A Bio-Psycho-Social Perspective on Anxiety Disorders Treated with Cognitive Behavioral Therapy

A Multilevel Approach to Understanding the Relationship Between Biomarkers and Fear Exposure, as well as the Importance of Attachment Style and Therapeutic Alliance

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Abbreviations

ACTH	Adrenocorticotropic Hormone
AD	Anxiety Disorder
ARG1	Arginase 1
B-Cells	B Lymphocytes
CBT	Cognitive Behavioral Therapy
CD4+ T Cells	T Helper Cells
CD8+ T Cells	T Cytotoxic Cells
CRH	Corticotropin-Releasing Hormone
DNA	Deoxyribonucleic Acid
DSM	Diagnostic Statistical Manual of Mental Disorders
ET	Exposure Therapy
EWAS	Epigenome-Wide Association Study
FKBP5	FK506 binding protein 5
FHIT	Fragile Histidine Triad
HECA	Hdc Homolog, Cell Cycle Regulator
HPA axis	Hypothalamic-Pituitary-Adrenal Axis
HTR	Hydroxy Tryptamine Receptor
MAD1L1	Mitotic Arrest Deficient-Like 1
mRNA	Messenger Ribonucleic Acid
PACAP	Pituitary Adenylate Cyclase Activating Polypeptide
PD	Panic Disorder
RNA	Ribonucleic Acid
SGK1	Serum Glucocorticoid Regulated Kinase 1
SNS	Sympathetic Nervous System

List of Publications

Publication I

Lange, J., & Erhardt-Lehmann, A. (2025). HPA system in anxiety disorder patients treated with cognitive behavioural therapy: A review. *Biomarkers in Neuropsychiatry, 12*, 100116. https://doi.org/10.1016/j.bionps.2024.100116

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Publication III

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Publication IV

Moser, S., Martins, J., Czamara, D., **Lange, J.**, Müller-Myhsok, B., & Erhardt, A. (2022). DNA-methylation dynamics across short-term, exposure-containing CBT in patients with panic disorder. *Translational Psychiatry, 12*(1), 46. https://doi.org/10.1038/s41398-022-01802-7

Publication V

Lange, J., Goerigk, S., Nowak, K., Rosner, R., & Erhardt, A. (2021). Attachment style change and working alliance in panic disorder patients treated with cognitive behavioral therapy. *Psychotherapy (Chic), 58*(2), 206-218. https://doi.org/10.1037/pst0000365

1. Summary

Anxiety disorders (ADs) are one of the most prevalent mental disorders, affecting up to around 30% of the population worldwide (Jacobi et al., 2014; Szuhany & Simon, 2022). They represent pathological anxiety and fear, marked by disproportionate emotional and physiological responses in the absence of real threat, leading to avoidance behavior or safety strategies. They often include panic attacks with cardiovascular and respiratory symptoms. ADs impair functioning, reduce quality of life, and impose a major socioeconomic burden (Baxter et al., 2014; Santomauro et al., 2021). The pathogenesis of AD is multifactorial, including biological, psychological, and environmental causes. Cognitive behavioral therapy (CBT), integrating exposure to feared situations and targeting maladaptive beliefs, is a widely used and highly effective treatment for various AD (Bandelow et al., 2023; Hofmann et al., 2025). Nevertheless, approximately one-third of patients do not respond to treatment. Identifying underlying mechanisms of action for CBT treatments and disentangling biological and psychological risk factors would allow a better understanding of the etiology of ADs. Moreover, it would allow to determine the utility of biomarkers in predicting therapy response and relapse risk as well as the development of a more personalized "precision" medicine to tailor treatments to the individual's needs, which has the potential to improve treatment response rates. Hence, recent research efforts have focused more on individual differences to identify relevant factors for the prediction of therapy response: biological mechanisms, such as (epi-)genetics and stress hormones, environmental factors, such as maltreatment experiences or resulting attachment styles, as well as therapy process variables, such as therapeutic alliance (Bandelow et al., 2016; Bandelow et al., 2017; Fava & Morton, 2009; Zilcha-Mano & Fisher, 2022). However, our understanding of how

psychotherapy might be influenced by, e.g., (epi-)genetics and the biological stress response, is still limited. Even less is known about the impact of CBT *on* biological processes. Understanding the impact of CBT as a positive and predictable environmental factor becomes even more relevant as many studies have highlighted how intertwined the environment, e.g., maltreatment experiences, is with biological mechanisms, e.g., related to stress response (Fischer et al., 2021) and epigenetics (Weber et al., 2025). Interestingly, attachment style has also emerged as a relevant risk factor for the development of ADs (Levy et al., 2011). Further, it seems likely that attachment style influences the psychotherapy success of AD via the therapeutic alliance (for a meta-analysis see Notsu et al., 2025). Hence, it seems important to understand how CBT for AD is influenced by and may influence biological mechanisms and relational processes. An integration of research results from various domains within a bio-psycho-social model could contribute to a better understanding of the contributions of several mechanisms in therapy. Aiming to address this research gap, we conducted the following studies.

In Publication I, we conducted a literature review of how CBT might be related to and might influence the functioning of the hypothalamic-pituitary-adrenal (HPA) axis in patients with ADs. Since it is one of the most relevant biological systems for stress response, understanding changes within this system is particularly relevant for patients suffering from ADs. Overall, we found evidence supporting the idea that cortisol levels have the potential to indicate the AD disease status and serve as a possible biomarker in therapy response prediction. In detail, the reviewed studies suggest that persistently elevated cortisol levels seem to impede therapy response. We further focused on how CBT, specifically therapeutic exposure to feared situations, might influence HPA axis functioning. A beneficial effect of cortisol elevation during exposure or, in reverse, effects of a blunted HPA system response on an unfavorable outcome are supported. Moreover, effective CBT treatment seems to normalize HPA system hyperfunction. Additionally, cognitive processes, e.g., positive anticipatory appraisal, attribution, perceived controllability, and coping, seem to have an impact on adaptive HPA system functioning.

Second, we conducted a therapy progress study with patients suffering from panic disorder (PD) undergoing CBT at the outpatient clinic of the Max Planck Institute of Psychiatry in Munich. We assessed dynamic changes in epigenetic (gene methylation and expression), metabolomic (small molecules produced by metabolism), and immune system biomarkers, as well as in relational patterns (attachment style, interpersonal distress, and therapeutic alliance). We applied several measures, including various questionnaires and taking blood samples, during a standardized short-term exposure-based CBT (12 sessions and two booster sessions) at several time points: pre-/ post-, mid-treatment, exposure sessions, and follow-up.

Biological markers on different levels were analyzed: Publication II identified specific DNA methylation signatures (HECA gene), which, as time-stable heritable gene-regulatory mechanisms, might serve as biomarkers for PD, thereby revealing potential epigenetic mechanisms. Publication III found that exposure to a feared situation induced significant changes in a plasma metabolite, namely glyoxylate, linked to the experienced anxiety level. These findings suggest that the metabolome might serve as a dynamic marker for different anxiety states. The related gene expression changes during exposure therapy were subtle but may indicate biological correlates of acute panic attacks and therapy success. Publication IV found that specific DNA methylation, such as within the gene for the serotonin receptor 3A related to the regulation of panic states and fear circuitry, as well as immune system patterns related to PD and acute fear (including arginase 1 gene methylation, CD4+ T cells, CD8+ T cells, B cells, and granulocytes), were altered during and after successful exposure therapy. Further, these changes were related to therapy response. In combination, the findings of studies III and IV highlight the possible role of epigenetics in the effects of CBT and its potential as a biomarker for treatment outcome.

In publication V, we analyzed psychological process variables throughout the course of the therapy, namely the relationship between attachment style changes and the working alliance. We found that an insecure, specifically anxious attachment style was reduced after treatment. Further, decreased anxious attachment was associated with improvements in symptom severity, therapeutic alliance, and interpersonal difficulties, which in turn were related to the effectiveness of CBT. Furthermore, findings showed that general attachment style and interpersonal distress seem to moderate the relation between the therapeutic alliance and subsequent symptom severity. The study highlights the dynamics and potential positive effects of standardized short-term CBT on interpersonal patterns.

The publications provide insights into both the pathophysiology of AD and the treatment mechanisms involved in CBT, aiming to enhance understanding of effective treatments and personalized approaches for AD patients. They emphasize, on the one hand, biological mechanisms potentially influenced by CBT: changes in the HPA system (cortisol levels), genetics (DNA methylation and gene expression), metabolomic shifts (glyoxylate regulation), and immune system dynamics. On the other hand, psychological processes are corroborated, highlighting the importance of cognitive factors, interpersonal patterns, and therapeutic alliance in the treatment of PD. Overall, multilevel changes are shown, which support a bio-psycho-social approach to PD treatment. Despite its limitations, this work highlights the probable multifaceted impact of CBT on AD/ PD and advances understanding of treatment mechanisms which could guide a more integrative approach to mental health. Future progress lies in tailoring treatments to individual needs and tracking outcomes with both biomarkers and psychological assessments.

2. General Introduction

The most common anxiety disorders (AD) include panic disorder (PD) with/without agoraphobia, social anxiety disorder, generalized anxiety disorder, and specific phobias. In the following section, I present relevant definitions and models regarding ADs, focusing particularly on PD. Moreover, I will introduce the biological stress response system and (epi-)genetic factors correlated to ADs. Finally, I will focus on psychological factors affecting the therapy process.

ADs are the most common mental disorders, with a lifetime prevalence of up to 30 % worldwide (Eck et al., 2025; Jacobi et al., 2014; Penninx et al., 2021; Santomauro et al., 2021; Szuhany & Simon, 2022). ADs are pathological forms of anxiety and fear that are characterized by excessive emotional and physiological responses in the absence of real threats or danger. Often, they include panic attacks, which are defined in the Diagnostic Statistical Manual of Mental Disorders - DSM-5 (American Psychiatric Association, 2013) as uncontrollable anxiety with pronounced cardiovascular and respiratory symptoms (e.g., increased heart rate, dizziness, shortness of breath, trembling, etc.). In consequence, anxiety-related emotions and behavior lose their original purpose to protect the individual from harm. They lead to impaired functioning, a reduced quality of life, and contribute significantly to a societal socioeconomic burden (Baxter et al., 2014; Kavelaars et al., 2023; Santomauro et al., 2021). Their devastating impact is further corroborated by high comorbidity rates with other psychiatric (e.g., depression) and somatic diseases. Nevertheless, they are often not adequately treated, resulting in a chronic course and disability (Penninx et al., 2021; Szuhany & Simon, 2022; Wittchen et al., 2011).

A PD is diagnosed if unexpected panic attacks occur, accompanied by worries about recurrence and possible consequences, as well as a change in behavior with avoidance or safety strategies. The estimated lifetime prevalence of PD in Europe and other Western countries varies between 3 and 5% (Bandelow et al., 2023; Szuhany & Simon, 2022; Wittchen et al., 2010). In around 20 to 65% of those affected, PD is accompanied by agoraphobia (Roest et al., 2019; Wittchen et al., 2010). Patients suffering from agoraphobia have an elevated fear response to situations and places from which escape is difficult or in which a panic attack might be embarrassing. Hence, these situations and places are avoided or only endured with severe discomfort (ICD-10; Dilling et al., 2011). Table 1 provides an overview of the essential diagnostic criteria for PD and agoraphobia according to DSM-5 guidelines (American Psychiatric Association, 2013). Publications I - V still refer to the DSM-IV-TR (American Psychiatric Association, 2000). In DSM-5, PD and agoraphobia are diagnosed as separate conditions (in DSM-IV linked), with agoraphobia having its distinct criteria and a required duration of 6 months. Since panic attacks also occur in other anxiety disorders, such as phobias (e.g., social, heights, animals, flying), there is a new panic attack specifier applicable to various disorders.

Table 1

DSM-5 Diagnostic Criteria for Panic Disorder and Agoraphobia

Criterion	Description
A. Panic Attacks	Recurrent, unexpected panic attacks characterized by an abrupt surge of intense fear or discomfort that peaks within minutes. During the attack, four or more of the following symptoms occur: - Palpitations, pounding heart, or accelerated heart rate - Sweating - Trembling or shaking - Sensations of shortness of breath or smothering - Feeling of choking

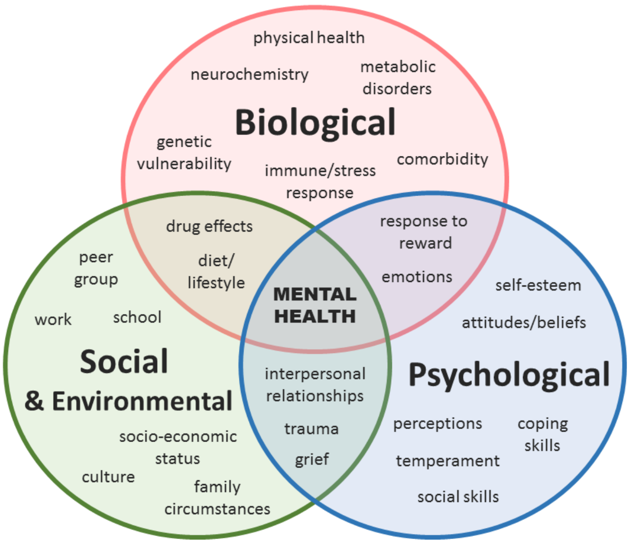
Criterion	Description
	- Chest pain or discomfort - Nausea or abdominal distress - Feeling dizzy, unsteady, lightheaded, or faint - Chills or heat sensations - Paresthesias (numbness or tingling sensations) - Derealization or depersonalization - Fear of losing control or “going crazy” - Fear of dying
B. Post-Attack Consequences	At least one panic attack is followed by 1 month or more of one or both of the following: - Persistent concern or worry about additional panic attacks or their consequences (e.g., losing control, having a heart attack). - Significant maladaptive changes in behavior related to the attacks (e.g., avoidance of exercise or unfamiliar situations)
C. Exclusion Criteria	The disturbance is not attributable to physiological effects of a substance (e.g., drugs, medications) or another medical condition (e.g., hyperthyroidism)
D. Comorbidity Rule-Out	The disturbance is not better explained by another mental disorder (e.g., social AD, post-traumatic stress disorder)
Agoraphobia	Marked fear or anxiety about at least 2 of the following 5 situations: - Using public transportation - Being in open spaces (e.g., parking lots, marketplaces) - Being in enclosed spaces (e.g., shops, theaters) - Standing in line or being in a crowd - Being outside the home alone Fear arises from thoughts that escape might be difficult or help unavailable during panic-like symptoms Situations are actively avoided, require a companion, or are endured with intense fear Fear/anxiety is disproportionate to actual danger and persists for 6 months or more

2.1. Bio-Psycho-Social Model

The pathogenesis of PD is multifactorial: Integrating biological and cognitive-behavioral perspectives as in the *bio-psycho-social-environmental model* of mental health (see Figure 1, Rezaie, 2025), there are multiple interconnected pathways involved in the onset and maintenance of PD, like genetic predispositions, neurochemical imbalances (e.g., disruptions in serotonin and neuropeptide systems), hormonal and neurophysiological dysregulation (e.g., sensitivity to CO_2), and dysfunctional neuroanatomical circuits involving regions like the amygdala. Alongside these biological factors, the model emphasizes environmental and individual factors such as childhood trauma and interpersonal difficulties that have a strong pathogenetic influence (Fava & Morton, 2009; Joraschky & Petrowski, 2008; Kay et al., 2024).

Figure 1

Bio-Psycho-Social-Environmental Model for Mental Health

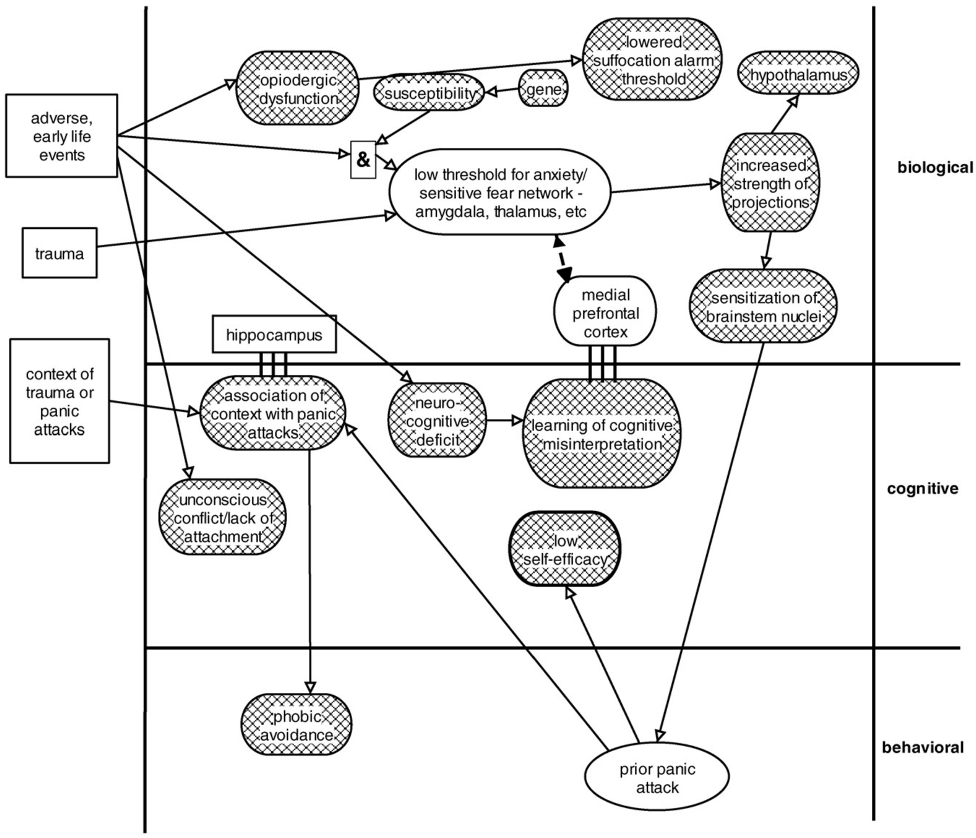


Note: From "Exploring the relationship between anxiety and depression," by P. Rezaie, 2025, *OpenLearn*, The Open University (https://www.open.edu/openlearn/science-maths-technology/exploring-the-relationship-between-anxiety-and-depression/content-section-2). Licensed under CC BY-NC-SA 4.0.

Cognitive models include self-efficacy, the catastrophic misinterpretation, and heightened anxiety sensitivity, which amplify fear responses and perpetuate panic attacks (Kyriakoulis & Kyrios, 2023). From a cognitive-behavioral perspective, acute panic attacks are caused by a psychobiological feedback loop between physical symptoms, their association with danger, the resulting anxiety response, and avoidance behavior (Margraf & Schneider, 2011). For an example of a model integrating many proposed factors, see Figure 2 by Fava and Morton (2009) and Kyriakoulis & Kyrios (2023).

Figure 2

Causal Model of Panic Disorder Theories



Note. Pre-existing states and conditions are indicated by oval, cross-hatched forms. The sensitive fear network is shaded. From "Causal modeling of panic disorder theories." by L. Fava and J. Morton, 2009, *Clin Psychol Rev, 29*(7), p. 635. © 2009 by Elsevier Ltd. Reprinted with permission from Elsevier.

The integration of various domains into a causal framework illustrates how environmental, genetic, neurobiological, respiratory, behavioral factors, and cognitive vulnerabilities interact dynamically, leading to a self-sustaining cycle of panic. The composite cognitive theory posits that distal antecedents such as genetic predispositions, neurobiological abnormalities (sensitive fear network, e.g., amygdala-hippocampus interactions), and past adverse experiences via a dysfunctional attachment and neurocognition, interact with proximal triggers, e.g., bodily sensations or contextual cues, to initiate panic attacks. Central to cognitive models of panic disorder is the catastrophic misinterpretation of bodily sensations ,the ‘perceived threat’, which creates a vicious cycle of escalating anxiety and physiological arousal (Robinaugh et al., 2024). Cognitive vulnerabilities like low self-efficacy (Breuninger et al., 2019; Southward et al., 2024) and attentional biases toward threat amplify this process, while avoidance and safety-seeking behaviors maintain the disorder (Robinaugh et al., 2024). Integrative models (Fava & Morton, 2009; Kyriakoulis & Kyrios, 2023) emphasize the need for a combined approach, incorporating cognitive-behavioral therapy to address misinterpretations and avoidance, as well as biological treatments to modulate vulnerabilities.

2.2. Treatment

Following the proposed pathogenesis, the most effective treatments are cognitive-behavioral therapy (CBT), which targets avoidance behavior and maladaptive thoughts through exposure to feared situations, as well as pharmacotherapy with antidepressants (For a review see Bandelow et al., 2023; Hofmann et al., 2025). Despite the demonstrated efficacy of both pharmacotherapy and CBT, these approaches remain largely unintegrated, and neither has sufficiently clarified the underlying mechanisms of action. Even with appropriate treatment, therapy is not always successful: up to 30 % of panic patients respond poorly to psychotherapy and the disorder becomes chronic (Hofmann et al., 2025). In addition to therapy process variables (e.g., therapeutic alliance), clinical factors such as severity of symptoms, comorbidity (e.g., personality disorders), demographic characteristics, and other psychological (e.g., attachment style) as well as biological patient variables (e.g., genetics, stress regulation) seem to be relevant for the prediction of therapy response (Compton et al., 2014; Diener & Monroe, 2011; Eley et al., 2012). However, even though findings highlight the potential importance of these factors, inconsistent and isolated results across studies prevent a unified theory. The underlying biological underpinnings for differential response to CBT are widely unexplored. Regarding the bio-psycho-social perspective, the likely interplay with CBT is as follows: Environmental factors, e.g., caregiving, social support, time of day, and individual factors (e.g., age, sex, and lifestyle), interact with biological factors like the endocrinological systems, e.g., stress hormones or oxytocin, which in turn influence cognitive-behavioral functioning, such as memory or emotion expression. Vice versa, psychotherapeutic processes like the therapeutic alliance, cognitive restructuring, and behavioral activation have an effect on the endocrinological system and thus, pathology (Fischer & Zilcha-Mano, 2022). Psychotherapy, specifically CBT, can be seen as a positive and predictable environment and is therefore ideal to study interactions with biological factors, allowing for prospective assessment of symptoms (Eley, 2014). A combination of biological and psychosocial variables has the potential to enable additive prediction of therapy outcome and determine the individual duration and intensity of CBT treatment.

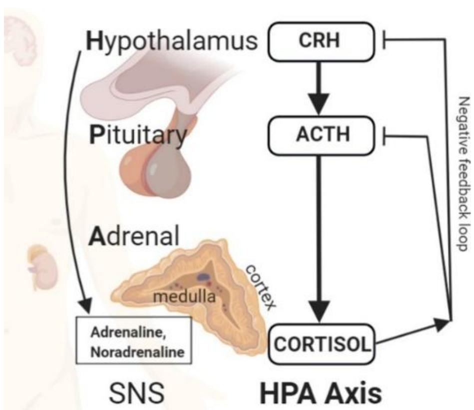
2.3. Biological Correlates

2.3.1. HPA Axis Regulation

The hypothalamic-pituitary-adrenal (HPA) axis mediates the stress response via cortisol secretion from the adrenal cortex. Elevated circulating glucocorticoids exert a negative feedback on the periventricular nucleus of the hypothalamus, inhibiting corticotropin-releasing hormone (CRH) synthesis and subsequent adrenocorticotropic hormone (ACTH) release from the anterior pituitary, thereby promoting homeostatic restoration (see Figure 3; Meir Drexler et al., 2020). Compared to the sympathetic nervous system (SNS), which activates a rapid neural pathway that releases norepinephrine and adrenaline for acute stress, the slower-acting HPA axis takes over for prolonged stress. Cortisol release activates the body and increases, for example, heart rate and blood pressure; it suppresses immune and reproductive functions and provides energy for a "fight or flight" response. After the end of a stress-inducing situation, the negative feedback loop reduces cortisol levels (see Figure 3).

Figure 3

The Stress Response



Note: From "Stress modulation of fear and extinction in psychopathology and treatment." by S. Meir Drexler, C. J. Merz, V. L. Jentsch, and O. T. Wolf, 2020, *Neuroforum, 26*(3), p. 134 (https://doi.org/doi:10.1515/nf-2020-0018). Licensed under CC BY 4.0.

The hypothesis that ADs involve a dysfunction of the HPA system has led to extensive research on both acute and chronic alterations of the HPA system. Findings on cortisol level, used as a biomarker of HPA axis activity, are mixed, with studies showing both elevated and reduced cortisol in AD subtypes, depending on factors such as time of day, disorder severity, or stress exposure (for a review see Bandelow et al., 2017; Elnazer & Baldwin, 2014; Elnazer et al., 2021; Hek et al., 2013; Plag et al., 2013; Vinkers et al., 2021; Łoś & Waszkiewicz, 2021). Experimental stress paradigms (e.g., Trier Social Stress Test [TSST]; Kirschbaum et al., 1993; cortisol suppression tests) revealed variable HPA system reactivity in AD patients: some show hypoactivation (blunted stress response), others hyperactivation, and some no difference compared to controls (for a review see Bandelow et al., 2017; Ising et al., 2012). Cortisol has been proposed as both a trait marker for early detection of pathological anxiety and a state marker, reflecting symptom severity over time (Łoś & Waszkiewicz, 2021). Initial cortisol elevations may drop with chronicity, potentially indicating HPA system exhaustion and desensitization. Hair cortisol studies support this view, with higher levels in single-episode AD and lower levels in recurrent cases (Elnazer et al., 2021). In general, an altered HPA system activity is linked to long-term outcomes, e.g., low cortisol awakening response or high pre-treatment cortisol predicting poor prognosis in AD (Bandelow et al., 2000; Coryell et al., 1991; Petrowski et al., 2012). Especially childhood maltreatment disrupts the normal development of the HPA axis, resulting in long-term changes in cortisol regulation, which can manifest as increased or dysregulated cortisol secretion, contributing to dysfunctional stress sensitivity and a greater risk for both mental and physical health throughout life (Heim & Nemeroff, 2001; Strüber et al., 2014). A two-pathway model is proposed by Strüber and colleagues (2014) that emphasizes that early adversity, accompanied by either high or low maternal care, can lead to distinct alterations in neurodevelopment and glucocorticoid regulation: either glucocorticoid hyperfunction, with higher emotionality and self-focus, or hypoactivation, with diminished self-reflection and empathy. Following this, hypocortisolism is often implicated in symptomatology related to trauma (for a review see Fischer et al., 2021). In general, HPA axis dysfunction, hypo- and hyperactivation, seems to be predictive of CBT non-response and might be modifiable alongside therapeutic improvement (Fischer & Zilcha-Mano, 2022). However, the complex dynamic of the HPA system regarding AD treated with CBT is still ambiguous and needs clarification.

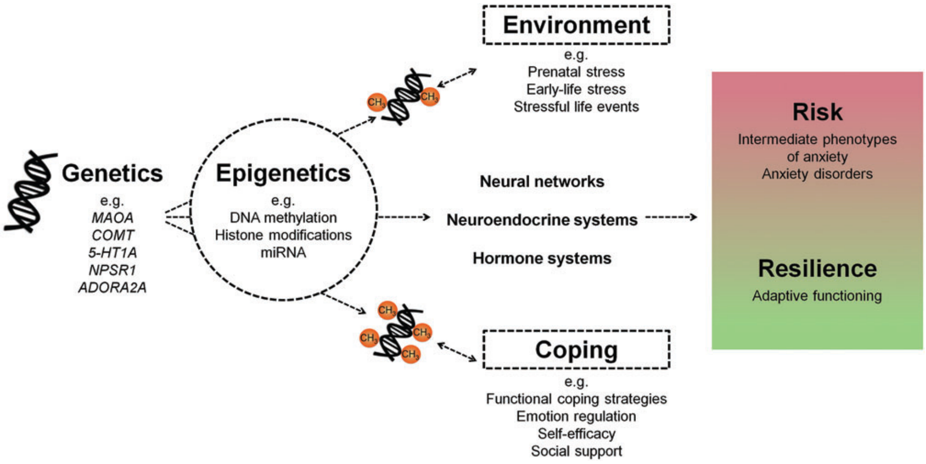
2.3.2. Genetics

Two research methods gained importance in the field of genetics in psychiatric disorders: *Genome-wide association* studies scan the entire genome to find genetic variations (usually single-nucleotide polymorphisms) that are associated with a particular trait or disorder. C*andidate gene approaches* focus on a few specific genes based on prior research or theory. The former enables the generation of new hypotheses but has lower statistical power, while the latter offers greater power when focusing on a predefined set of genes. The estimated genetic contribution for various AD ranges from 30 to 50% and is attributed to variable effects of multiple genes (Weber et al., 2025). Exposure to negative life events and stress, as well as gene–environment interactions, substantially moderate the risk for AD. According to the differential-susceptibility hypothesis, individuals generally vary in their developmental plasticity in response to both negative and positive environmental influences (Pluess & Belsky, 2013), which may be reflected in genetic factors: genotype and epigenetics (Eley, 2014). Unlike static deoxyribonucleic acid (DNA) sequence variations (genotype), epigenetic markers, such as histone modifications, small/ micro ribonucleic acid (RNA)-related gene silencing, and DNA methylation, can change with environmental exposures and possibly differ between individuals with AD and those without (Weber et al., 2025). DNA *methylation* involves the addition of methyl groups to DNA mediated through DNA methyltransferases (typically at CpG sites, regions where a guanine follows a cytosine), which can regulate gene activity without altering the genetic code. This addition usually happens near genes, and it can silence or reduce the activity of that gene by regulating the accessibility of transcription factors to their binding sites (Kribelbauer et al., 2020). Thus, it is a key mechanism through which factors like stress, trauma, or other environmental influences modulate *gene expression*, the process by which a gene is transcribed into protein. Methylation in the promoter region (a segment of DNA located before the gene's transcription start site) often leads to reduced gene expression, whereas decreased/ no methylation results in more gene expression (Jackson-Grusby et al., 2001).

Genetic factors play an important role in the development of psychiatric disorders and likely in response to therapy. Regarding the gene-environment interplay and in line with the model by Schiele and Domschke (2018), therapeutic environmental inputs like CBT promoting coping could modify gene regulation in a way that supports remission. Figure 4 summarizes the interaction of genetic factors, environmental stressors, and coping-related protective mechanisms in a multilevel model of risk and resilience in ADs. These interactions are mediated by epigenetic processes, which regulate neural networks, neuroendocrine systems, and hormonal pathways to either increase risk or enhance resilience (Schiele & Domschke, 2018).

Figure 4

Multilevel Model of Risk and Resilience in Anxiety Disorders



Note. From "Epigenetics at the crossroads between genes, environment and resilience in anxiety disorders." by M. A. Schiele, and K. Domschke, 2018, *Genes Brain Behav, 17*(3), p. 7 (https://doi.org/10.1111/gbb.12423). © 2017 John Wiley & Sons Ltd. Used with permission of John Wiley & Sons - Books; permission conveyed through Copyright Clearance Center, Inc.

