanisms linking depression and CVD are very complex and multifactorial, and not yet fully understood (Goldston & Baillie, 2008). However, patients suffering from MDD and CVD have a significantly worse prognosis in terms of cure. The link between depression and CVD is problematic as both disorders are associated with a long duration of illness, high relapse rates, ultimately more complex treatment and increased mortality (Carney et al., 1987; Fuller, 1935). Unfortunately, some antidepressant medications might even have unfavorable effects on heart rate and blood pressure (Licht et al., 2009). Thus, to develop and improve the treatment for both disorders it is important to learn more about the biological aspects of MDD and the bidirectional association to the cardiovascular system.

Sympathetic and parasympathetic neurons innervate the heart via the vagus nerve and the stellate ganglion (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Therefore, one possibility is to measure vagal activation as a part of parasympathetic activation (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). HRV has become an intensively-investigated research topic in health and disease research from 1960 up to now (Billman, 2011). HRV is the variation of the period between two consecutive heartbeats over time. It indicates the adaptivity of the heartbeat to changing inner and outer conditions and thus reflects the adaptivity of the ANS (Shaffer & Ginsberg, 2017). As a measure of cardiac-autonomic control, HRV serves as an indicator of the efficiency of cortico-cardiac interactions and adaptability to inner and outer circumstances (Thayer et al., 2012). In their model of neurovisceral integration, Thayer and colleagues described HRV in a dynamic systems framework (Thayer & Brosschot, 2005; Thayer & Lane, 2000). Within this cardiac-autonomic network (CAN, based on Benarroch, 1993), cognitive, emotional, behavioral and physiological responses are regulated and reflected by excitatory and inhibitory innervation of the heart (Thayer & Siegle, 2002). This reciprocal cortico-cardiac interaction is synaptically mediated by the brain, the sympathetic nervous system and the vagal branch of the parasympathetic nervous system in the periphery. This sympathetic-parasympathetic interplay is modulated by prefrontal cortical (PFC) areas and expressed as HRV (Thayer et al., 2012).

Low or decreased HRV has been associated with increased susceptibility to stress and emotional instability (Koval et al., 2013), increased risk of cardiovascular and mental disorder (e.g. depression) (Karavidas et al., 2007; Thayer & Lane, 2009) and all-cause mortality (Kluttig et al., 2010; Thayer & Lane, 2007; Thayer et al., 2010). With regard to depressed patients, HRV also appears to provide information about the severity of the disorder. The severity of depressive symptoms appears to be inversely related to HRV and this association does not seem to be attributable to medication side effects (Alvares et al., 2016).

Reviewing the scarce empirical evidence on HRV changes following depression treatments, the results appear contradictory. First research results in a naturalistic setting reported that severely depressed CVD patients benefit from CBT twice, namely by reduced depressive symptoms and an increased HRV (Carney et al., 2000). Riffer and colleagues (2016) supplement the findings to the effect that the psychological improvement was mainly associated with changes in the parasympathetically innervated parameters of the HRV. Two studies found no improvement in HRV variables even though the psychotherapy (mindfulness-based psychotherapy) was successful (Wheeler et al., 2014). Similarly, Brunoni and colleagues (2013) found

no HRV improvement after an antidepressant treatment with Selective Serotonin Reuptake Inhibitors (SSRI) or transcranial direct current stimulation regardless of clinical response. Those studies reporting no changes in HRV following symptom reduction explained this absence mostly with a delay of physiological changes following psychological changes or with sample characteristics (Licht et al., 2010). Only severely depressed patients seem to profit twice: through reduction of depressive symptoms and increase of HRV (Agelink et al., 2002, Carney et al., 2000). Brunoni and colleagues (2013) suspect that an increased HRV is more of a trait-marker for MDD which, even after responding to treatment, can predispose patients to various conditions and disorders. In conclusion, these few studies analyzing the specific link between the psychotherapy of MDD and corresponding HRV improvement do not allow for a final judgement due to the inconclusive results.

Besides these inconclusive results regarding changes of biological variables, the effects and side effects of antidepressant medication on HRV also show contradictory findings. Individual studies suggest that there could be a tendency whereby antidepressants further lower HRV (Agelink et al., 2001; Lehofer et al., 1997; Licht et al., 2009; O'Regan et al., 2015). However, a meta-analysis by Kemp and colleagues (2010) concluded that typically used antidepressants had no significant impact on HRV, apart from tricyclic antidepressants, which decreased HRV.

First results from experimental studies show that, apart from change sensitivity, HRV might be a possible candidate to predict treatment outcome. Lang and colleagues (1998) show that for patients suffering from anxiety disorder a higher heart rate reactivity during an anxious imagination is able to predict the response to CBT. For patients suffering from depression, Rottenberg and colleagues (2005) show that a higher vagal reaction while watching sad movies is able to predict treatment outcome. A pilot study from Ehrental and colleagues (2010) shows that cardiovascular parameters like blood pressure reactivity at intake are able to predict treatment outcome after 12 weeks of inpatient psychotherapy treatment. The sample consisted of persons with various depressive disorders such as moderate and severe episodes, double depression with various comorbidities, but the sample of 21 patients was rather small considering this heterogeneous symptom constellations. Even if the HRV reactivity is not directly comparable with resting HRV, which was used in chapter 2, it is another indicator that HRV might be useful to predict treatment outcomes in mental disorders.

In conclusion, the recording of HRV seems to provide important information about treatment response. However, studies with large samples which analyze HRV at two time points in a naturalistic setting are still scarce.

1.2.3 Predictive biomarker: CAR

One of the most important neurobiological abnormalities found in the context of depression is a change of the HPA axis (Jauch-Chara & Hohagen, 2009). Research has shown that the stress-responsive HPA axis is related to the pathophysiology of depression and cognitive function (Keller et al., 2017; Pariante & Lightman, 2008). The HPA axis consists of forward and inhibition loops which involve the brain, pituitary and adrenal gland to regulate glucocorticoid production. More than 40-60 % of MDD patients suffer from hypercortisolism (Murphy, 1991; Nemeroff & Vale, 2005) or other disturbances of the HPA axis (Deuschle et al., 1997; Pfohl et al., 1985; Yehuda et al., 1996). Paraiante and Lightman (2008) show that hypercortisolism is not only a consequence of MDD but also a risk factor for the development of MDD episodes. The authors point out that early life stress is associated with disturbances of HPA axis functioning and a higher risk for MDD in later life. Thus, changes in the HPA axis seem to be relevant not only in the pathogenesis but also in the further course of the disorder. Numerous studies have dealt with the interaction between MDD, psychotherapy and HPA axis activity over many years (McKnight et al., 1992; Stetler & Miller, 2011; Thase et al., 1996). Increased HPA axis activity seems to represent a predictive factor for a poorer response to psychotherapy (Thase et al., 1996). At the same time, it must be taken into account that various antidepressants have a direct or indirect influence on the functioning of the HPA axis (Vreeburg et al., 2009).

The cortisol secretion in the morning is one of the most relevant indicators to quantify the HPA axis function in recent literature (Chida & Steptoe, 2009; Stalder et al., 2016). During the night the cortisol level is low, rises very sharply after awakening and peaks after approximately 30-45 minutes. This change in cortisol level is called the “cortisol awakening response” (CAR) (Fries et al., 2009; Pruessner et al., 1997, Wilhelm et al., 2007). Pruessner and colleagues (2003) describe two basic values to quantify the CAR: (1) overall cortisol secretion during the waking-up period calculated by integrating the area under the curve (AUC); or (2) the total cortisol increase after awakening by subtracting cortisol after awakening from the maximum level during the waking-up period. Both measures have been related to the anticipations for the upcoming day and “mobilizing” of energy resources (Adam et al., 2006; Fries et al., 2009). This approach is supported by studies examining CAR on different days. CAR seems to be higher during weekdays than during weekends because on weekdays, demands and stressful

anticipations are usually higher (Schlotz et al., 2004). Another study analyzing CAR before and on a competition-day found similar results (MacDonald & Wetherell, 2019).

Many studies show that CAR represents a reliable endocrine biomarker of the HPA axis reactivity that is sensitive to different psychosocial and health factors such as job stress, life stress and fatigue (e.g. Chida & Steptoe, 2009; Schmidt-Reinwald et al., 1999; Wüst et al., 2000a). On the one hand, psychosocial stress (e.g. work overload or financial strain) has been associated with an increased CAR in cross-sectional studies (Adam et al., 2006; Schlotz et al., 2004; Steptoe, 2007). On the other hand, a blunted CAR has been associated with posttraumatic stress syndrome and fatigue (Chida & Steptoe, 2009). Steptoe (2007) points out that there are also indications that a reduced CAR is associated with resilience and positive emotions (e.g. happiness and wellbeing). The increasing number of studies analyzing CAR and psychological variables show inconsistent findings (Bhagwagar et al., 2003; Chida & Steptoe, 2009; Fischer et al., 2017; Pruessner et al., 2003). Particularly for MDD and depressed mood, the results seem to be very heterogeneous. Some studies point out that MDD is associated with a blunted CAR (e.g. Dedovic & Ngiam, 2015) while others showed results where MDD is associated with an increased CAR (e.g. Bhagwagar et al., 2005). Possible explanations for these different results are that CAR might be dependent on depressive severity (Chida & Steptoe, 2009). Mild to moderate degrees of depressive symptoms have been related to a heightened CAR whereas severe depression has been related to a blunted CAR.

A meta-analysis from Stetler and Miller (2011) suggests that cortisol levels differ between subgroups of MDD. Higher cortisol levels seem to be associated with melancholic or psychotic depression symptoms while lower levels were associated with atypical depressive symptoms. Further factors must be taken into account when interpreting the results of CAR studies (e.g. with respect to medications or comorbidities that can influence cortisol production; Chida & Steptoe, 2009; Manthey et al., 2011).

Up to now it has not been fully clarified if CAR is a trait biomarker for MDD or a state marker of depressive mood. On the one hand there is evidence from a large cohort study that increased CAR is associated with a higher risk of MDD episodes even in individuals in remission (Vreeburg et al., 2009). On the other hand there is evidence, from studies analyzing emotions in healthy persons with comparable cortisol results, suggesting CAR is rather a descriptive state marker than a trait biomarker (Schlotz et al., 2004; Shibuya et al., 2014). Despite all the ambiguous findings, a current meta-analysis, including 35 studies, showed that increased

cortisol might be a potential predictor for MDD onset, recurrence or relapse (Kennis et al., 2020).

1.3 Research Question and Dissertation Outline

A recent meta-analysis by Cuijpers and colleagues (2019) includes 696 studies analyzing the effects of psychotherapy on depression. The authors show that psychotherapy and antidepressant therapy are able to reduce depressive symptoms (g=0.31). However, the high rate of relapse, treatment-resistant depression and long-term course of MDD shows that further research is needed to improve treatment and diagnosis of MDD. Apart from that, the effects of psychotherapy on biological symptoms or biological markers of depression such as HRV and CAR remain unclear. Valid sensitive and specific biological markers that are able to help in description and prediction of the course of MDD might be promising but are still scarce. Despite great research efforts no general robust biological markers have been found for MDD patients in a naturalistic setting up to now. Most studies in this field focus on subclinical groups under laboratory conditions with small sample sizes (Young et al., 2016). Participants in these studies show mild depression symptoms without any complex mental comorbidities, different antidepressant medications, severe physical comorbidities, or chronic or recurrent depressive episodes (Fischer et al., 2017). Therefore, the results of these investigations cannot be transferred to patients in a naturalistic clinical setting. Studies in naturalistic settings are still scarce or include very specific small samples.

Recent research shows that biological markers can reflect inflammatory processes, neurotransmitters, neurotrophic, neuroendocrine and metabolic systems, but as described above there are inconsistent findings about the association between biological markers and the course of MDD. Consequently, there is a call for further research to verify the results in a naturalistic setting (Burman et al., 2010). Furthermore, there seem to be indications it is possible to predict treatment effects with the help of biomarkers, but here, too, there are strong differences between studies (Jani et al., 2015). One explanation for the aforementioned inconsistencies is the lack of standardized measurement protocols for many biomarkers such as CAR or HRV. The CAR is very susceptible to measurement inaccuracies, e.g. taking the first saliva probe, some minutes after awakening, can significantly falsify the results (for details see Stalder et al., 2016). Especially with regard to HRV, research shows that HRV can be described by very different variables, each representing different aspects of ANS: e.g. vagal activation (Root Mean Sum of

Squared Distance (RMSSD), High frequency power (HF-power)) or total ANS activity (Standard Deviation of RR-Intervals (SDNN)) (for an overview see Shaffer & Ginsberg, 2017). These different variables are not directly comparable to each other.

Apart from the different HRV variables, HRV values have been shown to be highly state-dependent due to situational context factors (Bertsch et al., 2012) and tend to display large day-to-day random variations, which makes it difficult to discover intervention effects within individuals (Pinna et al., 2007). In addition, the reliability of HRV values collected by a single measurement is low (Bertsch et al., 2012; Pinna et al., 2007). Eikeseth and colleagues (2020) show that, especially in psychiatry research with mentally ill patients, the reliability is even lower. The authors show that HRV variables are highly state dependent rather than trait dependent in psychiatric samples. Multiple HRV measurements are therefore required to compensate for situational variance and to match the robustness that is also required from depressive symptom patterns (Bertsch et al., 2012; Eikeseth et al., 2020).

Last but not least Young and colleagues (2016) pointed out that the large number of confounding variables and the diverse pathophysiology of MDD makes it even more difficult to interpret research results of biomarkers like HRV or CAR. Therefore, replication studies with large sample sizes are necessary to verify first putative research results regarding biomarkers for the course of MDD.

In sum, the purpose of this dissertation is to investigate the association between selected biomarkers (CAR and HRV) and depressive symptoms before, at the end and at long term after an intensive psychiatric psychotherapeutic treatment. The focus is on two aspects: First, are biomarkers for MDD sensitive to change after an inpatient treatment? Second, is it possible to predict depressive symptom deterioration with the help of biomarkers after inpatient therapy? Both aspects are examined in a naturalistic clinical setting in this dissertation. In addition, the methodological goals of this dissertation are to improve biomarker measurement protocols and to reduce the influence of confounding variables. Both aspects are very important to consider in a naturalistic setting. Therefore multiple measurement designs and a detailed description of the measurement protocols are used in all of the following studies. Furthermore, one study is a replication study with a stricter measurement protocol in order to assess additional methodological considerations.

2. Reduction of depressive symptoms during inpatient treatment is not associated with changes in heart rate variability[1]

[1] This chapter is based on the following manuscript:

Neyer, S., [redacted], [redacted]., [redacted], [redacted]., [redacted], [redacted]., [redacted], [redacted]. [redacted]., & [redacted], [redacted]. (2021). Reduction of depressive symptoms during inpatient treatment is not associated with changes in heart rate variability. *PloS one*, *16*(3), e0248686.

2.1 Introduction

MDD is one of the most common, and highly debilitating mental disorders, affecting an estimated 264 million people in 2020 (World Health Organization, 2020). MDD is typically associated with a significant reduction in quality of life (Andrade et al., 2003; Kemp & Quintana, 2013) and several physical illnesses especially cardiovascular diseases (CVD) (Carney et al., 1987; Nicholson et al., 2006; Pan et al., 2011; Pratt et al., 1996; van der Kooy et al., 2007). To optimize the treatment for both conditions it is important to learn more about the biological aspects of MDD and its association with the cardiovascular system.

2.1.1 HRV and autonomic nervous system (ANS)

One relevant indicator for psychopathological abnormalities is autonomic imbalance (Beauchaine & Thayer, 2015), which is reflected in a reduced heart rate variability (HRV) (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). HRV is the variation of the period between two consecutive heartbeats over time. It indicates the adaptivity of the heartbeat to changing inner and outer conditions and thus reflects the adaptivity of the ANS (Shaffer & Ginsberg, 2017). Sympathetic and parasympathetic neurons innervate the heart via the vagus nerve and the stellate ganglion (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). In their model of neurovisceral integration, Thayer and colleagues describe HRV in a dynamical systems framework (Thayer & Brosschot, 2005; Thayer & Lane, 2000). Within this model, cognitive, emotional, behavioral and physiological responses are regulated and reflected by excitatory and inhibitory innervation of the heart (Benarroch, 1993; Thayer & Siegle, 2002). A low adaptivity is an indicator of a relative sympathetic dominance and relative restricted parasympathetic tone. Low vagally mediated HRV has been associated with all-cause mortality (Kluttig et al., 2010; Thayer & Lane, 2009; Thayer et al., 2010), increased susceptibility to stress, emotional instability (Koval et al., 2013) and increased risk of cardiovascular diseases and mental disorders (e.g. depression) (Karavidas et al., 2007; Thayer & Lane, 2009).

2.1.2 HRV and Depression Therapy

The results of two meta-analyses of MDD patients free of heart disease suggest that MDD patients have a reduced HRV compared to healthy controls (Alvares et al., 2016; Kemp et al., 2010). Furthermore, the severity of depressive symptoms appears to be inversely related

to HRV and this association does not seem to be attributable to medication side effects (Alvares et al., 2016).

Psychotherapy reduces depressive symptoms (g=0.31) (Cuijpers et al., 2019), but its effect on HRV remains unclear. Carney and colleagues (2000) reported that severely depressed CVD patients benefit from cognitive behavioral psychotherapy (CBT) via depressive symptom reduction, heart rate reduction and increased HRV. In addition, a greater treatment response in depression was found to be associated with an increase in vagally mediated cardiac variability following acupuncture treatment (Chambers & Allen, 2002). It is worth noting that the sample was homogenous and does not represent a naturalistic clinical depressive sample. Nonetheless, Kim and colleagues (2009) reported a significant improvement in HRV after a successful CBT/ meditation intervention in physically healthy depressed patients but not for CBT alone.

In contrast to the aforementioned findings, there are also indications that HRV may not significantly change following interventions despite the observed improvement in self-reported depressive symptoms. Carney and colleagues (2000) reported that moderately or mildly depressed CVD patients show reduced depressive symptoms without concomitant changes in HRV following CBT. Wheeler and colleagues (2014) showed that a mindfulness based cognitive therapy can reduce depressive symptoms but did not affect HRV. Similarly, Brunoni and colleagues (2013) found no improvement in HRV after a non-pharmacological (transcranial direct current stimulation) or pharmacological (Selective Serotonin Reuptake Inhibitor, SSRI) treatment of unipolar depression.

Besides these inconclusive results, the effects and side effects of antidepressant medication on HRV similarly shows contradictory findings (Agelink, 2001; Glassman et al., 2007; Kemp et al., 2010; Lehofer et al., 1997; Licht et al., 2010; Rechlin, 1994; Terhardt et al., 2013; Yeragani et al., 2002). A meta-analysis by Kemp and colleagues (2010) concluded that typically used antidepressants had no significant impact on HRV, apart from tricyclic antidepressants that decreased HRV. However, individual studies suggest that there could be a tendency that antidepressants lower HRV (Agelink, 2001; Lehofer et al., 1997; Licht et al., 2009; O'Regan et al., 2015).

2.1.3 Research gap

The question of whether HRV could represent a biomarker not only for depression before treatment, but also for therapeutic change remains open. One possible explanation for the

inconsistent findings may be that even after successful intervention, psychophysiological correlates of depression remain stable (Berkman et al., 2003; Post, 1992; Rees et al., 2004). Greenberg and colleagues (2015) suggest that this observed lack of HRV improvement in depressive patients might be one reason for the high relapse rates. Those studies, reporting no changes in HRV following symptom reduction, explained this absence mostly with a delay of physiological changes following psychological changes (Licht et al., 2010). Overall, the evidence that psychotherapy might be able to increase HRV in patients with MDD remains ambiguous.

2.1.4 Methodological problems of HRV measurements

Regardless of the inconsistent findings, the current available studies contain some methodological shortcomings. Firstly, they focus on too specific samples (often persons with mild levels of depression and without any medication) which mostly do not reflect the regular patients in clinical psychotherapy settings (Chambers & Allen, 2002; Kim et al., 2009). Therefore, the generalizability of these findings to patients with severe depressive disorders is limited. Second, the few existing studies comparing pre- and post-intervention HRV do not show any intervention effects between depressive symptom reduction and HRV values (Brunoni et al., 2013; Licht et al., 2008). Third, there are only a few studies comparing pre and post intervention HRV values but all of them have used single short-term recordings for each timepoint (Brunoni et al., 2013; Chambers & Allen, 2002; Kim et al., 2009). This is problematic because HRV values have been shown to be highly state-dependent due to situational context factors (Bertsch et al., 2012; Uhlig et al., 2020) and they tend to display large day-to-day random variations, which makes it difficult to discover intervention effects within individuals (Pinna et al., 2007). Fourth, it is difficult to compare the results of different studies because of the use of different HRV parameters, measurement hard- and software across and within each study (Laborde et al., 2017; Quintana et al., 2016).

2.1.5 Contribution

The purpose of the present study is to investigate the association between HRV and depressive symptoms before and after an intensive psychiatric psychotherapeutic treatment. From a methodological perspective, we considered it important to achieve a robust HRV measurement by using multiple measurements to compensate for situational variance (Bertsch et al., 2012; Uhlig et al., 2020). In addition, we sampled MDD patients in a naturalistic inpatient setting.

2.2 Methods

2.2.1 Participants

The sample consisted of 50 inpatients (*N*=34 females, *N*=16 males) admitted for psychotherapy in a German psychosomatic hospital, with a *Mea*n age of 39.51 years (*SD*=14.97; *Range*=17.5-67.8 years). Twenty-six percent of the participants identified themselves as smokers, smoking an average of 13.75 cigarettes per day (*SD*=12.48; min<1; max=40).

The admission criteria for clinical treatment were serious depressive symptoms or serious social impairment so that everyday requirements could no longer be met; patients experiencing treatment resistant depression, or where an outpatient therapy did not lead to an improvement of depressive symptoms (Deutsche Gesellschaft für Psychiatrie und Psychotherapie, Psychosomatik und Nervenheilkunde, 2015). The participants' diagnoses were assessed through a structured clinical interview (SCID I, II) (Fydrich et al., 1997; Wittchen et al., 1997a; Wittchen et al., 1997b). Inclusion criteria for the present study was a diagnosed MDD. Exclusion criteria were evidence of comorbid excessive substance or alcohol use, psychosis, autoimmune-thyroiditis, anorexia nervosa, BMI<18.0, respiratory, hormone or heart diseases. Seventy-six percent of the inpatient sample fulfilled the criteria for at least one additional comorbid mental disorder. The number of comorbid diagnoses ranged from 0 to 6 (*M*=1.86, *SD*=1.48). Table 2.1 shows the distribution of comorbid diagnoses.

Table 2.1.

Comorbid disorders

	N	%
Personality Disorders	29	58
Eating Disorders	13	26
Posttraumatic Stress Disorder	8	16
Somatoform Disorders	6	12
Anxiety Disorders	6	12
Others	5	10
No comorbidities	12	24
	50	100

Note. Number of patients with a comorbid diagnosed disorder (e.g. attention deficit hyperactive disorder or inorganic sleeping disorders).

Sixty-eight percent of patients were prescribed antidepressant medication during treatment. Average onset of depression was 10.72 years before their current inpatient treatment (*SD*=9.59; *Rang*e=1-35 years). Fifty-eight percent of the participants had been previously hospitalized, while for 30% this was their first time as inpatients (for the remaining 12% this information was missing). The *Mean* number of previous inpatient therapies was 1.42 (*SD*=1.47; *Range*=0-5 previous inpatient therapies). This study was approved by the Ethics Committee of the "Medical Association Westfalen-Lippe" and written informed consent was obtained from all participants prior to data collection.

2.2.2 Design

This study utilized a longitudinal naturalistic pre-post-design. All patients completed routine computer based self-report questionnaires during their first and last week of inpatient therapy, while a clinical psychologist conducted the Hamilton-Interview during the first and last week. The patients stayed between 6 and 12 weeks (*M*=8.80; *SD*=2.5) as inpatients and attended an individual psychotherapy session five times per week and at least one group therapy per weekday (e.g. Mindfulness based therapy, Mentalization based Therapy, social skills training, Psychoeducation for Depressive Disorders). Psychopharmacotherapy prescriptions were reviewed at least once a week and adapted if necessary. The CBT interventions differed between patients to accommodate for the heterogeneity of depressive disorders and symptoms. HRV assessment took place during the first and last week of therapy on three days (normally Monday, Wednesday and Friday morning between 9 and 11 am). Three assessments at the beginning and at the end of therapy were used to reduce the high impact of situational confounders and to increase the transsituational variance from about 49% following one-time assessments up to 75% for two or three assessments (Bertsch et al., 2012).

2.2.3 Instruments

2.2.3.1 Short term HRV assessment

At the beginning of each individual HRV assessment, the experimenter checked whether the following exclusion criteria were met: refraining from smoking or drinking caffeinated beverages at least three hours before the measurement and not participating in morning exercise on measurement days. The time period of three hours was based on the daily clinical routine and also applied in comparable studies (see: Agelink et al., 2002; De Rubeis et al., 2016; Kiviniemi et al., 2007). If necessary, the assessment was postponed to the following day.

The experimenter explained the procedure and gave a short information about basic functions of the ANS. Participants were requested to switch off their mobile phones before measurement started. The experimenter assisted with administering the electrocardiogram (ECG) electrodes (disposable ECG-electrodes with fluidity impairment foamed material from Dahlhausen, Köln, Germany) correctly (Einthoven's triangle: Lead III). After administering the measurement hardware and before starting the recording, the experimenter checked if the equipment worked properly and asked if the patient felt well enough to proceed. After this short stabilization period (Flatt & Esco, 2016; Krejci et al., 2018) the actual HRV measurement began. During each measurement the patient was asked to sit still in a comfortable chair and breathe normally for the next five minutes to assess the ECG baseline. The electrocardiogram (Biosign, 2009) was recorded at a 500 Hz sampling rate. The experimenter was seated outside in front of the room during the recording.

2.2.3.2 Hamilton Rating Scale of Depression

The Hamilton Rating Scale of Depression (HRSD) is an assessment tool to record the severity of depressive symptoms (Miller et al., 1985; Schramm et al., 2011). It consists of 24 items, scored from 0 to 4. It is sensitive to change and therefore suitable for use in clinical trials. The internal consistency (Cronbach's alpha) of the HRSD at intake and at discharge was adequate to very good: HRSD_Intake=.77 (*N*=49); HRSD_Discharge=.91 (*N*=37). Both are comparable to a previous validation of the German HRSD version (Drieling et al., 2007).

2.2.3.3 Beck Depression Inventory-II

The Beck Depression Inventory-II (BDI-II) is a 21-item self-report questionnaire used to assess the severity of depressive symptoms (Beck et al, 1996a; Hautzinger et al., 2006). Each item consists of four response statements that are rated from 0 to 3 representing ascending severity of depressive symptoms. A total value of 0-9 indicates minimal depression, 10-18 indicates mild depression, 19-29 indicates moderate depression and 30-63 indicates severe depression. The internal consistency (Cronbach's alpha) of the BDI-II at intake and discharge were very good: BDI-II_Intake=.93, BDI-II_Discharge=.96 (N=50) and these values are comparable to a published study conducted with the German BDI-II version (Kühner et al., 2007).

2.2.4 Data reduction

ARTiiFACT software (Kaufmann et al., 2011) was used to extract QRS complexes, determine interbeat intervals, and to detect and correct the raw ECG. In line with common research standards, HRV measures indicating vagally mediated HRV were extracted. For the time value, we used the Root Mean Square of Successive Differences (RMSSD) (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). RMSSD reflects the variance of successive beat to beat intervals and is a reliable indicator for vagal activity during 5-minute short-term recordings with spontaneous breathing (Williams et al., 2015). In addition, the RMSSD appears to be more robust against state influences compared to other HRV values. As a frequency measure, power of the high frequency band (HF power, 0.15-0.4Hz) was derived (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). HF power is also indicative of parasympathetic activity (Bassett, 2016; Williams et al., 2015).

2.2.5 Statistical analyses

Prior to analysis, all variables were checked for accuracy of data entry and missing values. Little's MCAR Test for all psychometric and biometric data showed a statistically non-significant result ($\chi 2(140)=118.71$, $p=.91$) indicating that values missing completely at random could be inferred (Tabachnick & Fidell, 2013).

All variables were checked for univariate outliers by identifying cases with *z*-score above 3.29 or below -3.29, and these were dealt with by deletion. Consequently, two individual's HRV measurements (*HF_T1_Intake* and *RMSSD_T1_Intake*) at intake, and five individual's measurement at discharge (*HF_T1_Discharge*, *RMSSD_T1_Discharge*, *HF_T2_Discharge*, *RMSSD_T2_Discharge* and *HF_T3_Discharge*) were identified as outliers and deleted without replacement. Other missing values (in total: 40 values) result from premature discharge, non- compliance or technical problems.

The HRV variable HF was normalized via log(n)-transformation (Field, 2013). All other variables were normally distributed without transformation. Normalization might have an impact on the data analysis, so we checked all analyses without normalization of data. No significant change of results occurred within this additional step of analysis. *Mean* HRV indices were calculated for all three respective measurement points at intake (RMSSD_Intake,

lnHF_Intake) and all three respective measurement points at discharge (RMSSD_Discharge, lnHF_Discharge).

Cronbach's alpha was calculated to test the reliability of all four indices (RMSSD_Intake, lnHF_Intake, RMSSD_Discharge and lnHF_Discharge) based on the respective three measurement points. Cronbach's alpha values were .73-.89 for each index and therefore can be considered good to very good. The associations between HRV variables and questionnaire measurements (intake, discharge) were assessed using Pearson's correlations and *T*-Tests. Statistical analysis was performed using SPSS 25 (IBM Corporation, 2016).

2.3 Results

2.3.1 Descriptive statistics

We examined the association between MDD symptoms and HRV before and after inpatient therapy. The descriptive statistics and psychometric results at intake and discharge are presented in Table 2.2 including *Mean* intake HRV and *Mean* discharge HRV indices.

Table 2.2.

Summary of descriptive psychometrics and HRV values

		N	*M*	*SD*	Min	Max
Descriptive data and symptom severeness	Age (years)	50	39.51	14.97	17.49	67.75
	BMI	50	24.26	3.97	18.0	34.6
	Duration of CBT (weeks)	50	8.8	2.5	6	12
	Previous stationary therapies	50	1.42	1.47	0	5
	HRSD Intake	45	23.51	7.15	10.00	37.00
	HRSD Discharge	40	14.33	10.49	1.00	49.00
	BDI-II Intake	49	28.41	12.94	5.00	52.00
	BDI-II Discharge	50	16.70	14.78	0.00	53.00
HRV values	RMSSD T1 Intake (ms)	49	22.17	11.37	3.75	43.97
	RMSSD T2 Intake (ms)	49	21.92	14.43	2.76	63.41
	RMSSD T3 Intake (ms)	48	21.08	11.77	5.76	48.34
	RMSSD Mean Intake (ms)	50	21.62	11.34	5.38	49.42
	RMSSD T1 Discharge (ms)	49	22.03	13.38	4.63	62.76
	RMSSD T2 Discharge (ms)	49	20.97	11.64	4.92	54.03
	RMSSD T3 Discharge (ms)	42	20.21	11.20	3.99	53.21
	RMSSD Mean Discharge (ms)	50	21.62	10.56	4.99	48.84
	Ln HF_T1_Intake (ms^2)	49	3.96	1.30	0.76	6.12
	Ln HF_T2_Intake (ms^2)	49	3.89	1.38	-0.11	6.08
	Ln HF_T3_Intake (ms^2)	48	3.89	1.36	1.11	6.02
	Ln HF_Mean Intake (ms^2)	50	3.89	1.22	1.34	5.74
	Ln HF_T1_Discharge (ms^2)	49	3.96	1.24	0.73	6.28
	Ln HF_T2_Discharge (ms^2)	49	3.77	1.20	0.89	5.88
	Ln HF_T3_Discharge (ms^2)	41	3.80	1.18	0.99	5.92
	Ln HF_Mean_Discharge (ms^2)	50	3.90	1.06	0.93	5.88

Note. Abbreviations: M = Mean, SD = Standard deviation, Min = Minimum, Max = Maximum, HRSD = Sum score of Hamilton Rating Scale of Depression, BDI-II = Sum score of Beck Depression Inventory, RMSSD = Root Mean Square of Successive Differences, Ln HF = Power of High Frequency Band.

The HRV values at intake were comparable to previous studies with MDD patients (e.g. RMSSD *M*=27.16 (Khandoker et al., 2017) or RMSSD *M*=23.50 (Rechlin, 1994)) and notably lower than normed HRV values of healthy persons (RMSSD *M*=42, *SD*=15 (Nunan et al.,

2010)). The intake self-reported data was comparable to other studies investigating psychotherapy outcomes of CBT after an MDD inpatient treatment (Carney et al., 2000; Whisman et al., 1991).

2.3.2 Correlation between HRV values and psychometric results

There was a significant positive correlation (Pearson's *r*) between the two psychometric indices (BDI-II & HRSD) (for all min. $r>.37$, $p<.05$) and a significant positive correlation between the two HRV indices (RMSSD & HF) (for all $r>.49$, $p<.001$) (see Table 2.3).

Table 2.3.
Correlation between HRV values and Depression symptom severeness

			HRV values			Symptom severeness				
			RMSSD Discharge (ms)	Ln HF Intake (ms^2)	Ln HF Discharge (ms^2)	BDI-II Intake	BDI-II Discharge	HRSD Intake	HRSD Discharge	Stationary Therapies
HRV values	RMSSD Intake (ms)	*r*	**.522*** **	**.872*** **	**.586*** **	**-.293***	-.186	**-.352***	-.270	**-.303***
		p	**<.001**	**<.001**	**<.001**	**.041**	.200	**.019**	.111	**.049**
		N	50	50	50	49	49	44	36	43
	RMSSD Discharge (ms)	*r*		**.486*** **	**.811*** **	-.125	-.055	-.133	-.180	-.208
		p		**<.001**	**<.001**	.391	.708	.391	.294	.181
		N		50	50	49	49	44	36	43
	Ln HF Intake (ms^2)	*r*			**.697*** **	-.209	-.156	**-.360***	-.182	**-.363***
		p			.001	.150	.285	**.017**	.288	**.017**
		N			50	49	49	44	36	43
	Ln HF Discharge (ms^2)	*r*				-.102	-.076	-.185	-.121	**-.304***
		p				.487	.603	.229	.482	**.004**
		N				49	49	44	36	43
Symptom values	BDI-II Intake	*r*					**.605*** **	**.714*** **	**.529** **	**.434** **
		p					**<.001**	**<.001**	**.001**	**.004**
		N					48	43	36	42
	BDI-II Discharge	*r*						**.483** **	**.738*** **	**.366***
		p						**.001**	**<.001**	**.017**
		N						43	35	42
	HRSD Intake	*r*							**.369***	**.342***
		p							**.038**	**.033**
		N							32	39
	HRSD Discharge	*r*								.312
		p								.082
		N								32

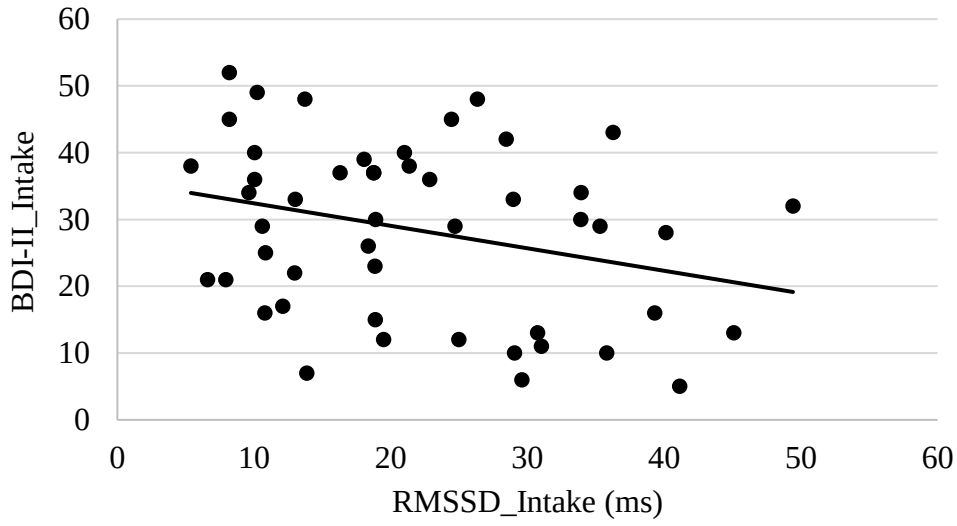
Note: Abbreviations: HRSD = Sum score of Hamilton Rating Scale of Depression, BDI-II = Sum score of Beck Depression Inventory, RMSSD = Root Mean Square of Successive Differences, Ln HF = Power of High Frequency Band.