A novel field of interest named "therapygenetics" has been developed, where genetic markers are used to predict response to psychotherapy (Lester & Eley, 2013). However, pinpointing specific molecular signatures as 'susceptibility biomarkers' has been challenging. A few studies have found some evidence that genetic differences contribute to the variance in response to psychological interventions (For a review see Bandelow et al., 2016; Deckert & Erhardt, 2019; Lester & Eley, 2013) others found no single nucleotide polymorphisms strongly associated with therapy response (Coleman, Lester, Keers, et al., 2016; Coleman, Lester, Roberts, et al., 2016; Rayner et al., 2019). Whilst there is still no evidence that a specific dysfunction of a neurotransmitter system is the main cause for ADs, some research indicates that changes in the serotonergic system seem to play a role in ADs (for a review see Bandelow et al., 2017). However, this assumption needs further evaluation as the results are inconsistent across studies (for a meta-analysis see Schiele et al., 2021; for a review see Vashishtha et al., 2023). In summary, current evidence does not support the use of individual genotyping in clinical routine for psychotherapy response prediction in AD at this stage (for a review see Weber et al., 2025).

Current studies investigating changes in epigenetic alterations seem more promising (Domschke et al., 2025; Hudson et al., 2013; Murphy & Singewald, 2019; Roberts et al., 2014; Roberts et al., 2017; Schiele et al., 2018; Wang et al., 2015; Ziegler et al., 2016). First epigenome-wide findings imply that DNA-methylation changes can serve as markers for stress effects and differential clinical therapy response in ADs (for a review see Weber et al., 2025). Recently, in a large EWAS an association of DNA methylation with a favorable CBT outcome was found with gene sites, which were previously found to be involved in synaptic plasticity, cognition, and the regulation of stress (Domschke et al., 2025). Taken together, these findings are promising in terms of the potential predictive use of epigenetic markers in CBT. They add to a growing body of research, indicating that response to CBT may be linked to biological changes. As the effect sizes seem to be modest for single markers, a combination of genetic variations, epigenetic changes, and further molecular markers might have the best predictive values (Deckert & Erhardt, 2019).

2.4. Attachment Style and Therapeutic Alliance

2.4.1. Attachment Style

Following Ainsworth et al. (1979) categorical approach, adult attachment style is grouped in *secure* (comfortable with intimacy and autonomy, valuing of intimate friendships), *preoccupied* with relationships (overinvolvement, dependence on other people's acceptance, tendency to idealize other people), *dismissing* (downplaying of the importance of close relationships, restricted emotionality, an emphasis on independence and self-reliance) and *fearful* of intimacy (socially avoidant, sense of personal insecurity, distrust of others; Bartholomew & Horowitz, 1991). These categories fall along two dimensions: *avoidance*, which results in distancing oneself emotionally, and *anxiety*, which means seeking constant reassurance and proximity. The nomenclature used in the measurement of adult attachment varies (Ravitz et al., 2010). Common ground for the many attachment measures is the classification of a secure attachment and several categories of an insecure attachment style that define attachment either on a categorical or dimensional basis.

Based on Bowlby (1969), attachment theory postulates a primary need for a bond with a person that is essential for survival. From the environmental perspective, attachment orientation is “conceptualized as the psychological residue of an individual’s unique attachment history” (Mikulincer et al., 2013). These patterns influence expectations, emotions, and behaviors in adult relationships: Repeated experiences with dismissing or fearful attachment figures strengthen the expectation of unreliable and unpredictable reactions. In consequence, individuals tend to become more mistrusting, fearful, and hypervigilant for danger (Nolte et al., 2011). Findings support the idea that childhood maltreatment broadly impacts impairments in adult personality functioning (relational capacities, identity integration, self-control, and social concordance), primarily via insecure (anxious) attachment pathways (Cohen et al., 2017). In individuals with childhood adversity, an insecure attachment style has been found to partially mediate the relationship with depression and anxiety (Bifulco et al., 2006). Moreover, there is a relationship between an insecure attachment style and the internalization of psychological problems (Colonnesi et al., 2011). Research suggests that a secure attachment style protects against, whilst an insecure attachment style is a risk and a maintaining factor of anxiety (Cassidy et al., 2009; Colonnesi et al., 2011; Esbjorn et al., 2012; Levy et al., 2011; Manning et al., 2016; Pini et al., 2014) and vice versa (Davila et al., 1997; Horesh et al., 2014). Earlier studies show that AD patients often have an insecure and mainly preoccupied/anxious attachment style (de Ruiter & Van Ijzendoorn, 1992; Nielsen et al., 2017) and that attachment anxiety contains a greater risk for developing an AD than attachment avoidance (Nolte et al., 2011). However, a study in SAD showed that a fearful attachment style mediated the association between childhood adversities and symptom severity (Elling et al., 2022). Further, attachment style is shown as an unspecific factor of general vulnerability for AD, which has to interact with covariates like temperament and psychosocial stress to trigger a disorder (de Ruiter & Van Ijzendoorn, 1992; Mikulincer & Shaver, 2012). Some studies have even assessed potential genetic biomarkers for attachment style. For example, candidate gene studies suggest that insecure attachment might be partially explained by specific genes (for a review see Erkoreka et al., 2021; Gillath et al., 2008). Moreover, the literature consistently indicates a connection between early childhood adversity, attachment processes, and epigenetic modifications (For a review see Darling Rasmussen & Storebø, 2021). Attachment style is also known to correlate with neurobiological factors and the endocrine stress system (Adams et al., 2020; Ketay & Beck, 2017; Toumbelekis et al., 2021). One study combined domains and found that FK506 binding protein 5 (FKBP5) methylation moderates the associations of FKBP5 genotype and resistant (anxious) attachment with cortisol reactivity (Mulder et al., 2017). However, evidence from studies remains limited.

Although attachment style shows general stability ("trait-like"), it can be revised through significant relational experiences or life events. The *prototype hypothesis* suggests that early-established attachment orientations persist as a stable core influencing relational behavior, though new experiences can temporarily shift attachment (Fraley et al., 2011; Jones et al., 2018; for a review see Ravitz et al., 2010). Further, attachment working models are conceptualized as hierarchical by nature with relationship-specific styles (e.g., parent, romantic, friend) derived from particular episodic memories existing beneath a more global style with generic representations of attachment relationships (Mikulincer & Shaver, 2007a). Fraley and colleagues (2011) stated that the adult attachment toward a romantic partner is less stable than toward one's parents, since stability depends on the duration and hence the consolidation of the representational systems, and there are probably more opportunities to revise the inner working models due to more frequent contact. A high number of anxiety patients with agoraphobia rely strongly on their partner, since they avoid being alone or need an accompanying “safe person” (Zalaznik et al., 2019). Anxiety may serve to preserve the relationship, e.g., the attention, or may even reduce an internal attachment-autonomy conflict, sustaining dysfunctional relationship patterns. Further, it might strengthen the dynamics of a complementary union by granting psychological reward for the care provider. On the other hand, it is a strain on the relationship (Marcaurelle et al., 2003;

Zaider et al., 2010). Relying on a "safe person" to cope with anxiety might further decrease the individual's self-efficacy, which has been shown to be a mediator of outcome in CBT for PD (Fentz et al., 2014). This loss of self-efficacy reinforces anxiety in relationships, generating a vicious cycle that increases the pathology (Zalaznik et al., 2019).

Psychotherapy, particularly CBT, can potentially alter attachment style by changing automatic relational patterns (for a review see Levy et al., 2018). Previous research indicates inconsistent evidence regarding whether CBT produces lasting changes in attachment style among AD patients (Belanger et al., 2011; Madigan et al., 2015; Murphy et al., 2016; Müller & Rosenkranz, 2009; Rimane et al., 2020; Stovall-McClough & Cloitre, 2003; Strauß et al., 2018). An important question is which factors targeted by the therapy might mediate the change of the inner working model of attachment. A possible effect of psychotherapy could be a growth in self-efficacy following the successful overcoming of their fears and gaining a feeling of control (Breuninger et al., 2019; Hoffart, 2016; Southward et al., 2024). Another possibility could be an experience of a new secure bond, which changes the inner working model of relationships (Levy & Johnson, 2018; Mikulincer et al., 2013; Skourteli & Lennie, 2011; Zuroff & Blatt, 2006). Further, CBT, including interoceptive and in vivo exposure, has been shown to improve deficits in emotion regulation (Meine et al., 2024), which seems not only to predict outcome (Strauss et al., 2019) but to be a catalyst for attachment insecurity (Esbjorn et al., 2012; Zalaznik et al., 2019) and has been shown to mediate attachment anxiety and AD (Nielsen et al., 2017). Further, negative self-representations, anxiety sensitivity, problems in interpersonal relations, and reflective function have been proposed as potential mediators between attachment insecurity (anxiety and/or avoidance) and psychopathology (Levy & Johnson, 2018; Mikulincer & Shaver, 2012, 2018; Zalaznik et al., 2019; Zalaznik et al., 2022). Overall, the question remains open as to whether the general attachment style can be permanently changed and how such a change can be achieved.

Furthermore, an important issue is how attachment style influences the psychotherapeutic process and the interaction of patient and therapist. Importantly, an insecure attachment style (especially anxious) has been shown to have a detrimental effect on psychotherapy response by affecting interpersonal functioning and thus, therapeutic alliance quality (for a meta-analysis see Levy et al., 2018).

2.4.2. Therapeutic Alliance

According to Grawe (1992), the relationship between patient and therapist is one of the most important factors in psychotherapy. The therapeutic alliance is an essential component of the relationship between therapist and patient and represents its interactive and collaborative aspects (bond, common goals, and tasks) in the context of an appreciative and empathic approach (Castonguay et al., 2006). Rooted in attachment theory, the therapeutic alliance serves as a secure relational base, providing emotional safety, validation, thus promoting exploration and change (Mikulincer et al., 2013). It is an effective factor across therapy methods, as demonstrated by moderate but robust effects in various therapy contexts (Flückiger et al., 2018; Horvath et al., 2011). Findings suggest that clients with insecure attachment styles (for a meta-analysis see Notsu et al., 2025) and with interpersonal problems ($r = -.12$; for a meta-analysis see Iovoli et al., 2024) tend to have weaker therapeutic alliances, which negatively affects outcomes. The therapeutic alliance includes both stable "trait-like" factors that reflect a patient's general interpersonal capability and dynamic "state-like" factors responsive to therapeutic interactions. Enduring trait-like alliance is thought to result from the intrapersonal and interpersonal characteristics of patients and therapists. State-like alliance, on the other hand, displays the evolving therapeutic process in session-to-session fluctuations (Zilcha-Mano & Fisher, 2022). Studies suggest these state-like alliance variations often precede symptom and attachment style improvement, highlighting alliance as potentially therapeutic in itself (Zilcha-Mano, 2017). However, the directionality of these relationships remains unclear, underscoring the need for further longitudinal studies to disentangle the interactive dynamics among attachment style, therapeutic alliance, and therapy outcomes.

3. Research Questions and Objectives

'Diagnostic biomarkers' detect or confirm a disorder, 'susceptibility biomarkers' estimate the probability of developing a disorder, and 'monitoring biomarkers' estimate the status of a disorder. In distinction, response to CBT is most likely explained by molecular 'predictive biomarkers' that assess the effect of CBT (Domschke et al., 2025). From findings in previous research of dysfunctional alterations of genetic markers and the stress response system in patients suffering from AD in the sense of diagnostic and susceptibility biomarkers, the logical inquiry is whether therapy outcome can be predicted, whether therapy itself can induce beneficial changes, and whether it influences other biological systems during acute fear. If certain biological patterns change in response to successful CBT, it could indicate a biological embedding of therapeutic effects. Moreover, observing interpersonal process variables in therapy will allow conclusions about the interaction of therapeutic intervention, time, individual relational patterns, and therapeutic alliance (Gloster et al., 2011). An assessment of endocrinological dysfunctions, genetic risk factors (Hudson et al., 2013), immune system profiles, and metabolomics (small molecules found in blood plasma that are involved in or produced by metabolism, e.g., amino acids, lipids, carbohydrates), as well as therapy process-related factors, such as attachment and alliance, has the potential to enable additive prediction of therapy outcomes, inform research about potential mechanisms of action, and adapt the individual duration and intensity of CBT treatment. Understanding the complex interplay of these factors, identifying measurable parameters as disease markers, and disentangling risk factors, causes, and consequences remains a significant challenge for psychiatric research. Investigation is required regarding the impact of treatments themselves, as well as patient factors in the sense of an individual therapy-patient fit. Given the multifaceted nature of ADs, the present cumulative thesis synthesizes five peer-reviewed studies that examine both biological and attachment-related correlates of CBT for AD, with a focus on PD. The overall goal is to advance our understanding of how effective treatment for AD may produce changes across different levels – from stress hormones and gene regulation to interpersonal functioning – and how these changes relate to therapeutic outcomes. Each study addresses a specific domain of interest, as outlined below:

(1) **HPA axis function in ADs treated with CBT (Literature Review):** Previous reviews have only briefly addressed the dynamic HPA axis response to cognitive behavioral therapy (CBT), particularly exposure-based CBT. Our review (publication I) is the first to focus specifically on cortisol-based HPA system dynamics before, during, and after exposure-based CBT in AD patients. It categorizes existing research into three parts: baseline cortisol as a predictor of treatment outcome, cortisol response during therapy sessions, and pre-/post-treatment cortisol changes.

(2) **DNA methylation signatures in PD:** An epigenome-wide association study (publication II) examines DNA methylation differences between PD patients and healthy controls. It investigates whether PD is associated with specific epigenetic markers in blood plasma DNA.

(3) **DNA methylation dynamics and gene expression during CBT for PD:** While baseline epigenetic differences can indicate underlying biological traits of PD, an equally important question is how those epigenetic markers might change during therapy. Studies III and IV are based on the hypothesis that epigenetic mechanisms mediate the interaction of genetics and environment in PD. They are tracking changes

in DNA methylation, gene expression, as well as immune cell composition, and plasma metabolome over the course of short-term CBT in a sample of patients with PD, specifically during exposure to feared situations.

(4) **Attachment style change and therapeutic alliance in CBT for PD:** Attachment style and therapeutic alliance across the course of CBT and follow-up are investigated. Study V aims to determine if attachment insecurity can decrease with CBT. Furthermore, the study examines the relationships between attachment changes, the development of therapeutic alliance, interpersonal difficulties, and clinical outcomes.

4. General Methods

In the literature review (publication I), we present a summary of research examining the HPA axis in relation to AD, along with initial evidence regarding its potential for modification through CBT. Our focus is on experimental studies that have investigated cortisol levels before, during, and after exposure-based CBT. To identify relevant literature, we conducted a systematic search of MEDLINE/PubMed, PsycINFO, Web of Science, and the reference lists of retrieved articles up to April 2024. Studies were included if they contained keywords pertaining to the HPA axis, anxiety disorders, and CBT techniques.

The therapy progress study (publications II to V) was conducted in the outpatient clinic for anxiety disorders of the Max Planck Institute for Psychiatry in Munich. Study protocols were approved by the ethics committee of the Ludwig-Maximilian University in Munich. Participants were recruited from a pool of patients seeking treatment for anxiety disorders at the clinic who met the inclusion criteria. Written consent was obtained from each participant. The results reported in this thesis were attained through the interdisciplinary collaboration of psychotherapists, psychiatrists, researchers, and laboratory technicians. Statistical analyses were conducted using RStudio (RStudioTeam, 2020). The psychotherapeutic treatment of PD (studies III to V) was conducted according to a research-based manual by Lang and colleagues (2011) and includes interoceptive (e.g., hyperventilation) and in vivo exposures. Following an initial interview, CBT consisted of 12 sessions (six to eight weeks) with a frequency of two sessions per week, and two booster sessions after two and four months. Individuals underwent at least two in vivo exposure sessions accompanied by a therapist (see Table 2 and Figure 5).

Table 2

Session Procedure of CBT for Panic Disorder with/without Agoraphobia

	Session	Content	
Psychoeducation	1	Information about anxiety, vicious cycle model	Overview of the treatment
			What are anxiety and panic?
			Introduction to the development model of panic disorder and agoraphobia
			Introduction to the vicious cycle of anxiety
	2	Development model and behavioral analysis	Development of an individual model
			Behavioral analysis based on a recent panic attack
			Collection of additional anxiety-provoking situations and symptoms
	3	Effects of avoidance behavior & deriving treatment goals	Identification of situational factors influencing anxiety
			Significance and effects of thoughts
			Significance and effects of avoidance behavior
			Development of an individual vicious-cycle model
			Derivation of treatment goals
Interoceptive Exposure	4	Conducting interoceptive exposure exercises	Creating a fear hierarchy related of bodily sensations
			Conducting the first interoceptive exposure exercises
	5	Repetition of interoceptive exposure exercises	Identifying changes after interoceptive exposure
In-vivo-Exposure		Treatment rationale for in-vivo exposure	Creating a fear hierarchy related to feared situations
			Conducting a thought experiment to derive the rationale for in-vivo exposure
	6 - 8	Conducting exposure exercises	Preparation for the exposure exercise
			If necessary, building motivation for exposure

<table>
<tr><th></th><th>Session</th><th>Content</th><th></th><th></th></tr>
<tr><td rowspan="11">In-vivo-Exposure</td><td rowspan="3">6 - 8</td><td rowspan="3">Conducting exposure exercises</td><td rowspan="2">From the fear hierarchy</td><td>1. Individual exercise (e.g., bus/tram)</td></tr>
<tr><td>2. Individual exercise (e.g., department store, tower)</td></tr>
<tr><td colspan="2">Debriefing after the exercises</td></tr>
<tr><td rowspan="5">9</td><td rowspan="5">Changes in anticipatory anxiety & challenges in exposure</td><td colspan="2">Identifying changes after the exposure situations</td></tr>
<tr><td colspan="2">Evaluation of learning experiences</td></tr>
<tr><td colspan="2">Assessment of remaining symptoms</td></tr>
<tr><td colspan="2">Identifying changes in anticipatory anxiety</td></tr>
<tr><td colspan="2">Preparing for unaccompanied exposure exercises</td></tr>
<tr><td rowspan="3">10 -11</td><td rowspan="3">Conducting exposure exercises</td><td colspan="2">Preparation for the first unaccompanied exposure exercise</td></tr>
<tr><td colspan="2">Conducting the first unaccompanied exposure exercise</td></tr>
<tr><td colspan="2">Debriefing after the exposure exercise</td></tr>
<tr><td rowspan="8">Relapse Prevention</td><td rowspan="4">12</td><td rowspan="4">Learning experience, practice plans</td><td colspan="2">Identifying changes that occurred after the exercises</td></tr>
<tr><td colspan="2">Evaluation of previous learning experiences</td></tr>
<tr><td colspan="2">Identification of possible relapse risks</td></tr>
<tr><td colspan="2">Developing a practice plan</td></tr>
<tr><td rowspan="4">2 Booster Sessions</td><td rowspan="4">Learning experience</td><td colspan="2">Evaluation of the self-directed exercises</td></tr>
<tr><td colspan="2">Changes in existing relapse risks</td></tr>
<tr><td colspan="2">Agreement on/recommendation of further exercises</td></tr>
<tr><td colspan="2">Conclusions from the treatment (only in second booster session)</td></tr>
</table>

Note: Session procedure of CBT for panic disorder with/ without agoraphobia according the manual of Lang and colleagues (2011).

Figure 5

Course of CBT

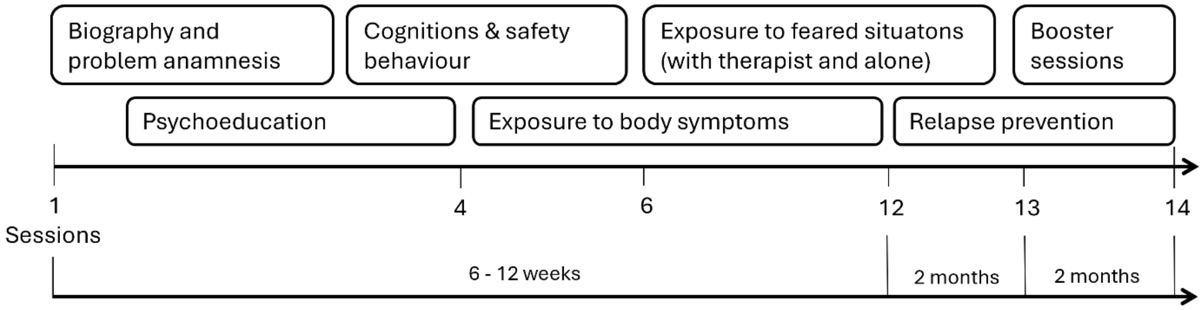
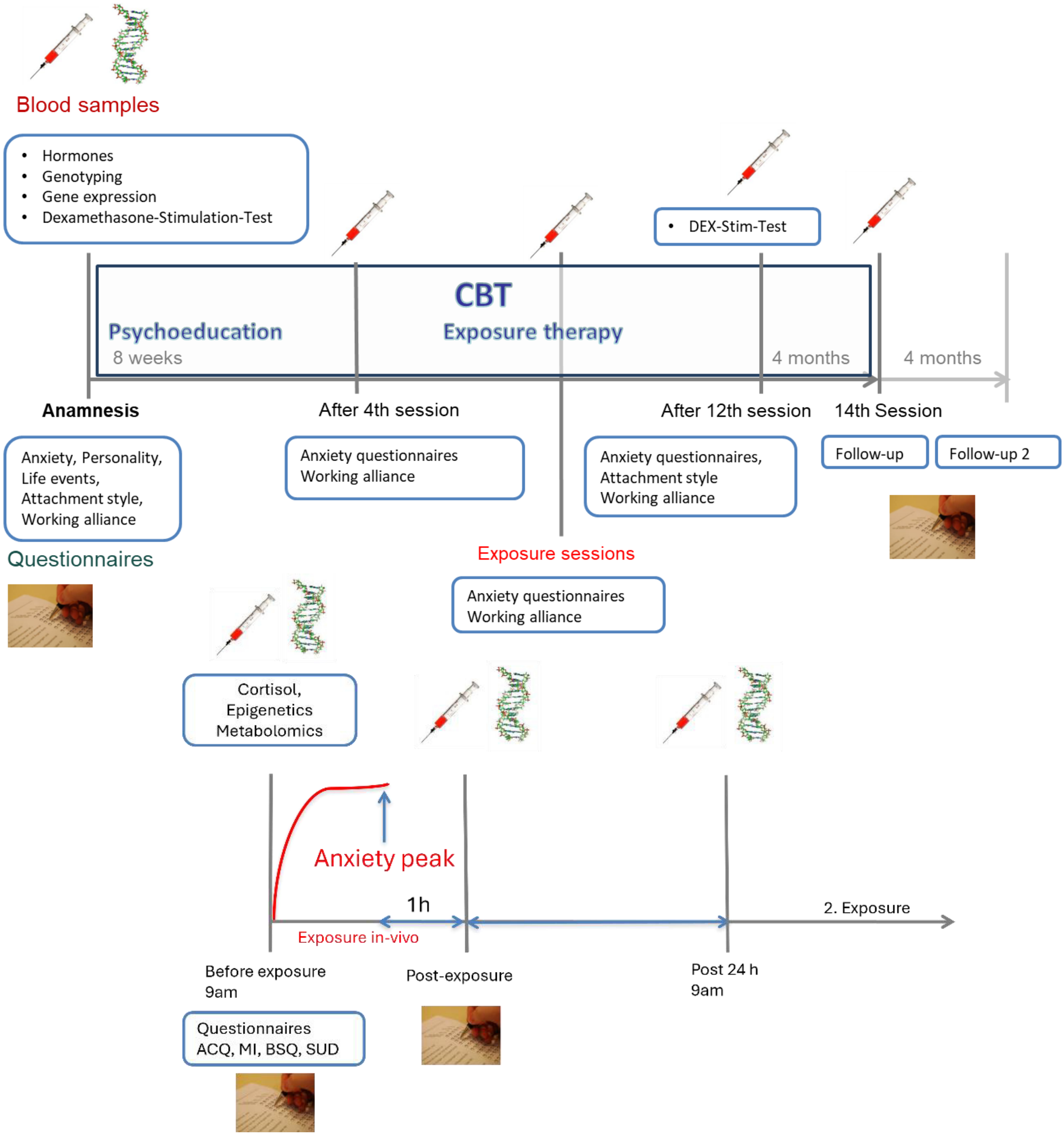


Figure 5 shows the phases and timeline of the CBT. Figure 6 illustrates the parallel study design for the intervention study, during which we collected the data for publications II to V. The diagnostic questionnaires, as detailed in Table 3, and blood tests, e.g., assessing epigenetics, were carried out before the beginning of treatment, before the fifth session of the treatment, and after the end of treatment. Due to changes in cortisol levels throughout the day, assessments took place in the morning. In addition, a subsample of 25 participants was examined during exposure sessions with blood tests and questionnaires to assess cortisol, epigenetic profiles, and metabolomics. After the end of therapy, a follow-up survey was carried out after four (second booster session) and eight months.

Figure 6

Study Design



Note: ACQ = Anxiety Cognitions Questionnaire, MI = Mobility Inventory , BSQ = Body Sensations Questionnaire, SUD = Subjective units of distress.

Table 3

Questionnaires

<table>
<tr><th colspan="2">Self-ratings</th><th>Base line</th><th>After 4th session</th><th>Post</th><th>Follow-up 1</th><th>Follow-up 2</th></tr>
<tr><td>Anxiety symptoms</td><td>Mobility Inventory (MI; Ehlers & Margraf, 2001)</td><td>x</td><td>x</td><td>x</td><td>x</td><td>x</td></tr>
<tr><td></td><td>Anxiety Cognitions Questionnaire (ACQ; Ehlers & Margraf, 2001)</td><td>x</td><td>x</td><td>x</td><td>x</td><td>x</td></tr>
<tr><td></td><td>Body Sensations Questionnaire (BSQ; Ehlers & Margraf, 2001)</td><td>x</td><td>x</td><td>x</td><td>x</td><td>x</td></tr>
<tr><td></td><td>Panic and Agoraphobia Scale (PAS; Bandelow, 1997)</td><td>x</td><td>x</td><td>x</td><td>x</td><td>x</td></tr>
<tr><td></td><td>State Trait Anxiety Inventory (STAI; Laux et al., 1981)</td><td>x</td><td></td><td>x</td><td></td><td></td></tr>
<tr><td></td><td>Anxiety Sensitivity Index (ASI-R; Hoyer et al., 2005)</td><td>x</td><td></td><td>x</td><td>x</td><td>x</td></tr>
<tr><td>Other symptoms</td><td>Symptom-Checklist (SCL-90-R; Franke, 1995)</td><td>x</td><td></td><td>x</td><td>x</td><td>x</td></tr>
<tr><td></td><td>Beck Depression Inventory (BDI; Hautzinger et al., 2000)</td><td>x</td><td>x</td><td>x</td><td>x</td><td>x</td></tr>
<tr><td>Relational patterns</td><td>Working Alliance Inventory (for patients, WAIp; Wilmers, 2008)</td><td colspan="2">After sessions
1 4 8</td><td>x</td><td>x</td><td></td></tr>
<tr><td></td><td>Relationship Scales Questionnaire (RSQ; Steffanowski, 2001)</td><td>x</td><td></td><td>x</td><td>x</td><td>x</td></tr>
<tr><td></td><td>Inventory of Interpersonal Problems (IIP-D; Horowitz et al., 2000)</td><td>x</td><td></td><td>x</td><td>x</td><td>x</td></tr>
<tr><td>Personality</td><td>Tridimensional Personality Questionnaire (TPQ; Dufeu et al., 1995)</td><td>x</td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td>Eysenck Personality Questionnaire (EPQ-RK; Ruch, 1999)</td><td>x</td><td></td><td></td><td></td><td></td></tr>
</table>

Self-ratings		Base line	After 4th session	Post	Follow-up 1	Follow-up 2
Live events	Impact of Event Scale (IES; Maercker & Schützwohl, 1998)	x				
	Kurz-Inventar zur Erfassung früher traumatischer Lebensereignisse (K-IFTL; Heim, 2000)	x				
	Ereignisliste[1]	x				
	Childhood Trauma Questionnaire (CTQ; Klinitzke et al., 2011)	x				
Therapist ratings						
Anxiety symptoms	Hamilton Anxiety Scale (HAMA; Hamilton, 1996a)	x	x	x	x	x
	Panic and Agoraphobia Scale (PAS; Bandelow, 1997)	x	x	x	x	x
Other symptoms	Hamilton Depression Scale (HAMD; Hamilton, 1996b)	x		x	x	x
	Structured Clinical Interview für DSM-IV – II (SCID-II; Wittchen et al., 1997)	x				
Relational patterns	Working Alliance Inventory (for therapists; WAIt; Horvath & Greenberg, 1989)	After sessions 1	4 8	x	x	

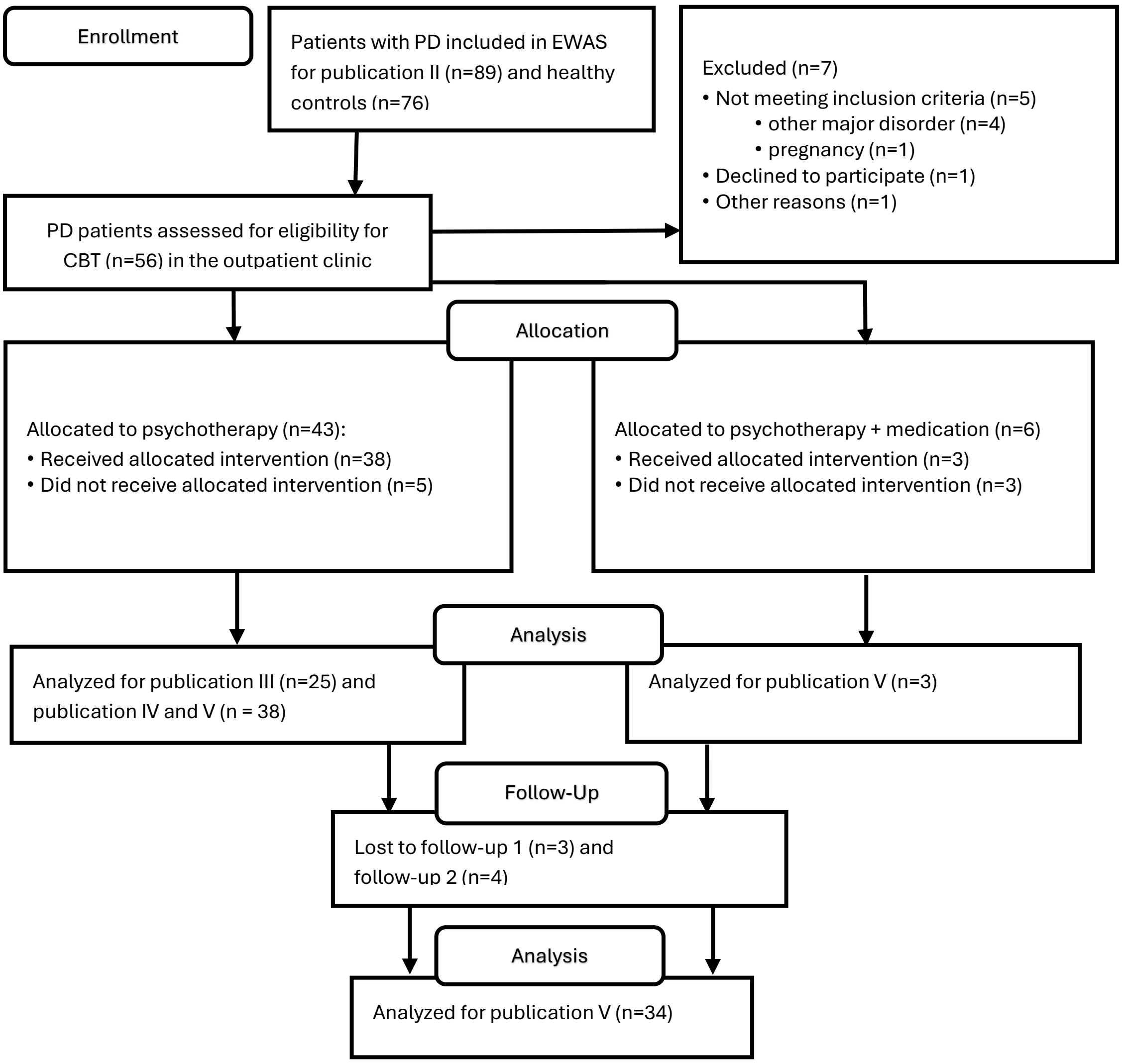
Note: All questionnaires were provided in a German version.