Significance (two-tailed), * $p < .05$. ** $p < .01$. *** $p < .001$.

There was a significant negative correlation between RMSSD_Intake and psychometric intake measurements (r=-.29, p<.05). Ln_HF_Intake only showed a significant correlation with HRSD_Intake. At discharge there was no significant correlation between HRV and psychometric indices. None of the aforementioned significant correlations reached significance at discharge (e.g. see Figure 2.1).

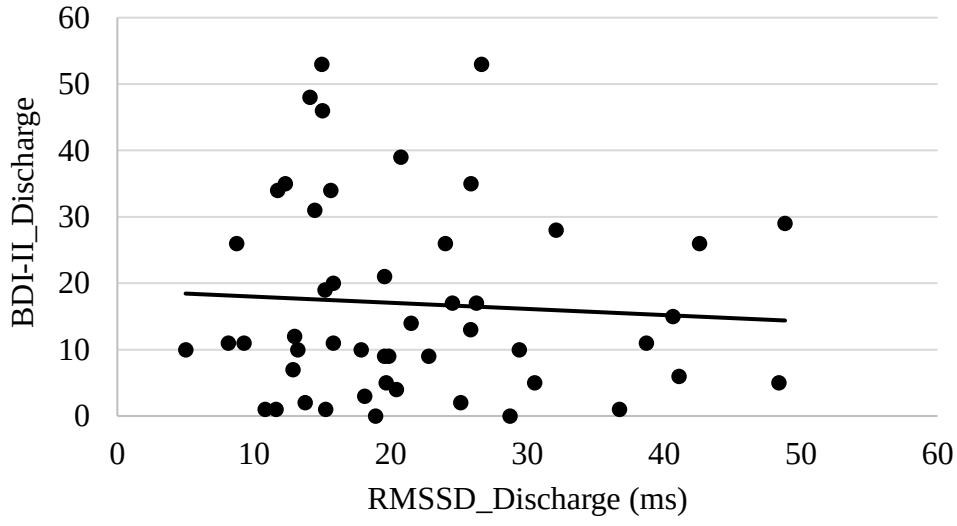
Figure 2.1

a. Association between BDI-II and RMSSD during the first week of an inpatient treatment



Note. r=-.29, p<05. Abbreviations: BDI-II = Sum score of Beck Depression Inventory, RMSSD = Root Mean Square of Successive Differences.

b. Association between BDI-II and RMSSD during the last week of an inpatient treatment



Note. r=-.06, p=.71. Abbreviations: BDI-II = Sum score of Beck Depression Inventory, RMSSD = Root Mean Square of Successive Differences.

There was no significant correlation between HRV-change indices and symptom-reduction indices (discharge minus intake values) (for all p>.16). The change of intake HRV indices

and discharge HRV indices was not significantly associated with symptom-reduction variables either (for all $p>.20$).

Symptom severity was significantly positively correlated with the duration of psychotherapy (Intake: $r=.56$, $p<.001$ and Discharge: $r=.38$, $p<.01$). *T*-Test comparisons of subgroups showed that smokers and non-smokers did not differ on depression severity (e.g. Smokers: BDI-II_Intake $M=33.83$, $SD=16.45$; Nonsmokers: BDI-II_Intake $M=26.65$, $SD=11.30$; $T=1.70$, $p=.10$) or HRV values (Smokers: RMSSD_Intake $M=20.53$, $SD=13.62$; Nonsmokers: RMSSD_Intake $M=22.01$, $SD=10.61$; $T=.40$, $p=.69$). There was no significant correlation between smoking behavior and HRV (RMSSD_Intake $r=-.06$; $p=.69$; RMSSD_Discharge $r=.03$, $p=.86$) or Depression values (BDI-II_Intake $r=.16$; $p=.27$, BDI-II_discharge $r=.24$, $p=.10$). T-Test comparisons of subgroups showed that normal weight (female BMI 18-24, male 19-25) persons did not differ from overweight (female BMI>24, male BMI>25) persons in depression severity or HRV values (for all comparisons $T<1.24$, $p>.22$).

The repetition of all calculations with only the first measurement at intake and the first measurement at discharge showed no significant association between physiological and psychological values at intake or at discharge. Consistent with the results based on *Mean* HRV values, there were no significant differences between admission and discharge measurements. Other indicators (Heart Rate, SDNN, pNN50 or LF/HF Ratio) were calculated but did not lead to different results and did not change between admission and discharge (see S1 and S2 Table)[2].

2.3.3 Symptom reduction

All psychometric questionnaires for the assessment of MDD symptoms showed significant reductions (see Table 2.4). On average, the BDI-II_Discharge values were reduced by 11 BDI-II-points compared to BDI-II_Intake. Following therapy, 20% of patients continued to display severe BDI-II symptom severity, 16% as moderate, with 60% of patients demonstrating no, minimal or mild depressive symptoms. The HRSD_Discharge values were reduced on average by 7 points.

[2] *Note: S1 and S2 refers to supplemental material that was required for submission of this study.*

Table 2.4.

Paired T-Tests for Depression and HRV indices at Intake and Discharge of Psychotherapy

	N	Intake	Discharge	*Df*	*t*	*p*	*d*
HRSD	36	23.39 (7.24)	14.14 (10.93)	35	5.11	<.001	.874
BDI-II	49	28.41 (12.94)	16.84 (14.90)	48	6.58	<.001	.837
RMSSD (ms)	50	21.62 (11.34)	21.62 (10.56)	49	.00	.997	.0001
Ln HF (ms^2)	50	3.96 (1.30)	3.96 (1.22)	49	-.06	.95	-.007

Note. Abbreviations: HRSD = Sum score of Hamilton Rating Scale of Depression, BDI-II = Sum score of Beck Depression Inventory, RMSSD = Root Mean Square of Successive Differences, Ln HF Band = Power of High Frequency Band.

The HRV indices showed no significant changes after inpatient psychotherapy and the *SD*s did not vary between intake and discharge (for all indices $p>.95$). Nevertheless, intraindividual *SD*s showed fluctuations within the three measurements at intake (RMSSD: *Mean_SD*=5.51; *SD_SD*=4.01; ln_HF: *Mean_SD*=.58; *SD_SD*=.39) and within the three measurements at discharge (RMSSD: *Mean_SD*=6.87, *SD_SD*=4.45; ln_HF: *Mean_SD*=.64; *SD_SD*=.46). Paired *T*-Tests showed no significant difference between intake variability of HRV values and discharge variability of HRV values (for all intraindividual HRV values $p>.45$).

2.3.4 Gender analysis

A *T*-Test of HRSD showed that women (HRSD_Intake *M*=25.23; *SD*=7.12) had higher HRSD depression scores than men (HRSD_Intake *M*=20.07; *SD*=6.05) at the beginning of psychotherapy ($t(43)=2.41$, $p=.02$). All other psychometrics showed similar differences between men and women at intake and discharge. There was no significant difference between men and women in HRV values (for all HRV values $p>.12$).

2.4 Discussion

The aim of the present study was to investigate - by applying robust HRV estimations - the association between HRV and depressive symptoms before and after an intensive inpatient treatment in a sample of MDD inpatients. This study combines self-reported, third-party and psychophysiological data and is one of the first based on a naturalistic sample. It is also one of the first studies to utilize average multiple HRV assessments to obtain trait-like characteristics in a clinical sample.

2.4.1 Association between MDD and HRV at intake

In line with previous controlled studies patients with MDD showed lower than average HRV indices at intake (Nahshoni et al., 2004). Levels of depression were comparable to published inpatient samples at intake for CBT (Caldwell & Steffen, 2018; Rechlin, 1994; Whisman et al., 1991). As expected, a positive association between symptom severity and duration of psychotherapy was observed. At the time of intake before treatment onset, MDD symptom severity and HRV indices were negatively associated, replicating previous clinical studies (Kemp et al., 2010). These findings support the notion that depressed patients show reduced vagal activation, underlining the role of vagally mediated HRV as a biomarker for mental health. The current state of research suggests different explanations for these findings at intake: It can be assumed that somatic symptoms of MDD (e.g. sleeping problems, changes in appetite, fatigue, pain) are more pronounced in severe depression and are associated with a decrease in HRV (De Jonge et al., 2007). An alternative explanation could be the processing of socially threatening stimuli, which are perceived stronger by depressive persons and therefore lead to a stronger autonomous reaction (De Rubeis et al., 2016). Patients experience worries and hypervigilance have particularly severe difficulties in deactivating threatening stimuli (Kemp et al., 2012). This lack of inhibition leads to chronic overactivity of the sympathetic nervous system and reduced parasympathetic withdrawal, which both decrease the ability of ANS to adapt to inner and outer circumstances because of a defect in noradrenaline reuptake (Barton et al., 2007; Hausberg et al., 2007; Schiweck et al., 2019), resulting in lower HRV. An implication of sustained SNS overactivity is an autonomous imbalance with a relatively low parasympathetic activation associated with depressive symptoms and - in nonclinical samples - emotional dysregulation (Appelhans & Luecken, 2006).

2.4.2 No association between MDD and HRV at discharge

Following intervention, depressive symptoms showed significant reductions on all the self- and third-party assessments. However, the HRV indices as estimates of vagal activation remained unchanged. The significant association between HRV indices and MDD symptoms (self-reported and externally assessed) at the beginning of psychotherapy disappeared post-intervention. Thus, the symptom alleviation during treatment does not seem accompanied by a simultaneous improvement of HRV. The assumption that HRV might be a specific biomarker for current depressive symptoms cannot be supported in regards to post-treatment situations. In line with previous findings (Agelink et al., 2001; Kemp et al., 2010; Kim et al., 2009), our

results rather suggest that inpatient CBT and psychiatric treatment significantly reduce depressive symptoms without changing short term HRV values at the same time.

One explanation for the dissociation between the longitudinal development of depression and HRV, might be that psychotherapy helps depressive patients gain more insight into dealing with depression, and helps them become more self-compassionate. Patients learn how to behave and think in different situations. HRV has been reported to reflect inhibitory and emotion regulatory capacity (Thayer & Lane, 2009; Thayer & Siegle, 2002). It could be speculated that this capacity might be triggered by psychological interventions (Schneider & Kuhl, 2012). The assumption of a better use of regulatory cognitive strategies obtained during the psychotherapeutic treatment, i.e., a more efficient use of existing neural capacities, does not require the assumption of an increased HRV baseline. In line with this suggestion, Brunonni and colleagues (2013) suggest that a reduced parasympathetic activity might be a trait factor for depression which explains the high relapse rates rather than a state marker for depressive symptoms (Pintor et al., 2003). Consequently, low resting HRV might not be a state-like indicator of a current depression level, but an endophenotype of the underlying vulnerability and thus persisting beyond successful treatment and symptom alleviation. In depressed persons, vulnerability and symptoms coincide. This view on HRV as a vulnerability marker is supported by evidence showing low HRV to be a risk factor for the later development of depression and a marker for various risk factors contributing to depression, such as dysfunctional emotion regulation or perseverative thinking (Cropley et al., 2017; Di Simplicio et al., 2012: Thayer & Lane, 2000). In this sense, HRV should be seen as a transdiagnostic marker for stress and psychopathological vulnerability that can coincide with clinical manifestation in untreated individuals, but not as a specific biomarker for depression. This result is in line with Beauchaine and Thayer (2015) who suggest that especially HF-HRV can be considered a “transdiagnostic biomarker of psychopathology” (Beauchaine & Thayer, 2015, p. 345). Apart from that the high portion of within-subject variance of HRV values also makes it difficult to detect changes in HRV values over time (Uhlig et al., 2020).

This explanatory approach in the tradition of vulnerability-stress-models remains to date speculative due to a lack of empirical evidence on patients' pre-morbid HRV status. It is, however, compatible with the notion that low HRV is a risk factor for a wide range of mental disorders. Furthermore, it is compatible with the observation of successful symptom alleviation in

the absence of HRV changes. The extent to which this explanation accounts for symptom alleviation can only be tested in conditions that are structurally (in terms of frequency, intensity and set-up) identical with the actual treatment condition.

Importantly, it should be mentioned that reduced HRV is not a specific feature of MDD but rather a transdiagnostic factor which relates to several stress-related states, conditions and behavioral factors as well as to medical conditions and antidepressant medication (Gidron et al., 2018). Psychiatric and psychotherapeutic interventions may therefore not be sufficient to change neurobiological processes, which may only take place after a global change in life and behavior. Despite the improved mood, there may still be unfavorable life-style factors (e.g. smoking, sleep disorders, lack of activities, overweight, etc.) that could explain the consistently reduced HRV values (Carney & Freedland, 2017; Dinas et al., 2013). This hypothesis is supported by the meta-analysis by Gan and colleagues (2014) supporting the effects of unfavorable lifestyle factors on the risk of developing CVD.

With regard to specific lifestyle factors research shows that nicotine disturbs normal ANS functioning by increasing SNS activity and reducing PNS modulation (Dinas et a., 2013). Similar mechanisms have been shown for the impact of overweight (Karason et al., 1999). Especially for depressed patients Harte and colleagues (2013) showed that depressed smokers had significantly increased sympathetic tone which manifests in reduced HRV values compared to depressed non-smokers. In our sample, there were people who continued to have unfavorable lifestyle factors e.g. smoking. Since this change is not the main goal of inpatient therapy and a global change in lifestyle and behavior would only become apparent in the outpatient setting in the long term. HRV improvement might take more time because of the necessary neurological changes in the central autonomic network (CAN; Thayer & Siegle, 2002).

Another possible explanation for the constantly reduced HRV values could be the intake of psychotropic drugs, which were prescribed, adjusted or discontinued individually throughout the therapy. There might be an association between HRV values and antidepressant medication which is responsible for the autonomic disbalance (Licht et al., 2008). In addition, Licht and colleagues (2010) found that HRV values were lower in people taking antidepressant medication compared to people without medication regardless of the success of the therapy. Furthermore, Brunoni and colleagues (2013) found that HRV scores did not change following treatment with either a non-pharmacological (tDCS) or pharmacological (sertraline) intervention, nor did HRV increase with clinical response to treatment.

2.4.3 Methodological considerations

The variability of HRV values is considerable between the various intraindividual measurement points. This instability of HRV short term recordings should not be confused with low reliability, as short-term recordings are considered to be quite accurate estimations of vagal activation (Sandercock et al., 2005; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). The temporal volatility rather reflects the imminent state characteristics of the ANS, and the vagal activity of depressive persons in particular. Consistently, Bertsch and colleagues (2012) have shown that the state dependent variance in single measurements is 49% and can be reduced to 25% by using multiple measurements. Therefore it seems prudent to measure HRV from multiple measurements to reduce the situational influences, especially for naturalistic settings (Bertsch et al., 2012; Eikeseth et al., 2020; Sandercock et al., 2005). Especially for a clinical naturalistic sample, HRV measurement is influenced by multiple confounders, and at present, no clear guidelines or comparative values for depressive patients exist. This lack of comparable studies may be due to a considerable publication bias because we would expect more studies reporting no significant associations between MDD and HRV especially after therapy, or a higher variability within the different study findings. Another explanation could be the high effort to use a robust research design in a clinical naturalistic setting. In addition, the comparability of published HRV values between studies is difficult because there are a large number of different HRV indices with different implications and interpretations of each indices (Heathers, 2014; Quintana & Heathers, 2014).

2.4.4 Limitations

When interpreting the findings of the present study, the following three limitations need to be considered. Firstly, the possible effects of antidepressant medication on HRV have been widely discussed in the current literature, without any overall agreement (Kemp et al., 2010; Licht et al., 2010; O'Regan et al., 2015). Due to the severity and duration of their disorders, patients within our dataset were prescribed antidepressant medication. In addition, adjustments and terminations of medication during inpatient therapy are common within this natural setting, and thus lead to changes in individual medication combinations. These factors could not be calculated as control variables since the respective subgroups were too small. However, potential interaction between antidepressant medication and HRV values cannot be ruled out.

Second, in addition to the diagnosis of depression, other mental illnesses, e.g. anxiety disorders (Chalmers et al., 2014) or personality disorders (Carr et al., 2018; Meyer et al., 2016) seem to be associated with reduced HRV values. Our sample consists of seriously depressed patients with a large variety of comorbidities, which may have affected the results. These possible effects cannot be statistically isolated due to the many combinations and resulting homogeneous sub-groups. Additionally, depression itself is a very heterogeneous disorder and strongly different subgroups exist (Rechlin, 1994) in our naturalistic sample recruited directly in a psychosomatic hospital. Lastly our study does not include a healthy control group, so that comparable studies (e.g. with normed values or similar measurement and calculation methods) were used to classify the HRV values (Nunan et al., 2010). Besides, the absolute HRV values are not of primary importance but rather the relationship between HRV and depression.

Third, although we controlled for the most significant situational confounders, we were not able to control for all possible confounders. For example, there is evidence that the menstrual cycle or Body Mass Index can affect HRV levels (Vallejo et al., 2005) or that ruminative thoughts might lower HRV (Cropley et al., 2017). We tried to minimize these effects with three measurements on different days but there might be still confounders impacting our findings.

2.4.5 Future research

Future studies investigating the association between HRV and MDD before and after psychotherapy should consider subgroups within the (naturalistic) sample. The most relevant subgroups in this sense are based on comorbidities and medications. Regarding comorbidities, Kircanski and colleagues (2019) showed that only in anxious depressed patients, HRV can predict the treatment outcome. Consequently, a three-group design (patients with depression, patients with comorbid depression and anxiety and patients with anxiety disorders only) with a large sample to separate these comorbidities, seems necessary. Regarding medications, despite a large evidence base, there are no clear conclusions of confounding effects on HRV measurement. It cannot be precluded that changes of the autonomic nervous system after a successful MDD therapy are masked by the effects of antidepressants. Consequently, a subgroup design considering the medications and including a control group without medications seems necessary.

From a methodological perspective, multiple HRV measurements should be used in future investigations to obtain more valid and reliable data compared to one-time measurements. Alternatively, long-term HRV measurements could be used to further analyze potential long-

term effects. In addition, future studies could augment the study design with a follow-up measurement after discharge (e.g. six months later) to examine if there is a lag effect of HRV, at the end of therapy.

2.5 Conclusion

The present study is among the first to examine HRV before and after a psychotherapy inpatient treatment in a naturalistic sample. By measuring HRV multiple times at intake and multiple times at discharge and considering situational factors, we collected reliable and valid psychophysiological data. In summary, we observed an association between MDD and HRV values at intake, but not at discharge, even though depressive symptoms improved significantly. Therefore, HRV does not appear to be suitable as a change-sensitive biomarker for depression. This means that even after successful psychotherapy, the autonomic imbalance remains the same and can still be treated as a risk factor for diseases like CVD or an additional depressive episode. For this reason, in addition to psychotherapy, behavioral change techniques should be promoted, that are known to have beneficial effects on the autonomic nervous system, e.g. physical exercise, smoking cessation and healthy eating.

3. The Cortisol Awakening Response at Admission to Hospital predicts Depression Severity After Discharge in MDD patients[3]

[3] This chapter is based on the following manuscript:

Eikeseth, S. S., Denninghaus, S., Cropley, M., Witthöft, M., Pawelzik, M., & Sütterlin, S. (2019). The cortisol awakening response at admission to hospital predicts depression severity after discharge in MDD patients. *Journal of Psychiatric Research, 111*, 44-50.

3.1 Introduction

Biological markers that can help in diagnosis and predict post treatment symptom deterioration in Major Depression Disorder (MDD) are still scarce. This may be due to the large heterogeneity of physical symptoms associated with depression and the insufficient measurement validity of many biomarkers (Mayeux, 2004). Whether treatment is to become more effective in the future will depend on gaining a deeper understanding of the etiology and pathophysiology of depression (Saveanu & Nemeroff, 2012). Alterations in the hypothalamic-pituitary-adrenal (HPA) axis is a frequently reported and replicated finding in a large proportion of patients with MDD (Varghese & Brown 2001). Typically, MDD patients demonstrate hypersecretion of cortisol partly due to an impaired endogenous glucocorticoid feedback regulation of HPA axis activity (Pariante & Lightman, 2008; Sachar et al., 1970; Saveanu & Nemeroff, 2012; Stetler & Miller, 2011). Moreover, evidence suggests that HPA axis hyperactivity may not be a consequence of depression, but may rather be a predisposing factor for developing depression as a result of adverse early life experiences, sensitizing the individual to stress by causing enduring alterations in HPA axis functioning from early on (Pariante & Lightman, 2008).

The CAR refers to the sharp rise in cortisol secretion in response to awakening that peaks after approximately 30 to 45 minutes (Fries et al., 2009; Pruessner et al., 1997). This distinct rise in cortisol in response to awakening has been related to one's anticipations for the upcoming day and the corresponding "mobilizing" of energy resources to meet these demands (Fries et al., 2009). This hypothesis is supported by observations of the CAR being higher during weekdays than during weekends, because demands are usually higher during weekdays (Schlotz et al., 2004), and elevated when experiencing higher levels of social stress, worry, and lack of social recognition (Wüst et al., 2000a). The CAR reflects one aspect of HPA axis reactivity that is sensitive to certain psychosocial and health factors such as job stress, life stress and fatigue (Chida & Steptoe, 2009; Schmidt-Reinwald et al., 1999; Wüst et al., 2000a).

In spite of the large body of literature relating hypercortisolism to MDD, studies have also reported a blunted CAR in MDD (Dedovic & Ngiam, 2015), suggesting that hypocortisolism may occur in cases of MDD. In the recent years it has been suggested that the CAR is related to depression severity, in which moderate degrees of depression have been associated with heightened CAR, and severe depression with blunted CAR (Chida & Steptoe, 2009; Wardenaar et al., 2011). Several mechanisms for the development of hypocortisolism from hyper-

cortisolism have been outlined (see Fries et al., 2005; Heim et al., 2000). HPA axis downregulation is known to follow persisting chronic stress (Heim et al., 2000), and is related to burnout (Oosterholt et al., 2015), which is a state considered to conceptually overlap with depression (Bianchi et al., 2015).

The symptom deterioration that is typically observed between treatment offset and follow-up assessments may be influenced by the continuation of pretreatment stressors (Monroe et al., 2009). Such pretreatment stressors may be driven by interactions between the individual and the environment. For instance, depressed individuals may elicit social rejection through maladaptive social behavior (van Orden & Joiner, 2013; see also De Rubeis et al., 2017), and it seems plausible that these effects persist even after discharge in remitted MDD patients. Moreover, a higher number of depressive episodes, more residual symptomatology, and daily hassles were related to symptom deterioration after treatment over a 2-year period (Bockting et al., 2006). A follow-up of this study, extending to 5.5 years, identified two maladaptive coping strategies as additional predictors of symptom deterioration: avoidant problem solving and lower ability to distract oneself from negative thoughts (Bockting et al., 2009). Environmental characteristics may include factors related to socio-economic conditions (Monroe et al., 2009).

We argue that — as an index of physiological stress and HPA reactivity — the CAR reflects the body's cumulated response to enduring environmental stressors and is thus an index of an individual's overall level of psychosocial stress prior to hospitalization. Moreover, recent research suggests that the CAR is a predictor for treatment outcomes in MDD (Jones et al., 2015).

This article presents two studies examining the value of the CAR assessed during the first days of hospitalization prior to psychotherapeutic intervention in predicting long-term treatment outcome in patients with MDD. Whereas the first study was exploratory in nature, the second study aimed at replicating and support the findings of Study 1. In Study 1, the association between the CAR at time of intake and depressive symptoms six weeks after discharge was examined in a sample of 101 inpatients. Study 2 was an extension of Study 1, involving a larger sample of MDD inpatients (n = 127) with an extended follow-up period of six months after discharge. Based on the results of Study 1 and previous research suggesting that a blunted CAR is associated with higher depressive symptom severity, lower treatment response, less favorable prognosis, and chronic stress, (Chida & Steptoe, 2009; Jones et al., 2015; Vreeburg et al., 2013; Wardenaar et al., 2011), we hypothesized in Study 2 that a lower

CAR at intake to hospitalized treatment for MDD would predict a higher depressive symptom deterioration six weeks and six months following discharge. To our knowledge these are the first studies investigating the CAR as a biomarker for predicting long-term post treatment depressive symptom deterioration in an inpatient setting.

Both studies were approved by the Ethics committee of the "Medical Association Westfalen-Lippe" and written informed consent was obtained from all participants prior to data collection.

3.2 Study 1

3.2.1 Method

3.2.1.1 Participants

One hundred and one inpatients (57 % females) admitted for psychotherapy treatment in a German psychosomatic hospital were recruited between 2011 and 2014. Inclusion criteria was MDD as a main diagnosis. Exclusion criteria were glucocorticoid medication use, comorbid addiction disorder, excessive substance abuse, psychosis, autoimmune-thyroiditis, personality disorders due to medical conditions, respiratory disease, hormone or heart conditions. The participants' diagnoses were determined through a structured clinical interview (SCID I, II; Fydrich et al., 1997; Wittchen et al., 1997a; Wittchen et al., 1997b). At intake, 36 patients (35.6 %) were on at least one psychotropic drug (Table 3.1).

3.2.1.2. Materials and procedures

3.2.1.2.1. Beck Depression Inventory-I. The Beck Depression Inventory (BDI) is a 21-item self-report questionnaire assessing the severity of depressive symptoms. Items consist of four response statements that are scored from 0 to 3 representing ascending severity of depressive symptoms (Beck & Steer, 1993; German version: Hautzinger et al., 1995). A score of 0-9 indicates minimal depression, 10-18 indicates mild depression, 19-29 indicates moderate depression, and 30-63 indicates severe depression. Scores were assessed within the first five days after admission (BDI Intake), and during the last five days before discharge (BDI Discharge). The treatment consisted of cognitive behavioral therapy (CBT) with daily individual sessions, and additional group therapies and pharmacotherapy. The BDI follow-up assessment was completed via an online questionnaire with patients being personally contacted by a member of the research/clinical team by email six weeks (BDI 6WF) after discharge. After completing the online follow-up assessment the participants were also invited back to the clinic to assess their

wellbeing and to discuss the results with a clinician. The internal consistency (Cronbach's alpha) of the BDI at all timepoints was good to very good: BDI Intake α = .88 (*N* = 94); BDI Discharge α = .87 (*N* = 75); BDI 6WF α = .93 (*N* = 62) and comparable to a previous validation of the German BDI version (Kühner et al., 2007).

***3.2.1.2.2. Assessment of CAR*.** Cortisol secretion was assessed during the first five days of admission through saliva sampling using cotton salivettes (manufacturer: Sarstedt AG & Co., Nümbrecht/Germany) at two time points: Directly after awakening in the morning, and 30 minutes after awakening. The samples were sent to a medical laboratory for Enzyme Immunoassay after collection. The CAR was calculated by subtracting the cortisol measurement 30 minutes after awakening from the cortisol measurement directly after awakening, such that higher values would indicate a higher CAR[4]. Salivettes were handed out by the therapists the day before the measurements along with instructions about using the first salivette directly after awakening and the second salivette 30 minutes after awakening. Participants were advised to refrain from brushing their teeth, sucking on drops, exercise, caffeine consumption and smoking between awakening and the second cortisol assessment at 30 minutes after awakening.

3.2.1.3. Statistical analyses

Statistical analysis was performed using IBM SPSS Statistics version 24. Prior to analysis, all variables were checked for accuracy of data entry and missing values. The differences in sample size within the tables reflect missing values. Little's MCAR (missing completely at random) Test showed a statistically non-significant result (χ^2 = 28.36, *df* = 27, *p* =.393) indicating that values missing completely at random could be inferred (Tabachnick & Fidell, 2013). All variables were checked for univariate outliers by identifying cases with *z*-values above 3.29 or below -3.29, and dealt with by deletion. One individual's measurement at awakening and one individual's measurement 30 minutes after awakening were identified as outliers and deleted. All variables were normally distributed.

The associations between CAR and BDI measurements (intake, discharge and 6WF) were assessed using Pearson's correlations. One patient had bipolar depression (F31.4), and therefore a second correlation analysis was performed excluding this patient to explore how

[4] *Following a request from one of the reviewers, the analysis was repeated using the Area Under the Curve group (AUGg) method, in line with (Pruessner et al., 2003), but the overall pattern of results remained unchanged. For this reason and for ease of exposition, these results are not reported.*

this would affect the results. The results from this second correlation analysis are reported separately.

3.2.2. Results

Descriptive statistics are presented in Table 3.1. No significant associations were found between the CAR at intake and BDI Intake, BDI Discharge, and BDI 6WF (Table 3.2). However, the strength of the associations increased at each time point, and a tendency towards a negative linear association emerged between CAR and BDI 6WF. Age was negatively associated with BDI Discharge. Consistent with the literature, neither age (r = -.028, p = .801, two-tailed) nor sex (r = -.037, p = .741, two-tailed) were associated with the CAR (see Fries et al., 2009). The results from the separate correlation analysis with the exclusion of one patient with bipolar depression yielded similar non-significant associations between CAR and the BDI assessments: CAR*BDI Intake: r = -.096, p = .400, two-tailed; CAR*BDI Discharge: r = -.157, p = .212, two-tailed; and CAR*BDI 6WF: r = -.249, p = .069, two-tailed.

Table 3.1.

Summary of descriptive statistics and cortisol levels in Study 1

		N	*M*	*SD*	Min	Max
Survey and Demographics	Age	101	40.3	13.7	15.9	68.7
	BDI Intake	94	23.8	10.7	0	52.0
	BDI Discharge	78	11.6	8.3	0	40.0
	BDI 6WF	65	13.7	10.6	0	45.2
	Valid N (listwise)	55				
Psychotropic medication use	Total	36 (35.6 %)				
	SSRI	13				
	SNRI	12				
	Tricyclics	2				
	MAO inhibitors	8				
	Antipsychotics	6				
	Anticonvulsants	8				
	Other	4				
Cortisol	Cortisol awakening	87	15.7	8.6	1.6	40.4
	Cortisol +30	86	23.2	11.1	2.9	55.0
	CAR	85	7.5	9.5	-21.3	30.9
	Valid N (listwise)	85				

Note. The percentage of total psychotropic medication use refers to the percentage of the total sample. The total number of each psychotropic drug does not add up to the total number of patients taking psychotropic medication because 14 patients were taking more than one drug. Cortisol Values in nmol/L. Abbreviations: *M* = Mean, *SD* = Standard deviation, Min = Minimum, Max = Maximum, WF = Week Follow-up.

Table 3.2.

Correlation between CAR and depressive pathology in Study 1

Measure		1. CAR	2. BDI Intake	3. BDI Discharge	4. BDI 6WF
1. CAR	Pearson Correlation	-	-.097 (-.310 - .125)	-.163 (-.389 - .082)	-.255 (-.487 - .011)
	Sig. (2-tailed)		.391	.191	.060
	N		80	66	55
2. BDI Intake	Pearson Correlation	-.097 (-.310 - .125)	-	**.528** ** **(.343 - .674)**	**.520** ** **(.311 - .681)**
	Sig. (2-tailed)	.391		.000	.000
	N	80		75	62
3. BDI Discharge	Pearson Correlation	-.163 (-.389 - .082)	**.528** ** **(.343 - .674)**	-	**.666** ** **(.493 - .788)**
	Sig. (2-tailed)	.191	.000		.000
	N	66	75		58
4. BDI 6WF	Pearson Correlation	-.255 (-.487 - .011)	**.520** ** **(.311 - .681)**	**.666** ** **(.493 - .788)**	-
	Sig. (2-tailed)	.060	.000	.000	
	N	55	62	58	

Note. * $p < .05$, ** $p < .001$. Two-tailed 95% confidence intervals in parentheses.

3.2.3. Discussion

The tendency towards a negative association between CAR and BDI 6WF did not support the CAR as a predictor of follow-up symptom deterioration, but indicated the need for further research on this topic. A blunted CAR is suggested to be associated with high depression severity (Dedovic & Ngiam, 2015), and the low proportion of severely depressed MDD patients in the present sample might account for the relatively weak association between CAR and depression (Dedovich & Ngiam, 2015; Wardenaar et al., 2011). Furthermore, it is unclear at which time after discharge from inpatient treatment the cumulative effect of exposure to internal and external risk factors maximizes. We therefore replicated the previous study with a larger sample of depressed inpatients, and utilized a longer follow-up period of six months after discharge. We were also able to retrieve data on body mass index (BMI) from the sample in Study 2 which was not possible in Study 1.

3.3. Study 2

Study 2 is a replication and extension of Study 1 where we increased the number of participants, added an additional follow-up measure of BDI at six months after discharge (BDI 6MF), updated the diagnostic tool from BDI-I to BDI-II, collected data on BMI, and retrieved inter- and inter assay coefficients for the cortisol analyses which were not available for Study 1. Improvements and differences from BDI to BDI-II are outlined below. The same procedures for measuring CAR and BDI at different timepoints were used as described in Study 1. Based on the findings of Study 1, we hypothesized that the CAR at intake would be negatively associated with follow-up depressive symptoms and predict symptom deterioration six weeks and six months following discharge.

3.3.1. Method

3.3.1.1. Participants

One hundred and twenty-seven patients (57.7 % females) admitted to the same inpatient psychotherapeutic treatment as in Study 1 were recruited between 2014 and 2015. Study 2 had the same inclusion and exclusion criteria and assessed the patients' clinical diagnoses as in Study 1. None of the participants had a bipolar disorder diagnosis. At intake, 71 (57.7 %) patients were on at least one psychotropic drug (Table 3.3).

3.3.1.2. Materials and procedures

3.3.1.2.1. Beck Depression Inventory-II. The Beck Depression Inventory-II (BDI-II) measures the intensity of depressive symptoms and attitudes in accordance with the DSM 4 criteria (Beck et al., 1996a; German version: Kühner et al., 2007). Several changes were made in the BDI-II to increase its psychometric properties, such as eliminating four items (e.g. items involving changes in body image), adding four diagnostic specific items (e.g. items involving concentration difficulties, agitation, loss of energy and worthlessness), and reformulating items involving eating and sleeping problems (Beck et al., 1996b). Participants were asked to rate the past two weeks instead of the past week as in BDI I (Beck et al., 1996a). The cutoff scores differ in BDI-I and BDI-II. In BDI-II, scores between 0-13 indicate minimal depression, 14-19 indicate mild depression, 20-28 indicate moderate depression, and 29-63 indicate severe depression. In our sample, the scale had very good internal consistency at all timepoints: BDI-II Intake $\alpha = .92$ ($N = 127$); BDI-II Discharge $\alpha = .93$ ($N = 120$); BDI-II 6WF $\alpha = .94$ ($N = 96$); BDI-II 6MF $\alpha = .95$ ($N = 83$).

As in Study 1, the follow-up assessments were completed via an online questionnaire with patients being personally contacted by a member of the research/clinical team by email six weeks (BDI 6WF) and six months (BDI 6MF) after discharge. After completing the online follow-up assessments, the participants were invited back to the clinic to discuss their results with a clinician.

3.3.1.2.2. Assessment of CAR. The same materials, measurements, and procedures as described in Study 1 were used to assess the CAR. Intra- and inter assay coefficients were 3.05 % and 4.15 %, respectively.

3.3.1.3. Statistical analyses

Statistical analysis was performed using IBM SPSS Statistics version 24. The same procedures as in Study 1 were followed prior to analysis, in addition to checking the assumptions for multiple regression analysis. The differences in sample size within the tables reflect missing values. Little's MCAR (missing completely at random) Test showed a statistically non-significant result ($\chi 2 = 80.54$, $df = 78$, $p = .400$) indicating that values missing completely at random could be inferred (Tabachnick & Fidell, 2013). Four individuals' cortisol measurement after awakening, one individual's cortisol measurement 30 minutes after awakening and one CAR difference value were identified as univariate outliers and deleted. All variables were normally distributed.