[1] The event list consists of 37 relevant life events (e.g., birth of a child, divorce), which are surveyed together with their subjective stress level

Figure 7 describes the procedure and enrollment of participants in the different publications. Individual methods and materials are described in detail in the publications below.

Figure 7

Flowchart of Participants



5. Results

Theoretical background, methodology, results, and discussion are outlined separately for each manuscript in executive summaries below, with an overarching synthesis in Chapter 6.

5.1. Publication I: HPA system in anxiety disorder patients treated with cognitive behavioural therapy: A Review

Lange, J., & Erhardt-Lehmann, A. (2025). HPA system in anxiety disorder patients treated with cognitive behavioural therapy: A review. *Biomarkers in Neuropsychiatry*, *12*, 100116. https://doi.org/10.1016/j.bionps.2024.100116

Executive Summary

The HPA axis is central to the physiological stress response, which culminates in the release of cortisol. Understanding how the body's stress-response system serves as a biomarker for disease status during psychotherapy and affects the response to treatment is important for a more holistic perspective on treatment mechanisms. Dysregulated HPA axis activity has been observed in various ADs: Chronic stress and anxiety can lead to either hyperactivation or, conversely, blunted reactivity due to exhaustion of the stress system. Cortisol has thus been studied as a potential biomarker of symptom severity and recovery. Baseline HPA system characteristics might predict response to therapy. Further, if CBT effectively reduces a patient's pathological fear, corresponding changes in HPA axis regulation could be expected.

Study I addressed these questions through a systematic literature review of HPA axis findings in AD patients treated with CBT. It compiled findings from experimental studies that measured cortisol levels before, during, and after CBT, particularly focusing on those treatments that included exposure therapy. First, we summarized studies using just one baseline measurement of cortisol predicting response. Second, examinations during exposure were reviewed, and third, studies with pre-/post-assessments were researched.

The summarized studies, though inconsistent, suggest first that cortisol levels may reflect disease status and serve as potential biomarkers for outcome prediction. Global HPA system dysfunction, characterized by chronically elevated cortisol concentrations and hyporeactivity to stress situations (conceivably due to an already exhausted HPA axis), appears to be linked to a greater symptom burden and poorer CBT response. Second, and in line with the latter, low cortisol responses during exposure exercises were repeatedly found to be a risk marker for non-response to therapy. These findings suggest that patients whose stress system does not adequately activate during exposure tend to benefit less from CBT, pointing to the necessity of a flexible and adaptive cortisol response during fear-disconfirming exposure. An adaptive cortisol response probably facilitates extinction learning by promoting neuroplasticity. Third, some evidence indicates that successful CBT can normalize HPA system functioning: patients who responded to therapy showed reductions in excessive cortisol reactivity to fear-related stimuli over time and a return toward normal basal cortisol levels. This finding aligns with the idea that effective fear reduction through CBT not only alleviates psychological distress but also normalizes the physiological stress system. Importantly, the findings also indicate that cognitive processes inherent to CBT, e.g., appraisal, attribution, perceived controllability, and coping, may contribute to the modulation of HPA system activity.

The review concludes that cortisol is a potential candidate biomarker for several aspects of ADs and their treatment. Cortisol measures might be used to determine current AD severity, to predict which patients are likely to respond to CBT, to monitor treatment progress objectively, and even to augment therapy pharmacologically. However, findings across studies have been mixed. Interpretation of current study results is limited due to small sample sizes, variability in HPA system assessment methods, differences in experimental conditions, inconsistencies in symptom ratings, and variations in treatment procedures, including the use of medication, treatment duration, and follow-up periods. Additional factors such as childhood adversity, biological influences, diurnal rhythms, disorder duration, and psychiatric comorbidities may further contribute to inconsistent findings and should be considered. Ideally, future studies would employ more uniform and comprehensive cortisol measurements. Assessing multiple parameters of HPA axis dynamics, including global function, cortisol awakening response, diurnal slope, acute and long-term cumulative cortisol output on consecutive days at various points in therapy, would allow a more nuanced understanding of changes within the cortisol feedback loop. Differential approaches may enhance outcomes by targeting distinct phases and types of ADs: reducing acute stress responses or elevated cortisol in the early phase, and normalizing a downregulated HPA system in chronic AD, through exposure, cognitive techniques, coping strategies, and probably glucocorticoid augmentation.

5.2. Publication II: DNA Methylation signatures in panic disorder

Iurato, S., Carrillo-Roa, T., Arloth, J., Czamara, D., Diener-Holzl, L., **Lange, J.**, Muller-Myhsok, B., Binder, E. B., & Erhardt, A. (2017). "DNA Methylation signatures in panic disorder". *Translational Psychiatry*, *7*(12), 1287. https://doi.org/10.1038/s41398-017-0026-1

Executive Summary

The etiopathogenesis of PD remains largely uncertain, though both genetic predisposition and environmental exposures are known to play a role. Investigating DNA methylation could provide an integrated perspective on these combined risk factors. So far, studies in the European population are lacking. Although epigenetic modifications, such as DNA methylation, are generally tissue-specific, certain sites exhibit cross-tissue relevance. Alterations in peripheral tissues like blood may serve as potential biomarkers for disease risk.

Study II aimed to identify methylation changes that could serve as biomarkers of the disorder or provide insight into its underlying biology. It focused on identifying DNA methylation differences in blood samples associated with PD by carrying out an epigenome-wide association study (EWAS) comparing medication-free patients (n=89) to healthy controls (n=76) and assessing an independent replication sample of cases (n=131) and controls (n=169). Using the Illumina HumanMethylation BeadChip arrays, approximately 450,000 CpG sites across the genome were assessed for differential methylation patterns using linear regression models. The analysis was stratified by sex to detect any gender-specific effects. Further, in an independent set of female subjects (n=71), gene functionality was tested by measuring related messenger RNA (mRNA) expression at baseline and after a dexamethasone suppression test using Illumina

Human Expression BeadChip arrays and analyzed with a linear mixed effects model. Additionally, a list of candidate genes was evaluated that previous studies had implicated in ADs.

Key findings included a notable sex-specific epigenetic signature: only in female PD patients did one particular CpG site reach genome-wide significance for differential methylation compared to controls. This CpG (identified as cg07308824) is located in an enhancer region of the Hdc homolog (HECA*)* gene, which is a cell cycle regulator, possibly playing an important role in human carcinoma. Importantly, this finding was replicated in the second independent sample. In a combined meta-analysis across samples, the association became even more statistically robust (adjusted after multiple testing, p=0.004). The study went further to explore the functional relevance of this epigenetic hit in an independent set of female patients: Methylation at the PD-associated CpG site was found to be correlated with mRNA expression of the *HECA* gene, both at baseline and after a dexamethasone suppression test. This finding suggests that the methylation change may have a regulatory effect on gene function. Aside from this genome-wide significant finding, out of 15 candidate genes examined, five showed nominally significant methylation differences in PD patients (after gene-wise correction): SGK1, FHIT, ADCYAP1/PACAP, HTR1A, and HTR2A, all of which had been reported by prior research to relate to AD or PD risk. For example, *SGK1* (serum glucocorticoid regulated kinase 1) and *PACAP* (pituitary adenylate cyclase activating polypeptide) are involved in stress-hormone signaling and fear response, while *HTR1A/2A* encode serotonin receptors implicated in anxiety modulation.

Overall, Study II found limited but notable DNA methylation differences in patients affected by PD, with the most compelling evidence for a female-specific methylation change in the *HECA* gene. However, PD is not associated with extensive methylation alterations in blood. As genome-wide studies examining epigenetic markers in patients with PD are still very limited, this study contributes to establishing a foundation for subsequent research.

5.3. Publication III: Exposure-induced changes of plasma metabolome and gene expression in patients with panic disorder

Martins, J., Czamara, D., **Lange, J.**, Dethloff, F., Binder, E. B., Turck, C. W., & Erhardt, A. (2019). Exposure-induced changes of plasma metabolome and gene expression in patients with panic disorder. *Depression and Anxiety*, *36*(12), 1173-1181. https://doi.org/10.1002/da.22946

Executive Summary

Only a few studies have been published regarding the neurobiological pathways and molecular mechanisms involved in and activated by panic attacks in the context of ADs. Biomarkers such as gene expression and plasma metabolites (small molecules that are involved in or produced by metabolism), which represent molecular pathway activities, are promising candidates for assessing treatment efficacy.

Study III focused on dynamic molecular changes in metabolites and gene expression in individuals with PD (n=25) during two repeated exposure-induced panic states during therapist-guided exposure sessions to feared situations embedded in short-term CBT. Blood samples were taken at three time points: before exposure/ baseline reflecting anticipatory anxiety, one hour after the peak of anxiety, and one day later following the exposure (24h after baseline) to assess if these changes could serve as markers of the panic state or might be linked to the patient's clinical symptoms. Using liquid chromatography-mass spectrometry, the levels of numerous small-molecule metabolites in plasma were analyzed, and gene expression levels (mRNA) were measured using Illumina Expression BeadChip arrays, providing insight into both metabolic and transcriptomic responses to acute panic reactions. Metabolites with significant changes were identified with a two-way analysis of variance for repeated measures. Gene expression was analyzed using linear mixed effects models.

The results identified one metabolite that showed a notable change: the metabolite *glyoxylate* was dynamically regulated over the three time points. Glyoxylate is a biochemical intermediate that is part of the degradation pathway of cholecystokinin, a neuropeptide known to be involved in ADs and used to induce panic attacks in research settings (panicogenic peptide). Interestingly, glyoxylate levels at baseline correlated most pronounced with the peak anxiety level experienced by the patients during exposure (p=.03, adjusted R^2=28%), suggesting they might predict subjective distress during the intervention and hence could be an indicator of the panic state. Furthermore, glyoxylate levels prior to exposure showed gender-dependent differences, with higher levels in male patients (p=.006). Moreover, cholecystokinin gene expression was significantly elevated pre-exposure in patients who later achieved remission (p=.03). However, general gene expression exhibited minimal fold changes (magnitude of change between two measurements as a ratio) throughout all three time points of the exposure experiment (<0.09), indicating limited clinical utility.

In summary, Study III provided initial evidence that metabolites can serve as dynamic markers of acute panic states. The dynamical regulation of glyoxylate during panic attacks is a novel finding, pointing to the possible role of the cholecystokinin neuropeptide system during exposure-induced panic states, bridging a molecular mechanism with the phenomenology of panic. The found correlation with peak anxiety suggests a role for cholecystokinin in anticipatory anxiety, potentially modulating anxiety intensity during exposure. If confirmed, such metabolic markers could aid in monitoring patients' acute stress levels or responses during exposure exercises and have the potential to predict anxiety states. Meanwhile, the minimal gene expression changes suggest that transient panic might not allow for large transcriptional changes to manifest. However, these preliminary results, based on a small sample, suggest that peripheral blood gene expression may not reliably reflect acute fear states, emphasizing the need for further research into its clinical relevance and replication in larger samples. The higher cholecystokinin gene expression pre-exposure in remitters further implicates cholecystokinin in anticipatory processes and the engagement of neural circuits critical for learning. However, these findings warrant validation in larger cohorts with direct assessment of cholecystokinin-related neural pathways.

5.4. Publication IV: DNA-methylation dynamics across short-term, exposure-containing CBT in patients with panic disorder

Moser, S., Martins, J., Czamara, D., **Lange, J.**, Müller-Myhsok, B., & Erhardt, A. (2022). DNA-methylation dynamics across short-term, exposure-containing CBT in patients with panic disorder. *Translational Psychiatry, 12*(1), 46. https://doi.org/10.1038/s41398-022-01802-7

Executive Summary

Since epigenetics describes time-stable heritable gene-regulatory mechanisms that are also highly dynamic and reactive to environmental influences, they are likely candidates to mediate the effects of exposure-based CBT. Previous studies showed evidence for DNA methylation and immune phenotype regulation in PD, but the temporal dynamics of these changes during CBT and exposure have not yet been studied sufficiently.

Study IV investigated this by conducting a longitudinal investigation of DNA methylation dynamics before and after short-term CBT. The CBT intervention with PD patients (n=38) consisted of an 8-week program that included exposure therapy sessions. We collected blood samples prior to the intervention, after the fourth session, and after therapy completion. A subsample of these patients (n=21) was monitored during exposure sessions, and additional blood samples were taken to capture acute change (before exposure, one hour after peak of anxiety, and 24 hours after). This design allowed the examination of two levels of change: short-term, state-dependent changes occurring immediately during an exposure-induced panic episode, and long-term changes developing across the full course of treatment. We assessed therapy-related changes with linear mixed models and paired-sample *t* tests in a DNA methylation epigenome-wide and in a candidate gene approach, as well as modulations of immune cell profiles (estimated from the DNA methylation data) using Illumina bead chip arrays.

One major finding was that immune cell proportions, specifically CD4+T-cells, CD8+T-cells, B-cells, and Granulocytes, changed from pre- to post-treatment to a healthier "control-like" status. Furthermore, immune profiles, such as CD4+ T-Cells, Natural Killer cells, and Granulocytes, were temporarily regulated during acute fear provocation in exposure sessions, reflecting an acute stress immune response, without differences between responders and non-responders.

Importantly, an epigenome-wide analysis of DNA methylation across therapy was performed and identified a few specific CpG sites that exhibited significant methylation changes either during the exposure or over the course of therapy. One standout result was the discovery of a CpG site (cg01586609), located in a CpG island within the gene for the serotonin receptor 3A (HTR3A; product: 5-HT3A receptor). This serotonin receptor is known to play a role in the regulation of panic states and fear circuitry. The 5-HT3A receptor subtype is localized in limbic brain regions, including the amygdala, hippocampus, and cortex, which are involved in the regulation of panic. In the context of fear provocation, HTR3A showed differential methylation and gene expression related to therapy response. During the exposure session, methylation at this HTR3A-associated site changed temporarily, suggesting fear elevation can alter methylation even in the short term. The study also found that changes in methylation corresponded with a decrease in HTR3A gene expression one hour after the peak of anxiety. Interestingly, the degree of HTR3A expression change differed between remitters and non-remitters (p=0.028 for the interaction of time and remission status), with decreased HTR3A receptor production in remitters, suggesting a pronounced anxiolytic effect.

Another CpG (cg01699630), annotated to the arginase 1 (ARG1) gene, which is related to the urea cycle, neurology, and immunometabolism, was identified as undergoing significant methylation changes from pre- to post-treatment (paired *t* test, genome-wide adjusted *p*=0.02), indicating an epigenetic alteration as a result of CBT.

In a candidate analysis, two CpGs in the mitotic arrest deficient 1-like (1MAD1L1) gene, which is associated with chromosomal instability and linked to anxiety-related psychopathology and psychiatric disorders, were found to be nominally regulated during the therapy. Further analysis of the methylation dynamics revealed a consistent pattern across individuals, i.e., an initial decrease at the start of therapy, followed by an increase in anticipation of the fear exposure, which persisted during the exposure itself before a gradual decrease to the subsequent therapy timepoints.

Study IV provides the first evidence of longitudinal changes in the context of CBT and acute fear exposure for PD in immune cell-type composition and at the epigenetic level, specifically ARG1 and HTR3A methylation and expression. The results add to a growing body of evidence linking the immune system with PD. Our results indicate that exposure induces an acute immune system stress response, while the therapy overall appears to promote a gradual shift toward a “less-stressed” immune state. Therapy potentially normalizes some of the immune dysregulation associated with chronic stress or anxiety, without knowing if this is a marker for therapy success or just a physiological reaction to stress. The HTR3A result is quite novel, pointing to a potential biomarker for therapy success: it implies that how a patient’s epigenome reacts to an early exposure challenge could predict therapy outcome. Furthermore, the finding is in line with research showing improved efficacy of serotonin-reuptake inhibitors after blockade of 5-HT3AR. The results and those of study II (HTR1A and HTR2A) suggest a contribution of the serotonin system in the etiology of PD and response to acute fear, with a potential for clinical application of 5HT3AR in therapeutic exposure and pharmacological treatment. Taken together, these findings support the notion that successful psychotherapy can induce measurable changes in gene regulation. Additional studies are needed to corroborate our findings and understand the mechanism of action in detail before exploring clinical potential.

5.5. Publication V: Attachment style change and working alliance in panic disorder patients treated with cognitive behavioral therapy

Lange, J., Goerigk, S., Nowak, K., Rosner, R., & Erhardt, A. (2021). Attachment style change and working alliance in panic disorder patients treated with cognitive behavioral therapy. *Psychotherapy (Chic), 58*(2), 206-218.
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Executive Summary

Attachment style, based on Bowlby's (1969) theory, refers to systematic patterns of reciprocal social behavior in relationships based on relational expectations and emotions shaped by early caregiving experiences and heritable personality traits, which together stabilize internalized attachment orientations over time. They are described in two dimensions: anxious attachment, which means to be preoccupied with fears of abandonment and a negative "inner working model of self", and avoidant attachment, which refers to discomfort with closeness and dependence and a negative "inner working model of others". In adults, attachment style is often considered a stable trait, and an insecure attachment style is thought to persist over time. However, some studies indicate that attachment style can evolve, especially in the context of significant relational experiences or interventions. Psychotherapy, as a relationship-based intervention, might be one context in which attachment patterns could shift. In CBT for PD, although the working alliance is an important factor, the focus is typically on cognitive changes, such as disproving catastrophic misinterpretations, and behavioral changes, such as reducing avoidance behavior, rather than explicitly on relational dynamics. Yet, it remains possible that even standardized short-term therapy could foster changes in their attachment orientation. An insecure attachment style has been shown to have a detrimental influence on therapy outcome in previous studies. It was associated with more interpersonal problems and a weaker therapeutic alliance. Improvements in interpersonal patterns appear to result from alliance changes and, in turn, predict symptom severity. However, whether a secure alliance drives changes in attachment style or attachment shapes the alliance–outcome relationship remains unclear and requires long-term investigation.

Study V investigated the following questions: 1) Does attachment style change due to a short-term treatment and follow-up, 2) is improvement in attachment style preceded by alliance change, and does it predict subsequent improvement in outcome and interpersonal patterns, and 3) is the impact of improvement in alliance on subsequent outcome moderated by attachment style and interpersonal distress?

We examined PD patients' (n=49) attachment style, symptom severity, interpersonal distress, and the therapeutic alliance using self-report and therapist measures throughout the above described short-term CBT (12 sessions plus two booster sessions) at multiple time points. These included baseline, mid-treatment at fourth and eighth sessions, post-treatment, and follow-up after four and eight months after treatment. We used linear mixed effects models to analyze changes *within* patients over time (intra-individual change) as well as *between* patients. For the between-patient effects, we used the individual patient's mean, which is reflective of "trait-like" differences in patient characteristics. Significant within-patient effects reflect divergence from the subject's specific mean. Dependent variables were lagged (t+1).

First, patients showed overall a strong decrease in attachment anxiety over the course of therapy (research question 1). The reduction in anxious attachment was statistically significant at the end of the main treatment, and improvements remained stable throughout the four and eight-month follow-up. Attachment avoidance, on the other hand, already not very high from the start, showed less pronounced improvements and showed a trend effect only at the follow-up assessments. The improvement of attachment style was accompanied by less interpersonal distress, an increase in therapeutic alliance, as well as symptom improvement.

Second, the study found reciprocal relationships between attachment style changes and clinical outcomes within individuals (research question 2). When a given patient experienced a reduction in attachment anxiety at a certain time point compared with their mean level, this predicted improvements in panic symptoms (e.g., agoraphobic cognitions), therapeutic alliance, and interpersonal distress at the subsequent measurement. In the reverse direction, an increase in alliance quality or a decrease in symptoms predicted later decreases in attachment anxiety. Additionally, decreases in interpersonal distress preceded reductions in attachment avoidance at follow-up.

At the between-person level, generally (mean) higher anxious attachment and interpersonal distress were associated with greater symptom severity, indicating risk and maintenance factors for poorer outcomes. More insecure attachment styles overall were further linked to greater interpersonal difficulties. In line with our assumptions, a generally stronger therapeutic alliance was associated with lower symptom severity.

The study examined further how average levels of attachment and interpersonal distress influenced the relationship between within-patient alliance and subsequent outcomes (research question 3). We found that *mean* attachment anxiety and interpersonal distress *moderated* the association between early alliance changes and later outcomes. Patients who were generally more anxiously attached or who had higher interpersonal distress benefited more from a growing alliance in terms of subsequent depressive symptom improvement.

The findings provide evidence that even a short, exposure-focused CBT might lead to significant changes in attachment style and interpersonal problems. Short-term CBT for PD primarily uses standardized, exposure-based interventions and does not specifically target relationship quality. Thus, directly addressing attachment style may not be necessary; focusing on overcoming fear alone might be sufficient to promote lasting improvements in anxious attachment and the "inner working model of self." However, the results suggest that improvement in anxious attachment may have facilitated the ability to form a relationship with the therapist, and with an increasing therapeutic bond, probably enabling treatment to be effective. On the other hand, they suggest that improvement in alliance fosters more subsequent secure attachment. Thus, strengthening the therapeutic alliance appears crucial for symptom reduction, especially in patients with anxious attachment and interpersonal difficulties. Possibly, targeting the therapeutic alliance in addition to the standard CBT protocol might yield further beneficial effects. Monitoring attachment styles and interpersonal function as potential risk factors for symptom severity is recommended for clinical utility.

6. General Discussion

This cumulative thesis explored multiple levels in the treatment of PD, investigating biological markers (endocrine, epigenetic, transcriptomic, metabolomic, and immune system; publications I to IV) and psychological processes (attachment style and alliance; publication V). The HPA axis review (publication I) highlighted how stress hormone regulation correlates with and responds to exposure-based CBT in AD. The genetics studies (publications II to IV) showed that both pre-existing and exposure-induced changes in gene regulation, such as DNA methylation and gene expression, accompany PD and improvement. Publication III also suggests an acute metabolomic mechanism of panic attacks. Further, the investigation in publication IV found changes in immune system-related processes. Publication V shows the modifiability and reciprocal relationship between attachment style, interpersonal problems, symptom severity, and the therapeutic alliance, whose importance for anxious attached individuals is emphasized.

A unifying theme across the studies is the idea that successful CBT for PD does not merely improve anxiety symptoms alone, but creates a change that resonates through the organism, from physiology to personal development. Each study provided a piece of the broader picture of the bio-psycho-social model. Many of the changes observed can be interpreted as moving from a dysregulated state associated with mental illness toward a more balanced physiological and psychological state associated with health and well-being. This pattern suggests that effective therapy might reverse or mitigate some of the physiological and interpersonal conditions and manifestations of PD. The results are discussed in more detail in the following chapters.

6.1. Multi-Level Biological Changes

This dissertation aimed to shed light on the role of biomarkers in the development and recovery from psychiatric disorders across different biological levels. Studies I, III, and IV show that patients with PD treated with CBT exhibit a coherent pattern of biological changes during exposure-based CBT: normalization in HPA axis hyperregulation, modulation of DNA methylation and gene expression of specific genes, e.g., related to the serotonergic system (HTR3A), immune system (ARG1), and cholecystokinin, as well as acute immune cell profile and metabolic fluctuations such as glyoxylate linked to the cholecystokinin degradation pathway.

The review of the HPA system in AD along CBT (publication I) confirms the potential role of cortisol levels as a 'predictive biomarker' of outcome and as a 'monitoring biomarker' for the indication of disease status. HPA axis dysfunction seems to correlate with greater symptom severity and poorer CBT response. Low cortisol responses during exposure interventions consistently predict risk for non-response.

The results of study II point to a possible epigenetic mechanism, a potential 'susceptibility biomarker', contributing to PD in women. This mechanism could relate to sex-specific risk or resilience factors. The association with differences in HECA mRNA expression levels in another female sample after a dexamethasone suppression test suggests that the methylation change may potentially be a part of a pathway in the context of stress hormone response, given that dexamethasone is a glucocorticoid analog. In a recent study, HECA emerged as central in RNA metabolism and cellular adaptation to stress (Ricolo et al., 2025). The findings highlight both the promise and the complexity of using blood DNA methylation as a biomarker. While a frequently found epigenetic marker, such as the HECA locus, might eventually contribute to identifying and understanding PD, the overall small number of significant methylation sites underscores that any epigenetic contributions to PD are likely nuanced and might represent only a small incremental aspect in understanding PD.

Our findings regarding epigenetics in the context of CBT point towards small alterations in DNA methylation (study IV) as potential predictive candidate biomarkers for assessing stress-related effects, physiological changes due to therapeutic intervention, and predicting differences in therapeutic response. This is corroborated by a review of genomics research in AD by Weber and colleagues (2025) regarding therapygenetics, which aims to quantify the genetic influence on response to psychotherapy. Furthermore, a recent EWAS (Domschke et al., 2025) found dynamic epigenome-wide DNA methylation changes along with CBT response at four CpGs (ADIPOR2, EIF3B, OCA2, TMCC1), previously linked to processes related to anxiety, contextual fear conditioning, or neuronal synaptic plasticity. Additionally, changes in gene expression (studies II to IV) might be more subtle and harder to measure. Targeting biologically plausible gene sets, e.g., inflammation, immune, neural, or endocrine, shows promise, while genome-wide findings of singular methylation sites from small, unreplicated samples lack clinical utility. Moving forward requires large-scale collaborations, streamlined blood collection, diverse samples, multiple therapies, and standardized protocols to yield actionable insights (Ricon-Becker & Cole, 2024).

The results regarding the immune system dynamics (study IV) support the neuroinflammation hypothesis in anxiety, that chronic anxiety may have an inflammatory component that can be alleviated with treatment (Nelles et al., 2025). The metabolomic finding (study III) identified glyoxylate as a candidate state-marker. As an intermediate biochemical product of degradation, it specifically points to the cholecystokinergic system, reinforcing older theories that cholecystokinin hyperactivity contributes to panic attacks (Zwanzger et al., 2012). Cholecystokinin is densely present in brain regions regulating fear and anxiety, acting as both a neurotransmitter and a neuromodulator. Its role in human anxiety is well established. Cholecystokinin agonists (e.g., pentagastrin) reliably induce panic attacks in a dose-dependent manner, especially in individuals with PD, who show heightened sensitivity and distinct cholecystokinin metabolism (Bradwejn & Koszycki, 2025).

Our findings of multi-system changes underscore the possible integrative biological impact of CBT, linking symptom improvement in PD to quantifiable changes in endocrine, gene, metabolic, and immune system regulation. Collectively, these findings broaden our understanding of PD's pathophysiology and recovery. They point toward promising predictive and monitoring biomarkers – from cortisol dynamics to specific methylation sites – that could inform and enhance future personalized interventions. This approach establishes the foundation for viewing therapeutic change not just in psychological terms but also as measurable biological changes, reinforcing a biopsychological understanding of treatment.

6.2. Therapy Process Factors

The attachment style study (publication V) demonstrated that standardized short-term CBT may effectively reduce anxious attachment, symptom severity, and interpersonal problems in patients with PD long term. Attachment style improvement seems to be reciprocally related to symptom severity, alliance change, and interpersonal difficulties. Furthermore, general attachment style and interpersonal distress appear to moderate the relation of the therapeutic alliance and subsequent depressive symptom severity, with more anxiously attached and interpersonal distressed patients benefiting more from an improvement of the alliance.

The most significant aspect of Study V is that short-term CBT for PD might positively affect certain factors, such as attachment style and interpersonal problems, which have been thought of as rather unchangeable traits. These shifts were intricately linked with the therapy process and outcomes. This challenges the notion that a brief, manualized treatment cannot affect change beyond symptom alleviation. The decrease in attachment anxiety is notable because CBT for PD does not directly target attachment or interpersonal beliefs. Interestingly, our results are corroborated by a later study finding improvements in anxious, but not avoidant attachment, in PD patients treated with internet-based CBT (Zalaznik et al., 2022). Both results suggest that even when therapy does not explicitly focus on relationships, patients may internalize the experience in a way that updates their internal working models of attachment. The improvement likely emerged indirectly, perhaps as patients gained confidence and self-efficacy by mastering their fear and experiencing more control, they improved their self-representations (Renner et al., 2025; Zalaznik et al., 2019). In the study of Zalaznik and colleagues (2022), most of the change in anxious attachment was during phases that focus on cognitive work, which suggests that reinterpretations of the sensations as harmless, improvement in anxiety sensitivity respectively, help patients to feel relief without depending on others, and a sense of self-competence. The reciprocal dynamic between attachment style, symptom severity, interpersonal problems, and alliance suggests a reinforcing cycle: as patients improve anxiety sensitivity, they become less anxiously attached and have fewer interpersonal difficulties, they may engage more openly with therapy and trust the process. As their symptoms and functioning improve, they might feel more interpersonally secure, reducing attachment anxiety further. These findings are corroborated by a study finding a reciprocal dynamic between interpersonal problems and therapeutic alliance, in which improvements in one construct were linked with beneficial changes in the other over time (Iovoli et al., 2025). Moreover, due to experiencing a positive, corrective relationship with the therapist, through experiencing a supportive, collaborative alliance, patients likely revise expectations of others' availability (Mikulincer et al., 2013). Change in avoidant attachment via various psychotherapies has, for example, been shown in other disorders (Kirchmann et al., 2012; Strauß et al., 2018; Zalaznik et al., 2019; Zalaznik et al., 2025), but with mixed results (Taylor et al., 2015). However, concluding from our findings, exposure therapy likely modifies more self-related beliefs associated with anxious attachment, but might have a limited impact on beliefs related to others, linked to avoidant attachment. It seems likely that a stronger focus on interpersonal healing might have more impact, as suggested by our finding of reduced interpersonal distress preceding reductions in attachment avoidance at follow-up. Additionally, a change in internal models of others might take more time, as could be hypothesized from the found trend in attachment avoidance reduction at follow-up. Notably, avoidant attachment was overall not high from the start in our PD sample, similar to the sample in the study of Zalaznik and colleagues (2022), thus, conclusions are limited.

The therapeutic alliance, known to be critical for outcome in general (Flückiger et al., 2018), is confirmed here as a mechanism for change for individuals with interpersonal difficulties. The finding that more anxiously attached and interpersonal distressed patients benefit more from an improvement of the alliance makes intuitive sense. Those who start therapy with interpersonal insecurities or difficulties stand to gain the most from forming a strong bond with the therapist. It might not only facilitate the technical aspects of therapy but also directly help heal some relational wounds, translating into broader symptom improvement, including mood, as noted by depressive symptoms in the moderation effect. Meanwhile, patients who were already more secure or less interpersonally distressed might improve regardless of alliance or need less of that relational factor to progress. In summary, effective fear-focused treatment and a good alliance may suffice to bring about positive interpersonal changes.

6.3. Cognitive Aspects

By targeting anticipatory anxiety and maladaptive appraisals, CBT effectively reduces the perceived threat of bodily sensations and situations. One clear outcome observed is a reduction in the "fear of fear." As patients learn that panic symptoms are not dangerous, the anticipatory anxiety is diminished, and in turn, patients often feel more confident confronting situations they avoided, creating a positive cycle: facing situations that disprove catastrophic expectations and further reducing anticipatory anxiety.

An important cognitive factor in this regard, as mentioned above and previously described in the causal model of PD theories by Fava & Morton (2009), is self-efficacy (Breuninger et al., 2019). Evidence from CBT for ADs suggests that improvement in self-efficacy mediates treatment effects and temporally precedes symptom reductions, supporting its role as a primary mechanism of therapeutic change (Southward et al., 2024). Self-efficacy gained through therapy also helps break the dependency on safety behaviors. As described above, our finding (study V) implies that a newfound self-reliance and control can improve interpersonal functioning. This suggests that once patients gained confidence in handling anxiety independently, their anxious attachment in close relationships diminished. No longer overly reliant on others for a sense of safety, they probably became more secure in themselves. Related, a recent study using CBT for PD found also self-esteem to change significantly from pre- to post-treatment as well as predicting overall therapy outcome (Renner et al., 2025). This underscores how profoundly a sense of control over one's fear can potentially influence broader aspects of one's life (Mikulincer et al., 2013; Zalaznik et al., 2019; Zalaznik et al., 2022). Vice versa, a reduction in anxious attachment was shown to precede a decrease in agoraphobic cognitions, suggesting that more positive self-representations reduce fear of fear and enhance perceived bodily control (Fentz et al., 2014).

Notably, improved cognitive coping can be observed at a biological level. The HPA system findings tie into learning models of anxiety. Patients who fail to show a cortisol spike in exposure might not be experiencing enough fear activation to drive new learning: According to the emotional processing theory, a certain level of fear arousal is needed in exposure therapy for maximal benefit. This assumption aligns with the observation that a blunted stress response can indicate treatment resistance (publication I). Looking more closely at the interplay of cognitive processes and HPA system activity during exposure, studies have observed that physiological arousal, like stress hormone release, increases in anticipation of a feared event (Coplan et al., 1998; Meuret et al., 2014). Importantly, cortisol has been shown to promote plasticity at the synaptic structural level (Liston et al., 2013). According to the *memory reconsolidation hypothesis*, each time a fear memory is reactivated, it becomes unstable due to induced plasticity, creating a temporary window during which the memory is vulnerable to disruption and can be modified into a fear-inhibitory memory (Dudai, 2006; Meir Drexler et al., 2015; Monfils et al., 2009). It is important to note that a chronically hyperaroused HPA system, long-term stress respectively, seems to diminish neuroplasticity, e.g., by suppressing the brain-derived neurotrophic factors and subsequently coping abilities. In contrast, acute stress stimulates brain-derived neurotrophic factors and neuroplasticity and thus improves learning and memory (Bandelow et al., 2017). Investigations also demonstrated that reminding of a task ahead and encouraging anticipation or presenting a fear-activating stimulus before successful exposure can lead to persistent attenuation of conditioned fear (Johnson & Casey, 2015; Maples-Keller et al., 2017). Thus, acute cortisol release probably enhances the effect of exposure therapy by augmenting the consolidation of a new extinction memory. However, it does not seem to change the original fear memory (Bouton & Todd, 2014; Myers & Davis, 2002; Todd et al., 2014). An activated HPA system during exposure seems beneficial, emphasizing the essential "adaptive and protective function of cortisol", which is often assessed and interpreted in terms of negative consequences of stress (McEwen, 2015).

Interestingly, none of the studies we reviewed tested or found whether a blunted response can be normalized, although hypocortisolism is found in some studies in AD (Bandelow et al., 2017). There are probably subgroups with high life adversity and a decrease of the initially elevated cortisol due to enhanced negative feedback sensitivity and thus, inhibition of the HPA axis (Fischer et al., 2021). Possibly, these individuals need strategies to better engage with their fear during exposure. Importantly, the studies reviewed imply that cognitive processes in CBT, e.g., positive anticipatory appraisal, attribution, perceived controllability, and coping, seem to have a beneficial influence on adaptive HPA axis regulation (Abelson et al., 2005; Gaab et al., 2005; 2017; Pulopulos et al., 2019; Tafet et al., 2005; Wang et al., 2013). A recent study corroborates cognitive reappraisal as a promising psychological intervention for restoring HPA system dysregulation, particularly an increase in cortisol response, in individuals with high early-life adversity (Bentele et al., 2024). There is also evidence that adopting active coping strategies can favorably influence the body's stress systems, promoting a healthier physiological balance. For instance, individuals who engage in problem-solving or approach-oriented coping when stressed, show more adaptive stress hormone patterns compared to those who cope by internalizing, worrying, or avoiding (Bakouni et al., 2020; Gilbert et al., 2017; Ma et al., 2019). In ADs, chronic dysfunctional passive coping has been linked to hypocortisolism in chronic anxiety states, whereas functional active coping may normalize adaptive HPA axis reactivity. Thus, changes in these cognitive processes during CBT not only relieve subjective anxiety but may also normalize dysfunctional stress reactions associated with PD. The implication would be that whenever a person learns to face their fears and manages them successfully, the body registers safety rather than a persistent threat. Over time, biological markers would shift towards a non-anxious state. In short, coping may not just be a consequence of symptom reduction – it can actively drive both psychological and physiological remission.

In our investigations (studies III and IV), patients undergoing exposure therapy also showed measurable changes in other biological systems before the exposure even began, e.g., DNA methylation in MAD1L1, e.g. linked to anxiety-related psychopathology, most likely reflecting how strongly the expectation of fear activates the body. Interestingly, MAD1 (the gene product of MAD1L1*)* was recently shown to be an essential regulator of neuronal development (Goo et al., 2023). Further, temporary covalent changes in gene expression due to methylation changes of HTR3A were found, which were previously shown to modulate memory formation (Miller & Sweatt, 2007). Moreover, a recent longitudinal EWAS showed that among those who responded to CBT, shifts in methylation from baseline to six-month follow-up occurred at CpG sites near genes previously associated with anxiety, fear conditioning, and neuronal synaptic plasticity (Domschke et al., 2025). The mechanisms driving effective CBT likely involve fear extinction and broader learning processes that are not exclusive to ADs. For example, the divergence between CpG markers of acute versus follow-up response likely reflects temporally distinct molecular pathways: while short-term effects are driven by cognitive restructuring and new associative learning, long-term outcomes likely involve the consolidation and stabilization of these therapeutic gains. Since lasting behavioral change depends on memory-related processes, including fear extinction, it is plausible that distinct epigenetic changes contribute to early versus sustained treatment success (Domschke et al., 2025). Taken together, these epigenetic results introduce the idea of neuronal plasticity as an essential part of therapeutic change.

In our findings (study III), the baseline glyoxylate levels were indicative of the severity of experienced fear during the exposure. As it is a metabolite of cholecystokinin, we hypothesize that this system might be intrinsically involved in anticipatory exposure-induced anxiety. This idea is in line with findings that highlight the relevance of cholecystokinin in the formation of hippocampal memory and neuroplasticity (for a review see Asim et al., 2024). Analyzing the trajectory of cholecystokinin gene expression demonstrated a significant pre-exposure elevation among *remitters*, suggesting that cholecystokinin may facilitate anticipatory anxiety and the engagement of brain networks critical for learning in exposure-based interventions. Again, an activated biological system due to anticipation of a feared situation seems to be beneficial even before exposure and may promote consolidation of an inhibitory memory.

These results underscore the importance of cognitive factors, which are an essential part of CBT, in explaining "recalibration" of bodily systems from the HPA axis regulating cortisol to certain neurochemical pathways. 'Predictive biomarkers', to assess the effect of CBT, seem to be linked to neurotransmitter systems and pathways involved in learning and memory and intracellular mechanisms of synaptic plasticity (Domschke et al., 2025).

6.4. Implications for Future Research

The interplay between the biological and psychological factors deserves attention. Replication of the results and further evaluation of specific genetic effects and modified treatment interventions for individuals with unfavorable genetic markers for CBT are, for example, important tasks for future research.

Importantly, while most studies focus on one domain, in reality, these domains interact. Our studies did not directly test these cross-domain correlations, but forthcoming research should. Future directions could include analyzing several of these domains simultaneously, e.g., combining measures of cortisol, methylation, and attachment to examine how they interrelate and determine which combination best predicts outcome. This would advance an integrated model of how change occurs.

Notably, the question arises of how to address a blunted cortisol response in therapy and whether it can be changed by treatment. So far, only a few studies have investigated this subject, particularly in persons with high early adversity or posttraumatic stress disorder (Bentele et al., 2024; for a review see Fischer et al., 2021; Shakiba & Raby, 2023). An interesting field of research would be the possible link between attachment style and cortisol response. A hypothesis regarding attachment style and the

HPA system would derive from combining the aforementioned two-pathway model of glucocorticoid regulation by Strüber and colleagues (2014) and the model of development of different relational coping strategies (Mikulincer & Shaver, 2012): Deprivation in the face of childhood adversity may result in deactivation of the HPA axis and likely the attachment system (avoidance), as well as less self-reflection and empathy. However, accompanied by high care, adversity might result in hyperactivation of the HPA axis and also the attachment system (anxiety), as well as higher self-focus and emotionality. This is corroborated by a recent study showing that adopted children with a high risk of life adversity who showed high attachment avoidance had a blunted cortisol response, while those who sought proximity and contact with their adoptive parents showed an increased cortisol reactivity (Shakiba & Raby, 2023). Thus, for persons with avoidant attachment and possibly higher risk for a blunted cortisol response, threat-activating exposure might be less successful. Conversely, for persons with hyperactivation of the attachment system and hence hypercortisolism, exposure therapy might be more effective in the sense of beneficial HPA activation and coping strategies. However, previous studies have mainly shown that individuals with insecure attachment patterns in general exhibit *heightened* cortisol reactivity, also indicating less adaptive stress regulation (for a meta-analysis Groh & Narayan, 2019). For example, insecure attachment style was found to affect the hormonal stress system in interpersonal stress situations (Ketay & Beck, 2017; Monteleone et al., 2019) and to impact the cortisol awakening response (Adams et al., 2020). Further studies showed that babies with an insecure attachment had an increased cortisol reactivity a few years later (Müller et al., 2021). Additionally, secure attachment primes have been shown to reduce fear consolidation after fear conditioning tasks, which was also associated with cortisol regulation (Toumbelekis et al., 2021). In this regard, the role of social buffering, which refers to the attenuation of psychological stress perception and HPA axis activation through the presence of social support, might be an area of interest (Gustison & Phelps, 2022). It is plausible that those who internalize the safety of therapy (improving attachment) are less stressed during exposures. Thus, likely facilitating both the changes associated with safety learning and beneficial clinical outcomes. Taken together, there seems to be a connection between attachment style and the HPA system, but further studies are required to determine the exact nature of this link.

Another stimulating field might certainly be translational research for personalized interventions. Future research might incorporate, for example, neuroimaging, physiological measures (e.g., electrodermal activity, electrocardiogram, or electromyogram) representing an influence of the sympathetic nervous system, or cerebrospinal fluid measures to link peripheral markers with central neural changes. It would be interesting, for example, to investigate if patients who show the HTR3A methylation change also show changes in amygdala activity or connectivity on functional magnetic resonance imaging during exposure. Given glyoxylate's implication of cholecystokinin, findings regarding 5HT3AR, and immune system involvement, pharmacologically enhanced trials of augmenting or blocking agents prior to exposure therapy could be explored as potential adjuncts to interventions, as has been done with multiple other agents (Hofmann et al., 2014; Singewald et al., 2014).

Further illuminating research would be to expand the current findings to research on other disorders or therapy types. A different therapy type, such as pharmacotherapy or psychodynamic therapy, could produce similar biological changes. Comparing modalities could reveal common versus specific mechanisms. Exposure therapy might have unique effects on fear circuitry, or the opposite, any effective treatment might normalize the HPA system and attachment security.

Although only indicative, this integrated view suggests a model in which therapeutic change in PD is a holistic process where behavioral, cognitive, emotional, and physiological changes feed into each other in a positive feedback loop: e.g., facing fears, reducing catastrophic misinterpretations and anxiety, promoting coping, self-efficacy, attachment security, an adaptive hormonal stress response, and beneficial DNA methylation. This idea aligns with bio-psycho-social models of mental health, where intervention at one level cascades to improvements at other levels. This dissertation highlights once more that future research and treatment must adopt an integrated approach. It could guide more personalized, effective, and potentially more durable interventions for individuals suffering from PD, and might even foster the development of novel, synergistic therapies for PD (Kyriakoulis & Kyrios, 2023).

6.5. Clinical Implications

From a theoretical standpoint, these studies enrich our understanding of PD. Clinically, one implication is the potential for enhanced assessment: The identification of biomarkers like cortisol or methylation sites raises the potential for objective measures to guide therapy. For instance, a cortisol assessment before therapy or during early sessions and exposure might provide prognostic information, or monitoring a marker like glyoxylate during exposure could one day validate that a panic response was adequately induced. Measuring cortisol or certain gene markers is not yet routine, but could be part of research trials to stratify patients. Importantly, several studies identified factors that distinguish responders from non-responders to CBT. For example, in the HPA system review, low cortisol reactivity during exposure emerged as a risk for poor response. In the epigenetic study, HTR3A methylation dynamics during a single exposure were associated with long-term CBT outcome, with a significant difference between responders and non-responders. In the gene expression study, cholecystokinin expression was elevated pre-exposure among remitters. In the attachment study, those with higher attachment anxiety initially were the ones for whom alliance-building was most critical to outcome. These insights imply that we can start to sketch a profile of patients who might need augmented or tailored interventions: perhaps patients with a blunted stress response (indicating an exhausted HPA axis or ineffective engagement with the exposure), or specific epigenetic profiles might benefit from adjunctive biological treatments, such as D-cycloserine or cortisol augmentation to enhance exposure (for a review see Hofmann et al., 2015; Singewald et al., 2014). Also, anti-inflammatory approaches might be considered (Nelles et al., 2025). Patients with insecure attachment might benefit from extra attention to alliance or an integrated approach that addresses interpersonal difficulties alongside CBT techniques. The finding regarding alliance suggests that therapists in CBT training should continue to emphasize interpersonal skills and responsiveness, not just technical protocol adherence. Assessing attachment style at intake could alert a therapist that a patient may require more attention to the alliance or may benefit from strategies to address interpersonal difficulties. In this regard, additional treatment might be useful (Levy & Johnson, 2018). Studies show that targeting specific components, e.g., emotion regulation (Meine et al., 2024; Nielsen et al., 2017) or to conduct interventions informed by attachment theory are promising, e.g., security priming (Mikulincer & Shaver, 2007b), mentalization-based approach (Fonagy & Luyten, 2009), interpersonal therapy (Gunlicks-Stoessel et al., 2017; Spence et al., 2016), transference-focused psychotherapy (Buchheim et al., 2017), interpersonal and emotional processing therapy (Newman et al., 2015), schema therapy (Young, 2003), and cognitive behavioral analysis system of psychotherapy (McCullough, 2000).

Last but not least, an important implication is the validation biomarkers provide to patients: knowing that “your therapy not only helps you feel better, it actually is changing your brain and body chemistry in measurable ways” can be a powerful motivator and reduce stigma. It reinforces that PD is a real, multifaceted condition, and that recovery can restore balance in one’s physiology.

6.6. Limitations

While the studies offer valuable insights, there are several limitations to acknowledge. Many findings, especially on the biological side, are preliminary or exploratory. The HPA system review pointed out the inconsistencies and small sample sizes in the literature; this limitation also applies to our studies. Replications in larger, well-controlled trials are urgently needed before any biomarker can be deemed clinically actionable. The epigenetic changes need replication and deeper investigation to understand the mechanism.

Our design assessing the impact of exposure on biological markers was conducted in a naturalistic therapeutic setting, with participants confronting their individual fear-inducing situations, such as riding the subway, visiting malls, or entering supermarkets. While this real-life approach enhances ecological validity and mirrors actual therapeutic practice, it introduces practical challenges for precise biological data collection, which had to occur approximately one hour after peak anxiety. Future studies, potentially using virtual reality, could aim to capture biological responses during peak anxiety and consider multiple time-point assessments.

Another limitation is that most biological measures were taken from peripheral blood, which is an accessible but indirect insight into brain processes. Not all changes in blood reflect changes in the central nervous system. Cortisol is a hormone that reflects HPA axis central regulation, but DNA methylation in blood cells or metabolite levels in plasma might not mirror what's happening in the brain during panic or therapy, although overlap seems likely.

The timeframes of measurement varied and could be expanded. The metabolomic study looked at very acute changes; the methylation therapy study looked at pre- to post-treatment effects; the attachment study extended to an eight-month follow-up. We still don't know how stable some of the biological changes are long term. For example, if the epigenetic changes persist four months later, along with psychological variables. Long-term follow-ups for biological variables would be informative. On the attachment side, while eight months is useful, it would be worth seeing if those attachment gains remain years later or if patients need continued maintenance or additional relational experiences to consolidate them.

Another limitation might be the use of self-report measures for attachment style, which are economical and examine current conscious attitudes and memories of experiences relating to present relationships. More extensive interviews that are based on the activation of the attachment system might bring different or more insightful results.

All studies were observational or associative, except that therapy was an active intervention but not randomized against a control group in these contexts, so causality is inferred but not proven. For example, we assume CBT caused the changes, but conceivably, some coincidental factors, like simply the passage of time, diet, or seasonal changes, could have impacted them. Future work could include control groups (e.g., a waitlist or another treatment) to isolate the effects of CBT on these measures.

6.7. Conclusion

Overall, the thesis supports a bio-psycho-social perspective on PD treatment. Effective short-term CBT is multi-dimensional: next to reducing anxiety symptoms, it potentially regulates the hormonal stress system, the epigenome, and the molecular associations of fear in the immune system and metabolome, as well as psychological growth in reducing maladaptive beliefs and fostering healthier relational patterns. Together, this work, despite limitations, underscores the multifaceted impact of CBT on PD and contributes to an understanding of treatment mechanisms, which can inform an integrative approach to mental health. This holistic improvement is encouraging: it means recovery is not only feeling less anxious, but the body and brain themselves are recovering a state of balance, and the person is emerging more resilient and socially connected. In the future, the hope is to use this knowledge to enhance outcomes by identifying which patients need what kind of treatment, by tracking progress with objective biomarkers alongside subjective reports, taking the whole person into account.

References

Abelson, J. L., Liberzon, I., Young, E. A., & Khan, S. (2005). Cognitive modulation of the endocrine stress response to a pharmacological challenge in normal and panic disorder subjects. *Arch Gen Psychiatry*, *62*(6), 668-675. https://doi.org/10.1001/archpsyc.62.6.668

Adams, G. C., Wrath, A. J., von Dewitz, B., Marciniuk, K., Roesler, A., & Napper, S. (2020). Attachment impacts cortisol awakening response in chronically depressed individuals. *Psychoneuroendocrinology*, *120*, 104778. https://doi.org/10.1016/j.psyneuen.2020.104778

Ainsworth, M. D., Blehar, M. C., Waters, E., & Wall, S. (1979). *Patterns of attachment. A psychological study of the strange situation*. Lawrence Erlbaum Associates Inc.

American Psychiatric Association. (2000). *Diagnostic and statistical manual of mental disorders (4th ed., text rev.)*. American Psychiatric Publishing, Inc.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders: DSM-5™, 5th ed*. American Psychiatric Publishing, Inc.

Asim, M., Wang, H., & Chen, X. (2024). Shedding light on cholecystokinin's role in hippocampal neuroplasticity and memory formation. *Neurosci Biobehav Rev*, *159*, 105615. https://doi.org/10.1016/j.neubiorev.2024.105615

Bakouni, H., Ouimet, M. C., Forget, H., & Vasiliadis, H. M. (2020). Temporal patterns of anxiety disorders and cortisol activity in older adults. *J Affect Disord*, *277*, 235-243. https://doi.org/10.1016/j.jad.2020.08.020

Bandelow, B. (1997). *Panik und Agoraphobie-Skala (PAS). Handanweisung*. Hogrefe.

Bandelow, B., Allgulander, C., Baldwin, D. S., Costa, D., Denys, D., Dilbaz, N.,...Zohar, J. (2023). World Federation of Societies of Biological Psychiatry (WFSBP) guidelines for treatment of anxiety, obsessive-compulsive and posttraumatic stress disorders - Version 3. Part I: Anxiety disorders. *World J Biol Psychiatry*, *24*(2), 79-117. https://doi.org/10.1080/15622975.2022.2086295

Bandelow, B., Baldwin, D., Abelli, M., Altamura, C., Dell'Osso, B., Domschke, K.,...Riederer, P. (2016). Biological markers for anxiety disorders, OCD and PTSD - a consensus statement. Part I: Neuroimaging and genetics. *World J Biol Psychiatry*, *17*(5), 321-365. https://doi.org/10.1080/15622975.2016.1181783

Bandelow, B., Baldwin, D., Abelli, M., Bolea-Alamanac, B., Bourin, M., Chamberlain, S. R.,...Riederer, P. (2017). Biological markers for anxiety disorders, OCD and PTSD: A consensus statement. Part II: Neurochemistry, neurophysiology and neurocognition. *World J Biol Psychiatry*, *18*(3), 162-214. https://doi.org/10.1080/15622975.2016.1190867

Bandelow, B., Wedekind, D., Sandvoss, V., Broocks, A., Hajak, G., Pauls, J.,...Ruther, E. (2000). Diurnal variation of cortisol in panic disorder. *Psychiatry Res*, *95*(3), 245-250.

Bartholomew, K., & Horowitz, L. M. (1991). Attachment styles among young adults: a test of a four-category model. *Journal of Personality and Social Psychology*, *61*(2), 226-244.

Baxter, A. J., Vos, T., Scott, K. M., Ferrari, A. J., & Whiteford, H. A. (2014). The global burden of anxiety disorders in 2010. *Psychol Med*, *44*(11), 2363-2374. https://doi.org/10.1017/s0033291713003243

Belanger, C., Marcaurelle, R., Marchand, A., El-Baalbaki, G., Guay, S., & Pecknold, J. (2011). Effect of a marital communication training on treatment outcome in panic disorder with agoraphobia. In *Advances in Psychology Research* (pp. 141-163). Nova Publishers.

Bentele, U. U., Klink, E. S. C., Benz, A. B. E., Meier, M., Gaertner, R. J., Denk, B. F.,...Pruessner, J. C. (2024). The effect of cognitive reappraisal and early-life maternal care on neuroendocrine stress responses. *Sci Rep*, *14*(1), 6837. https://doi.org/10.1038/s41598-024-57106-x

Bifulco, A., Kwon, J., Jacobs, C., Moran, P. M., Bunn, A., & Beer, N. (2006). Adult attachment style as mediator between childhood neglect/abuse and adult depression and anxiety. *Soc Psychiatry Psychiatr Epidemiol*, *41*(10), 796-805. https://doi.org/10.1007/s00127-006-0101-z

Bouton, M. E., & Todd, T. P. (2014). A fundamental role for context in instrumental learning and extinction. *Behav Processes*, *104*, 13-19. https://doi.org/10.1016/j.beproc.2014.02.012

Bowlby, J. (1969). *Attachment and loss*. Basic Books.

Bradwejn, J., & Koszycki, D. (2025). Chapter 22 - Cholecystokinin and panic disorder. In C. Feinle-Bisset & J. F. Rehfeld (Eds.), *Cholecystokinin* (pp. 505-521). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-443-23720-1.00001-0

Breuninger, C., Tuschen-Caffier, B., & Svaldi, J. (2019). Dysfunctional cognition and self-efficacy as mediators of symptom change in exposure therapy for agoraphobia - Systematic review and meta-analysis. *Behav Res Ther*, *120*, 103443. https://doi.org/10.1016/j.brat.2019.103443

Buchheim, A., Horz-Sagstetter, S., Doering, S., Rentrop, M., Schuster, P., Buchheim, P.,...Fischer-Kern, M. (2017). Change of unresolved attachment in borderline personality disorder: Rct study of transference-focused psychotherapy. *Psychotherapy and Psychosomatics*, *86*(5), 314-316. https://doi.org/10.1159/000460257

Cassidy, J., Lichtenstein-Phelps, J., Sibrava, N. J., Thomas Jr, C. L., & Borkovec, T. D. (2009). Generalized anxiety disorder: Connections with self-reported attachment. *Behavior Therapy*, *40*(1), 23-38. https://doi.org/10.1016/j.beth.2007.12.004

Castonguay, L. G., Constantino, M. J., & Holtforth, M. G. (2006). The working alliance: Where are we and where should we go? *Psychotherapy (Chic)*, *43*(3), 271-279. https://doi.org/10.1037/0033-3204.43.3.271

Cohen, L. J., Ardalan, F., Tanis, T., Halmi, W., Galynker, I., Von Wyl, A., & Hengartner, M. P. (2017). Attachment anxiety and avoidance as mediators of the association between childhood maltreatment and adult personality dysfunction. *Attachment and Human Development*, *19*(1), 58-75. https://doi.org/10.1080/14616734.2016.1253639

Coleman, J. R., Lester, K. J., Keers, R., Roberts, S., Curtis, C., Arendt, K.,...Eley, T. C. (2016). Genome-wide association study of response to cognitive-behavioural therapy in children with anxiety disorders. *Br J Psychiatry*. https://doi.org/10.1192/bjp.bp.115.168229

Coleman, J. R., Lester, K. J., Roberts, S., Keers, R., Lee, S. H., De Jong, S.,...Eley, T. C. (2016). Separate and combined effects of genetic variants and pre-treatment whole blood gene expression on response to exposure-based cognitive behavioural therapy for anxiety disorders. *World J Biol Psychiatry*, 1-34. https://doi.org/10.1080/15622975.2016.1208841

Colonnesi, C., Draijer, E. M., Jan, J. M. S. G., Van der Bruggen, C. O., Bogels, S. M., & Noom, M. J. (2011). The relation between insecure attachment and child anxiety: a meta-analytic review. *Journal of Clinical Child and Adolescent Psychology*, *40*(4), 630-645. https://doi.org/10.1080/15374416.2011.581623

Compton, S. N., Peris, T. S., Almirall, D., Birmaher, B., Sherrill, J., Kendall, P. C.,...Albano, A. M. (2014). Predictors and moderators of treatment response in childhood anxiety disorders: results from the CAMS trial. *Journal of Consulting and Clinical Psychology*, *82*(2), 212-224. https://doi.org/10.1037/a0035458

Coplan, J. D., Goetz, R., Klein, D. F., Papp, L. A., Fyer, A. J., Liebowitz, M. R.,...Gorman, J. M. (1998). Plasma cortisol concentrations preceding lactate-induced panic. Psychological, biochemical, and physiological correlates. *Arch Gen Psychiatry*, *55*(2), 130-136.

Coryell, W., Noyes, R., Jr., & Reich, J. (1991). The prognostic significance of HPA-axis disturbance in panic disorder: a three-year follow-up. *Biol Psychiatry*, *29*(2), 96-102.

Darling Rasmussen, P., & Storebø, O. J. (2021). Attachment and Epigenetics: A Scoping Review of Recent Research and Current Knowledge. *Psychological Reports*, *124*(2), 479-501. https://doi.org/10.1177/0033294120901846

Davila, J., Burge, D., & Hammen, C. (1997). Why does attachment style change? *J Pers Soc Psychol*, *73*(4), 826-838.

de Ruiter, C., & Van Ijzendoorn, M. H. (1992). Agoraphobia and anxious-ambivalent attachment: An integrative review. *Journal of Anxiety Disorders*, *6*, 365-381.

Deckert, J., & Erhardt, A. (2019). Predicting treatment outcome for anxiety disorders with or without comorbid depression using clinical, imaging and (epi)genetic data. *Curr Opin Psychiatry*, *32*(1), 1-6. https://doi.org/10.1097/yco.0000000000000468

Diener, M. J., & Monroe, J. M. (2011). The Relationship Between Adult Attachment Style and Therapeutic Alliance in Individual Psychotherapy: A Meta-Analytic Review. *Psychotherapy*, *48*(3), 237-248. https://doi.org/10.1037/A0022425

Dilling, H., Mombour, W., Schmidt, M. H., & Schulte-Markwort, E. (2011). *Internationale Klassifikation psychischer Störungen. ICD-10 Kapitel V (F). Diagnostische Kriterien für Forschung und Praxis*. Huber.

Domschke, K., Schiele, M. A., Crespo Salvador, Ó., Zillich, L., Lipovsek, J., Pittig, A.,...Deckert, J. (2025). Epigenetic markers of disease risk and psychotherapy response in anxiety disorders – a longitudinal analysis of the DNA methylome. *Mol Psychiatry*. https://doi.org/10.1038/s41380-025-03038-5

Dudai, Y. (2006). Reconsolidation: the advantage of being refocused. *Curr Opin Neurobiol*, *16*(2), 174-178. https://doi.org/10.1016/j.conb.2006.03.010

Dufeu, P., Kuhn, S., & Schmidt, L. G. (1995). Prüfung der Gütekriterien einer deutschen Version des „Tridimentional Personality Questionnaire (TPQ)“ von Cloninger bei Alkoholabhängigen. *Sucht 41*, 395-407.

Eck, S., Dümmler, D., Aktürk, Z., Korman, M., Dawson, S., Schneider, A.,...Fomenko, A. (2025). Beck Anxiety Inventory (BAI) for detecting anxiety disorders in adults. *Cochrane Database Syst Rev*, *12*(12), Cd015457. https://doi.org/10.1002/14651858.cd015457

Ehlers, A., & Margraf, J. (2001). *Fragebogen zu körperbezogenen Ängsten, Kognitionen und Vermeidung (AKV). Manual* (2., überarb. und neunormierte Auflage ed.). Beltz Test GmbH.