The associations between CAR and the BDI-II measurements (BDI-II Intake, BDI-II Discharge, BDI-II 6WF, and BDI-II 6MF) were assessed using Pearson's correlations. In addition, four hierarchical multiple regression analyses were conducted to examine whether CAR predicted follow-up depression at six weeks and six months after discharge, controlled for initial depression (either at intake or at discharge). BMI was entered in step 1 in all hierarchical regression analyses.

3.3.2. Results

Descriptive statistics for age, BDI-II measurements and cortisol measurements are presented in Table 3.3. CAR was negatively associated with both BDI-II follow-ups (Table 3.4). As in Study 1, age (r = -.051, p = .595, two-tailed) and sex (r = -.039, p = .686, two-tailed) were not associated with CAR. BMI was correlated with age (r = .318, p < .001, two tailed), sex (r = .279, p = .002), BDI 6WF (r = .268, p = .011, two-tailed), and BDI 6MF (r = .269, p = .012, two-tailed), but not with the CAR (r = -.093, p = .332, two-tailed).

Table 3.3.

Summary of descriptive statistics and mean cortisol levels in Study 2

		N	*M*	*SD*	Min	Max
Survey and Demographics	Age	123	42.9	13.7	17.0	68.0
	BDI Intake	122	28.9	11.9	1.0	52.0
	BDI Discharge	118	13.3	10.7	0.0	47.0
	BDI 6WF	89	15.1	12.0	0.0	49.0
	BDI 6MF	88	15.9	12.6	0.0	53.0
	Valid N (listwise)	66				
Psychotropic medication use	Total	71 (57.7%)				
	SSRI	30				
	SNRI	23				
	Tricyclics	13				
	MAO inhibitors	9				
	Antipsychotics	7				
	Anticonvulsants	17				
	Other	9				
Cortisol	Cortisol awakening	114	26.2	14.3	2.9	81.1
	Cortisol +30	114	40.9	19.2	3.2	88.1
	CAR	112	14.4	16.6	-19.7	61.4
	Valid N (listwise)	112				

Note. The percentage of total psychotropic medication use refers to the percentage of the total sample. The total number of each psychotropic drug does not add up to the total number of patients taking psychotropic medication because 25 patients were taking more than one drug. Cortisol Values in nmol/L. Abbreviations: *M* = Mean, *SD* = Standard deviation, Min = Minimum, Max = Maximum, WF = Week Follow-up, MF = Month Follow-up.

Table 3.4.

Correlation between CAR and depressive pathology in Study 2

Measure		1. CAR	2. BDI-II Intake	3. BDI-II Discharge	4. BDI-II 6WF	5. BDI-II 6MF
1. CAR	Pearson Correlation	-	.012 (-.174 - .197)	-.090 (-.277 - .104)	**-.259* (-.451 - -.044)**	**-.223* (-.423 - -.002)**
	Sig. (2-tailed)		.897	.366	.020	.048
	N		111	104	81	79
2. BDI-II Intake	Pearson Correlation	.012 (-.174 - .197)	-	**.530** (.384 - .650)**	**.396** (.204 - .559)**	**.472** (.288 - .622)**
	Sig. (2-tailed)	.897		.000	.000	.000
	N	111		114	88	85
3. BDI-II Discharge	Pearson Correlation	-.090 (-.277 - .104)	**.530** (.384 - .650)**	-	**.797** (.703 - .863)**	**.760** (.652 - .837)**
	Sig. (2-tailed)	.366	.000		.000	.000
	N	104	114		84	84
4. BDI-II 6WF	Pearson Correlation	**-.259* (-.451 - -.044)**	**.396** (.204 - .559)**	**.797** (.703 - .863)**	-	**.842** (.758 - .898)**
	Sig. (2-tailed)	.020	.000	.000		.000
	N	81	88	84		71
5. BDI-II 6MF	Pearson Correlation	**-.223* (-.423 - -.002)**	**.472** (.288 - .622)**	**.760** (.652 - .837)**	**.842** (.758 - .898)**	-
	Sig. (2-tailed)	.048	.000	.000	.000	
	N	79	85	84	71	

Note. * $p < .05$, ** $p < .001$. Two-tailed 95% confidence intervals in parentheses.

Two hierarchical multiple regression analyses were conducted to predict BDI-II 6WF (Table 3.5), one controlling for BDI-II Intake (left column) and one controlling for BDI-II Discharge (right column). BDI-II Intake was entered in step 1 and the CAR at step 2, which added a significant amount of explained variance to the prediction of BDI-II 6WF. The final model as a whole was statistically significant: F (3, 77) = 9.10, $p < .001$. When BDI-II Discharge was entered in step 1, the CAR predicted less variance in BDI-II 6WF, but remained statistically significant. The final model was statistically significant: F (3, 77) = 56.78, $p < .001$.

In a similar vein, two hierarchical multiple regression analyses were conducted to predict BDI-II 6MF (Table 3.6). In the first hierarchical multiple regression model for BDI-II 6MF, BDI-II Intake was entered in step 1 and CAR was entered in step 2. Adding the CAR as an additional predictor to the model in step 2 significantly increased the model's explanatory power. The final model as a whole was statistically significant: F (3, 75) = 11.14, $p < .001$, where both BDI-II Intake and the CAR were statistically significant individual predictors of BDI-II 6MF. In the second hierarchical model BDI-II Discharge was entered in step 1 and the CAR at step 2 leading to further explained variance. The final model as a whole was statistically significant: F (3, 75) = 41.17, $p < .001$, and both variables made unique contributions to the prediction of BDI-II 6MF[5].

[5] *As requested by one of the reviewers, the associations between the CAR and anxiety at different time points were also explored. This was done by re-running the analyses in Study 1 and 2 by replacing the BDI-assessments with the anxiety subscale of the Symptom Checklist-90 (SCL-90). The CAR was only associated with SCL-90 Anxiety at the six month follow-up in Study 2: r = -.284, 95 % CIs: -.475 - -.068, p = .011. Two hierarchical multiple regressions were conducted predicting SCL-90 Anxiety at six month follow-up with BMI and either SCL-90 Anxiety at intake or discharge in Step 1 and CAR in Step 2. In both hierarchical regression models, the CAR was not a significant predictor of six month follow-up SCL-90 Anxiety.*

Table 3.5.

Summary of hierarchical multiple regression analyses predicting BDI-II 6WF

Controlling for BDI-II Intake			Controlling for BDI-II Discharge		
Predictor	ΔR^2	Standard. β	Predictor	ΔR^2	Standard. β
Step 1	.202**		Step 1	.657**	
BMI		.214*	BMI		.148*
BDI Intake		.365*	BDI Discharge		.774**
Step 2	.060*		Step 2	.031*	
BMI		.190	BMI		.134*
BDI Intake		.371**	BDI Discharge		.761**
CAR		-.246*	CAR		-.178*
Total R^2	.262**		Total R^2	.689**	
Total Adjusted R^2	.233**		Total Adjusted R^2	.677**	
N	80		*N*	80	

Note. * $p < .05$, ** $p < .001$.

Table 3.6.

Summary of hierarchical multiple regression analyses predicting BDI-II 6MF

Controlling for BDI-II Intake			Controlling for BDI-II Discharge		
Predictor	ΔR^2	Standard. β	Predictor	ΔR^2	Standard. β
Step 1	.264**		Step 1	.602**	
BMI		.204*	BMI		.156*
BDI Intake		.442**	BDI Discharge		.736**
Step 2	.044*		Step 2	.021*	
BMI		.183	BMI		.144*
BDI Intake		.448**	BDI Discharge		.725**
CAR		-.212*	CAR		-.145*
Total R^2	.308**		Total R^2	.622**	
Total Adjusted R^2	.280**		Total Adjusted R^2	.607**	
N	78		*N*	78	

Note. * $p < .05$, ** $p < .001$.

3.3.3. Discussion

The results supported our hypothesis that a lower CAR would predict a higher depressive symptom deterioration following discharge from hospitalized treatment in patients with MDD. We found that BDI-II scores at discharge explained more than half of the variance in both BDI-II 6WF and BDI-II 6MF. Adding the CAR into the model explained additional var-

iance beyond the self-reported data, illustrating its predictive value in both follow-up measurements after discharge. Beta weights indicate that the additional explanatory power of the CAR remained very similar over the 6 week- and 6 month-period.

It should be noted that the unique explained variance in follow-up depression by the CAR after controlling for BMI and BDI-II either at intake or discharge was small. However, considering the strong associations between the repeated BDI-II assessments, it is especially noteworthy that pretreatment CAR contributes with additional explained variance in posttreatment follow-up depression, regardless of the magnitude. Moreover, the association between the CAR and depression (Dedovic & Ngiam, 2015), implies that there will be a substantial overlap between the predictive values of pretreatment CAR and depressive symptoms assessed with the BDI-II.

3.4. General Discussion

The search for biomarkers to enhance diagnosis, treatment and prognosis of mental disorders has received increasing attention in recent years, but the findings are somewhat equivocal (Strawbridge et al., 2017). In this paper two studies were conducted to examine the value of the CAR in predicting follow-up depressive symptoms in hospitalized patients with MDD. In Study 1, the CAR was measured at intake before treatment initiation, with self-reported depressive symptoms (BDI-I) assessed at intake, discharge and six weeks after discharge. Study 2 was a replication and extension of Study 1 adding a follow-up assessment of depression severity at six months after discharge with a larger sample and the revised version of BDI (BDI-II).

In Study 1, the CAR had a tendency towards being negatively associated with depression severity at the six week follow-up. In Study 2, the CAR predicted depression severity at six weeks and six months adjusting for depression (BDI-II ratings) at intake or at discharge. The observed negative associations between the CAR and the BDI in both studies indicate that a blunted CAR is associated with greater severity of follow-up self-reported depressive symptoms. To our knowledge, this is the first study to investigate the value of the CAR in predicting post-treatment symptom deterioration in depressed inpatients. Nonetheless, our results are in line with previous work that has found the CAR to predict treatment outcome in inpatients with MDD, where a higher CAR at admission was associated with a greater treatment response (Jones et al., 2015). Despite the different follow-up periods, with depressive symptoms being

assessed at six weeks and six months after discharge in our study, rather than directly at discharge as in the study by Jones and colleagues (2015), both studies indicate that lower CAR is associated with less favorable outcomes in inpatients with MDD. Interestingly, in our study, the CAR was not associated with depressive symptoms at discharge. However, based on the premise that CAR reflects the accumulated levels of stress over time prior to hospitalization, it is not surprising to find that the association between CAR and depressive symptoms does not become evident until the patients have spent time in their home environments, where it is likely that pretreatment stressors still are present. Similarly, in a study with healthy controls and individuals with depression and/or anxiety disorders, a blunted CAR was associated with a less favorable prognosis over a two year period (Vreeburg et al., 2013). Taken together, these and our findings illustrate that a blunted CAR is associated with a less favorable prognosis in MDD.

Two reasons might explain the nonsignificant association between CAR and follow-up depression in Study 1. First, the relatively smaller sample size in Study 1 compared to Study 2 may have resulted in a lack of power preventing the association to reach statistical significance. Second, compared to the sample in Study 2, the severity of depression at intake in Study 1 was lower. When applying the BDI cut-off criteria for severe depression, Study 1 consisted of 25.8 % severely depressed patients, compared to 45.7 % in Study 2. Due to the various differences between the BDI-I and the later published BDI-II, a statistical comparison of both samples would be questionable. It could be speculated however, that the weaker and nonsignificant negative association between the CAR and the BDI 6WF in Study 1, was related to the lower proportion of severely depressed patients and thus a less accentuated profile of blunted CAR in Study 1.

3.4.1. Limitations

Findings of the present study should be interpreted in light of some limitations. A blunted CAR was observed in teachers high in work-related rumination (Cropley et al., 2015). The authors explained their findings in terms of the high ruminators having sleep disturbances, leading to the cortisol secretion occurring before the actual awakening (Cropley et al., 2015; see also Chida & Steptoe, 2009). Sleep disturbance is a symptom of Major Depressive Disorder (APA, 2013) and poor sleep quality may affect the CAR and therefore have influenced our results. As all patients were woken at the same time each morning, we did not assess actual sleep time or quality of sleep. Although the subjects received instructions for conducting the saliva samplings, compliance was not assessed. The CAR has shown to peak between 30 and

45 minutes after awakening, and by sampling only at awakening and 30 minutes after awakening, we cannot be sure to have captured the true CAR peak. However, at least 50 % mean cortisol increase occurs within the first 30 minutes after awakening (Pruessner et al., 1997; Wüst et al., 2000b) which indicates that a substantial proportion of the CAR magnitude was captured in the present studies. Even though the CAR is considered a reliable measure of HPA-axis functioning (Schmidt-Reinwald et al., 1999), we also know that it is influenced by short term changes in relation to anticipations for the upcoming day (see Schlotz, et al., 2004), and ideally, future research should assess the CAR on two consecutive days to reduce variance attributable to situational effects (Hucklebridge et al., 2005; Wüst et al., 2000b). Finally, as this study was based on a relatively heterogeneous naturalistic sample of depressed inpatients it was not possible to systematically examine comorbid mental disorders or subtypes of depressive disorders. However, the tradeoff from less control over such factors also entails the benefit of greater ecological validity with the naturalistic properties of our two studies.

3.4.2. Future directions

Further studies on the CAR as a predictor of future depressive symptoms should include measurements of the CAR in the follow-up periods or during treatment to examine how the CAR changes over time in relation to depressive symptoms, and how depression severity at intake influences this relationship over time. Such studies would benefit from incorporating diary reports to control for psychosocial predictors of symptom changes after discharge and thus have better control over the environments the patients return to after discharge. A main focus for future studies should lie in mapping the most important factors contributing to alterations in the CAR so that its relation to MDD can be fully understood. This may include comparing the CAR and depression severity between the broader diagnostic subtypes of depression (e.g. melancholic and atypical depression) and between degrees of illness chronicity. Indeed, different subtypes of depression have been associated with differences in HPA axis dysregulation, where melancholic, endogenous and psychotic subtypes have been associated with higher cortisol secretion while atypical depression has been associated with lower cortisol secretion (Stetler & Miller, 2011).

3.5. Conclusion

To our knowledge this is the first study investigating the CAR as a biomarker for predicting post treatment depressive symptom deterioration in an inpatient setting. The CAR assessed at intake before treatment initiation predicted depressive symptoms six weeks and six

months after discharge. In addition to being an index of depression severity, an abnormal CAR may also be a reflection of more adverse psychosocial factors prior to hospitalization that may increase the probability for symptom deterioration after discharge. The possibility of identifying at intake which patients will suffer from greater symptom deterioration in the follow-up period could contribute to lowering the high relapse and recurrent rates seen in MDD.

4. The Cortisol Awakening Response at Admission to Hospital predicts Depression Severity After Discharge in MDD patients – A Replication Study [6]

[6] This chapter is based on the following manuscript:

Neyer, S., [redacted], [redacted]., [redacted], [redacted]., [redacted], [redacted]., [redacted], [redacted]., & [redacted], [redacted]., (2021). The Cortisol Awakening Response at Admission to Hospital predicts Depression Severity After Discharge in MDD patients – A Replication Study. Submitted

4.1 Introduction

Major Depressive Disorder (MDD) affects 4.4% of the worldwide population with prevalence estimates rising steadily, yet there remains a lack of sufficiently effective and sustainable treatment options (World Health Organization, 2017). One possible explanation for this, is the heterogeneity of MDD and the associated heterogeneous diagnostic tools, treatment possibilities and different treatment results (Baumeister & Gordon, 2012; van Loo et al., 2012). Research is therefore trying to find markers that may help to classify and specify symptoms to adapt to individual therapy. The extent to which treatment can become more effective for MDD arguably depends on a deeper understanding of its etiology and pathophysiology (Saveanu & Nemeroff, 2012).

Valid biological markers which may help to specify the diagnosis and predict post treatment symptom deterioration are still scarce. This may be due to the large heterogeneity of physical symptoms associated with MDD (e.g., sleeping disorder, reduced appetite, pain, blood count changes and many more), the insufficient measurement validity of many biomarkers (Mayeux, 2004) or the lack of measurement guidelines.

4.1.1. Biological foundations

Some studies report that MDD patients have typical alterations in the hypothalamic-pituitary-adrenal (HPA) axis (Ehlert et al., 2001; Varghese & Brown, 2001). Most MDD patients demonstrate hypersecretion of cortisol partly due to an impaired endogenous glucocorticoid feedback regulation of HPA axis activity (Saveanu & Nemeroff, 2012; Stetler & Miller, 2011). Cortisol secretion in the morning is considered to be one of the most relevant measures to classify the function of the HPA axis (Stalder et al., 2016). In response to awakening, cortisol secretion rises sharply and peaks after approximately 30-45 minutes; a process termed the "Cortisol awakening response" (CAR) (Fries et al, 2009; Pruessner et al., 1997). The CAR has been related to the anticipations for the upcoming day and "mobilizing" of energy resources (Adam et al., 2006; Fries et al., 2009). The CAR represents a reliable measure of the HPA axis reactivity, that is sensitive to different psychosocial and health factors such as job stress, life stress and fatigue (Chida & Steptoe, 2009; Schmidt-Reinwald et al., 1999). Chronic and acute psychosocial stress has been associated with an increased CAR in cross-sectional studies (Adam et al., 2006; Schlotz et al., 2004).

4.1.2. CAR in MDD Patients

Studies examining the association between the CAR and depression have so far found that an unusual (higher or lower) CAR is significantly associated with depression; but the direction of this association seems unclear. Some studies report a reduced CAR in depressed patients (Dedovic & Ngiam, 2015; Stetler & Miller, 2011), whereas others report an increased CAR (Bhagwagar & Cowen, 2005; Pruessner et al., 2003). Recent research, however, suggests that the CAR association is moderated by depression severity, in which mild to moderate degrees of depression have been related to a heightened CAR, while severe or chronic depression to a blunted CAR (Chida & Steptoe, 2009; Wardenaar, et al., 2011). A long period of mental or physical stress leads to a downregulation of cortisol receptors so that the HPA axis becomes less responsive (Heim et al., 2000). Fries and colleagues (2005) suggest that hypocortisolism results from a long period of hypercortisolism.