Eley, T. C. (2014). THE FUTURE OF THERAPYGENETICS: WHERE WILL STUDIES PREDICTING PSYCHOLOGICAL TREATMENT RESPONSE FROM GENOMIC MARKERS LEAD? *Depress Anxiety*. https://doi.org/10.1002/da.22292

Eley, T. C., Hudson, J. L., Creswell, C., Tropeano, M., Lester, K. J., Cooper, P.,...Collier, D. A. (2012). Therapygenetics: the 5HTTLPR and response to psychological therapy. In *Mol Psychiatry* (Vol. 17, pp. 236-237). https://doi.org/10.1038/mp.2011.132

Elling, C., Forstner, A. J., Seib-Pfeifer, L.-E., Mücke, M., Stahl, J., Geiser, F.,...Conrad, R. (2022). Social anxiety disorder with comorbid major depression – why fearful attachment style is relevant. *Journal of Psychiatric Research*, *147*, 283-290. https://doi.org/https://doi.org/10.1016/j.jpsychires.2022.01.019

Elnazer, H. Y., & Baldwin, D. S. (2014). Investigation of Cortisol Levels in Patients with Anxiety Disorders: A Structured Review. *Curr Top Behav Neurosci*. https://doi.org/10.1007/7854_2014_299

Elnazer, H. Y., Lau, L. C. K., Amaro, H., & Baldwin, D. S. (2021). Hair cortisol concentration in anxiety disorders: exploration of relationships with symptom severity and inflammatory markers. *Acta Neuropsychiatr*, *33*(2), 104-110. https://doi.org/10.1017/neu.2020.35

Erkoreka, L., Zumarraga, M., Arrue, A., Zamalloa, M. I., Arnaiz, A., Olivas, O.,...Basterreche, N. (2021). Genetics of adult attachment: An updated review of the literature. *World J Psychiatry*, *11*(9), 530-542. https://doi.org/10.5498/wjp.v11.i9.530

Esbjorn, B. H., Bender, P. K., Reinholdt-Dunne, M. L., Munck, L. A., & Ollendick, T. H. (2012). The development of anxiety disorders: considering the contributions of attachment and emotion regulation. *Clinical Child and Family Psychology Review*, *15*(2), 129-143. https://doi.org/10.1007/s10567-011-0105-4

Fava, L., & Morton, J. (2009). Causal modeling of panic disorder theories. *Clin Psychol Rev*, *29*(7), 623-637. https://doi.org/10.1016/j.cpr.2009.08.002

Fentz, H. N., Arendt, M., O'Toole, M. S., Hoffart, A., & Hougaard, E. (2014). The mediational role of panic self-efficacy in cognitive behavioral therapy for panic disorder: a systematic review and meta-analysis. *Behav Res Ther*, *60*, 23-33. https://doi.org/10.1016/j.brat.2014.06.003

Fischer, S., Schumacher, T., Knaevelsrud, C., Ehlert, U., & Schumacher, S. (2021). Genes and hormones of the hypothalamic-pituitary-adrenal axis in post-traumatic stress disorder. What is their role in symptom expression and treatment response? *J Neural Transm (Vienna)*, *128*(9), 1279-1286. https://doi.org/10.1007/s00702-021-02330-2

Fischer, S., & Zilcha-Mano, S. (2022). Why Does Psychotherapy Work and for Whom? Hormonal Answers. *Biomedicines*, *10*(6). https://doi.org/10.3390/biomedicines10061361

Flückiger, C., Del Re, A. C., Wampold, B. E., & Horvath, A. O. (2018). The alliance in adult psychotherapy: A meta-analytic synthesis. *Psychotherapy (Chic)*. https://doi.org/10.1037/pst0000172

Fonagy, P., & Luyten, P. (2009). A developmental, mentalization-based approach to the understanding and treatment of borderline personality disorder. *Dev Psychopathol*, *21*(4), 1355-1381. https://doi.org/10.1017/s0954579409990198

Fraley, R. C., Vicary, A. M., Brumbaugh, C. C., & Roisman, G. I. (2011). Patterns of stability in adult attachment: an empirical test of two models of continuity and change. *J Pers Soc Psychol*, *101*(5), 974-992. https://doi.org/10.1037/a0024150

Franke, G. (1995). *Die Symptom-Checkliste von Derogatis - Deutsche Version - (SCL-90-R). Manual*. Beltz Test GmbH.

Gaab, J., Jucker, P., Staub, F., & Ehlert, U. (2005). Mind over matter. *Zeitschrift für Klinische Psychologie und Psychotherapie*, *34*(2), 121-132. https://doi.org/10.1026/1616-3443.34.2.121

Gilbert, K., Mineka, S., Zinbarg, R. E., Craske, M. G., & Adam, E. K. (2017). Emotion Regulation Regulates More than Emotion: Associations of Momentary Emotion Regulation with Diurnal Cortisol in Current and Past Depression and Anxiety. *Clin Psychol Sci*, *5*(1), 37-51. https://doi.org/10.1177/2167702616654437

Gillath, O., Shaver, P. R., Baek, J. M., & Chun, D. S. (2008). Genetic correlates of adult attachment style. *Pers Soc Psychol Bull*, *34*(10), 1396-1405. https://doi.org/10.1177/0146167208321484

Goo, B. S., Mun, D. J., Kim, S., Nhung, T. T. M., Lee, S. B., Woo, Y.,...Park, S. K. (2023). Schizophrenia-associated Mitotic Arrest Deficient-1 (MAD1) regulates the polarity of migrating neurons in the developing neocortex. *Mol Psychiatry*, *28*(2), 856-870. https://doi.org/10.1038/s41380-022-01856-5

Grawe, K. (1992). Komplementäre Beziehungsgestaltung als Mittel zur Herstellung einer guten Therapiebeziehung. In J. Margraf & J. Brengelmann (Eds.), *Die Therapeut-Patient-Beziehung in der Verhaltenstherapie* (pp. 215-244). Gerhard Röttger.

Groh, A. M., & Narayan, A. J. (2019). Infant Attachment Insecurity and Baseline Physiological Activity and Physiological Reactivity to Interpersonal Stress: A Meta-Analytic Review. *Child Dev*, *90*(3), 679-693. https://doi.org/10.1111/cdev.13205

Gunlicks-Stoessel, M., Westervelt, A., Reigstad, K., Mufson, L., & Lee, S. (2017). The role of attachment style in interpersonal psychotherapy for depressed adolescents. *Psychotherapy Research*, 1-8. https://doi.org/10.1080/10503307.2017.1315465

Gustison, M. L., & Phelps, S. M. (2022). Individual differences in social attachment: A multi-disciplinary perspective. *Genes Brain Behav*, *21*(3), e12792. https://doi.org/10.1111/gbb.12792

Hamilton, M. (1996a). Hamilton Anxiety Scale (HAMA). In CIPS (Ed.), *Internationale Skalen für Psychiatrie*. Beltz Test GmbH.

Hamilton, M. (1996b). Hamilton Depression Scale (HAMD). In CIPS (Ed.), *Internationale Skalen für Psychiatrie*. Beltz Test GmbH.

Hautzinger, M., Bailer, M., Worrall, H., & Keller, F. (2000). *Beck-Depressions-Inventar (BDI). Testhandbuch* (3. Aufl. ed.). Huber.

Heim, C. (2000). *Deutsche Version des Early Trauma Inventory: Inventar zur Erfassung früher traumatischer Lebensereignisse (IFTL). Unveröffentlichtes Manuskript*. Emory University School of Medicine.

Heim, C., & Nemeroff, C. B. (2001). The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry*, *49*(12), 1023-1039. https://doi.org/10.1016/s0006-3223(01)01157-x

Hek, K., Direk, N., Newson, R. S., Hofman, A., Hoogendijk, W. J. G., Mulder, C. L., & Tiemeier, H. (2013). Anxiety disorders and salivary cortisol levels in older adults: a population-based study. *Psychoneuroendocrinology*, *38*(2), 300-305. https://doi.org/https://doi.org/10.1016/j.psyneuen.2012.06.006

Hoffart, A. (2016). Cognitive models for panic disorder with agoraphobia: A study of disaggregated within-person effects. *Journal of Consulting and Clinical Psychology*, *84*(9), 839-844. https://doi.org/10.1037/ccp0000114

Hofmann, S. G., Fang, A., & Gutner, C. A. (2014). Cognitive enhancers for the treatment of anxiety disorders. *Restor Neurol Neurosci*, *32*(1), 183-195. https://doi.org/10.3233/rnn-139002

Hofmann, S. G., Kasch, C., & Reis, A. (2025). Effect sizes of randomized-controlled studies of cognitive behavioral therapy for anxiety disorders over the past 30 years. *Clin Psychol Rev*, *117*, 102553. https://doi.org/10.1016/j.cpr.2025.102553

Hofmann, S. G., Mundy, E. A., & Curtiss, J. (2015). Neuroenhancement of Exposure Therapy in Anxiety Disorders. *AIMS Neurosci*, *2*(3), 123-138. https://doi.org/10.3934/Neuroscience.2015.3.123

Horesh, D., Cohen-Zrihen, A., Ein Dor, T., & Solomon, Z. (2014). Stressful life events across the life span and insecure attachment following combat trauma. *Clinical Social Work Journal*, *42*(4), 375-384. https://doi.org/10.1007/s10615-014-0477-2

Horowitz, L. M., Strauß, B., & Kordy, H. (2000). *Inventar zur Erfassung interpersonaler Probleme. Deutsche Version. Manual* (2., überarbeitete und neu normierte Auflage ed.). Beltz Test GmbH.

Horvath, A. O., Del Re, A. C., Fluckiger, C., & Symonds, D. (2011). Alliance in individual psychotherapy. *Psychotherapy (Chic)*, *48*(1), 9-16. https://doi.org/10.1037/a0022186

Horvath, A. O., & Greenberg, L. S. (1989). Development and Validation of the Working Alliance Inventory. *Journal of Counseling Psychology*, *36*(2), 223-233. https://doi.org/10.1037//0022-0167.36.2.223

Hoyer, J., Helbig, S., & Margraf, J. (2005). *Diagnostik der Angststörungen: Kompendien, Psychologische Diagnostik*. Hogrefe.

Hudson, J. L., Lester, K. J., Lewis, C. M., Tropeano, M., Creswell, C., Collier, D. A.,...Eley, T. C. (2013). Predicting outcomes following cognitive behaviour therapy in child anxiety disorders: the influence of genetic, demographic and clinical information. *J Child Psychol Psychiatry*, *54*(10), 1086-1094. https://doi.org/10.1111/jcpp.12092

Iovoli, F., Flückiger, C., Gómez Penedo, J. M., Engelhardt, J. H., Kaschlaw, H. H., Lauterbach, R.,...Rubel, J. A. (2024). The relationship between interpersonal problems and therapeutic alliance in psychotherapy: A three-level mixed-effects meta-analysis. *Psychotherapy (Chic)*, *61*(3), 198-211. https://doi.org/10.1037/pst0000534

Iovoli, F., Gómez Penedo, J. M., Lutz, W., & Rubel, J. A. (2025). Temporal associations between interpersonal problems and therapeutic alliance in cognitive behavioral therapy. *Psychotherapy (Chic)*. https://doi.org/10.1037/pst0000566

Ising, M., Hohne, N., Siebertz, A., Parchmann, A. M., Erhardt, A., & Keck, M. (2012). Stress response regulation in panic disorder. *Curr Pharm Des*, *18*(35), 5675-5684. https://doi.org/10.2174/138161212803530880

Jackson-Grusby, L., Beard, C., Possemato, R., Tudor, M., Fambrough, D., Csankovszki, G.,...Jaenisch, R. (2001). Loss of genomic methylation causes p53-dependent apoptosis and epigenetic deregulation. *Nat Genet*, *27*(1), 31-39. https://doi.org/10.1038/83730

Jacobi, F., Hofler, M., Siegert, J., Mack, S., Gerschler, A., Scholl, L.,...Wittchen, H. U. (2014). Twelve-month prevalence, comorbidity and correlates of mental disorders in Germany: the Mental Health Module of the German Health Interview and Examination Survey for Adults (DEGS1-MH). *Int J Methods Psychiatr Res*, *23*(3), 304-319. https://doi.org/10.1002/mpr.1439

Johnson, D. C., & Casey, B. J. (2015). Easy to remember, difficult to forget: the development of fear regulation. *Dev Cogn Neurosci*, *11*, 42-55. https://doi.org/10.1016/j.dcn.2014.07.006

Jones, J. D., Fraley, R. C., Ehrlich, K. B., Stern, J. A., Lejuez, C. W., Shaver, P. R., & Cassidy, J. (2018). Stability of Attachment Style in Adolescence: An Empirical Test of Alternative Developmental Processes. *Child Dev*, *89*(3), 871-880. https://doi.org/10.1111/cdev.12775

Joraschky, P., & Petrowski, K. (2008). Angst und Bindung: bindungstheoretische Prozesse bei Angststörungen. In B. Strauß (Ed.), *Bindung und Psychopathologie* (pp. 354). Klett-Cotta.

Kavelaars, R., Ward, H., Mackie, D. S., Modi, K. M., & Mohandas, A. (2023). The burden of anxiety among a nationally representative US adult population. *J Affect Disord*, *336*, 81-91. https://doi.org/10.1016/j.jad.2023.04.069

Kay, S. J., Keefe, J. R., Milrod, B. L., & Barber, J. P. (2024). Childhood Trauma and Panic Disorder: The Impact of History of Child Abuse on Illness Severity and Treatment Response. *Am J Psychother*, *77*(3), 112-118. https://doi.org/10.1176/appi.psychotherapy.20230060

Ketay, S., & Beck, L. A. (2017). Attachment predicts cortisol response and closeness in dyadic social interaction. *Psychoneuroendocrinology*, *80*, 114-121. https://doi.org/10.1016/j.psyneuen.2017.03.009

Kirchmann, H., Steyer, R., Mayer, A., Joraschky, P., Schreiber-Willnow, K., & Strauss, B. (2012). Effects of adult inpatient group psychotherapy on attachment characteristics: an observational study comparing routine care to an untreated comparison group. *Psychotherapy Research*, *22*(1), 95-114. https://doi.org/10.1080/10503307.2011.626807

Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1993). The 'Trier Social Stress Test'--a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology*, *28*(1-2), 76-81. https://doi.org/119004

Klinitzke, G., Romppel, M., Häuser, W., Brähler, E., & Glaesmer, H. (2011). Die deutsche Version des Childhood Trauma Questionnaire (CTQ) – psychometrische Eigenschaften in einer bevölkerungsrepräsentativen Stichprobe. *PPmP - Psychotherapie · Psychosomatik · Medizinische Psychologie*, *62*, 47-51. https://doi.org/10.1055/s-0031-1295495

Kribelbauer, J. F., Lu, X.-J., Rohs, R., Mann, R. S., & Bussemaker, H. J. (2020). Toward a Mechanistic Understanding of DNA Methylation Readout by Transcription Factors. *Journal of Molecular Biology*, *432*(6), 1801-1815. https://doi.org/https://doi.org/10.1016/j.jmb.2019.10.021

Kyriakoulis, P., & Kyrios, M. (2023). Biological and cognitive theories explaining panic disorder: A narrative review. *Front Psychiatry*, *14*, 957515. https://doi.org/10.3389/fpsyt.2023.957515

Lang, T., Helbig-Lang, S., Westphal, D., Gloster, A. T., & Wittchen, H.-U. (2011). *Expositionsbasierte Therapie der Panikstörung mit Agoraphobie: Ein Behandlungsmanual mit CD-ROM* [Exposure-based therapy for panic disorder with agoraphobia: A treatment manual with CD-ROM]. Hogrefe.

Laux, L., Glanzmann, P., Schaffner, P., & Spielberger, C. D. (1981). *Das State-Trait-Angstinventar. Theoretische Grundlagen und Handanweisung*. Beltz Test GmbH.

Lester, K. J., & Eley, T. C. (2013). Therapygenetics: Using genetic markers to predict response to psychological treatment for mood and anxiety disorders. *Biol Mood Anxiety Disord*, *3*(1), 4. https://doi.org/10.1186/2045-5380-3-4

Levy, K. N., Ellison, W. D., Scott, L. N., & Bernecker, S. L. (2011). Attachment style. *J Clin Psychol*, *67*(2), 193-203. https://doi.org/10.1002/jclp.20756

Levy, K. N., & Johnson, B. (2018). Attachment and Psychotherapy: Implications From Empirical Research. *Canadian Psychology*. https://doi.org/10.1037/cap0000162

Levy, K. N., Kivity, Y., Johnson, B. N., & Gooch, C. V. (2018). Adult attachment as a predictor and moderator of psychotherapy outcome: A meta-analysis. *J Clin Psychol*, *74*(11), 1996-2013. https://doi.org/10.1002/jclp.22685

Liston, C., Cichon, J. M., Jeanneteau, F., Jia, Z., Chao, M. V., & Gan, W.-B. (2013). Circadian glucocorticoid oscillations promote learning-dependent synapse formation and maintenance [Article]. *Nat Neurosci*, *16*(6), 698-705. https://doi.org/10.1038/nn.3387 http://www.nature.com/neuro/journal/v16/n6/abs/nn.3387.html#supplementary-information

Ma, D., Serbin, L. A., & Stack, D. M. (2019). How children's anxiety symptoms impact the functioning of the hypothalamus-pituitary-adrenal axis over time: A cross-lagged panel approach using hierarchical linear modeling. *Dev Psychopathol*, *31*(1), 309-323. https://doi.org/10.1017/s0954579417001870

Madigan, S., Vaillancourt, K., McKibbon, A., & Benoit, D. (2015). Trauma and traumatic loss in pregnant adolescents: the impact of Trauma-Focused Cognitive Behavior Therapy on maternal unresolved states of mind and Posttraumatic Stress Disorder. *Attachment & Human Development*, *17*(2), 175-198. https://doi.org/10.1080/14616734.2015.1006386

Maercker, A., & Schützwohl, M. (1998). Erfassung von psychischen Belastungsfolgen: Die Impact of Event Skala - revidierte Version (IES-R). *Diagnostica*, *44*, 130-141.

Manning, R. P., Dickson, J. M., Palmier-Claus, J., Cunliffe, A., & Taylor, P. J. (2016). A systematic review of adult attachment and social anxiety. *J Affect Disord*, *211*, 44-59. https://doi.org/10.1016/j.jad.2016.12.020

Maples-Keller, J. L., Price, M., Jovanovic, T., Norrholm, S. D., Odenat, L., Post, L.,...Rothbaum, B. O. (2017). Targeting memory reconsolidation to prevent the return of fear in patients with fear of flying. *Depress Anxiety*. https://doi.org/10.1002/da.22626

Marcaurelle, R., Belanger, C., & Marchand, A. (2003). Marital relationship and the treatment of panic disorder with agoraphobia: a critical review. *Clin Psychol Rev*, *23*(2), 247-276.

Margraf, J., & Schneider, S. (2011). *Lehrbuch der Verhaltenstherapie* (Vol. 2). Springer.

Mayer, S. E., Snodgrass, M., Liberzon, I., Briggs, H., Curtis, G. C., & Abelson, J. L. (2017). The psychology of HPA axis activation: Examining subjective emotional distress and control in a phobic fear exposure model. *Psychoneuroendocrinology*, *82*, 189-198. https://doi.org/10.1016/j.psyneuen.2017.02.001

McCullough, J. P. (2000). *Treatment for chronic depression: cognitive behavioral analysis system of psychotherapy-CBASP*. Guilford Press.

McEwen, B. S. (2015). Biomarkers for assessing population and individual health and disease related to stress and adaptation. *Metabolism*, *64*(3 Suppl 1), S2-S10. https://doi.org/10.1016/j.metabol.2014.10.029

Meine, L. E., Müller-Bardorff, M., Recher, D., Paersch, C., Schulz, A., Spiller, T.,...Kleim, B. (2024). Network analyses of ecological momentary emotion and avoidance assessments before and after cognitive behavioral therapy for anxiety disorders. *J Anxiety Disord*, *106*, 102914. https://doi.org/10.1016/j.janxdis.2024.102914

Meir Drexler, S., Merz, C. J., Hamacher-Dang, T. C., Tegenthoff, M., & Wolf, O. T. (2015). Effects of Cortisol on Reconsolidation of Reactivated fear Memories. *Neuropsychopharmacology*. https://doi.org/10.1038/npp.2015.160

Meir Drexler, S., Merz, C. J., Jentsch, V. L., & Wolf, O. T. (2020). Stress modulation of fear and extinction in psychopathology and treatment. *Neuroforum*, *26*(3), 133-141. https://doi.org/doi:10.1515/nf-2020-0018

Meuret, A. E., Trueba, A. F., Abelson, J. L., Liberzon, I., Auchus, R., Bhaskara, L.,...Rosenfield, D. (2014). High cortisol awakening response and cortisol levels moderate exposure-based psychotherapy success. *Psychoneuroendocrinology*, *51c*, 331-340. https://doi.org/10.1016/j.psyneuen.2014.10.008

Mikulincer, M., & Shaver, P. R. (2007a). *Attachment in adulthood: Structure, dynamics, and change*. Guilford Press.

Mikulincer, M., & Shaver, P. R. (2007b). Boosting attachment security to promote mental health, prosocial values, and inter-group tolerance. *Psychological Inquiry*, *18*(3), 139-156. https://doi.org/10.1080/10478400701512646

Mikulincer, M., & Shaver, P. R. (2012). An attachment perspective on psychopathology. *World Psychiatry*, *11*(1), 11-15. https://doi.org/10.1016/j.wpsyc.2012.01.003

Mikulincer, M., & Shaver, P. R. (2018). Attachment orientations and emotion regulation. *Current Opinion in Psychology*, *25*, 6-10. https://doi.org/10.1016/j.copsyc.2018.02.006

Mikulincer, M., Shaver, P. R., & Berant, E. (2013). An attachment perspective on therapeutic processes and outcomes. *J Pers*, *81*(6), 606-616. https://doi.org/10.1111/j.1467-6494.2012.00806.x

Miller, C. A., & Sweatt, J. D. (2007). Covalent modification of DNA regulates memory formation. *Neuron*, *53*(6), 857-869. https://doi.org/10.1016/j.neuron.2007.02.022

Monfils, M. H., Cowansage, K. K., Klann, E., & LeDoux, J. E. (2009). Extinction-reconsolidation boundaries: key to persistent attenuation of fear memories. *Science*, *324*(5929), 951-955. https://doi.org/10.1126/science.1167975

Monteleone, A. M., Ruzzi, V., Pellegrino, F., Patriciello, G., Cascino, G., Del Giorno, C.,...Maj, M. (2019). The vulnerability to interpersonal stress in eating disorders: The role of insecure attachment in the emotional and cortisol responses to the trier social stress test. *Psychoneuroendocrinology*, *101*, 278-285. https://doi.org/10.1016/j.psyneuen.2018.12.232

Mulder, R. H., Rijlaarsdam, J., Luijk, M. P., Verhulst, F. C., Felix, J. F., Tiemeier, H.,...Van Ijzendoorn, M. H. (2017). Methylation matters: FK506 binding protein 51 (FKBP5) methylation moderates the associations of FKBP5 genotype and resistant attachment with stress regulation. *Dev Psychopathol*, *29*(2), 491-503. https://doi.org/10.1017/s095457941700013x

Murphy, C. P., & Singewald, N. (2019). Role of MicroRNAs in Anxiety and Anxiety-Related Disorders. *Curr Top Behav Neurosci*. https://doi.org/10.1007/7854_2019_109

Murphy, S., Elklit, A., Hyland, P., & Shevlin, M. (2016). Insecure attachment orientations and posttraumatic stress in a female treatment-seeking sample of survivors of childhood sexual abuse: A cross-lagged panel study. *Traumatology*, *22*(1), 48-55. https://doi.org/10.1037/trm0000060

Myers, K. M., & Davis, M. (2002). Behavioral and neural analysis of extinction. *Neuron*, *36*(4), 567-584.

Müller, M., Zietlow, A. L., Klauser, N., Woll, C., Nonnenmacher, N., Tronick, E., & Reck, C. (2021). From Early Micro-Temporal Interaction Patterns to Child Cortisol Levels: Toward the Role of Interactive Reparation and Infant Attachment in a Longitudinal Study. *Front Psychol*, *12*, 807157. https://doi.org/10.3389/fpsyg.2021.807157

Müller, R. T., & Rosenkranz, S. E. (2009). Attachment and treatment response among adults in inpatient treatment for posttraumatic stress disorder. *Psychotherapy (Chic)*, *46*(1), 82-96. https://doi.org/10.1037/a0015137

Nelles, P. A., Singewald, N., Sperner-Unterweger, B., & Hüfner, K. (2025). The “conflict avoidance theory of inflammation-induced anxiety” (CATIA): A psychoneuroimmunologic hypothesis. *Medical Hypotheses*, *196*, 111580. https://doi.org/https://doi.org/10.1016/j.mehy.2025.111580

Newman, M. G., Castonguay, L. G., Jacobson, N. C., & Moore, G. A. (2015). Adult attachment as a moderator of treatment outcome for generalized anxiety disorder: Comparison between cognitive-behavioral therapy (CBT) plus supportive listening and CBT plus interpersonal and emotional processing therapy. *J Consult Clin Psychol*, *83*(5), 915-925. https://doi.org/10.1037/a0039359

Nielsen, S. K. K., Lonfeldt, N., Wolitzky-Taylor, K. B., Hageman, I., Vangkilde, S., & Daniel, S. I. F. (2017). Adult attachment style and anxiety - The mediating role of emotion regulation. *J Affect Disord*, *218*, 253-259. https://doi.org/10.1016/j.jad.2017.04.047

Nolte, T., Guiney, J., Fonagy, P., Mayes, L. C., & Luyten, P. (2011). Interpersonal stress regulation and the development of anxiety disorders: an attachment-based developmental framework. *Frontiers in Behavioral Neuroscience*, *5*, 55. https://doi.org/10.3389/fnbeh.2011.00055

Notsu, H., Blansfield, R. E., Spina, D. S., & Levy, K. N. (2025). An updated meta-analysis of the relation between adult attachment style and working alliance. *Psychotherapy Research*, *35*(5), 721-734. https://doi.org/10.1080/10503307.2024.2370344

Penninx, B. W., Pine, D. S., Holmes, E. A., & Reif, A. (2021). Anxiety disorders. *Lancet*, *397*(10277), 914-927. https://doi.org/10.1016/s0140-6736(21)00359-7

Petrowski, K., Wintermann, G. B., Kirschbaum, C., & Bornstein, S. R. (2012). Dissociation between ACTH and cortisol response in DEX-CRH test in patients with panic disorder.

Psychoneuroendocrinology, *37*(8), 1199-1208. https://doi.org/10.1016/j.psyneuen.2011.12.013

Pini, S., Abelli, M., Troisi, A., Siracusano, A., Cassano, G. B., Shear, K. M., & Baldwin, D. (2014). The relationships among separation anxiety disorder, adult attachment style and agoraphobia in patients with panic disorder. *J Anxiety Disord*, *28*(8), 741-746. https://doi.org/10.1016/j.janxdis.2014.06.010

Plag, J., Schumacher, S., Schmid, U., & Ströhle, A. (2013). Baseline and acute changes in the HPA system in patients with anxiety disorders: the current state of research. *http://dx.doi.org/10.2217/npy.13.6*. https://doi.org/10.2217/npy.13.6

Pluess, M., & Belsky, J. (2013). Vantage sensitivity: individual differences in response to positive experiences. *Psychol Bull*, *139*(4), 901-916. https://doi.org/10.1037/a0030196

Pulopulos, M. M., Baeken, C., & De Raedt, R. (2019). Cortisol response to stress: The role of expectancy and anticipatory stress regulation. *Horm Behav*, 104587. https://doi.org/10.1016/j.yhbeh.2019.104587

Ravitz, P., Maunder, R., Hunter, J., Sthankiya, B., & Lancee, W. (2010). Adult attachment measures: a 25-year review. *Journal of Psychosomatic Research*, *69*(4), 419-432. https://doi.org/10.1016/j.jpsychores.2009.08.006

Rayner, C., Coleman, J. R. I., Purves, K. L., Hodsoll, J., Goldsmith, K., Alpers, G. W.,...Eley, T. C. (2019). A genome-wide association meta-analysis of prognostic outcomes following cognitive behavioural therapy in individuals with anxiety and depressive disorders. *Transl Psychiatry*, *9*(1), 150. https://doi.org/10.1038/s41398-019-0481-y

Renner, V., Duhm, J., Lorenz, T., Joraschky, P., & Petrowski, K. (2025). Positive Side-Effects of Psychotherapy: The Influence of Cognitive-Behavioral Therapy on Self-Concept in Patients With Panic Disorder. *J Clin Psychol*. https://doi.org/10.1002/jclp.70055

Rezaie, P. (2025). *The biopsychosocial model of mental health: revisited*. The Open University. Retrieved May 05, 2025 from https://www.open.edu/openlearn/science-maths-technology/exploring-the-relationship-between-anxiety-and-depression/content-section-2

Ricolo, D., Casanova, J., & Giannios, P. (2025). Drosophila and human Headcase define a new family of ribonucleotide granule proteins required for stress response. *Sci Adv*, *11*(13), eads2086. https://doi.org/10.1126/sciadv.ads2086

Rimane, E., Steil, R., Renneberg, B., & Rosner, R. (2020). Get secure soon: attachment in abused adolescents and young adults before and after trauma-focused cognitive processing therapy. *Eur Child Adolesc Psychiatry*. https://doi.org/10.1007/s00787-020-01637-x

Roberts, S., Lester, K. J., Hudson, J. L., Rapee, R. M., Creswell, C., Cooper, P. J.,...Eley, T. C. (2014). Serotonin transporter [corrected] methylation and response to cognitive behaviour therapy in children with anxiety disorders. *Transl Psychiatry*, *4*, e444. https://doi.org/10.1038/tp.2014.83

Roberts, S., Wong, C. C. Y., Breen, G., Coleman, J. R. I., De Jong, S., Johren, P.,...Eley, T. C. (2017). Genome-wide expression and response to exposure-based psychological therapy for anxiety disorders [Original Article]. *Transl Psychiatry*, *7*, e1219. https://doi.org/10.1038/tp.2017.177

Robinaugh, D. J., Haslbeck, J. M. B., Waldorp, L. J., Kossakowski, J. J., Fried, E. I., Millner, A. J.,...Borsboom, D. (2024). Advancing the network theory of mental disorders: A computational model of panic disorder. *Psychological Review*, *131*(6), 1482-1508. https://doi.org/10.1037/rev0000515

Roest, A. M., de Vries, Y. A., Lim, C. C. W., Wittchen, H. U., Stein, D. J., Adamowski, T.,...de Jonge, P. (2019). A comparison of DSM-5 and DSM-IV agoraphobia in the World Mental Health Surveys. *Depress Anxiety*, *36*(6), 499-510. https://doi.org/10.1002/da.22885

RStudioTeam. (2020). *RStudio: Integrated Development for R. RStudio, Inc.* In http://www.rstudio.com/

Ruch, W. (1999). Die revidierte Fassung des Eysenck Personality Questionnaire und die Konstruktion des deutschen EPQ-R bzw. EPQ-RK. *Zeitschrift für Differentielle und Diagnostische Psychologie*, *20*(1), 1-24. https://doi.org/10.1024//0170-1789.20.1.1

Santomauro, D. F., Mantilla Herrera, A. M., Shadid, J., Zheng, P., Ashbaugh, C., Pigott, D. M.,...Ferrari, A. J. (2021). Global prevalence and burden of depressive and anxiety disorders in 204 countries and territories in 2020 due to the COVID-19 pandemic. *The Lancet*, *398*(10312), 1700-1712. https://doi.org/10.1016/S0140-6736(21)02143-7

Schiele, M. A., & Domschke, K. (2018). Epigenetics at the crossroads between genes, environment and resilience in anxiety disorders. *Genes Brain Behav*, *17*(3), e12423. https://doi.org/10.1111/gbb.12423

Schiele, M. A., Reif, A., Lin, J., Alpers, G. W., Andersson, E., Andersson, G.,...Lueken, U. (2021). Therapygenetic effects of 5-HTTLPR on cognitive-behavioral therapy in anxiety disorders: A meta-analysis. *Eur Neuropsychopharmacol*, *44*, 105-120. https://doi.org/10.1016/j.euroneuro.2021.01.004

Schiele, M. A., Ziegler, C., Kollert, L., Katzorke, A., Schartner, C., Busch, Y.,...Domschke, K. (2018). Plasticity of Functional MAOA Gene Methylation in Acrophobia. *Int J Neuropsychopharmacol*, *21*(9), 822-827. https://doi.org/10.1093/ijnp/pyy050

Shakiba, N., & Raby, K. L. (2023). Attachment dimensions and cortisol responses during the strange situation among young children adopted internationally. *Attach Hum Dev*, *25*(1), 89-103. https://doi.org/10.1080/14616734.2021.1896445

Singewald, N., Schmuckermair, C., Whittle, N., Holmes, A., & Ressler, K. J. (2014). Pharmacology of cognitive enhancers for exposure-based therapy of fear, anxiety and trauma-related disorders. *Pharmacol Ther*. https://doi.org/10.1016/j.pharmthera.2014.12.004

Skourteli, M. C., & Lennie, C. (2011). The therapeutic relationship from an attachment theory perspective. *Counselling Psychology Review*, *26*(1), 20-33.

Southward, M. W., Kushner, M. L., Terrill, D. R., & Sauer-Zavala, S. (2024). A Review of Transdiagnostic Mechanisms in Cognitive Behavior Therapy. *Psychiatric Clinics of North America*, *47*(2), 343-354. https://doi.org/https://doi.org/10.1016/j.psc.2024.02.003

Spence, S. H., O'Shea, G., & Donovan, C. L. (2016). Improvements in interpersonal functioning following interpersonal psychotherapy (ipt) with adolescents and their association with change in depression. *Behav Cogn Psychother*, *44*(3), 257-272. https://doi.org/10.1017/s1352465815000442

Steffanowski, M. (2001). *Psychometrische Überprüfung einer deutschsprachigen Version des Relationship Scales Questionaire (RSQ)*. Psychosozial.

Stovall-McClough, K. C., & Cloitre, M. (2003). Reorganization of unresolved childhood traumatic memories following exposure therapy. *Ann N Y Acad Sci*, *1008*, 297-299. https://doi.org/10.1196/annals.1301.036

Strauss, A. Y., Kivity, Y., & Huppert, J. D. (2019). Emotion Regulation Strategies in Cognitive Behavioral Therapy for Panic Disorder. *Behav Ther*, *50*(3), 659-671. https://doi.org/10.1016/j.beth.2018.10.005

Strauß, B., Altmann, U., Manes, S., Tholl, A., Koranyi, S., Nolte, T.,...Kirchmann, H. (2018). Changes of attachment characteristics during psychotherapy of patients with social anxiety disorder: Results from the SOPHO-Net trial. *PLoS One*, *13*(3), e0192802. https://doi.org/10.1371/journal.pone.0192802

Strüber, N., Strüber, D., & Roth, G. (2014). Impact of early adversity on glucocorticoid regulation and later mental disorders. *Neurosci Biobehav Rev*, *38*, 17-37. https://doi.org/10.1016/j.neubiorev.2013.10.015

Szuhany, K. L., & Simon, N. M. (2022). Anxiety Disorders: A Review. *Jama*, *328*(24), 2431-2445. https://doi.org/10.1001/jama.2022.22744

Tafet, G. E., Feder, D. J., Abulafia, D. P., & Roffman, S. S. (2005). Regulation of hypothalamic-pituitary-adrenal activity in response to cognitive therapy in patients with generalized anxiety disorder. *Cogn Affect Behav Neurosci*, *5*(1), 37-40.

Taylor, P., Rietzschel, J., Danquah, A., & Berry, K. (2015). Changes in attachment representations during psychological therapy. *Psychotherapy Research*, *25*(2), 222-238. https://doi.org/10.1080/10503307.2014.886791

Todd, T. P., Vurbic, D., & Bouton, M. E. (2014). Behavioral and neurobiological mechanisms of extinction in Pavlovian and instrumental learning. *Neurobiol Learn Mem*, *108*, 52-64. https://doi.org/10.1016/j.nlm.2013.08.012

Toumbelekis, M., Liddell, B. J., & Bryant, R. A. (2021). Secure attachment primes reduce fear consolidation. *Depress Anxiety*, *38*(10), 1078-1086. https://doi.org/10.1002/da.23166

Vashishtha, S., Kloiber, S., & Zai, G. (2023). The therapygenetics of anxiety disorders. *Psychiatr Genet*, *33*(4), 123-133. https://doi.org/10.1097/ypg.0000000000000342

Vinkers, C. H., Kuzminskaite, E., Lamers, F., Giltay, E. J., & Penninx, B. (2021). An integrated approach to understand biological stress system dysregulation across depressive and anxiety disorders. *J Affect Disord*, *283*, 139-146. https://doi.org/10.1016/j.jad.2021.01.051

Wang, C., Zhang, N., Zhang, Y. L., Zhang, J., Yang, H., & Timothy, T. C. (2013). Comparison of the neurobiological effects of attribution retraining group therapy with those of selective serotonin reuptake inhibitors. *Braz J Med Biol Res*, *46*(3), 318-326.

Wang, X., Sundquist, K., Hedelius, A., Palmer, K., Memon, A. A., & Sundquist, J. (2015). Circulating microRNA-144-5p is associated with depressive disorders. *Clin Epigenetics*, *7*, 69. https://doi.org/10.1186/s13148-015-0099-8

Weaver, I. C., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R.,...Meaney, M. J. (2004). Epigenetic programming by maternal behavior. *Nat Neurosci*, *7*(8), 847-854. https://doi.org/10.1038/nn1276

Weber, H., Hettema, J. M., Deckert, J., & Erhardt-Lehmann, A. (2025). Genomics of Anxiety Disorders. *Psychiatric Clinics of North America*. https://doi.org/https://doi.org/10.1016/j.psc.2025.01.012

Wilmers, F. (2008). Die deutschsprachige Version des Working Alliance Inventory – short revised (WAI-SR) – Ein schulenübergreifendes, ökonomisches und empirisch validiertes Instrument zur Erfassung der therapeutischen Allianz. *Zeitschrift für Klinische Diagnostik und Evaluation*, *1*(3), 343–358.

Wittchen, H.-U., Zaudig, M., & Fydrich, T. (1997). *Strukturiertes Klinisches Interview für DSM-IV*. Hogrefe.

Wittchen, H. U., Gloster, A. T., Beesdo-Baum, K., Fava, G. A., & Craske, M. G. (2010). Agoraphobia: a review of the diagnostic classificatory position and criteria. *Depress Anxiety*, *27*(2), 113-133. https://doi.org/10.1002/da.20646

Wittchen, H. U., Jacobi, F., Rehm, J., Gustavsson, A., Svensson, M., Jonsson, B.,...Steinhausen, H. C. (2011). The size and burden of mental disorders and other disorders of the brain in Europe 2010. *Eur Neuropsychopharmacol*, *21*(9), 655-679. https://doi.org/10.1016/j.euroneuro.2011.07.018

Young, J. E., Klosko, J.S., & Weishaar, M.E. (2003). *Schema therapy: A practitioner's guide*. Guilford Press.

Zaider, T. I., Heimberg, R. G., & Iida, M. (2010). Anxiety disorders and intimate relationships: a study of daily processes in couples. *Journal of Abnormal Psychology*, *119*(1), 163-173. https://doi.org/10.1037/a0018473

Zalaznik, D., Michal, W., & and Huppert, J. D. (2019). Improvement in adult anxious and avoidant attachment during cognitive behavioral therapy for panic disorder. *Psychotherapy Research*, *29*(3), 337-353. https://doi.org/10.1080/10503307.2017.1365183

Zalaznik, D., Strauss, A. Y., Halaj, A., Fradkin, I., Ebert, D. D., Andersson, G., & Huppert, J. D. (2022). Anxious attachment improves and is predicted by anxiety sensitivity in internet-based, guided self-help cognitive behavioral treatment for panic disorder. *J Couns Psychol*, *69*(2), 211-221. https://doi.org/10.1037/cou0000579

Zalaznik, D., Zlotnick, E., Barzilay, S., Ganor, T., Sorka, H., Ebert, D. D.,...Huppert, J. D. (2025). Interpersonal factors in internet-based cognitive behavioral therapy for depression: Attachment style and alliance with the program and with the therapist. *Psychother Res*, *35*(4), 558-573. https://doi.org/10.1080/10503307.2024.2325510

Ziegler, C., Richter, J., Mahr, M., Gajewska, A., Schiele, M. A., Gehrmann, A.,...Domschke, K. (2016). MAOA gene hypomethylation in panic disorder-reversibility of an epigenetic risk pattern by psychotherapy. *Transl Psychiatry*, *6*, e773. https://doi.org/10.1038/tp.2016.41

Zilcha-Mano, S. (2017). Is the alliance really therapeutic? Revisiting this question in light of recent methodological advances. *American Psychologist*, *72*(4), 311-325. https://doi.org/10.1037/a0040435

Zilcha-Mano, S., & Fisher, H. (2022). Distinct roles of state-like and trait-like patient–therapist alliance in psychotherapy. *Nature Reviews Psychology*, *1*(4), 194-210. https://doi.org/10.1038/s44159-022-00029-z

Zuroff, D. C., & Blatt, S. J. (2006). The therapeutic relationship in the brief treatment of depression: contributions to clinical improvement and enhanced adaptive capacities.

Journal of Consulting and Clinical Psychology, *74*(1), 130-140. https://doi.org/10.1037/0022-006x.74.1.130

Zwanzger, P., Domschke, K., & Bradwejn, J. (2012). Neuronal network of panic disorder: the role of the neuropeptide cholecystokinin. *Depress Anxiety*, *29*(9), 762-774. https://doi.org/10.1002/da.21919

Łoś, K., & Waszkiewicz, N. (2021). Biological Markers in Anxiety Disorders. *Journal of clinical medicine*, *10*(8). https://doi.org/10.3390/jcm10081744

Appendix

Appendix A – Publication I: HPA system in anxiety disorder patients treated with cognitive behavioural therapy: A Review

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Review

HPA system in anxiety disorder patients treated with cognitive behavioural therapy: A review

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A R T I C L E I N F O

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A B S T R A C T

Anxiety disorders (AD) have a complex etiology involving genetic, psychophysiological and environmental factors. Recent biomarker research in AD shows alterations in anatomical, biochemical and physiological pathways as well as in endocrinological processes. Perceived stress is one of the factors often reported in relation to the onset and the course of AD. Hence, the function of the hypothalamus-pituitary-adrenal (HPA) axis, the endogenous system regulating the stress response homeostasis, may serve as biomarker in disease etiology and response to treatment. Vice versa, successful treatment could have an advantageous effect on the function of the HPA system. In the present review, we summarize findings on the HPA system in relation to AD and first results on its modifiability in response to cognitive behavioral therapy (CBT), one of the most efficacious psychotherapeutic treatments for AD. We specifically focus on findings of experimental studies that explored cortisol levels before, during and after exposure-based CBT. A systematic search was conducted in MEDLINE/PubMed, PsycINFO, Web of Science, and in references of retrieved studies until April 2024. The inclusion criteria were studies enclosing keywords related to the HPA axis, an anxiety disorder and CBT techniques. The results of the summarized studies suggest that cortisol levels have the potential to indicate the AD disease status and serve as possible biomarker in outcome prediction. Global dysfunction of the HPA system seems to point to higher symptom burden and less advantageous response to CBT. Furthermore, low cortisol levels elicited in relation to exposure interventions are repeatedly associated with risk for non-response. Additionally, some evidence suggests that successful CBT containing exposure sessions as well as cognitive techniques induces normalization of the HPA system by reducing acute response to fear related stimuli in parallel to normalizing basal cortisol levels. To conclude, cortisol seems to be a promising candidate to depict several aspects of AD-related disease status and CBT effects, however, additional prospective studies are needed to evaluate the mode of applications of this marker in the clinical routine.

1. Introduction

1.1. Anxiety disorders and CBT

Anxiety disorders (AD) such as Panic Disorder (PD) with/without Agoraphobia (AG), Social Anxiety Disorder (SAD), Generalized Anxiety Disorder (GAD) and Specific Phobias are pathological forms of fear and anxiety that are characterized by excessive emotional and physiological responses in the absence of real danger. Cognitive behavioral therapy (CBT) is one of the most efficacious psychotherapeutic treatments (Bandelow et al., 2022; Cuijpers et al., 2016; Hans & Hiller, 2013; Hofmann & Smits, 2008), which includes strategies for modifying dysfunctional cognitions and exposure therapy (ET; Carpenter et al., 2018). However, about a third of the patients do not respond to CBT treatment for unknown reasons (Springer et al., 2018). Since "cause should inform cure" (Uher, 2008), it is important to identify biomarkers that extend the knowledge about the pathology and assesses the severity of the disorder (For a review see Bandelow et al., 2016; Bandelow et al., 2017), as well as to explain and predict differential treatment response as intended in personalized medicine. The assumption that

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psychotherapy is able to target the underlying (biological) cause, not just changing the subjective psychological features (cognitions, emotions) or coping abilities is widely unexplored and needs confirmation. Endogenous stress hormones, e.g. cortisol, that partly mediate the fear reaction and have been found to be elevated during acute fear (Joëls & Baram, 2009), are promising candidates.

1.2. HPA system

The hypothalamic-pituitary-adrenal (HPA) axis has been repeatedly shown to play an important role in AD (Elnazer & Baldwin, 2014; Plag et al., 2013). One of the major modulators of the HPA system is the corticotropin releasing hormone (CRH; for a review see Binder & Nemeroff, 2010; Nemeroff & Vale, 2005), which elicits the release of pituitary adrenocorticotropic hormone (ACTH) and subsequently cortisol under basal conditions and in response to stress. Cortisol follows a 24-hour circadian rhythm with highest levels in the morning upon wakening, which is called cortisol awakening response (CAR) with the peak release 30–45 min post-awakening, and lowest levels about mid-night (Lupien et al., 2005; Pruessner et al., 1997). To maintain physiological and behavioral responsiveness to stress, oscillating levels of adrenal glucocorticoid hormones are necessary and were shown to have an ultradian frequency due to a pulsatile secretion pattern (For a review see Spiga et al., 2015; Walker et al., 2012). Latter is impaired by continuous and prolonged stress with exposing the body to regularly high levels of cortisol (Heim et al., 2000), e.g. caused by high-frequency panic attacks and anticipatory anxiety. While cortisol has been shown to basically promote plasticity at the synaptic structural level (Liston et al., 2013) and thus to improve learning and memory, a chronically hyperactivated HPA system respectively long-term stress diminishes neuroplasticity (Surget & Belzung, 2022). Additionally, an inverted-U relationship between activity of endogenous stress hormones and human memory has been shown, with low doses of cortisol not affecting memory, with high doses probably impairing and in between promoting consolidation (Andreano and Cahill, 2006). Elevated cortisol levels seem to inhibit memory retrieval and working memory during emotionally arousing situations, but to enhance memory consolidation of emotionally arousing experiences (For a review see de Quervain et al., 2009; Hood et al., 2014; Lupien et al., 2005; Wingenfeld and Wolf, 2014).

The majority of the available studies assess peripheral cortisol levels in different bio samples as a direct measure of the HPA system activity in humans and animal models. The traditional cortisol assessment strategies like saliva, plasma and urine samples reflect the highly volatile short-term secretory activity of the stress system. Hair steroid analysis (Elnazer and Baldwin, 2014) is a reliable measure of cumulative cortisol concentration over a period of months (Elnazer et al., 2021) and can be used as long-term marker of HPA system functioning. Additionally, distinct HPA system function can be actively tested by administration of 1) glucocorticoids, e.g. dexamethasone (Dex), which assesses the suppression properties of the HPA system (the Dexamethason-Suppression-test, DST) or 2) corticotropin-releasing hormone (CRH), which is used to investigate the activation properties of the HPA system mostly in combination with suppression testing - the combined Dexamethasone suppression/Corticotropin-Releasing Hormone stimulation (Dex/CRH test; Heuser et al., 1994).

1.3. HPA system and anxiety disorders

The hypothesis of the HPA system dysfunction in AD has led to a plethora of studies investigating acute and chronic HPA system changes and its effects on therapeutic interventions, to understand the underlying biological processes and to develop new therapeutic drugs. There is mixed evidence for basal and acute deviations in cortisol levels as well as an association with symptom severity, duration of the disorder, and age in AD patients (for a review see Bandelow et al., 2017; Elnazer and Baldwin, 2014; Elnazer et al., 2021; Hek et al., 2013; Łoś and Waszkiewicz, 2021; Plag et al., 2013; Vinkers et al., 2021). Cortisol elevations in PD patients were either not found compared to controls or shown to be more pronounced during the day or during the night as well as during (induced) panic attacks and to be associated with higher symptom severity. Also, whether GAD or SAD is associated with aberrant increased basal cortisol levels remains unclear (For a review see Bandelow et al., 2017; Caldiroli et al., 2023; Kische et al., 2021).

Studies investigating HPA system dynamics in AD under psychosocial as well as physiological stress induction demonstrated both, hypo- as well as hyperactivation, or no difference to control condition (for a review see Bandelow et al., 2017; Ising et al., 2012). For example, hyporeactivity of the HPA system to psychological stress tests (e.g. Trier Social Stress Test [TSST]; Kirschbaum et al., 1993) at baseline was seen in PD patients (Altamura et al., 2018; Petrowski et al., 2010; Petrowski et al., 2013; Wichmann, Kirschbaum, Bohme, et al., 2017) and SAD patients (Petrowski et al., 2021) as well as a hyperresponsiveness in SAD patients (Roelofs et al., 2009; van West et al., 2008) or no difference to healthy subjects (e.g. Asbrand et al., 2019; Elzinga et al., 2010; Faucher et al., 2016; Grace et al., 2022; Klumbies et al., 2014; Krämer et al., 2012). Most studies suggest an enhanced cortisol increase during exposure to phobic stimuli in specific phobias with no difference to controls in the absence of fear-related situations (Bandelow et al., 2017). The majority of investigations with suppression tests have shown a normal suppression of ACTH and cortisol in the standard DST and Dex/CRH test in AD patients (For a review see Ising et al., 2012; Plag et al., 2013; Vreeburg et al., 2013; Wichmann et al., 2018) and a few found non-suppression e.g. in GAD (Tiller et al., 1988) or in PD patients (Erhardt et al., 2006). Earlier Coryell et al. (1991) demonstrated that non-suppression after DST in 77 medicated and non-medicated PD patients indicated a more persistent and chronically disabling condition three years later. Also, Petrowski et al. (2012) showed with the Dex/CRH test, that the duration of the illness may be a risk factor for hyperreactivity of the HPA system in PD patients.

Łoś and Waszkiewicz (2021) concluded in their review that cortisol in the initial stage of the AD is elevated, which makes it possible to treat it as a trait biomarker and thus, can be used for early detection of pathological forms of anxiety. Elevated cortisol levels may be a marker for the stress load due to anxiety symptoms itself. They further hypothesized that the cortisol concentration decreases during the course of AD and therefore may be monitored to assess the progress of the disease as state marker showing the level of clinical symptoms. However, it is not clear whether dysfunction of the HPA system is a potential etiological factor, for example for the onset of PD, or a result of continuous stress, for example caused by recurring panic attacks (Łoś and Waszkiewicz, 2021). Also, in SAD an initial increased cortisol reactivity to social exposition may attenuate following chronic exposure to stressful situations (Caldiroli et al., 2023). Taking the assumption into account that AD states exert long-term effects on GC secretion, findings of a recent study assessing hair cortisol indicate an elevated concentration in patients with single-episode AD and low concentration in recurrent-episode AD with a potential desensitisation of HPA response to stress (Elnazer et al., 2021). An alteration of the HPA system activation has also been linked to a worse course of symptoms in AD (Bandelow et al., 2000; Coryell et al., 1991; Petrowski et al., 2012). For example, investigating 837 depressive and anxiety patients in a longitudinal study, a lower salivary cortisol awakening response (CAR), possibly indicative of an underlying exhaustion of the HPA axis, was found to predict an unfavourable course trajectory after 2 years (Vreeburg et al., 2013). Interestingly, a study in 232 healthy individuals showed prospective associations between a higher salivary CAR and first onset of AD in 25 patients, especially of SAD (N = 11), over a six-year period (Adam et al., 2014). Also, higher pre-treatment mean 24-h cortisol levels in PD patients were previously shown to predict a worse outcome two years later (Abelson and Curtis, 1996).

In conclusion, there are contradicting results in the relevant research findings regarding the HPA abnormalities in AD with mixed evidence for the dysfunction of the HPA system, either hyper- or hyporesponse, as underlying etiological process or as marker of state anxiety. Overall, the status of the HPA system may be indicative on individual level for treatment outcome in AD. In line with this, the present article summarized the findings related to cortisol as HPA axis marker in response to psychotherapy, particularly exposure-based CBT, that focusses on cognitive processes and avoidance behavior. As was pointed out in reviews before (Fischer and Zilcha-Mano, 2022; Laufer et al., 2018) due to the wide range of different cortisol sampling and analysis protocols, the studies existing so far are too heterogeneous for meta-analysis. Further, previous reviews reported only briefly on the specific topic of dynamics in the HPA system in regard to explicit CBT techniques and its implications as well as in context of a broader focus regarding therapy methods, psychiatric disorders, and assessed hormones and other biomarkers (Bandelow et al., 2017; Elnazer and Baldwin, 2014; Fischer and Zilcha-Mano, 2022; Laufer et al., 2018; Łoś and Waszkiewicz, 2021) or had a narrower focus – for example only prediction (e.g. Fischer and Cleare, 2017) not change. Additionally, several recently published studies were not included. Thus, this is the first review that focuses in detail on HPA system assessment with cortisol levels measurement before, during and after exposure-based CBT in AD patients. Here, we emphasize the dynamics over time and factors specific for CBT that may have an effect. First, we examined studies using just one baseline measurement of cortisol predicting response. Second, examinations during exposure are summarized and third, studies with pre/post assessments are reviewed.

2. Methods

The sources for the current review were journal articles. Methods were based on the checklist of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009). The inclusion criteria were studies examining the HPA axis, cortisol respectively, in all varieties of sampling methods (saliva, blood, hair) in patients with an anxiety disorder before, during, and after treatment with CBT techniques. English and German experimental studies published until April 2024 were considered. Articles using other psychotherapy methods (e.g. psychodynamic) or examining healthy subjects were excluded. Several steps were taken to identify usable studies. First, we went through the citations list of all relevant reviews mentioned here to identify studies that examined related topics. Next, a literature search was conducted using the database MEDLINE/PubMed (https://www.ncbi.nlm.nih.gov/pubmed/), PsycINFO (American Psychological Association), and Web of Science. The search strategy using MeSH (Medical Subject Headings) was based on the following different combinations of keywords: cortisol, hypothalamic-pituitary-adrenal axis or system, dexamethasone, behavior therapy (BT), cognitive therapy (CT), cognitive behavior therapy, exposure therapy and the anxiety disorders PD/AG, SAD, GAD as well as specific phobias. Also, the references of retrieved studies were screened in search of additional articles that met the topic. We repeated this process until no further study could be found. In total, 29 studies have been identified that investigated cortisol as marker for HPA system function within exposure-based CBT or related interventions which due to the narrative nature and range of the review were all included. The examinations were grouped by study designs: a) just one baseline measure of cortisol, b) examinations during exposure and c) studies with pre/post measure. For an overview and determination of type and quality, study details were collected by the first author and sorted by type of anxiety disorder, patient sample, treatment characteristics, cortisol measure and results.

3. Results

3.1. Baseline cortisol levels and stress reactivity as predictor of CBT outcome

Cortisol as HPA system biomarker with a basal measurement before treatment has been evaluated as outcome predictor for CBT in AD; studies are summarized in Table 1. Short-term salivary or plasma basal cortisol levels were not found to predict CBT response in AD in a meta-analysis (Fischer and Cleare, 2017) containing data of 6 studies also described below (Brand et al., 2011; Dierckx et al., 2012; Lass-Hennemann and Michael, 2014; Meuret et al., 2014; Siegmund et al., 2011; see Table 1). Other investigations showed an association of an attenuated cortisol stress response in PD patients to a stress task with CBT non-response in regard to agoraphobic avoidance behavior (Wichmann, Kirschbaum, Lorenz, et al., 2017) and in children with AD only with less decrease in depressive symptoms but not anxiety at one year follow up (Dieleman et al., 2016). Non-suppression to the Dex/CRH test, hypo-responsiveness of the HPA system to CRH respectively, was shown to predict an unfavorable CBT outcome in PD patients (Wichmann et al., 2018). One recent study demonstrated higher baseline cortisol plasma levels in 21 pharmacotherapy- and comorbidity-free PD patients to be significantly associated with a worse CBT outcome, especially in female participants (Masdrakis et al., 2021). However, the study included only a small number of PD patients having different phases of disease, which limits the generalizability of the findings. One study observed higher hair cortisol concentrations as a predictor of improved therapy response in a mixed sample of 89 depressive and AD patients, while lower cortisol levels might reflect a failure to raise sufficient cortisol responses over the course of treatment (Fischer et al., 2018). In another study in 36 spider phobic individuals hair and saliva cortisol at baseline did not predict CBT outcome (Steudte-Schmiedgen et al., 2021). In summary, results regarding basal and stress-exposure related cortisol as predictor of CBT outcome are inconsistent but point to a detrimental effect of insufficient HPA system responsiveness.

3.2. Cortisol dynamics during exposure sessions

Some studies examined cortisol levels directly before, during and after exposure to phobic situations with inconsistent results regarding cortisol reactivity: they either found elevated levels or no response (Abelson and Curtis, 1989; Alpers et al., 2003; Curtis et al., 1976; Curtis et al., 1978; Woods et al., 1987; see Table 1). In an earlier investigation with 10 animal phobic women, elevated cortisol response to an exposure session decreased in the second session, though not significantly (Nesse et al., 1985). A study in 15 patients with spider phobia receiving one to three exposure sessions found increased levels of cortisol compared to healthy controls as well as a normalization over treatment (Gaab et al., 2005). Interestingly, these psychobiological effects were mediated by changes of cognitive appraisal. Concordantly, Abelson et al. (2005) showed that a short cognitive intervention (9 Minutes) to reduce novelty, increase cognitive coping and providing a sense of control prior to stimulation with pentagastrin (experimental panicogenic compound) reduced blood cortisol as well as ACTH levels and anxiety responses of PD patients compared to no intervention. Siegmund et al. (2011) showed, that a blunted cortisol reaction of PD patients at repeated exposures despite high anxiety levels was related to a worse therapeutic outcome. Additionally, Meuret et al. (2014) found that absolute cortisol levels over a period of several hours in patients with PD/AG moderated the extinction of panic, fear and avoidance. In this study, elevated cortisol response to exposure was associated with better outcome. Higher pre-exposure levels and CAR had a positive impact on outcome. In contrast, when elevated on a “normal” control day, higher CAR was associated with poorer outcome. They point out, that anticipation of a demanding task and an according adaptive process of the HPA system seems to be advantageous. However, conclusions from this study are

Table 1
Summary of studies investigating associations between HPA system variation and course of symptoms in AD treated with cognitive and/or exposure therapy.