To date, there is a lack of clarity and debate as to whether a changed CAR is a risk factor for the development of depressive episodes or a consequence of the disorder. There is evidence from several prospective studies that a high CAR might be a biomarker in healthy young people to predict the onset of MDD (e.g. Adam et al., 2010; Mannie et al., 2007). But there is also research suggesting that a changed CAR might be a consequence of the disorder and also a predictor of the course of the disorder (Bhagwagar & Cowen, 2007; Vreeburg et al., 2013). For example, Bhagwagar and Cowen (2007) found elevated cortisol secretion in recovered depressed patients, while Vreeburg and colleagues (2013) found that a lower CAR in depressive patients, predicted an unfavorable course of disorder development over the following two years.

4.1.3. Changes of CAR after psychotherapy

Jones and colleagues (2015) argue that the CAR might be a predictor for treatment outcomes in MDD patients. Patients with a high CAR at intake showed a better treatment response at discharge after a four-week hospital stay. At follow-up measurement points, however, many MDD inpatients show a typical symptom deterioration which may be influenced by the renewed confrontation with pretreatment stressors in their environment (Monroe et al., 2009). Possible explanations for these findings could be that depressed individuals may elicit social rejection through maladaptive social behavior (van Orden & Joiner, 2013). To further investigate these results, our earlier study (Eikeseth et al., 2019) analyzed the association between CAR at intake and long term depressive symptoms after an inpatient therapy. We were able to show that a blunted CAR before an inpatient psychotherapy is related to the severity of

depression six weeks and six months after discharge in patients with MDD. However, the effect sizes were small and due to the observation period of 30 minutes after waking up, it remains questionable whether the true CAR peak was measured. Additionally the review from Bhagwagar and Cowen (2007) shows that many of the neurobiological abnormalities still persist after a depressive episode and that these abnormalities are associated with changes in emotional information processing. They show that in people where the risk of recurrent depression is increased, the brain still appears to be in a state that prefers the processsing of negative information.

4.1.4. Need of Replication Study

In summary, the CAR might be a possible candidate to provide some information on the course of MDD at the beginning of a psychotherapy, but so far there is a lack of studies that have examined the long term effects, particularly in naturalistic settings.There are concerns about the replicability of the relationship between CAR and depression and therefore there is a call for replication studies to get more information about the association and predictive power of CAR (De Weerd-Wilson & Gunn, 2017). Therefore the purpose of this study is to analyze the CAR during the first days of hospitalization prior to a naturalistic inpatient psychotherapeutic treatment to predict long-term treatment outcome in MDD. We do this by replicating our previous study from Eikeseth and colleagues (2019) following a now stricter measurement and monitoring protocol (CAR measurement and monitoring protocol as suggested by Stalder and colleagues, 2016). While Eikeseth and colleagues (2019) measured the CAR two times in the morning (awakening and 30 Minutes after awakening), this assessment protocol could be criticized for potentially missing the CAR preak, even though that at least 50 % mean cortisol increase occurs within the first 30 minutes after awakening (Pruessner et al., 1997; Wüst et al., 2000b). The replicating study uses a longer measurement interval and three samples to determine the more common way of calculating CAR as area under the curve to predict depressive symptoms six weeks and six months after discharge. In a written follow-up survey after the cortisol measurement, compliance with the measurement protocol, sleep, mood and wake-up times were ascertained.

Based on our previously reported findings (Eikeseth et al., 2019), we hypothesized that the CAR at intake would be significantly negatively associated with follow-up depressive symptoms and predict symptom deterioration six weeks and six months following an inpatient psychotherapy.

4.2. Method

4.2.1. Participants

The sample of this study is highly comparable to Eikeseth and colleagues (2019). One-hundred and forty nine inpatients (67.8% females) admitted for psychotherapy treatment in a German psychosomatic hospital were recruited between 2019 and December 2020. As in the study of Eikeseth and colleagues (2019) the inclusion criteria was MDD as a main diagnosis. Exclusion criteria were glucocorticoid medication use, comorbid addiction disorder, excessive substance abuse, psychosis, autoimmune-thyroiditis, personality disorders due to medical conditions, respiratory disease, hormone or heart conditions (N=21). The participants' diagnoses were determined through a structured clinical interview (SCID I, II; Wittchen et al., 1997a; Wittchen et al., 1997b) by a trained psychotherapist. Eighty-five percent (N=127) of the patients suffered from at least one additional mental disorder. Therefore, most patients were on at least one psychotropic drug at intake or started a medication during the psychotherapy. This study was approved by the Ethics committee of the "Medical Association Westfalen-Lippe" and written informed consent was obtained from all participants prior to data collection. This study was also pre-registered on Aspredicted.org (#45146).

4.2.2. Materials and procedures

4.2.2.1 Beck Depression Inventory-II

Consistent with Eikeseth and colleagues (2019) we used the Beck Depression Inventory-II (BDI-II, Beck et al., 1996a). This is a self-assessment questionnaire which indicates the intensity of depressive symptoms and attitudes in accordance with the DSM 4 criteria (Beck et al., 1996a; German version: Kühner et al., 2007). All assessments were completed via an online questionnaire. The patients were personally contacted by a member of the research/clinical team by email six weeks (BDI-II 6WF) and six months (BDI-II 6MF) after discharge. After completing each follow-up assessment, the participants were invited back to the clinic to discuss their results with their individual psychotherapist.

4.2.2.2. Assessment of CAR

As in the study by Eikeseth and colleagues (2019), the cortisol secretion was assessed during the first five days of admission through saliva sampling using cotton salivettes (manufacturer: Sarstedt AG & Co., Nümbrecht/Germany). In this study we assessed the cortisol level

at three time points in the morning (Eikeseth et al., 2019), which were directly after awakening in the morning, 30 minutes after awakening, and 45 minutes after awakening. The samples were sent to a medical laboratory for Enzyme Immunoassay after collection on the same day. The CAR was calculated by using the Area Under the Curve ground (AUCg) and the Area Under the Curve increase (AUCi) in line with Pruessner and colleagues (2003). Persons with an AUCg of at least .091μgdl were classified as CAR responder (Clow et al., 2004). The AUCg has been shown to be a reliable marker in terms of individual stability (Edwards et al., 2001). Additionally, we calculated the total cortisol increase, calculated as the difference between cortisol levels at 30 minutes or 45 minutes after awakening and cortisol right after awakening). The calculation of the more accurate and widely accepted AUC measure in addition to cortisol increases assessed as difference between two time points is an extension of the original investigation by Eikeseth and colleagues (2019).

Salivettes were handed out by the therapists the day before the measurements along with instructions about using the first salivette directly after awakening, the second salivette 30 minutes after awakening and the last one 45 minutes after awakening. Participants were advised to refrain from brushing their teeth, sucking on drops, doing exhausting exercise, caffeine consumption and smoking, between awakening and the following cortisol assessments after awakening.

In this study we added a follow up measure to ensure that the patients had fully and correctly implemented the instructions. Apart from that the patients had to indicate deviations from the survey protocol. In the event of serious deviations from the survey protocol, the measurement was repeated the following day or the patients were excluded from the study (N=6). Participants were asked to specify at what time in the morning they woke up and if they had any sleeping disorders during the night.

After checking all exclusion criteria N=122 patients were included into the study (N=80 females; N=42 males; M=39.33 years, SD=14.35)

4.2.3. Statistical analyses

Statistical analysis was performed using IBM SPSS Statistics version 27. Prior to analysis, all variables were checked for accuracy of data entry and missing values. The differences in sample size within the tables reflect missing values. Little's MCAR (missing completely at

random) Test showed a statistically non-significant result (χ^2=42.54, *df* = 48, *p* =.696) indicating that values missing completely at random could be inferred (Tabachnick & Fidell, 2013). All variables were checked for univariate outliers by identifying cases with *z*-values above 3.29 or below -3.29, and dealt with by deletion. One individual's measurement at the evening was identified as outlier and deleted.

The associations between CAR values and the BDI-II measurements (BDI-II Intake, BDI-II Discharge, BDI-II 6WF, and BDI-II 6MF) were assessed using Pearson's correlations (significance level *p*=.05).

4.3. Results

4.3.1. Descriptive statistics and Check for potential confounding variables

We analyze the correlation between cortisol values at the beginning of an inpatient psychotherapy and depressive symptoms after inpatient psychotherapy. The descriptive statistics are presented in Table 4.1 including the CAR values. CAR values were lower compared to studies with healthy participants (see Clow et al., 2004: Cortisol Awakening=.40, Awakening+30=.73, Awakening+45=.67) and lower than in the Study from Eikeseth and colleagues (2019).

Table 4.1.

Summary of descriptive statistics and mean cortisol levels

		Replication Study					Eikeseth et al., 2019 (Cortisol values transformed in µg/dl)				
		N	*M*	*SD*	Min	Max	*N*	*M*	*SD*	Min	Max
Survey and Demographics	Age	118	39.34	14.35	18	67	128	43.1	13.7	17.0	68.0
	BDI-II Intake	122	31.20	10.19	14	56	127	28.7	11.8	1.0	52.0
	BDI-II Dis-charge	122	16.80	11.69	0	51	123	13.2	10.6	0.0	47.0
	BDI-II 6WF	120	17.50	12.60	0	52	93	15.0	11.8	0.0	49.0
	BDI-II 6MF	86	19.86	15.25	1	61	92	15.6	12.5	0.0	53.0
	Valid N						62				
Biomarker	Cortisol awak-ening	117	.29	.18	.04	.82	118	.97	.52	.11	2.94
	Cortisol +30	118	.49	.28	.04	1.39	118	1.51	.72	.12	3.51
	Cortisol +45	119	.48	.28	.04	1.26					
	CAR +30	117	.20	.21	-.23	.91	116	.53	.61	-.71	2.23
	CAR +45	117	.19	.24	-.36	.89					
	AUCi	117	5.91	6.37	-7.87	27.15					
	AUCg	117	18.80	10.26	1.79	47.02					
	N	117					112				

Note. Cortisol Values in µg/dl. Abbreviations: BDI-II = Beck Depressive Inventory II, WF = Week Follow-up, MF = Month Follow-up, CAR = Cortisol awakening response, AUCi = Area under the curve increase, AUCg = Area under the curve ground, M = Mean, SD = Standard deviation, Min = Minimum, Max = Maximum.

The examination of potential confounding variables with the primary key variables of CAR (AUCi and AUCg) and the depression severity at the different time points was calculated with bivariate regressions. The results are presented in Table 4.2. and shows that none of the potential confounding variables was significantly associated with one of the CAR values.

Table 4.2.

Bivariate association between possible confounding variables and BDI-II values or CAR indices

	M	*SD*	*p* value for association with AUCg	*p* value for association with AUCi	*p* value for association with BDI_In-take	*p* value for association with BDI_ Discharge	*p* value for association with BDI_6WF	*p* value for association with BDI_6MF
Age (years)	40.00	15.27	.32	.54	.47	.06	**.04***	.57
Male	42 (34,4%)		.58	.44	.125	**.032***	.33	.47
Female	80 (65.6%)							
Amount secondary diagnosis	2.14	1.51	.28	.44	**.004****	**<.001*****	**<.001*****	**<.001*****
Length depressive episode (months)	30.51	92.41	.57	.77	.16	**<.001*****	**.017***	**.032***
Distance to the first mental illness (years)	18.69	14.47	.74	.58	.26	.70	.64	.11
Medication	106 (86.9%)		.53	.09	.08	.43	.44	**.05***
No Medication	8 (6.6%)							
Awakening Time	6.36	.65	.12	.57	.54	.38	.72	.13

Note. * $p < .05$, ** $p < .01$, *** $p < .001$. bivariate association between confounding variables and CAR indices or BDI -II. Abbreviations: M = Mean, BDI-II = Beck Depressive Inventory II, WF = Week Follow-up, MF = Month Follow-up , AUCi = Area under the curve increase, AUCg = Area under the curve ground.

4.3.2. CAR at Admission Predict follow up depression severity

There was no significant correlation between psychometrics (BDI-II at intake, discharge and 6WF and 6 MF) and CAR values (see Table 4.3), or between AUCg and age (r=.115, p=.23) or AUCi and age (r=-.034, p=.72).

Table 4.3.

Correlation between CAR and depressive pathology

Measure		1. AUCg	2. AUCi	3. CAR+30	4. CAR+45	5. BDI-II Intake	6. BDI-II Discharge	7. BDI-II 6WF
1. AUCg	Pearson Correlation							
	Sig. (2-tailed)							
	N							
2. AUCi	Pearson Correlation	**.61 (.71-.48)**						
	Sig. (2-tailed)	<.001***						
	N	117						
3. CAR+30	Pearson Correlation	**.64 (.73-.51)**	**.99 (.99-.98)**					
	Sig. (2-tailed)	<.001***	<.001***					
	N	117	117					
4. CAR+45	Pearson Correlation	**.46 (.59-.30)**	**.90 (.93-.86)**	**.82 (.88-.76)**				
	Sig. (2-tailed)	<.001***	<.001***	<.001***				
	N	117	117	117				
5. BDI-II	Pearson Correlation	-.15 (.03-(-.32))	-.02 (.17-(-.20))	.-.03 (.15-(-.21))	.03 (.21-(-.16))			
Intake	Sig. (2-tailed)	.11	.85	.73	.78			
	N	117	117	117	117			
6. BDI-II Dis-	Pearson Correlation	-.14 (.04-(-.31))	.06 (.24-(-.12)	.04 (.22-(-.14))	.11 (.28-(-.08))	**.46 (.60-.32)**		
charge	Sig. (2-tailed)	.13	.52	.67	.25	<.001***		
	N	117	117	117	117	122		
7. BDI-II 6WF	Pearson Correlation	-.15 (.04-(-.32))	.11 (.28-(-.08)	.09 (.27-(-.09))	.13 (.30-(-.06))	**.53 (.65-.39)**	**.76 (.82-.67)**	
	Sig. (2-tailed)	.12	.26	.33	.17	<.001***	<.001***	
	N	115	115	115	115	120	120	
8. BDI-II 6MF	Pearson Correlation	-.21 (.00-(-.41))	-.13 (.09-(-.33))	-.12 (.10-(-.33))	-.12 (.10-(-.33))	**.65 (.76-.50)**	**.61 (.73-.45)**	**.75 (.83-.64)**
	Sig. (2-tailed)	.05	.26	.29	.27	<.001***	<.001***	<.001***
	N	82	82	82	82	86	86	84

Note. * $p < .05$, ** $p < .01$, *** $p < .001$. Two-tailed 95% confidence occur in bracets. Abbreviations: M = Mean, BDI-II = Beck Depressive Inventory II, WF = Week Follow-up, MF = Month Follow-up, AUCi = Area under the curve increase, AUCg = Area under the curve ground.

Hierarchical multiple regression analyses with AUCg and AUCi were conducted to predict BDI-II 6MF, after controlling for BDI-II Intake (left column) and BDI-II at Discharge (right column), while controlling for BMI as in our previous study (Eikeseth et al., 2019). BDI-II Intake and BMI were entered in step 1 and the CAR at step 2. All models were significant for the first step where BDI-II intake and BMI were entered. For AUCi, significant results arose only in the model where both BDI-II Discharge (β=.753, p<.01) and AUCi (β=.202, p>.05) were statistically significant individual predictors of BDI-II 6MF (R^2=.578, F=17.82, p=.026). AUCi in all other regressions was not significant (see Table 4.4.a.-d.)

Table 4.4.a.-d.

Hierarchical Regressions

a. BDI-II Intake*AUCg

	BDI-II Discharge	BDI-II 6WF	BDI-II 6MF
BDI-II Intake	.471**	.532**	.631**
BMI	.018	.057	.045
AUCg	.067	.067	.121
F	11.40	14.99	19.33
R^2	.234	.290	.430
ΔR^2	.004	.004	.014

b. BDI-II Discharge*AUCg

	BDI-II 6WF	BDI-II 6MF
BDI-II Discharge	.753**	.591**
BMI	.019	.030
AUCg	.041	.148
F	49.76	16.56
R^2	.576	.392
ΔR^2	.002	.022

c. BDI-II Intake*AUCi

	BDI-II Discharge	BDI-II 6WF	BDI-II 6MF
BDI-II Intake	.481**	.543**	.654**
BMI	.006	.040	.065
AUCi	.070	.117	.145
F	11.43	15.66	19.81
R^2	.234	.299	.436
ΔR^2	.005	.013	.020

d. BDI-II Discharge*AUCi

	BDI-II 6WF	BDI-II 6MF
BDI-II Discharge	.755**	633**
BMI	.009	.004
AUCi	.063	.202
F	50.233	17.82*
R^2	.578	.410
ΔR^2	.004	.039

Note. BDI: Beck's Depression Inventory II; BMI: Body Mass Index; AUCg: Area Under the Curve Ground; AUCi: Area Under the Curve Increase

*p<.05; **p<.01.

4.3.3. Comparison with correlations of Eikeseth and colleagues (2019)

Eikeseth and colleagues (2019) found a negative correlation between BDI-II 6WF and CAR r=-.259, p=.02 and a negative correlation between BDI-II 6MF and CAR r=-.213, p=.048 (N=79). Compared to the correlations found in this study there was no significant difference in correlation coefficients between these two studies, z=0.065, p=.474.

The same analytical method (Total cortisol increases after 30 or 45 Minutes: Cortisol 30 or 45 minutes after awakening minus Cortisol awakening) used by Eikeseth and colleagues (2019), produced similar results. The total Cortisol increase between awakening and 30 or 45 minutes after awakening was not significantly correlated with BDI-II 6MF (after 30 minutes: r=-.12, p=.29; after 45 minutes: r=-.12, p=.27). All CAR values (AUCg, AUCi, total Cortisol increase after 30 minutes and total Cortisol increase after 45 minutes) are significantly positively correlated to each other (for all r>.634, p<.001).

4.4. Discussion

Using a stricter measurement protocol to examine the value of the CAR in predicting follow-up depressive symptoms in hospitalized patients, the aim of this paper was to replicate our earlier finding that a blunted CAR can predict mood deterioration after an inpatient treatment of severe MDD (Eikeseth et al., 2019). In contrast to Eikeseth et al., three cortisol samples were used to calculate the CAR. We also used a follow-up measure to check the compliance to the measurement protocol.