Studies with baseline cortisol levels and stress reactivity as predictor of CBT outcome

Authors	Diagnosis	Sample	Treatment	Measures	Results	Study Quality[a]	Effect size
Wichmann et al. (2017)	PD±AG	N = 28 (20 women, age: M=35.71 ± 13.18 yrs.) N = 32 matched HC (21 women, age: M=34.66 ± 12.07 yrs.)	CBT (five weeks individual and group sessions)	Plasma cortisol and ACTH before and during the TSST (within first two weeks of therapy) VAS, PASA, MI, BSQ, ACQ, BDI	Decreased cortisol concentrations in PD patients in comparison to HC and association of attenuated cortisol stress response with CBT non-response (avoidance behaviour)	+++	r = −0.345 to −0.396
Dieleman et al. (2016)	AD	N = 152 children (age: 8–12 yrs.)	CBT (10 child sessions and 4 separate parent sessions)	Salivary cortisol prior and post a stress task at baseline, SCL, HRV MASC, CDI	Lower cortisol reactivity predicted less decrease in depressive symptoms at one-year follow-up	+++	R^2_{change} = .03
Wichmann et al. (2018)	PD±AG	N = 34 patients (23 women; age: M=35.50 ± 12.74) N = 34 matched HC (25 women; age: M=33.82 ± 12.50)	CBT (five weeks individual and group sessions)	Cortisol and ACTH during DEX/CRH test at baseline PAS, ACQ, BSQ, MI	Patients with inadequate cortisol suppression showed less improvement in agoraphobic cognitions after CBT	+++	r = 0.489
Masdrakis et al. (2021)	PD±AG	N = 21 (16 women; age; M=35 yrs; medication-free)	CBT (8 weekly sessions)	Plasma cortisol and ACTH at baseline SCL−90; CGI, ACQ, MI	Non responders to CBT as compared to responders demonstrated significantly higher cortisol and ACTH basal plasma concentrations	++	-
Fischer et al. (2018)	AD, MDD	N = 89 (93 % women; age: M=34)	CBT	Hair cortisol at baseline CTQ, PHQ−9, GAD−7	Non-responders in terms of anxiety (48 %) had lower pre-treatment hair cortisol as well as higher levels of trauma	+++	η^2= .079
Steudte-Schmiedgen et al. (2021)	Spider Phobia	N = 36 (26 women; age: M=25)	ET (1 session)	Hair and saliva cortisol and cortisone at baseline SAS, FSQ, BAT, EAST	No association of cortisol and treatment response	+++	-

Studies with cortisol assessment during exposure sessions

Authors	Diagnosis	Sample	Treatment	Measures	Results	Study Quality[a]	Effect size
Abelson and Curtis (1989)	Height Phobia	N = 2 patients (men; age: 19 and 34 yrs.)	ET (17 and 9 sessions)	Plasma cortisol and norepinephrine, HR before and during ET sessions SUD	Both subjects showed rising cortisol responses and stable, non-extinguishing norepinephrine responses to height exposure	+	-
Alpers et al. (2003)	Driving Phobia	N = 11 patients (women; age: M=48.4 ± 9.4 yrs.) N = 13 matched HC (age: M=48.6 ± 98.6 yrs.)	ET (3 driving sessions) within 7–15 days	Salivary cortisol before, during, and after exposure Driving Self-efficacy Inventory, SUD, FQ, STAI	Phobics had significantly greater cortisol response scores during driving exposure and during quiet sitting periods before and afterward exposure	++	d= 1.14 to d= 1.29 (exposures 1 and 2)
Curtis et al. (1976)	Physical Objects Phobia	N = 7	2 ET sessions (3 and 4), 3 control sessions (1, 2 and 5) in the early evening	Plasma cortisol 10 times (20-minute intervals) during sessions SUD	Anxiety elevation and successful ET effect, but no cortisol response	+	-
Curtis et al. (1978)	Physical Objects Phobia	N = 6 (5 women; age: 23–52)	2 ET sessions (3 and 4), 3 control sessions (1, 2 and 5) in the morning	Plasma cortisol 10 times (20-minute intervals) during sessions SUD	Moderate elevations of plasma cortisol above control levels in some subjects	+	-
Woods et al. (1987)	PD±AG	N = 18 patients (14 women, age: M=40.5 yrs.; medication: placebo 4 weeks prior exposure) N = 13 matched HC	CBT (weekly group therapy and in-vivo exposure)	HR, BP, plasma free MHPG, growth hormone, and prolactin levels before, during, and after exposure session HAMA, CGI, PASS, VAS	No significant differences in cortisol between groups	++	-
Nesse et al. (1985)	Animal Phobia	N = 10 (women; age: 25–43 yrs.) N = 15 controls	2 ET sessions (2 and 3), 2 control sessions (1 and 4)	Plasma cortisol 10 times (20 minute intervals) during sessions, pulse, BP, epinephrine, norepinephrine, growth hormone, insulin, glucagon, and pancreatic	Almost all variables increased during anxiety/ ET. Cortisol response was significantly elevated during ET and decreased (n.s.) to second exposure session	++	-

(continued on next page)

Table 1 (*continued*)

Studies with cortisol assessment during exposure sessions

Authors	Diagnosis	Sample	Treatment	Measures	Results	Study Quality[a]	Effect size
				polypeptide SUD, STAI			
Gaab et al. (2005)	Spider Phobia	N = 15 (14 women; age: M=26.3, 20–40 yrs.) N = 15 controls	ET (3 sessions)	7 saliva cortisol samples and HR before and during ET SUD, FSQ, SPQ, STAI, avoidance behaviour	Significantly increased levels of cortisol compared to controls and heart rate during exposure normalized significantly over sessions	+++	Increase: f^2= 0.56; Reduction: f^2= 0.78
Abelson et al. (2005)	PD±AG	N = 14 Patients (9 men; age: M=27.5 ± 5.78 yrs.) N = 14 matched HC	9-minute cognitive intervention before pharmacological panicogenic stimulation for half the group (randomly assigned)	Blood cortisol and ACTH before and after pharmacological activation (pentagastrin) Panic symptom intensity, VAS	The cognitive intervention significantly reduced cortisol and ACTH levels, despite pentagastrin's robust stimulation of both hormones. Patients' exaggerated anxiety responses to pentagastrin were normalized by the intervention	++	-
Siegmund et al. (2011)	PD±AG	N = 10 (6 women; age: M=36.9 ± 9.7 yrs.; 3 medicated) N = 10 matched HC	CBT including 3 in-vivo exposures	Plasma cortisol and ACTH before, during and after exposure PAS, MI, BAI	Low concentration of cortisol during exposure was moderately correlated with poorer therapeutic outcome	++	mean r = −0.49
Meuret et al. (2014)	PD±AG	N = 26 (88.5 % women; age: M=33.1 ± 9.12 yrs.; >3 months stable medication=65.4 %)	ET (3 weekly sessions + 2 months follow-up session)	Salivary CAR and cortisol prior, during and after exposure; control day MI, BSQ, ASI	Higher absolute cortisol levels during exposures moderated clinical improvement. Greater morning rises in cortisol on exposure day predicted greater treatment gains, but greater rises on the control day were associated with poorer outcomes	++	-
Lass-Hennemann (2014)	Spider Phobia	N = 60 (women; age: M=25.40, 18–50 yrs.)	ET (one 3 h session)	Hair and saliva cortisol before, during and after ET in the morning (8 am group) vs. in the evening (6 pm group) SUD, FSQ, BAT before, 1 week after and 3 months after ET	Cortisol was significantly higher in the morning group. Patients treated in the morning showed significantly less fear in the BAT after treatment and at follow up	+++	Cortisol difference: partial η^2= .71
Sopp et al. (2024)	Snake Phobia	N = 71 (60 women; age: M=22.3, 18–40 yrs.)	ET (one-hour video session)	Sleep quality (actigraphy), vigilance, salivary CAR and cortisol before, during and after ET in the morning (8 am group) vs. in the evening (6 pm group); EDA, ECG SNAQ, STAI, ASI, PSQI, BDI-II, rMEQ, PHQ-D, BAT, PVT, BAT before, 1 and 4 weeks after ET	Cortisol levels were not found to predict treatment outcome. Higher vigilance was found to be correlated with lower post and follow-up snake anxiety. Pre-exposure sleep efficiency moderated follow-up decrease in snake anxiety	+++	-
Meuret et al. (2016)	PD±AG	N = 26 (87.5 % women; age: M=32.4 ± 9.1 yrs.; >3 months stable medication=62.5 %)	ET (3 weekly sessions + 2 months follow-up session)	Salivary cortisol prior, during and after exposure MI, ACS, PDSS and ASI	Earlier sessions were associated with higher pre-exposure cortisol levels and in turn greater clinical improvement	++	ds> 1.05 and ds> 0.95
Wintermann et al. (2022)	PD±AG	N = 20 patients (12 women; age: M=29.8, 22.7–43.3) N = 20 matched HC (8 women; age: M=24.1, 22.0–28.9)	CBT	Salivary cortisol and HR before and after interoceptive exposure (low intensity exercise) PAS, ACQ, BSQ, MI	Cortisol decreased over time, especially in male patients.Significant negative correlation of cortisol response with therapy outcome	++	η^2= .177 and r = −.554 to −.738
Kuhlman et al. (2020)	SAD	N = 60 (58.3 % women)	Virtual reality ET (7 sessions)	Scopolamine-augmentation or placebo during sessions. 4 saliva cortisol samples during sessions 1, 4, 7, and a post-treatment extinction test LSAS	Elevated endogenous in-session cortisol during exposure sessions was associated with less symptom improvement from pre- to post-treatment and at 1-month follow-up	+++	Fear: d= 3.27 and avoidance: d= 1.66

Studies with cortisol assessment pre/post CBT

Authors	Diagnosis	Sample	Treatment	Measures	Results	Study Quality[a]	Effect size
Brand et al. (2011)	Mask Phobia	N = 46 patients (male army recruits; age: M=19.46 ± 1.64) N = 39 HC (age: M=19.69 ± 1.54)	CBT (two-day intensive treatment course)	Salivary CAR before and after CBT; cortisol prior, during and after exposure in the afternoon and control day STAI, GAS	Cortisol secretion was significantly higher in patients and decreased after therapy	+ +	AUC total: d= 0.86 AUC basal: d= 0.43 AUC net: d= 0.66
Hemyari et al. (2020)	Rat Phobia	N = 40 students (women; age: M=20.97, 18–24 yrs.)	One- (n = 20) and multi-session (n = 20) exposure based CBT	Plasma cortisol, pro-inflammatory cytokines, and interleukin−6 at baseline and during a BAT before and after CBT DPSS-R, FRQ, STAI, BAT	Cortisol levels and cytokines were significantly reduced after CBT in both types of treatment	++	-
Tafet et al. (2005)	GAD	N = 17 outpatients (9 women; age: M=42.4 ± 8.5 yrs.), N = 8 waitlist controls (4 women)	CT (24 sessions), no medication	Plasma cortisol before and after CT or after 24 weeks in the morning and afternoon HAMA	Significant decrease in afternoon cortisol levels along decrease in anxiety symptoms	++	-
Wang et al. (2013)	GAD, MDD, OCD	N = 54 patients (30 women; age: M=29.31 ± 9.78 yrs.) N = 55 medicated controls (29 women; age: M=30.95 ± 10.18 yrs.)	CT (attribution retraining group therapy, 8 sessions/weeks) or SSRI	Plasma cortisol in the morning before and after treatment HAMA, HAMD	Patients in the CT group had significantly lower plasma cortisol concentrations compared to baseline	+++	-
Dierckx et al. (2012)	AD	N = 116 children and adolescents (male ≈ 50 %; age: 8–16 yrs.; no medication)	CBT (10 child sessions and 4 separate parent sessions)	Salivary CAR and daytime cortisol before, 3 months and 1 year after treatment MASC	Compared to baseline, one-year follow-up high daytime cortisol production was associated with persistence of symptoms, high CAR with remission	++	-
Tiller et al. (1988)	GAD	N = 15 patients (8 men; age: M=38, 25–65 yrs.; 8 DST suppressors, 7 matched non suppressors), N = 13 matched HC (5 suppressors, 8 non suppressors)	BT (5 weeks)	DST 1 week before and 1 week after treatment HAMA, STAI	After treatment all patients were DST suppressors, but dexamethasone concentrations remained significantly lower in initial non-suppressors	+	Non-suppressors DST 1 × 2: r = 0.964
Faucher et al. (2016)	SAD	N = 13 CBT (38.5 % women; age: M=39.31 ± 10.40 yrs.) N = 14 MBSR (35.7 % women; age: M=36.64 ± 16.15 yrs.) N = 30 HC (36.7 % women; age: M=29.77 ± 12.77 yrs.)	Group CBT and MBSR (12 weeks)	6 saliva cortisol samples and HRV during a speech task before and after treatment (HC only baseline) LSAS-T, SIS, SPS, VAS	No change in physiological measurers	++	-
Asbrand et a. (2019)	SAD	N = 65 children (WL: N = 26; female=63,6 %; age: M=11.3, 9–13 yrs.) N = 55 HC (female=60 %; age: M=11.7, 9–13 yrs.)	Group CBT (12 weeks) and waitlist	6 saliva samples during the TSST (cortisol and alpha amylase) before and after CBT or no treatment SPAI-C	Children with SAD in the waitlist group showed stronger cortisol reactivity and a higher cortisol response rate compared to children in the CBT group after treatment	+++	d= 0.71–0.74

AD = anxiety disorders; PD±AG = panic disorder with or without agoraphobia; GAD = generalized anxiety disorder; SAD = social anxiety disorder; MDD = major depressive disorder; OCD = obsessive-compulsive disorder; HC = healthy controls; CBT = cognitive behavioural therapy; ET = exposure therapy; CT = cognitive therapy; BT = behavioural therapy; MBSR = mindfulness-based stress reduction; SSRI = selective serotonin reuptake inhibitor; ACTH = adrenocorticotropic hormone; BP = blood pressure; CAR = cortisol awakening response; ECG = electrocardiography; EDA = electrodermal activity; HR = heart rate; HRV = heart rate variability; MHPG = 3-Methoxy-4-Hydroxyphenylglycol; SCL = skin conductance level; BAT = behavioral avoidance test; SUD = subjective units of distress; VAS = visual analog scale ranging from 0 (no fear) to 10; CGI = Clinical Global Impressions-Improvement; GAS = Goal Attainment Scale; SCL-90 = Symptom Checklist–90; ASI = Anxiety Sensitivity Index; BAI = Beck Anxiety Inventory; HAMA = Hamilton Anxiety Rating Scale; STAI = State-Trait Anxiety Inventory; MASC = Multidimensional Anxiety Scale for Children; ACS = Anxiety Control Scale; FQ = Fear Questionnaire; FRQ = Fear of Rat Questionnaire; FSQ = Fear of Spiders Questionnaire; SAS = Spider Anxiety Screening; SPQ = Spider Phobia Questionnaire; SNAQ = Snake Questionnaire; LSAS = Liebowitz Social Anxiety Scale; SIS = Social Interaction Scale, SPAI-C = Social Phobia and Anxiety Inventory for Children; SPS = Social Phobia Scale; TSST = Trier Social Stress Test; ACQ = Agoraphobic Cognitions Questionnaire; BSQ = Body Sensations Questionnaire; MI = Mobility Inventory; PAS = Panic and Agoraphobia Scale; PASS = Panic Attack Symptom Scale; PDSS = Panic Disorder Severity Scale; GAD = Generalized Anxiety Disorder Scale; PASA = Primary Appraisal and Secondary Appraisal Questionnaire; BDI = Beck Depression Inventory; HAMD = Hamilton Depression Scale; CDI = Children's Depression Inventory; CTQ = Childhood Trauma Questionnaire; PHQ = Patient Health Questionnaire; DPSS-R = Disgust Propensity and Sensitivity Scale-Revised; EAST = Extrinsic Affective Simon Task; PSQI = Pittsburgh Sleep Quality Index; rMEQ = reduced Morningness-Eveningness-Questionnaire; PVT = Psychomotor Vigilance Task; r = Pearson's correlation; d = Cohen's D; AUC = area under the time curve; f^2 = multivariate f^2: small = 0.02, moderate = 0.15, large = 0.35; η^2: small ~.01, medium ~.06, large .> .14.

+ = weak
++ = moderate
+++ = strong

[a] according to Quality Assessment Tool for Quantitative Studies (Armijo-Olivo et al., 2012; Thomas et al., 2004).

limited by concomitant psychopharmacological treatment of some patients, especially intake of benzodiazepines, different time schedules for exposure sessions and cortisol measurements, not considering the circadian related cortisol secretion variability. Interestingly, another study compared the effects of one-session exposure in the morning (high endogenous cortisol) to exposure in the evening (low endogenous cortisol) in 60 spider phobic patients (Lass-Hennemann and Michael, 2014). Assessment of anxiety symptoms before the therapy, one week as well as three months after CBT showed that treatment in the morning was significantly more effective than in the evening. A recent study in 71 patients with snake phobia did not replicate these findings despite higher cortisol levels in the morning group, but found that rather vigilance and sleep quality play a role as moderators of pre-post changes in fear reduction (Sopp et al., 2024), which points to more complex diurnal effects on exposure therapy. In the above mentioned study with 10 animal phobic women, response to an exposure session in the morning also showed an overall higher cortisol level than in the evening, though increase from naturally lower levels was much higher in the evening; but blunted cortisol responses might be anticipated when levels are already elevated (Nesse et al., 1985). Consistently, in a study with 24 PD/AG patients receiving 72 exposure sessions (3 per week), earlier sessions were associated with higher pre-exposure cortisol levels, which mediated the effect of time of day on greater clinical improvement (Meuret et al., 2016). Similarly, in a study with 20 PD/AG patients and 20 matched healthy controls, a higher symptom severity before and unfavorable therapeutic outcome after CBT, were associated with a significantly lower cortisol response under interoceptive exposure (Wintermann et al., 2022). Whether higher cortisol concentrations during exposure sessions in AD patients are associated with better treatment outcomes remained unclear in the beforementioned meta-analysis by Fischer and Cleare (2017). In a study of Kuhlman et al. (2020) with SAD patients, elevated endogenous in-session cortisol during ET predicted attenuated symptom improvement. The authors state that their cortisol indices represent rather chronically elevated cortisol with little variation during ET since less than one third of the samples exhibited at least a 20 % increase in cortisol during exposure sessions and no significant change across the trial (Kuhlman et al., 2020). Taken together, these findings emphasize the probable detrimental effect of sustained elevations in cortisol levels for treatment outcome, compared to acute increase and thus, beneficial responsiveness.

3.3. *CBT effects on cortisol levels*

The studies assessing the relationship between CBT effects and HPA system function in AD before, during and after intervention are summarized in Table 1. A few studies investigated changes in cortisol levels and response after CBT treatment in AD: Brand et al. (2011) investigated army recruits with protective mask phobia. They found heightened basal morning salivary cortisol levels compared to controls as well as the association of a significant reduction in afternoon cortisol levels during exposure to the phobic stimulus with symptom reduction after a two-day intensive treatment course of CBT. Similar, a study investigating responses to one- and multi-session exposure-based therapy in rat phobia, showed a reduction in cortisol levels during a behavioral avoidance test conducted pre and after successful treatment (Hemyari et al., 2020). Tafet et al. (2005) showed that a significant decrease in symptom severity after CBT in 17 GAD patients was associated with a significant reduction in elevated afternoon plasma cortisol levels, while plasma cortisol in an untreated group remained high. Comparable, in a study of Wang et al. (2013) 19 GAD patients treated with attribution retraining group therapy for 8 weeks had significantly lower morning plasma cortisol concentrations compared to baseline and in contrast to a medicated group (SSRI; $N = 55$). Further, a prospective study showed that remission of children with AD one year after CBT was associated with lower daytime cortisol and that low CAR levels were associated with a poorer outcome at one year follow up (Dierckx et al., 2012). Additionally, Tiller et al. (1988) found in a sample of 15 non-medicated GAD patients that non suppressors in the DST compared to suppressors at baseline had an expected normalized suppression after 5 weeks of behavioral therapy. Regarding SAD, a study investigating effects of CBT versus MBSR treatment on stress reaction to the TSST found no changes in cortisol levels after treatment (Faucher et al., 2016). Another study in children with SAD comparing a CBT group to waitlist controls showed that in children who had not received treatment a repeated social stress task led to a sensitization and stronger cortisol reactivity, concluding that a potential vulnerability to stress-related diseases, possibly leading to an enhanced cortisol stress response, might be improved with CBT (Asbrand et al., 2019). Taken together, some evidence suggests that successful CBT induces normalization of an aberrant HPA system.

4. Discussion

We reviewed studies investigating the potential use of hormones related to the endogenic stress system as biological marker for the outcome of CBT in anxiety disorders. We focused on studies assessing basal levels and specifically before, during and after exposures sessions as well as changes in cortisol levels over the course of CBT and beyond. In summary, despite the methodological weaknesses, heterogeneity of study protocols, and mostly preliminary results, findings indicate on the one hand a detrimental effect of insufficient HPA system responsiveness regarding basal and stress-exposure related cortisol as predictor of CBT outcome as well as a beneficial effect of acute cortisol elevation, with most indicative parameters of morning cortisol response and increase before and during exposure. On the other hand, monitoring cortisol levels may function as biomarker for CBT effects, since effective treatment seems to normalize HPA system abnormalities. Moreover, findings imply that cognitive processes which are an essential part of CBT may play an important role in modulating the HPA system.

First, chronic dysregulation of the HPA system function, persistently elevated cortisol concentrations and hyporeactivity to phobic situations, possibly due to neuroendocrine adaptation processes in response to prolonged stress or due to the disorder itself, indicates a less favorable outcome of CBT in patients with AD and thus, represents a possible causal and maintaining factor. A blunted response to acute stress is associated with poorer outcome across studies in AD using CBT (Meuret et al., 2014; Siegmund et al., 2011), except for SAD, where elevated levels during exposure may be detrimental or less beneficial to outcome than in other AD (Asbrand et al., 2019; Kuhlman et al., 2020), but studies are small and conclusions are preliminary. Also, this may represent chronically elevated cortisol levels and is contradicted by a recent study with psychodynamic psychotherapy (Schmalbach et al., 2024). Following this, heightened cortisol might be beneficial by weakening the originally learned fear memory and consolidating a newly formed fear-inhibitory memory. The *memory reconsolidation hypothesis* proposes that every time the original fear memory is retrieved by a brief memory reactivation it becomes unstable through induced plasticity that opens up a transient period during which a memory becomes susceptible to disruption and can be modified (impaired, enhanced, or updated), which allows for transition into a fear-inhibitory memory (Dudai, 2006; Meir Drexler et al., 2015; Monfils et al., 2009). Investigations showed that reminding of a task ahead and encouraging anticipation or presenting a fear activating stimulus before, successful exposure can lead to persistent attenuation of conditioned fear (Johnson and Casey, 2015; Maples-Keller et al., 2017). Moreover, the impairing effect of glucocorticoids on reconsolidation is supported by animal extinction studies (Cai et al., 2006; Yang et al., 2006; Zohar et al., 2011). However, the occurrence of spontaneous recovery, renewal, rapid acquisition, and reinstatement after extinction, suggests that it does not seem to change the original fear memory (Bouton and Todd, 2014; Myers and Davis, 2002; Todd et al., 2014), which is corroborated by the inhibitory learning approach (Craske et al., 2014). Concurring, Fischer and Zilcha-Mano (2022) review that in phobias low cortisol during initial exposure sessions seem to facilitate retrieval of fear memories and to impede the formation of extinction memories, resulting in suboptimal treatment outcomes. Some studies have already investigated the potential use for pharmacological enhancement of exposure effects with glucocorticoids and various other substances (Singewald et al., 2014). Investigations augmenting exposure by cortisol administration found a significantly greater reduction in fear until follow up applying glucocorticoids prior to, but not post, ET (de Quervain et al., 2011; Fitzgerald et al., 2014; For a review see Hofmann et al., 2015; Nakataki et al., 2017; Raeder et al., 2019; For a review see Singewald et al., 2014; Soravia et al., 2006; Soravia et al., 2014; Steudte-Schmiedgen et al., 2021). Two recent studies with spider phobia patients showed a fear reducing effect of cortisol administration due to improving an aberrant functional connectivity of the amygdala (Nakataki et al., 2017) and restoration of the salience network activity (Soravia et al., 2018) when exposed to phobia-related stimuli. Results are encouraging but have to be further evaluated. Furthermore, an enhancing effect of cortisol on reconsolidation of the reactivated original fear memory was shown if this former memory is updated (Meir Drexler et al., 2015). This would explain the reinforcement of anxiety through fear confirming actions like avoidance and safety behavior of anxiety patients, ineffective exposure and lacking disprove of fears, respectively. Additionally, in a situation of high stress, cortisol may impair the retrieval of the new acquired extinction memory and to promote relapses and the return of fear after successful extinction learning by increasing activation of the fear network (Hagedorn et al., 2021). In Summary, not only discordant experience by the use of CBT may play a role in overcoming fear, but, despite the known impairing effect of a chronically elevated cortisol level on memory, acute stress before and during ET sessions, an activated HPA system respectively, interpreted as a flexible and adaptive response to fear-related situations, seems to be favorable for the extinction learning during fear disconfirming ET (Meuret et al., 2016), and hence a decreased cortisol response after.

Second, effective treatment seems to normalize HPA system abnormalities in AD patients which was found on different levels. First, a decrease in an elevated cortisol response over recurrent exposure sessions or repeated BATs was found in several studies (Brand et al., 2011; Gaab et al., 2005; Hemyari et al., 2020; Lass-Hennemann and Michael, 2014). Second, a few studies showed a decrease in elevated basal cortisol levels after treatment (Dierckx et al., 2012; Tafet et al., 2005; Wang et al., 2013). In this, the cortisol awakening response also plays a role with inconsistent findings whether it should be high (Dierckx et al., 2012) or low (Brand et al., 2011; Meuret et al., 2014) for beneficial therapy response, and which is probably depending on the upcoming day's demands and anticipation of it. Third, one study showed a normalization of cortisol hyporesponsiveness after CBT in the DST (Tiller et al., 1988). Taken together, cortisol appears to be one possible indicator of CBT effects on anxiety symptoms, specifically heightened basal and daytime cortisol levels before treatment as well as its dynamics in response to fear related stimuli and dexamethasone stimulation.

Moreover, the reviewed studies imply that cognitive processes, e.g. appraisal, attribution, perceived controllability, and coping, seem to have a beneficial influence on adaptive HPA system functioning and related cortisol changes (Abelson et al., 2005; Gaab et al., 2005; Tafet et al., 2005; Wang et al., 2013). Cortisol seems to be heightened during anticipating of a demanding task (Coplan et al., 1998; Meuret et al., 2014), e.g. exposure to a feared situation, whereas a more positive anticipatory cognitive stress appraisal and regulation seems to decrease the cortisol response to stress stimuli (Pulopulos et al., 2019). Further, distracting GAD patients from their anxious thoughts by refocusing the attention was demonstrated to reduce acute cortisol levels (Rosnick et al., 2013). Mayer et al., (2017) showed in a phobic fear exposure model that actual and perceived control was the determining factor for lower cortisol levels. In line with the inhibitory learning model (Craske et al., 2014), flexible responses, e.g. metacognitions, to environmental stimuli may facilitate extinction by inhibiting a conditioned reaction (Arch and Abramowitz, 2015). However, as explained above, an adaptive response of the HPA system to demands seems to be required. An investigation of the association of momentary emotion regulation and diurnal cortisol in depression and anxiety showed that current internalizing disorders seem to be associated with downregulated HPA axis functioning, while engaging in problem solving was associated with elevated cortisol reactivity (Gilbert et al., 2017). Similar, a recent longitudinal study showed that higher physiological symptoms in children with anxiety symptoms were related to hypercortisolism during the day and in contrast, that chronic worry and social concerns predicted hypocortisolism with a blunted diurnal cortisol pattern (Ma et al., 2019). Comparable, an increase in cortisol activity in older patients in remission of GAD, but not with PD and phobias, and a decrease in cortisol activity in those with persistent anxiety was found, which may be linked to symptoms of constant worry (Bakouni et al., 2020). This fits with findings in GAD with lower hair cortisol concentrations compared with healthy controls (Steudte et al., 2011). Thus, dysfunctional cognitive coping strategies might explain reduced cortisol response in chronic anxiety states, compared to acute exposure to a fear-related stimulus and adaptive HPA response. These results underscore the importance of cognitive factors in explaining HPA functioning and improvement of anxiety symptomatology, which are an essential part of CBT.

4.1. Conclusion and future directions

In summary, cortisol levels may have the potential to serve as biomarker for current anxiety severity and effectiveness of treatment. However, the interpretation of the results from currently available studies are clearly limited by small sample sizes, diverse type of cortisol assessment (e.g. salivary, plasma, hair) and methods to assess the HPA system function (e.g. cortisol awakening response, diurnal decline, stress response), different experimental conditions (e.g. time of day, natural or induced panic attacks, type of stressor), differentially performed ratings (self-ratings, therapist-ratings, specific vs. general anxiety symptoms) and treatment procedures (e.g. group, individual, virtual reality), including the combination with medication, different duration of treatment (e.g. 1–24 sessions) and follow up periods. Further variables like childhood adversity, biological (e.g. sex, genes, medication, oral contraceptives, etc.) or diurnal moderators (e.g. sleep efficiency, arousal, chronotype), duration of the disorder and comorbidity with other psychiatric disorders may contribute to inconsistencies and should be considered. For instance, smoking status, depression, especially female gender and the use of the contraceptive pill were shown to be related to a mainly blunted HPA axis reactivity (Bakouni et al., 2020; Kische et al., 2021; Wintermann et al., 2016).

In conclusion and in line with Laufer et al. (2018), the measurement of the global HPA system function, CAR, diurnal slope, acute and long-term cumulative cortisol output on consecutive days as well as before, during and after exposure in CBT might be useful to monitor the disease status, severity, and treatment effect along the CBT depending on the effect that is investigated. Moreover, differential approaches may be beneficial for the improvement in different states of the AD as well as for specific AD: reducing stress response to a fear-related stimulus or chronically elevated cortisol levels in the initial phase of an AD and activating/normalizing a downregulated, exhausted HPA system in persistent AD, like GAD and SAD, through exposure and cognitive techniques and possibly glucocorticoid augmentation. Importantly, cognitive processes and coping strategies, such as appraisal, sense of control, focus of attention, vigilance and self-monitoring, as well as avoidance behavior during the CBT may mediate the differential effects on the HPA system and should be included in future investigations. Finally, further reviews may include other forms of psychotherapy and determine common or different mechanisms of actions.

Based on the evidence available to date, we deduce the following recommendations for future studies to clarify the role of cortisol in specific AD and on CBT effects: 1) assessment of CAR, daily cortisol and hair cortisol before and after the psychotherapeutic intervention including at minimum one follow up after 3 month to measure hair cortisol levels, 2) assessment of cortisol before and after the exposure intervention by keeping the time of measurement comparable within and between the individuals, 3) ensuring diagnostic properties and specifically the comorbidity of AD and depression, 4) applying a global and AD-specific assessment of anxiety dimensions and of the therapy-related effects, such as cognitive processes, 5) assessing first onset and duration of AD symptoms as well as childhood adversity as putative moderating factor of HPA system response, 6) correcting for medication use, sex and age, and 7) considering further putative moderating variables on exposure, such as sleep quality. As HPA system abnormalities seem not to be a general pattern of AD, within subject-analyses could help to better identify those who most profit from a priori and concomitant HPA system measurements related to clinical outcomes to improve decision making for clinicians in terms of best fitting therapy.

CRediT authorship contribution statement

Angelika Erhardt-Lehmann: Writing – review & editing, Supervision. **Jennifer Lange:** Writing – original draft, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abelson, J.L., Curtis, G.C., 1989. Cardiac and neuroendocrine responses to exposure therapy in height phobics: desynchrony within the 'physiological response system. Behav. Res Ther. 27 (5), 561–567.

Abelson, J.L., Curtis, G.C., 1996. Hypothalamic-pituitary-adrenal axis activity in panic disorder: prediction of long-term outcome by pretreatment cortisol levels. Am. J. Psychiatry 153 (1), 69–73.

Abelson, J.L., Liberzon, I., Young, E.A., Khan, S., 2005. Cognitive modulation of the endocrine stress response to a pharmacological challenge in normal and panic disorder subjects. Arch. Gen. Psychiatry 62 (6), 668–675. https://doi.org/10.1001/archpsyc.62.6.668.

Adam, E.K., Vrshek-Schallhorn, S., Kendall, A.D., Mineka, S., Zinbarg, R.E., Craske, M.G., 2014. Prospective associations between the cortisol awakening response and first onsets of anxiety disorders over a six-year follow-up–2013 Curt Richter Award Winner. Psychoneuroendocrinology 44, 47–59. https://doi.org/10.1016/j.psyneuen.2014.02.014.

Alpers, G.W., Abelson, J.L., Wilhelm, F.H., Roth, W.T., 2003. Salivary cortisol response during exposure treatment in driving phobics. Psychosom. Med 65 (4), 679–687.

Altamura, M., Iuso, S., Balzotti, A., Francavilla, G., Dimitri, A., Cibelli, G., Bellomo, A., Petito, A., 2018. Salivary alpha-amylase and cortisol responsiveness to stress in first episode, drug-naive patients with panic disorder. Neurosci. Res. https://doi.org/10.1016/j.neures.2018.03.003.

Andreano, J.M., Cahill, L., 2006. Glucocorticoid release and memory consolidation in men and women. Psychol. Sci. 17 (6), 466–470. https://doi.org/10.1111/j.1467-9280.2006.01729.x.

Arch, J.J., Abramowitz, J.S., 2015. Exposure therapy for obsessive–compulsive disorder: an optimizing inhibitory learning approach. J. Obsessive-Compuls. Relat. Disord. 6, 174–182. https://doi.org/10.1016/j.jocrd.2014.12.002.

Armijo-Olivo, S., Stiles, C.R., Hagen, N.A., Biondo, P.D., Cummings, G.G., 2012. Assessment of study quality for systematic reviews: a comparison of the cochrane collaboration risk of bias tool and the effective public health practice project quality assessment tool: methodological research. J. Eval. Clin. Pr. 18 (1), 12–18. https://doi.org/10.1111/j.1365-2753.2010.01516.x.

Asbrand, J., Heinrichs, N., Nitschke, K., Wolf, O.T., Schmidtendorf, S., Tuschen-Caffier, B., 2019. Repeated stress leads to enhanced cortisol stress response in child social anxiety disorder but this effect can be prevented with CBT. Psychoneuroendocrinology 109, 104352. https://doi.org/10.1016/j.psyneuen.2019.06.003.

Bakouni, H., Ouimet, M.C., Forget, H., Vasiliadis, H.M., 2020. Temporal patterns of anxiety disorders and cortisol activity in older adults. J. Affect Disord. 277, 235–243. https://doi.org/10.1016/j.jad.2020.08.020.