Our former reported results (Eikeseth et al., 2019) have been partly replicated. There is a negative association between CAR at the beginning of an inpatient treatment and long term depressive symptoms. Controlling for initial CAR levels for symptom levels at 6 weeks and at 6 months following discharge do not reach statistical significance levels on a 5% level, the observed effect size is comparable to the original study.

In this replication study we examined a naturalistic sample of MDD patients with approximately comparable depressive symptoms (BDI-II Intake +2 BDI-II points compared to the original study), the measurement protocol was stricter and the evaluation methods were more accurate and yet we found a comparable association and comparable effects. The Cortisol-values were a little lower and the cortisol awakening response seems to be weaker than in the original study (see Table 4.1). Apart from that the clinical naturalistic setting was identical (number of individual therapy units, length of inpatient treatment, amount and type of group therapy, complexity of diagnoses etc.). The replication of association and effects show that a blunted CAR is associated with a higher depressive symptom deterioration 6-months after discharge of an inpatient treatment (Table 4.4.a.-d.). However, the lack of statistical significance in addition to the rather small effect sizes for CAR measurements, suggest only a minor clinical meaningfulness. Other variables, for example depression severity at intake, or at discharge have a higher predictive power for the long term course of MDD symptoms. At the same time,

the replicated effect sizes show that the CAR can be seen as a biomarker for depressive severity and predicts the probability for a relapse after discharge.

To the best of our knowledge, this is the first study to investigate the association between CAR and a long-term follow-up after discharge in a naturalistic setting. These findings are in line with previous work indicating that a CAR at the beginning of a therapeutic intervention is associated with a greater treatment outcome after a 4-week inpatient program (Jones et al., 2015). With only 25 included patients, however, these findings generalizability is rather limited. A higher baseline CAR may predict depressive episode recurrence (Vrshek-Schallhorn et al., 2013). What appears to be contradictory to our findings, may be explained by the dependence of the association between CAR and depression severity and the definition of MDD as categorical or dimensional. Previous research suggested that the association between CAR and depressive symptoms may be described as an inverted U function (Veen et al., 2010; Wardenaar et al., 2011). Patients with mild depressive symptoms show a rather low CAR, which is comparable to healthy control persons. Patients with moderate depressive symptoms show an increased CAR whereas patients suffering from severe depression show a blunted CAR.

Apart from that, a higher and flexible CAR could be associated with successful coping (Dedovic & Ngiam, 2015). For example, resilient psychological profiles (low stress symptoms) appear to be associated with a flexible CAR: low CAR on weekends, higher CAR on weekdays (Schlotz et al., 2004), while vulnerable psychological profiles (high stress) appear to be associated with a rigid CAR (same magnitude during weekdays and weekends). Reduced, inflexible CAR values seem to indicate exhaustion of the stress system. These inflexible profils may explain the difficulties of the HPA axis of depressive patients to adapt to different situations (Dedovic & Ngiam, 2015).

Other values seem to have a stronger predictive power for longterm depressive symptoms. For instance, Dedovic and Ngiam (2015) show that a negative attributional style can predict depressive symptoms and they found a negative association between CAR and hopelessness. A family history of depression, moreover, seems to be associated with higher CAR values even if the participants themselves showed no depressive symptoms (Dedovic & Ngiam, 2015).

4.4.1. Limitations

The findings of this study should be interpreted in light of some limitations. First, Cortisol seems to have a high day to day variation. In addition, adherence to the measurement protocol was not checked with electronic monitoring or a repeated measurement on the next day, there was only a self assessment questionnaire to prove the participant's "adherence to instruction". Hellhammer and colleagues (2007) stated that at least six days of measurement are needed to assess the CAR increase and two days for CAR AUC. However, in this study, all patients participated in the same daily clinical routines so that potential confounders such as increased anxiety or anticipation over the day's activities, can be neglected.

Second, sleep disturbances are one of the most common symptoms of MDD (APA, 2013), but the subjective self reported assessment of sleep quantity and quality is not very meaningful (Tsuchiyama et al., 2003). Additionally, poor sleep can affect the CAR and therefore may have influenced our results (Elder et al., 2014). However, since sleep problems are common in depressed patients it can be seen as part of MDD and could not be controlled in a naturalistic setting. Especially because patients often suffer from very heterogeneous sleep problems (sleeping too much, sleeping too little, difficulty falling asleep or staying asleep).

Finally, as this study was based on a naturalistic sample of depressed inpatients it was not possible to systematically control for the individual comorbid mental disorders or medication use/dose changes etc. Findings of MDD and anxiety patients showed either heightened (Greaves-Lord et al., 2007) or lowered CARs (Kallen et al., 2008), therefore it would be interesting to examine differences between the subgroups of these mental disorders in future studies (Kudielka & Wüst, 2010).

4.4.2. Future directions

To the best of our knowledge this is the first study to investigate the association between CAR and depressive symptom deterioration after an inpatient psychotherapy. Future studies analysing the predictive power of CAR in MDD patients should consider combining the measurement of CAR with an assessment of sleeping quality and quality, for example by including actigraphy measures during sleep. Sleeping disorders may lead to a disruption in cortisol production which then itself might result in altered cortisol levels after awakening (Chida & Steptoe, 2009; Dockray et al., 2008). The assessment of physical activity could provide a measure

for a sedentary lifestyle, which is often found in depressive patients (Zhai et al., 2015) and could have an impact on cortisol levels (Adam et al., 2010).

MDD is a disorder with many different symptom constellations and subtypes that are associated with distinct alteration of the HPA axis (Antonijevic, 2006). While a strength of this study is its naturalistic design implemented in a psychosomatic hospital with a patient population resembling a very typical profile for German institutions, future research on more homogeneous samples could differentiate clearer between individual variables contributing to the observed effect. Sub-samples of MDD patients with blunted or increased CAR could be further differentiated. Future studies could also focus on a more precise symptom dependent sample formation (i.e. subgroups of depression with main symptoms like rumination, hopelessness, anxiety, exhausting, restlessness, depressive severity) instead of sample formation depending on diagnosis only.

4.5. Conclusion

This replication resulted in statistically non-significant moderate predictive power of CAR on depressive severity 6-weeks and 6-months after discharge, thus largely replicating previous (significant) findings. Improved assessment and monitoring protocols add to the robustness of these findings. Therefore, the CAR at intake appears to be a (limited) predictive biomarker for global depressive symptoms after treatment and discharge. A blunted CAR at the beginning of a psychotherapy seems to be associated with a higher long term symptom load post treatment.

5. General Discussion

The results described in chapters 2 to 4 examined the relationship between physiological measures and the course of MDD. The overall research question that guided each individual paper was whether there are biomarkers that can provide additional information about the course of MDD. Two typical biomarkers for MDD were selected for this dissertation: First, HRV, which represents the ANS functioning and emotion regulation capacity (Williams et al., 2015) and is considered as a response biomarker. Second, CAR, that represents HPA axis functioning and is considered as a predictive biomarker. It was first investigated whether these two biomarkers are sensitive to change after an inpatient psychotherapy treatment and second whether these biomarkers have an additional predictive power for the further course of the disorder after an inpatient psychiatric psychotherapy. All studies included in this work are based on naturalistic samples of MDD patients in a German psychosomatic hospital.

In the second chapter it could be shown that there is a negative association between HRV indices and self-reported MDD symptom severity at the beginning of an inpatient antidepressant treatment. The more depressed a patient is, the worse the average HRV. This result replicates previous research results that show that MDD is negatively associated with HRV (Kemp et al., 2010). At the end of the treatment this association between psychophysiological indicators and symptom severity no longer exists. While the patients showed less depressive symptom burden, the analyzed HRV indices do not change accordingly. Therefore, the assumption that HRV might be a "sensitive" biomarker for depressive severity cannot be applied to post-treatment situations.

CAR was used in the studies described in the third and fourth chapters to test the predictive power of a biomarker for the course of MDD. It was shown that a blunted CAR can predict self-reported depressive symptoms six weeks and six months after discharge. MDD patients with a blunted CAR at the beginning of a psychotherapy inpatient treatment showed more symptom deterioration six weeks and six months post treatment. The study shown in chapter 3 was the first to analyze the predictive power of CAR for the course of MDD. The lack of comparable studies, together with methodical limitations and a low effect size made it seem reasonable to perform a replication of this first study with a more precise measurement protocol, which is reported in the fourth chapter. Using an updated measurement protocol (three instead of two saliva measurements in the morning and an additional follow up questionnaire to check compliance with the measurement protocol), the original results were replicated. Even the effect sizes of the replication study were very similar to the original study. This reinforces the findings

of the original study and shows that CAR can provide additional information about the course of depression at the beginning of an inpatient psychotherapy.

This dissertation addresses different research questions regarding response and predictive biomarkers for inpatients suffering from MDD in a naturalistic setting. Benefits and limits of biomarkers for MDD in psychological and psychiatry research are discussed in the following, as well as implications for MDD diagnosis. In addition, limitations of this dissertation will be addressed and possible research implications as well as implications for treatment of MDD will be discussed.

5.1 Properties of specific Biomarkers for MDD

The results of all three studies show that there is an association between psychopathology and potential biological markers in patients suffering from MDD. Both biological markers used in this study are analyzed at different time points and seem to have significant associations with MDD symptom severity. These results are in line with previous research which demonstrates that MDD patients show changes in different biological systems (Penninx et al., 2013). A changed HPA axis and autonomic disbalance are well-studied findings in psychiatry research (Holsboer, 2000; Rottenberg et al., 2007; Vreeburg et al., 2009). Patients suffering from severe depression symptoms show a blunted cortisol awakening response (e.g. Vreeburg et al., 2009) and an increased HRV (Kemp et al., 2010). The studies in this work complement the findings to the effect that CAR seems to be a potential biomarker at intake to assess symptom deterioration in the long term. Nevertheless, the biomarkers analyzed in this dissertation do not seem to be sensitive and specific enough to reflect the severity of depression. This finding corresponds to the results of Thase (2014), who describes that there is still no clinically valid biomarker to predict the course of depression. In the study presented in chapter 2, HRV has not changed after discharge even though the depression severity was reduced. An abnormal CAR implies an abnormal stress reactivity that correlates with a greater severity of depression even after successful treatment (Doane et al., 2013). Current research suggests that the cortisol values show a U-shaped course rather than a linear association (Veen et al., 2010; Wardenaar et al., 2011). That U-shaped association means that healthy people and severely depressed patients show similarly low values whereas patients with moderate depressive symptoms show higher values (Veen et al., 2010; Wardenaar et al., 2011). Therefore, absolute values of biomarkers are not, on their own, suitable for making statements about depression severity. They are, rather, transdiagnostic markers for (psychological) stress (Boksa, 2013). Apart from

that, the results of these studies show that both biomarkers demonstrate a large inter- or intra-individual variance (see chapters 2 to 4). Possible explanations for this high variance are that there are many other confounding variables (like mental or physical comorbidities, number of previous depressive episodes, duration of disorder, medication, etc.) which influence both MDD symptom severity and neurobiological systems. Especially for the course of MDD, other aspects like the number of previous depressive episodes seems to have a high impact (Mueller et al., 1999). The number and duration of depressive symptoms seems to influence biological systems, too. Depression first results in an acute stress reaction with associated hypercortisolism, if this condition persists over a long period of time it might lead to a downregulation of the HPA axis with the consequence of hypocortisolism (Fries et al., 2005; Heim et al., 2000).

Until now it has not been fully understood if or how neurobiological improvements (e.g. HRV) change after a successful antidepressant therapy. There is evidence from biofeedback studies that therapy utilizing HRV-biofeedback is able to reduce depressive symptoms and to increase HRV during the biofeedback sessions (Wheat & Larkin, 2010). However, the authors pointed out critically that the long-term carry-over effects on the baroreflex gain are not concurrent in all cases. One possible explanation might be that physiological improvements (like baseline improvement of HRV) need more time beyond treatment to be expressed because of the necessary neurological changes in the central autonomic network (Thayer & Siegle, 2002). In addition, there is evidence from antidepressant medication studies that some drugs are able to improve HRV values parallel to symptom reduction, but without sustainably changing the underlying physiological processes (Kemp et al., 2010; Licht et al., 2008).

Apart from that, there are some symptom constellations of MDD which are more strongly associated with biological changes than others. In particular, motor activity (Volkers et al., 2003), rumination (Carnevali et al., 2018) and fatigue (Freeman & Komaroff, 1997) are strongly associated with changes in autonomic nervous functioning.

Lastly, there are comorbid disorders of MDD, that influence both biological systems and the course of MDD. A prominent example is anxiety. MDD patients with a comorbid anxiety disorder seem to differ in terms of MDD symptoms, treatment outcomes and HRV indices (Hofmann et al., 2010; Kircanski et al., 2019). Kircanski and colleagues (2019) describe that patients suffering from anxious depression and higher HRV had better outcomes than those with lower HRV (which shows a similar association as the results in chapter 2). Interestingly, patients suffering from non-anxious depression and lower HRV had better treatment outcomes than those with non-anxious depression and higher HRV within the same study. This seems

contradictory at first but again shows the high relevance of considering comorbidities when interpreting biomarker results. Also, recent research shows that lifestyle factors like smoking (Harte et al., 2013), overweight (Holsboer, 2000), or social networks (Hallgren et al., 2017) have a high impact on the course of MDD and biomarkers. For example, Harte and colleagues (2013) pointed out that depressed smokers showed lower HRV compared to depressed non-smokers, which can be seen as an indicator for dysregulation of the ANS, and it has also been found that smoking behavior has a negative impact on the long term course of depression (Colman et al., 2011). Apart from smoking, being overweight and obesity have unfavorable effects for treatment outcomes of MDD and HPA axis functioning (Holsboer, 2000). It is important to consider these confounding variables when interpreting the absolute results of HRV values.

5.2 Benefits and limits of biomarkers in psychiatry research

The benefits of including biomarkers in psychotherapy or psychiatry research depend on the type of biomarker and the intention to use. For both analyzed biomarkers for the course of MDD in this dissertation there are three main benefits. First HRV and CAR are able to contain additional information about MDD and psychological coping mechanisms related stress. Together with psychometric data and information about medical history it may be possible to predict the risk of a difficult (i.e. recurrent or chronic) MDD course and thus to improve the diagnostic process. Secondly, both are noninvasive and easy-to-use markers which reflect autonomic control and HPA axis functioning. This information about both systems can be added to an individual treatment plan, for example to enhance clinical decision-making by choosing the most appropriate treatment or treatment combination for a specific individual (Lopresti et al., 2014). Recent research indicates that depressed patients with reduced HRV benefit twice from a combination of HRV biofeedback and CBT compared with CBT alone (Caldwell & Steffen, 2018). The combination leads to an increase in HRV values and a decrease in depressive symptoms compared to treatment as usual. Third, the consideration of biological systems related to MDD could increase patients' commitment because it corresponds to a somatic disorder understanding of many patients (Tylee & Gandhi, 2005). A better fit between the patient's understanding of the disorder and the treatment could have a positive effect on the course, since patients may get faster effective therapy and show greater engagement with their treatment (Härter et al., 2017). Also, recent therapy guidelines suggest there should be a focus not only on psychological symptoms but also on physiological and social aspects of depression (Härter et al., 2017).

There are three main limits to HRV and CAR. First, there are no generally applicable norms or cutoff values for depressed patients (Strawbridge al., 2017). Therefore, the identification and interpretation of pathologic values is very difficult. For example, CAR possibly has a U-shaped profile. That means that severly depressed patients show a blunted CAR, whereas mildly to moderately depressed patients have an increased CAR. For HRV, in a recent study from Heiss and colleagues (2020) the authors quantify an ideal range of HRV: HRV deviations from this ideal range seems to be associated more or less with different mental disorders. These results can be seen as a start to overcome the aforementioned limitation - however, robust HRV norms for different mental disorders are still needed. Second, the high number of confounding variables (internal and external variables) and the individual interactions of them could lead to abnormal values, and must also be taken into account (Mayeux, 2004). If possible, the potential influence of confounding variables should be investigated separately before the study. However, this procedure increases the effort required immensely and is therefore not affordable or feasible for many examinations (Mayeux, 2004). As a result, there is a risk of biased results, especially for those variables which cannot be controlled for or which are unidentified variables that influence both biological and psychological results. Third, in biomarker research potential biases (e.g., time course, storage of biomarker probes, analytic techniques) have to be meticulously observed and checked. As described in chapters 3 and 4, the time at which the saliva sample is taken has a major impact on the results. Even a slight shift in the sampling times can significantly change the dynamic course of the measured CAR (Stalder et al., 2016). Therefore strict compliance to the measurement protocol is mandatory to get valid results, and comparability of results between different studies is again hampered.

In sum, for an accurate interpretation of biomarkers it seems to be necessary to combine the absolute values with additional information like medical history or analysis of aspects of lifestyle. In addition, potential confounding variables should be checked before starting the investigation, or at least documented in as much detail as possible for future replications and validations, especially in the case of naturalistic research settings.

5.3 Implications for diagnosis and treatment of MDD

5.3.1 Implications for categorical diagnosis of MDD

One central problem of the diagnosis of mental disorders is that until now there have been no valid discrete entities found to distinguish between mentally ill patients and healthy persons (Jablensky, 2016). The author shows that it is even more complicated to distinguish between different mental disorders because there are several overlaps between diagnoses (e.g. anxiety and depression have several symptoms in common; DSM 5). In combination with the aforementioned problems of self-assessment and interviews, the high number of misdiagnoses is not surprising. Although psychiatric and psychotherapy research has invested a lot in biological investigations, up to now there are no laboratory tests to diagnose patients with psychiatric disorders (Lakhan et al., 2010). This leads to the question, 'To what extent can current diagnoses classify homogeneous groups or to what extent should the concept of mental diagnoses be revised?'

The problem becomes very clear using the example of depression. The DSM 5 criteria are structured in such a way that it is possible for two people to receive the same diagnosis (MDD) without suffering from even one common symptom and sometimes even to suffer from completely opposite symptoms (Zimmermann et al., 2015). This leads to a large heterogeneity within one diagnosis.

So far it has not been possible to reliably place patients in homogeneous subgroups within MDD on the basis of symptoms or treatment responsiveness (Arnow et al., 2015). The DSM-5 only differs in terms of the severity of MDD, depending on the number of different depressive symptoms a patient is suffering from (APA, 2013). Another approach could be to create subgroups depending on the cause of the depressive mood, e.g. depression as a result of physical illness, social problems, or overwhelming depression (Goldberg et al., 2011). Strawbridge and colleagues (2017) suggest cohesive subgroups of patients suffering with depression may be identifiable through a combination of psychological and biological factors. However, until now - and also taking into account the results of this dissertation - there has been no exclusive depression biomarker for the categorial disorder MDD. Even though chapters 3 and 4 show that CAR, at intake, might be able to provide information about the further course of MDD, it can only be seen as an additional source of information. CAR alone could not predict the course of the illness with sufficient validity in these samples.

All these approaches lead to the question of how useful and clinically relevant dichotomous divisions and categorical diagnostic systems can be or whether a completely different classification approach is needed. Fekadu and colleagues (2009) propose an approach based on looking at the depressive mood rather on a continuum between less depressive and severe depressive mood especially for treatment-resistant depression, instead of dichotomous categorical approaches. The Hierarchical Taxonomy Of Psychopathology (HiTOP) model could represent an alternative consideration of psychological abnormalities (Kotov et al., 2017). The authors describe a dimensional classification which is more clinically informative than diagnostic systems in actual use, like DSM 5. The HiTOP model defines empirically-derived syndromes (e.g. internalizing and externalizing) to describe psychopathology. The HiTOP model thus represents an economical way to grasp the dimensional nature of mental disorders (Clack & Ward, 2019). It includes information on risk factors, etiology, pathophysiology and illness course (Conway et al., 2019). Comorbid disorders (e.g. MDD and PTSD) are recorded as co-occuring syndromes and can sometimes be grouped under the same spectrum (Clack & Ward, 2019). The analysis of biomarkers on the basis of a classification of patients using the HiTOP model instead of DSM-5 diagnoses may provide new insights into the type of complaints from which patients suffer. So far, however, this model has been promising only for psychiatry research; in healthcare systems, the diagnoses according to DSM or International Statistical Classification of Diseases and Related Health Problems (ICD, see Graubner, 2013) are still mandatory, e.g., in order to claim treatment.

5.3.2 Biomarker implication for MDD treatment

The hope of explanatory reductionism research is to identify the biological abnormalities of mental disorders, so that treatment plans can be developed which restore these abnormalities (Borsboom et al., 2019). In consequence, the symptoms that mentally ill patients suffer from should be removed, but in the case of mental disorders the authors conclude that this aim falls short. The findings presented in this dissertation support their conclusion and show that a monocausal assumption is too simplified. Both aspects (biological and psychological) are strongly intertwined and should be considered to assess the course of MDD. However, they are not yet sufficiently understood to reliably predict the individual course of disease.

Nevertheless, the results presented in chapters 2 to 4 suggest that biological aspects should be taken into account in the diagnosis of MDD and its therapy, equally to psychological aspects. Analyses of biological variables, biofeedback interventions, interventions to cope with stress, relaxation techniques, analysis and improvement of unfavorable lifestyle factors are just

some possibilities in this sense. Doane and colleagues (2013) show that an abnormal cortisol response implies an abnormal stress reactivity that correlates with a greater severity of depression and a worse course of MDD. With respect to the studies presented in chapters 3 and 4, the evaluation of CAR might be an additional source to identify high-risk patients for a chronic or recurrent course of depression. The identification of such high-risk subgroups might be helpful to adapt therapy interventions, e.g., in case of chronic or therapy-resistant depression instead of using manual-based antidepressant therapy for all patients (Labermaier et al., 2013).

In particular, for depressive patients with low HRV there is an increased risk for cardiac death (Carney et al., 2005). For these patients HRV-biofeedback interventions are very promising, since they have a positive influence on the course of MDD (Karavidas et al., 2007). Furthermore, the authors describe an acute increase in HRV during biofeedback sessions, although the baseline remains unchanged. However, taking only biological aspects into account is not sufficient. They are a supplement to the psychological aspects and external factors. As described in the model by Beck and Bredemeier (2016), there are numerous feedback loops influence both negative thoughts and feelings as well as the biological correlates of depression. As Borsboom and colleagues (2019) describe, it is more productive to be distanced from the monocausal way of thinking and "accept the ideas that (1) mental disorders are massively multifactorial in their causal background; (2) many mechanisms that sustain disorders are transdiagnostic; and (3) mental disorders require pluralist explanatory accounts" (Borsboom et al., 2019, p. 3). The network model of mental illnesses is based on these assumptions (Borsboom, 2017; Borsboom & Cramer, 2013; Fried & Cramer, 2017).

5.4 Methodological considerations

5.4.1 Statistical vs clinical significance

Besides the aforementioned content-related aspects of biomarkers for MDD, this dissertation also focuses on methodological aspects to improve biomarker measurement protocols for clinical samples. Since in this work patients were surveyed in a naturalistic setting, the focus was on applying robust research designs.

Eikeseth and colleagues (2020) showed that, especially in clinical samples, there is a high situation-specific state portion in single HRV measurements. Apart from the confounding variables, the HRV study of this work (chapter 2) also demonstrates the high number of day-to-day variation of biomarker values. These results are in line with Bertsch and colleagues

(2012), who show that HRV is highly state-dependent due to random situational context factors in healthy participants. The authors recommend at least two measurements when using HRV as a (trait-) biomarker. Therefore, and due to a high intra-individual variance in HRV, an average of three measurements at each time point was used in the HRV study. Similar methodological considerations also apply for CAR (see chapters 3 and 4). Since the exact temporal development of cortisol levels after awakening differs interindividual, at least three saliva samples should be taken into account. Furthermore, research shows that daily fluctuations can be assumed for CAR as well and should be taken into account (Schlotz et al., 2004). If possible multiple measurement designs should be used in future research to reduce the day-to-day fluctuations (Hellhammer et al., 2007). Even though multiple measurements were used in chapter 2, the clinical relevance of single biomarkers seems to be rather low. There was no association between HRV and depressive symptoms at discharge of an inpatient following treatment even though there was a strong association at intake, so that HRV cannot be seen as a specific response biomarker for MDD.

CAR was significantly associated with symptom deterioration, but the additional explanation of variance was very slight. But even if the effect sizes are rather small, the findings could be proven in two studies. Rather small effect sizes are common in psychiatry research, especially in naturalistic settings (Singh & Rose, 2009). In line with previous research, the findings show that research interpretation should focus more on effect sizes than just on significance levels (Kraemer et al., 2002). A sufficiently large N is able to "produce" significant results, no matter how small the effect size is (Quatember, 2005).

For both analyzed biomarkers there is a lack of generally accepted guidelines, norms for mentally ill patients or cut-off values available (Strawbridge et al., 2017). Apart from that, dealing with confounding variables is also challenging due to the large number of possibilities and combinations. All of this leads to the fact that the study designs are hardly comparable, due to very different measurement protocols and samples. There are hardly any studies with naturalistic samples and if they do exist, only with very small case numbers (e.g., Ehrentahl et al., 2010, Peeters et al., 2003, Thase et al., 1996).

In sum, the small effect sizes and low comparability are not surprising because of the high number of potentially confounding variables in naturalistic biomarker research designs. This raises the question of the extent to which statistically significant results are of clinical relevance. As mentioned above, these results show that single biomarker analyses are not specific and effective enough to get valid data about the course of MDD alone, but, in addition to

otherer information, can be useful for MDD treatment and MDD course estimation (Singh & Rose, 2009. Furthermore, the aforementioned research direction regarding sets of biomarkers instead of studies analyzing a single biomarker becomes even more relevant.

5.4.2 Categorization of different biomarkers for MDD

It has been shown that difficulties in dealing with high life stress is also a major risk factor for depression or further depressive episodes (Plieger et al., 2015). Therefore a changed CAR might be more a trait marker for MDD rather than a state marker of the severity of depression (Vreeburg et al., 2009).

The dichotomous division of biomarkers into state and trait markers seems to be too global (Lema et al., 2018). The studies of this dissertation also provide initial indications that the markers examined have both state and trait components. Inconclusive research results show that there is evidence for both in the case of HRV. Hartmann and colleagues (2019) suggest that HRV is a biomarker for clinical state in unmediated MDD patients, whereas Brunoni and colleagues (2013) conclude that HRV can be seen as a trait marker for MDD.

Results of biofeedback studies show possibilities of enhancing HRV in combination with psychotherapy (e.g. Caldwell & Steffen, 2018; Karavidas et al., 2007; Siepmann et al., 2008). Without these biological treatments HRV adjustment may take longer, even after a successful psychotherapy. However, the fluctuating changeability of the HRV after MDD interventions and results from reliability studies of HRV seem to speak in favor of a state proportion and a trait proportion (Bertsch et al., 2012).

Psychotherapy itself can help patients make better use of their own abilities rather than constantly over-confessing or subjecting themselves to others. They learn how to behave and think in different situations. HRV has been reported to reflect inhibitory capacity (Thayer & Siegle, 2002; Thayer & Lane, 2009). It could be speculated that this capacity (reflected in HRV at rest) might be triggered by psychological intervention (Schneider & Kuhl, 2012). This process does not require sustained neuronal plasticity resulting in heightened baseline HRV since it is “just” a mobilization of existing resources. In this sense, low resting HRV might not be the consequence of a psychological disorder, but could be a sign of vulnerability that persists even after successful treatment and the easing of clinical symptoms. In this sense, HRV should be seen as a transdiagnostic marker for stress and psychopathological abnormalities and not as a specific biomarker for depression (which is also in line with Beauchaine & Thayer, 2015).

Finally, the sample selection of most studies (including those in this dissertation) represents a further difficulty in finally answering the question about state or trait biomarkers. Most studies analyze depressive patients at intake for a treatment, compared to healthy persons. Therefore, there is no valid information about the individual preclinical values of biomarkers before the onset of depression or after remission in naturalistic samples. Without this information it is difficult to get a comprehensive biological profile of an individual disorder course. The few existing studies analyzing these biological profiles in long-term designs come to different results. Vreeburg and colleagues (2009) found increased CAR also in acute and remitted depressive patients and conclude that CAR might be a vulnerability marker for MDD. In contrast, Hellhammer and colleagues (2007) pointed out that CAR is determined by larger state-dependent factors than trait factors. These results, and results from reliability studies, show that CAR is partly able to adapt to situational factors and daily stress load (Fries et al., 2009). Therefore it is assumed that CAR is determined by both state and trait factors. The research design can be adapted to measure and focus the proportion of state or trait according to the research question.

5.5 Limitations and implications for future research

Although the current dissertation combines different biomarkers and multiple measurement protocols to examine the course of depression in a naturalistic setting, some limitations need to be discussed that indicate implications for future research. The results presented in chapters 2 to 4 come from the same setting, therefore the limitations and implications for future research discussed here cover a broader scope, for example with respect to the use of categorial diagnostics for sample formation, than limitations already addressed in chapters 2 to 4.

5.5.1 Limitations

First, the studies within this dissertation are based on the typically used categorical diagnostic system (DSM 5) and do not include a control group design. As a result, there are no comparative values from healthy control persons or a waiting list control group. All classifications of values are based on results from other studies. However, due to the sometimes very different measurement methods, these cannot be directly compared and norms for depressed patients do not exist at this time. In this dissertation the focus lies on the association between biomarkers and depressive symptom severity, so that the absolute values are not primarily relevant. At the same time, however, the symptoms from which the depressed patients suffer are

very heterogeneous. A subdivision into MDD subgroups is not possible due to the insufficient number of cases and the excessively large number of symptoms in a naturalistic setting.

Second, the course of depression depends on many different factors which are difficult to measure and even more difficult to control in a naturalistic setting. Some examples are discussed in chapters 2 to 4 but there are several more predictors for the course of depression. Age, living conditions, relationships, comorbid mental or physical disorder are some examples for risk factors to which patients are exposed in outpatient settings (for an overview see Härter et al., 2017). There is no information about these conditions and therefore, they are not controlled for. In addition, there is no information about baseline biomarker values before the onset of depressive symptoms.

Third, only two typically used biomarkers are analyzed to get more information about the course of depression. But the interactions between mental states and biological systems are very complex and include different biological systems and networks. In this dissertation, the focus lies on one response and one predictive biomarker for the course of depression. This might not be enough to describe these complex interactions and networks. Recent research suggests measuring at least a set of different biomarkers at the same time to depict these complex networks and interactions slightly better (Brand et al., 2015). However, the value of even this approach of using a set of biomarkers is doubtful, since the prediction of the course of MDD solely based on a patient's biological states has not yet been conclusively validated (Borsboom et al., 2019). Representatives of network theory are skeptical of this assumption and suspect that even if the biological correlates are perfectly described, this is not sufficient to enable a valid statement about the individual case of a patient (Borsboom et al., 2019, Borsboom & Cramer 2013; Fried & Cramer 2017). In this sense, taking mental states and the world outside the body into account as well would enhance the possibility of making valid statements about the disorder and its course.

5.5.2 Future research

Future research regarding biomarkers for MDD should include study designs with a combination of different biomarkers. A combination between CAR and a polysomnography during the night before CAR measurement could reduce the influence of sleep disorders on the results. Schmidt and colleagues (2011) go further and suggest collecting biomarker sets. Brand and colleagues (2015) outlined a draft panel with 16 “strong” biomarkers especially for the diagnosis of MDD. This approach could enable a more accurate viewpoint into the complex

web of biological systems or networks. Apart from that, the analysis of different biological systems makes it possible to discover interactions among them and gain more detailed information about the nature of the association between biological systems and psychological symptoms.

Another aspect that should be taken into account in future studies is the collection of biomarkers at different measurement times, including long-term aspects. Psychophysiological variables may take longer to adapt than mental states. This would provide information about the extent to which biological systems can return to a normal state after a remission or whether stable pathological values have an influence on the course of a depression. Especially after an inpatient therapy, the stability of treatment effects in the everyday life of the patients is important. Here, too, future studies should additionally analyze the biological perspective (not only self-reported questionnaires) in order to detect any symptom deterioration (e.g., high allostatic load) at an early stage.

Lastly, future research should focus on a translational approach. The classification of patient groups should not be based on categorical diagnoses but rather on symptoms across different diagnoses. In order to identify a baseline at the same time, future studies should include a control group. The diagnosis of depression, in particular, seems to contain too many different, sometimes contradictory, symptoms for it to be possible to form homogeneous groups (Goldberg, 2011). It is therefore not surprising that, despite decades of effort in searching for a clearly diagnostically relevant biomarker, not a single one has yet been found that can be used for diagnosis and treatment of MDD (Gururajan et al., 2016; Lakhan et al., 2010). This translational approach may help to create symptom cluster groups to identify specific biomarker profiles which can help to specify the treatment and to identify risk groups for a recurrent or chronic mental disorder.

6. Conclusion

Taken together, the current results can be condensed into two broader conclusions: First, there are associations between biological markers and the course of depression, but the effects of single biomarkers seem to be too small to have clinical impact. The analysis of single biomarkers' absolute values is not sufficient to predict the course of depression after an inpatient therapy or in the long term. However, they can be viewed as a further source of information (in addition to psychosocial, demographic, and therapy course variables) in order to create a more reliable predictive model for the course of depression. An enhanced scientific understanding regarding the psychophysiological correlates of mental disorders is needed to fully evaluate the clinical application.

Second, due to the lack of generally applicable guidelines, it is crucial to describe the results and measurement methods in great detail in order to be able to compare the results across studies or to replicate earlier findings. This also includes using measurement methods that are as valid as possible in order to minimize situational influences and the impact of confounding variables. One possibility is applied designs with multiple measurements on different time points. In addition, various sources of information should be combined to assess the treatment effect, e.g., self-assessment, external assessment and biological aspects.

To the best of my knowledge this is the first dissertation which focuses on biomarkers with multiple measurement designs for the course of MDD in a naturalistic setting. From a methodological point of view, more studies are needed which validate the previous results and verify them with regard to clinical significance. Holistic research designs, including at least biological, social and psychological aspects of MDD, are needed. Such analyses with severely depressed patients are complex and the effect sizes are rather small, but they are still necessary to improve the diagnostic process and treatment options, especially for seriously ill patients.

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ERKLÄRUNG

gemäß § 6 Absatz 2 g) und gemäß §6 Absatz 2 h) der Promotionsordnung der Fachbereiche 02, 05, 06, 07, 09 und 10 vom 04. April 2016.

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Hiermit erkläre ich, dass ich die eingereichte Dissertation selbstständig, ohne fremde Hilfe verfasst und mit keinen anderen als den darin angegebenen Hilfsmitteln angefertigt habe, dass die wörtlichen oder dem Inhalt nach aus fremden Arbeiten entnommenen Stellen, Zeichnungen, Skizzen, bildlichen Darstellungen und dergleichen als solche genau kenntlich gemacht sind.

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Gemäß § 10 Abs. 5 und Abschnitt F des fachspezifischen Anhangs für Psychologie werden die folgende Teile der Dissertation als in einem wissenschaftlichen Fachjournal publiziert oder zur Publikation eingereicht kenntlich gemacht.

Kapitel 2 basiert auf folgendem Manuskript:
Neyer, S., Witthöft, M., Cropley, M., Pawelzik, M., Lugo, R. G., & Sütterlin, S. (2021). Reduction of depressive symptoms during inpatient treatment is not associated with changes in heart rate variability. *PloS one, 16*(3), e0248686.

Kapitel 3 basiert auf folgendem Manuskript:
Eikeseth, F. F., Denninghaus, S., Cropley, M., Witthöft, M., Pawelzik, M., & Sütterlin, S. (2019). The cortisol awakening response at admission to hospital predicts depression severity after discharge in MDD patients. *Journal of Psychiatric Research, 111,* 44-50.

Kapitel 4 basiert auf folgendem Manuskript:
Neyer, S., Witthöft, M., Cropley, M., Pawelzik, M., Sütterlin, S., & Lugo, R. The Cortisol Awakening Response at Admission to Hospital predicts Depression Severity After Discharge in MDD patients – A Replication Study. Submitted.

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_Münster, 04.09.2022_____ ____S. Neyer_____

Ort, Datum Unterschrift