Bandelow, B., Allgulander, C., Baldwin, D.S., Costa, D., Denys, D., Dilbaz, N., Domschke, K., Eriksson, E., Fineberg, N.A., Hättenschwiler, J., Hollander, E., Kaiya, H., Karavaeva, T., Kasper, S., Katzman, M., Kim, Y.K., Inoue, T., Lim, L., Masdrakis, V., Zohar, J., 2022. World federation of societies of biological psychiatry (WFSBP) guidelines for treatment of anxiety, obsessive-compulsive and posttraumatic stress disorders - Version 3. Part I: anxiety disorders. World J. Biol. Psychiatry 1–39. https://doi.org/10.1080/15622975.2022.2086295.

Bandelow, B., Baldwin, D., Abelli, M., Altamura, C., Dell'Osso, B., Domschke, K., Fineberg, N.A., Grunblatt, E., Jarema, M., Maron, E., Nutt, D., Pini, S., Vaghi, M.M., Wichniak, A., Zai, G., Riederer, P., 2016. Biological markers for anxiety disorders, OCD and PTSD - a consensus statement. Part I: neuroimaging and genetics. World J. Biol. Psychiatry 17 (5), 321–365. https://doi.org/10.1080/15622975.2016.1181783.

Bandelow, B., Baldwin, D., Abelli, M., Bolea-Alamanac, B., Bourin, M., Chamberlain, S. R., Cinosi, E., Davies, S., Domschke, K., Fineberg, N., Grunblatt, E., Jarema, M., Kim, Y.K., Maron, E., Masdrakis, V., Mikova, O., Nutt, D., Pallanti, S., Pini, S., Riederer, P., 2017. Biological markers for anxiety disorders, OCD and PTSD: a consensus statement. Part II: neurochemistry, neurophysiology and neurocognition. World J. Biol. Psychiatry 18 (3), 162–214. https://doi.org/10.1080/15622975.2016.1190867.

Bandelow, B., Wedekind, D., Sandvoss, V., Broocks, A., Hajak, G., Pauls, J., Peter, H., Ruther, E., 2000. Diurnal variation of cortisol in panic disorder. Psychiatry Res 95 (3), 245–250.

Binder, E.B., Nemeroff, C.B., 2010. The CRF system, stress, depression and anxiety-insights from human genetic studies. Mol. Psychiatry 15 (6), 574–588. https://doi.org/10.1038/mp.2009.141.

Bouton, M.E., Todd, T.P., 2014. A fundamental role for context in instrumental learning and extinction. Behav. Process. 104, 13–19. https://doi.org/10.1016/j.beproc.2014.02.012.

Brand, S., Annen, H., Holsboer-Trachsler, E., Blaser, A., 2011. Intensive two-day cognitive-behavioral intervention decreases cortisol secretion in soldiers suffering from specific phobia to wear protective mask. J. Psychiatr. Res 45 (10), 1337–1345. https://doi.org/10.1016/j.jpsychires.2011.04.010.

Cai, W.H., Blundell, J., Han, J., Greene, R.W., Powell, C.M., 2006. Postreactivation glucocorticoids impair recall of established fear memory. J. Neurosci. 26 (37), 9560–9566. https://doi.org/10.1523/jneurosci.2397-06.2006.

Caldiroli, A., Capuzzi, E., Affaticati, L.M., Surace, T., Di Forti, C.L., Dakanalis, A., Clerici, M., Buoli, M., 2023. Candidate biological markers for social anxiety disorder: a systematic review. Int J. Mol. Sci. 24 (1). https://doi.org/10.3390/ijms24010835.

Carpenter, J.K., Andrews, L.A., Witcraft, S.M., Powers, M.B., Smits, J.A.J., Hofmann, S. G., 2018. Cognitive behavioral therapy for anxiety and related disorders: a meta-analysis of randomized placebo-controlled trials. Depress Anxiety 35 (6), 502–514. https://doi.org/10.1002/da.22728.

Coplan, J.D., Goetz, R., Klein, D.F., Papp, L.A., Fyer, A.J., Liebowitz, M.R., Davies, S.O., Gorman, J.M., 1998. Plasma cortisol concentrations preceding lactate-induced panic. Psychological, biochemical, and physiological correlates. Arch. Gen. Psychiatry 55 (2), 130–136.

Coryell, W., Noyes Jr., R., Reich, J., 1991. The prognostic significance of HPA-axis disturbance in panic disorder: a three-year follow-up. Biol. Psychiatry 29 (2), 96–102.

Craske, M.G., Treanor, M., Conway, C.C., Zbozinek, T., Vervliet, B., 2014. Maximizing exposure therapy: an inhibitory learning approach. Behav. Res Ther. 58, 10–23. https://doi.org/10.1016/j.brat.2014.04.006.

Cuijpers, P., Gentili, C., Banos, R.M., Garcia-Campayo, J., Botella, C., Cristea, I.A., 2016. Relative effects of cognitive and behavioral therapies on generalized anxiety disorder, social anxiety disorder and panic disorder: a meta-analysis. J. Anxiety Disord. 43, 79–89. https://doi.org/10.1016/j.janxdis.2016.09.003.

Curtis, G., Buxton, M., Lippman, D., Nesse, R., Wright, J., 1976. "Flooding in vivo" during the circadian phase of minimal cortisol secretion: anxiety and therapeutic success without adrenal cortical activation. Biol. Psychiatry 11 (1), 101–107.

Curtis, G.C., Nesse, R., Buxton, M., Lippman, D., 1978. Anxiety and plasma cortisol at the crest of the circadian cycle:reappraisal of a classical hypothesis. Psychosom. Med 40 (5), 368–378. https://doi.org/10.1097/00006842-197808000-00002.

Dieleman, G.C., Huizink, A.C., Tulen, J.H., Utens, E.M., Tiemeier, H., 2016. Stress reactivity predicts symptom improvement in children with anxiety disorders. J. Affect Disord. 196, 190–199. https://doi.org/10.1016/j.jad.2016.02.022.

Dierckx, B., Dieleman, G., Tulen, J.H., Treffers, P.D., Utens, E.M., Verhulst, F.C., Tiemeier, H., 2012. Persistence of anxiety disorders and concomitant changes in cortisol. J. Anxiety Disord. 26 (6), 635–641. https://doi.org/10.1016/j.janxdis.2012.04.001.

Dudai, Y., 2006. Reconsolidation: the advantage of being refocused. Curr. Opin. Neurobiol. 16 (2), 174–178. https://doi.org/10.1016/j.conb.2006.03.010.

Elnazer, H.Y., Baldwin, D.S., 2014. Investigation of cortisol levels in patients with anxiety disorders: a structured review. Curr. Top. Behav. Neurosci. https://doi.org/10.1007/7854_2014_299.

Elnazer, H.Y., Lau, L.C.K., Amaro, H., Baldwin, D.S., 2021. Hair cortisol concentration in anxiety disorders: exploration of relationships with symptom severity and inflammatory markers. Acta Neuropsychiatr. 33 (2), 104–110. https://doi.org/10.1017/neu.2020.35.

Elzinga, B.M., Spinhoven, P., Berretty, E., de Jong, P., Roelofs, K., 2010. The role of childhood abuse in HPA-axis reactivity in social anxiety disorder: a pilot study. Biol. Psychol. 83 (1), 1–6. https://doi.org/10.1016/j.biopsycho.2009.09.006.

Erhardt, A., Ising, M., Unschuld, P.G., Kern, N., Lucae, S., Putz, B., Uhr, M., Binder, E.B., Holsboer, F., Keck, M.E., 2006. Regulation of the hypothalamic-pituitary-adrenocortical system in patients with panic disorder. Neuropsychopharmacology 31 (11), 2515–2522. https://doi.org/10.1038/sj.npp.1301168.

Faucher, J., Koszycki, D., Bradwejn, J., Merali, Z., Bielajew, C., 2016. Effects of CBT versus MBSR treatment on social stress reactions in social anxiety disorder. Mindfulness 7 (2), 514–526. https://doi.org/10.1007/s12671-015-0486-4.

Fischer, S., Cleare, A.J., 2017. Cortisol as a predictor of psychological therapy response in anxiety disorders-systematic review and meta-analysis. J. Anxiety Disord. 47, 60–68. https://doi.org/10.1016/j.janxdis.2017.02.007.

Fischer, S., King, S., Papadopoulos, A., Hotopf, M., Young, A.H., Cleare, A.J., 2018. Hair cortisol and childhood trauma predict psychological therapy response in depression and anxiety disorders. Acta Psychiatr. Scand. 138 (6), 526–535. https://doi.org/10.1111/acps.12970.

Fischer, S., Zilcha-Mano, S., 2022. Why does psychotherapy work and for whom? hormonal answers. Biomedicines 10 (6). https://doi.org/10.3390/biomedicines10061361.

Fitzgerald, P.J., Seemann, J.R., Maren, S., 2014. Can fear extinction be enhanced? A review of pharmacological and behavioral findings. Brain Res Bull. 105, 46–60. https://doi.org/10.1016/j.brainresbull.2013.12.007.

Gaab, J., Jucker, P., Staub, F., Ehlert, U., 2005. Mind over matter. Z. F. üR. Klin. Psychol. und Psychother. 34 (2), 121–132. https://doi.org/10.1026/1616-3443.34.2.121.

Gilbert, K., Mineka, S., Zinbarg, R.E., Craske, M.G., Adam, E.K., 2017. Emotion regulation regulates more than emotion: associations of momentary emotion regulation with diurnal cortisol in current and past depression and anxiety. Clin. Psychol. Sci. 5 (1), 37–51. https://doi.org/10.1177/2167702616654437.

Grace, C., Heinrichs, M., Koval, P., Gorelik, A., von Dawans, B., Terrett, G., Rendell, P., Labuschagne, I., 2022. Concordance in salivary cortisol and subjective anxiety to the trier social stress test in social anxiety disorder. Biol. Psychol. 175, 108444. https://doi.org/10.1016/j.biopsycho.2022.108444.

Hagedorn, B., Wolf, O.T., Merz, C.J., 2021. Stimulus-based extinction generalization: neural correlates and modulation by cortisol. Int J. Neuropsychopharmacol. 24 (4), 354–365. https://doi.org/10.1093/ijnp/pyaa085.

Hans, E., Hiller, W., 2013. A meta-analysis of nonrandomized effectiveness studies on outpatient cognitive behavioral therapy for adult anxiety disorders. Clin. Psychol. Rev. 33 (8), 954–964. https://doi.org/10.1016/j.cpr.2013.07.003.

Heim, C., Ehlert, U., Hellhammer, D.H., 2000. The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. Psychoneuroendocrinology 25 (1), 1–35.

Hek, K., Direk, N., Newson, R.S., Hofman, A., Hoogendijk, W.J.G., Mulder, C.L., Tiemeier, H., 2013. Anxiety disorders and salivary cortisol levels in older adults: a population-based study. Psychoneuroendocrinology 38 (2), 300–305. https://doi.org/10.1016/j.psyneuen.2012.06.006.

Hemyari, C., Dolatshahi, B., Sahraian, A., Koohi-Hosseinabadi, O., Zomorodian, K., 2020. Evaluation of the effectiveness of one- and multi-session exposure-based treatments in reducing biological and psychological responses to rat phobia among students. Psychol. Res Behav. Manag 13, 665–679. https://doi.org/10.2147/prbm.s256781.

Heuser, I., Yassouridis, A., Holsboer, F., 1994. The combined dexamethasone/CRH test: a refined laboratory test for psychiatric disorders. J. Psychiatr. Res 28 (4), 341–356.

Hofmann, S.G., Mundy, E.A., Curtiss, J., 2015. Neuroenhancement of exposure therapy in anxiety disorders. AIMS Neurosci. 2 (3), 123–138. https://doi.org/10.3934/Neuroscience.2015.3.123.

Hofmann, S.G., Smits, J.A., 2008. Cognitive-behavioral therapy for adult anxiety disorders: a meta-analysis of randomized placebo-controlled trials. J. Clin. Psychiatry 69 (4), 621–632.

Hood, A., Pulvers, K., Spady, T.J., Kliebenstein, A., Bachand, J., 2014. Anxiety mediates the effect of acute stress on working memory performance when cortisol levels are high: a moderated mediation analysis. Anxiety Stress Coping 1–37. https://doi.org/10.1080/10615806.2014.1000880.

Ising, M., Hohne, N., Siebertz, A., Parchmann, A.M., Erhardt, A., Keck, M., 2012. Stress response regulation in panic disorder. Curr. Pharm. Des. 18 (35), 5675–5684. https://doi.org/10.2174/138161212803530880.

Joëls, M., Baram, T.Z., 2009. The neuro-symphony of stress. Nat. Rev. Neurosci. 10 (6), 459–466. https://doi.org/10.1038/nrn2632.

Johnson, D.C., Casey, B.J., 2015. Easy to remember, difficult to forget: the development of fear regulation. Dev. Cogn. Neurosci. 11, 42–55. https://doi.org/10.1016/j.dcn.2014.07.006.

Kirschbaum, C., Pirke, K.M., Hellhammer, D.H., 1993. The 'trier social stress test'–a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 28 (1-2), 76–81.

Kische, H., Ollmann, T.M., Voss, C., Hoyer, J., Rückert, F., Pieper, L., Kirschbaum, C., Beesdo-Baum, K., 2021. Associations of saliva cortisol and hair cortisol with generalized anxiety, social anxiety, and major depressive disorder: an epidemiological cohort study in adolescents and young adults. Psychoneuroendocrinology 126, 105167. https://doi.org/10.1016/j.psyneuen.2021.105167.

Klumbies, E., Braeuer, D., Hoyer, J., Kirschbaum, C., 2014. The reaction to social stress in social phobia: Discordance between physiological and subjective parameters. PLoS One 9 (8), e105670. https://doi.org/10.1371/journal.pone.0105670.

Krämer, M., Seefeldt, W.L., Heinrichs, N., Tuschen-Caffier, B., Schmitz, J., Wolf, O.T., Blechert, J., 2012. Subjective, autonomic, and endocrine reactivity during social stress in children with social phobia. J. Abnorm Child Psychol. 40 (1), 95–104. https://doi.org/10.1007/s10802-011-9548-9.

Kuhlman, K.R., Treanor, M., Imbriano, G., Craske, M.G., 2020. Endogenous in-session cortisol during exposure therapy predicts symptom improvement: preliminary results from a scopolamine-augmentation trial. Psychoneuroendocrinology 116, 104657. https://doi.org/10.1016/j.psyneuen.2020.104657.

Lass-Hennemann, J., Michael, T., 2014. Endogenous cortisol levels influence exposure therapy in spider phobia. Behav. Res Ther. 60c, 39–45. https://doi.org/10.1016/j.brat.2014.06.009.

Laufer, S., Engel, S., Knaevelsrud, C., Schumacher, S., 2018. Cortisol and alpha-amylase assessment in psychotherapeutic intervention studies: a systematic review. Neurosci. Biobehav Rev. 95, 235–262. https://doi.org/10.1016/j.neubiorev.2018.09.023.

Liston, C., Cichon, J.M., Jeanneteau, F., Jia, Z., Chao, M.V., Gan, W.-B., 2013. Circadian glucocorticoid oscillations promote learning-dependent synapse formation and maintenance [Article]. Nat. Neurosci. 16 (6), 698–705. https://doi.org/10.1038/nn.3387. ⟨http://www.nature.com/neuro/journal/v16/n6/abs/nn.3387.html#supplementary-information⟩.

Łoś, K., Waszkiewicz, N., 2021. Biological markers in anxiety disorders. J. Clin. Med. 10 (8). https://doi.org/10.3390/jcm10081744.

Lupien, S.J., Fiocco, A., Wan, N., Maheu, F., Lord, C., Schramek, T., Tu, M.T., 2005. Stress hormones and human memory function across the lifespan. Psychoneuroendocrinology 30 (3), 225–242. https://doi.org/10.1016/j.psyneuen.2004.08.003.

Ma, D., Serbin, L.A., Stack, D.M., 2019. How children's anxiety symptoms impact the functioning of the hypothalamus-pituitary-adrenal axis over time: a cross-lagged panel approach using hierarchical linear modeling. Dev. Psychopathol. 31 (1), 309–323. https://doi.org/10.1017/s0954579417001870.

Maples-Keller, J.L., Price, M., Jovanovic, T., Norrholm, S.D., Odenat, L., Post, L., Zwiebach, L., Breazeale, K., Gross, R., Kim, S.J., Rothbaum, B.O., 2017. Targeting memory reconsolidation to prevent the return of fear in patients with fear of flying. Depress Anxiety. https://doi.org/10.1002/da.22626.

Masdrakis, V.G., Legaki, E.M., Papageorgiou, C., Markianos, M., 2021. Stress hormones as predictors of response to cognitive behavior therapy in panic disorder. Neuropsychobiology 1–10. https://doi.org/10.1159/000514073.

Mayer, S.E., Snodgrass, M., Liberzon, I., Briggs, H., Curtis, G.C., Abelson, J.L., 2017. The psychology of HPA axis activation: examining subjective emotional distress and control in a phobic fear exposure model. Psychoneuroendocrinology 82, 189–198. https://doi.org/10.1016/j.psyneuen.2017.02.001.

Meir Drexler, S., Merz, C.J., Hamacher-Dang, T.C., Tegenthoff, M., Wolf, O.T., 2015. Effects of cortisol on reconsolidation of reactivated fear memories. Neuropsychopharmacology. https://doi.org/10.1038/npp.2015.160.

Meuret, A.E., Rosenfield, D., Bhaskara, L., Auchus, R., Liberzon, I., Ritz, T., Abelson, J.L., 2016. Timing matters: Endogenous cortisol mediates benefits from early-day psychotherapy. Psychoneuroendocrinology 74, 197–202. https://doi.org/10.1016/j.psyneuen.2016.09.008.

Meuret, A.E., Trueba, A.F., Abelson, J.L., Liberzon, I., Auchus, R., Bhaskara, L., Ritz, T., Rosenfield, D., 2014. High cortisol awakening response and cortisol levels moderate exposure-based psychotherapy success. Psychoneuroendocrinology 51c, 331–340. https://doi.org/10.1016/j.psyneuen.2014.10.008.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G., The, P.G., 2009. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLOS Med. 6 (7), e1000097. https://doi.org/10.1371/journal.pmed.1000097.

Monfils, M.H., Cowansage, K.K., Klann, E., LeDoux, J.E., 2009. Extinction-reconsolidation boundaries: key to persistent attenuation of fear memories. Science 324 (5929), 951–955. https://doi.org/10.1126/science.1167975.

Myers, K.M., Davis, M., 2002. Behavioral and neural analysis of extinction. Neuron 36 (4), 567–584.

Nakataki, M., Soravia, L.M., Schwab, S., Horn, H., Dierks, T., Strik, W., Wiest, R., Heinrichs, M., de Quervain, D.J., Federspiel, A., Morishima, Y., 2017. Glucocorticoid administration improves aberrant fear-processing networks in spider phobia. Neuropsychopharmacology 42 (2), 485–494. https://doi.org/10.1038/npp.2016.207.

Nemeroff, C.B., Vale, W.W., 2005. The neurobiology of depression: inroads to treatment and new drug discovery. J. Clin. Psychiatry 66 (7), 5–13.

Nesse, R.M., Curtis, G.C., Thyer, B.A., McCann, D.S., Huber-Smith, M.J., Knopf, R.F., 1985. Endocrine and cardiovascular responses during phobic anxiety. Psychosom. Med 47 (4), 320–332. https://doi.org/10.1097/00006842-198507000-00002.

Petrowski, K., Herold, U., Joraschky, P., Wittchen, H.U., Kirschbaum, C., 2010. A striking pattern of cortisol non-responsiveness to psychosocial stress in patients with panic disorder with concurrent normal cortisol awakening responses. Psychoneuroendocrinology 35 (3), 414–421. https://doi.org/10.1016/j.psyneuen.2009.08.003.

Petrowski, K., Schmalbach, I., Strunk, A., Hoyer, J., Kirschbaum, C., Joraschky, P., 2021. Cortisol reactivity in social anxiety disorder: a highly standardized and controlled study. Psychoneuroendocrinology 123, 104913. https://doi.org/10.1016/j.psyneuen.2020.104913.

Petrowski, K., Wintermann, G.B., Kirschbaum, C., Bornstein, S.R., 2012. Dissociation between ACTH and cortisol response in DEX-CRH test in patients with panic disorder. Psychoneuroendocrinology 37 (8), 1199–1208. https://doi.org/10.1016/j.psyneuen.2011.12.013.

Petrowski, K., Wintermann, G.B., Schaarschmidt, M., Bornstein, S.R., Kirschbaum, C., 2013. Blunted salivary and plasma cortisol response in patients with panic disorder under psychosocial stress. Int J. Psychophysiol. 88 (1), 35–39. https://doi.org/10.1016/j.ijpsycho.2013.01.002.

Plag, J., Schumacher, S., Schmid, U., Ströhle, A., 2013. Baseline and acute changes in the HPA system in patients with anxiety disorders: the current state of research. https://doi.org/10.2217/npy.13.6. https://doi.org/10.2217/npy.13.6.

Pruessner, J.C., Wolf, O.T., Hellhammer, D.H., Buske-Kirschbaum, A., von Auer, K., Jobst, S., Kaspers, F., Kirschbaum, C., 1997. Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. Life Sci. 61 (26), 2539–2549. https://doi.org/10.1016/s0024-3205(97)01008-4.

Pulopulos, M.M., Baeken, C., De Raedt, R., 2019. Cortisol response to stress: the role of expectancy and anticipatory stress regulation. Horm. Behav., 104587 https://doi.org/10.1016/j.yhbeh.2019.104587.

de Quervain, D.J.F., Aerni, A., Schelling, G., Roozendaal, B., 2009. Glucocorticoids and the regulation of memory in health and disease. Front. Neuroendocrinol. 30 (3), 358–370. https://doi.org/10.1016/j.yfrne.2009.03.002.

de Quervain, D.J.F., Bentz, D., Michael, T., Bolt, O.C., Wiederhold, B.K., Margraf, J., Wilhelm, F.H., 2011. Glucocorticoids enhance extinction-based psychotherapy. Proc. Natl. Acad. Sci. USA 108 (16), 6621–6625. https://doi.org/10.1073/pnas.1018214108.

Raeder, F., Merz, C.J., Tegenthoff, M., Wolf, O.T., Margraf, J., Zlomuzica, A., 2019. Post-exposure cortisol administration does not augment the success of exposure therapy: a randomized placebo-controlled study. Psychoneuroendocrinology 99, 174–182. https://doi.org/10.1016/j.psyneuen.2018.09.015.

Roelofs, K., van Peer, J., Berretty, E., Jong, P., Spinhoven, P., Elzinga, B.M., 2009. Hypothalamus-pituitary-adrenal axis hyperresponsiveness is associated with increased social avoidance behavior in social phobia. Biol. Psychiatry 65 (4), 336–343. https://doi.org/10.1016/j.biopsych.2008.08.022.

Rosnick, C.B., Rawson, K.S., Butters, M.A., Lenze, E.J., 2013. Association of cortisol with neuropsychological assessment in older adults with generalized anxiety disorder. Aging Ment. Health 17 (4), 432–440. https://doi.org/10.1080/13607863.2012.761673.

Schmalbach, I., Witthöft, M., Strauß, B., Joraschky, P., Petrowski, K., 2024. The predictive value of cortisol in psychodynamic psychotherapy for social anxiety disorder: extended results of the SOPHONET-Study. Transl. Psychiatry 14 (1), 188. https://doi.org/10.1038/s41398-024-02882-3.

Siegmund, A., Koster, L., Meves, A.M., Plag, J., Stoy, M., Strohle, A., 2011. Stress hormones during flooding therapy and their relationship to therapy outcome in patients with panic disorder and agoraphobia. J. Psychiatr. Res. 45 (3), 339–346. https://doi.org/10.1016/j.jpsychires.2010.07.002.

Singewald, N., Schmuckermair, C., Whittle, N., Holmes, A., Ressler, K.J., 2014. Pharmacology of cognitive enhancers for exposure-based therapy of fear, anxiety and trauma-related disorders. Pharm. Ther. https://doi.org/10.1016/j.pharmthera.2014.12.004.

Sopp, M.R., Schäfer, S.K., Michael, T., Equit, M., Ferreira de Sá, D.S., Lass-Hennemann, J., 2024. Endogenous cortisol levels, sleep or vigilance: which factors contribute to better exposure therapy outcomes in the morning? Cogn. Ther. Res. 48 (4), 704–719. https://doi.org/10.1007/s10608-023-10463-9.

Soravia, L.M., Heinrichs, M., Aerni, A., Maroni, C., Schelling, G., Ehlert, U., Roozendaal, B., de Quervain, D.J., 2006. Glucocorticoids reduce phobic fear in humans. Proc. Natl. Acad. Sci. USA 103 (14), 5585–5590. https://doi.org/10.1073/pnas.0509184103.

Soravia, L.M., Heinrichs, M., Winzeler, L., Fisler, M., Schmitt, W., Horn, H., Dierks, T., Strik, W., Hofmann, S.G., de Quervain, D.J., 2014. Glucocorticoids enhance in vivo exposure-based therapy of spider phobia. Depress Anxiety 31 (5), 429–435. https://doi.org/10.1002/da.22219.

Soravia, L.M., Schwab, S., Weber, N., Nakataki, M., Wiest, R., Strik, W., Heinrichs, M., de Quervain, D., Federspiel, A., 2018. Glucocorticoid administration restores salience network activity in patients with spider phobia. Depress Anxiety. https://doi.org/10.1002/da.22806.

Spiga, F., Walker, J.J., Gupta, R., Terry, J.R., Lightman, S.L., 2015. 60 years of neuroendocrinology: glucocorticoid dynamics: insights from mathematical, experimental and clinical studies. J. Endocrinol. 226 (2), T55–T66. https://doi.org/10.1530/joe-15-0132.

Springer, K.S., Levy, H.C., Tolin, D.F., 2018. Remission in CBT for adult anxiety disorders: a meta-analysis. Clin. Psychol. Rev. 61, 1–8. https://doi.org/10.1016/j.cpr.2018.03.002.

Steudte, S., Stalder, T., Dettenborn, L., Klumbies, E., Foley, P., Beesdo-Baum, K., Kirschbaum, C., 2011. Decreased hair cortisol concentrations in generalised anxiety disorder. Psychiatry Res 186 (2-3), 310–314. https://doi.org/10.1016/j.psychres.2010.09.002.

Steudte-Schmiedgen, S., Fay, E., Capitao, L., Kirschbaum, C., Reinecke, A., 2021. Hydrocortisone as an adjunct to brief cognitive-behavioural therapy for specific fear: endocrine and cognitive biomarkers as predictors of symptom improvement. J. Psychopharmacol. 35 (6), 641–651. https://doi.org/10.1177/02698811211001087.

Surget, A., Belzung, C., 2022. Adult hippocampal neurogenesis shapes adaptation and improves stress response: a mechanistic and integrative perspective. Mol. Psychiatry 27 (1), 403–421. https://doi.org/10.1038/s41380-021-01136-8.

Tafet, G.E., Feder, D.J., Abulafia, D.P., Roffman, S.S., 2005. Regulation of hypothalamic-pituitary-adrenal activity in response to cognitive therapy in patients with generalized anxiety disorder. Cogn. Affect Behav. Neurosci. 5 (1), 37–40.

Thomas, B.H., Ciliska, D., Dobbins, M., & Micucci, S. (2004). A Process for Systematically Reviewing the Literature: Providing the Research Evidence for Public Health Nursing Interventions [https://doi.org/10.1111/j.1524-475X.2004.04006.x]. *Worldviews on Evidence-Based Nursing*, 1(3), 176-184. https://doi.org/https://doi.org/⟨10.1111/j.1524-475X.2004.04006.x⟩.

Tiller, J.W., Biddle, N., Maguire, K.P., Davies, B.M., 1988. The dexamethasone suppression test and plasma dexamethasone in generalized anxiety disorder. Biol. Psychiatry 23 (3), 261–270.

Todd, T.P., Vurbic, D., Bouton, M.E., 2014. Behavioral and neurobiological mechanisms of extinction in Pavlovian and instrumental learning. Neurobiol. Learn Mem. 108, 52–64. https://doi.org/10.1016/j.nlm.2013.08.012.

Uher, R., 2008. The implications of gene-environment interactions in depression: will cause inform cure? Mol. Psychiatry 13 (12), 1070–1078. https://doi.org/10.1038/mp.2008.92.

Vinkers, C.H., Kuzminskaite, E., Lamers, F., Giltay, E.J., Penninx, B., 2021. An integrated approach to understand biological stress system dysregulation across depressive and anxiety disorders. J. Affect Disord. 283, 139–146. https://doi.org/10.1016/j.jad.2021.01.051.

Vreeburg, S.A., Hoogendijk, W.J., DeRijk, R.H., van Dyck, R., Smit, J.H., Zitman, F.G., Penninx, B.W., 2013. Salivary cortisol levels and the 2-year course of depressive and anxiety disorders. Psychoneuroendocrinology 38 (9), 1494–1502. https://doi.org/10.1016/j.psyneuen.2012.12.017.

Walker, J.J., Spiga, F., Waite, E., Zhao, Z., Kershaw, Y., Terry, J.R., Lightman, S.L., 2012. The origin of glucocorticoid hormone oscillations. PLoS Biol. 10 (6), e1001341. https://doi.org/10.1371/journal.pbio.1001341.

Wang, C., Zhang, N., Zhang, Y.L., Zhang, J., Yang, H., Timothy, T.C., 2013. Comparison of the neurobiological effects of attribution retraining group therapy with those of selective serotonin reuptake inhibitors. Braz. J. Med Biol. Res 46 (3), 318–326.

van West, D., Claes, S., Sulon, J., Deboutte, D., 2008. Hypothalamic-pituitary-adrenal reactivity in prepubertal children with social phobia. J. Affect. Disord. 111 (2), 281–290. https://doi.org/10.1016/j.jad.2008.03.006.

Wichmann, S., Bornstein, S.R., Lorenz, T., Petrowski, K., 2018. Stress hormone response to the DEX-CRH test and its relation to psychotherapy outcome in panic disorder patients with and without agoraphobia. Transl. Psychiatry 8 (1), 37. https://doi.org/10.1038/s41398-017-0081-7.

Wichmann, S., Kirschbaum, C., Bohme, C., Petrowski, K., 2017. Cortisol stress response in post-traumatic stress disorder, panic disorder, and major depressive disorder patients. Psychoneuroendocrinology 83, 135–141. https://doi.org/10.1016/j.psyneuen.2017.06.005.

Wichmann, S., Kirschbaum, C., Lorenz, T., Petrowski, K., 2017. Effects of the cortisol stress response on the psychotherapy outcome of panic disorder patients. Psychoneuroendocrinology 77, 9–17. https://doi.org/10.1016/j.psyneuen.2016.11.030.

Wingenfeld, K., Wolf, O.T., 2014. Stress, memory, and the hippocampus. Front Neurol. Neurosci. 34, 109–120. https://doi.org/10.1159/000356423.

Wintermann, G.B., Kirschbaum, C., Petrowski, K., 2016. Predisposition or side effect of the duration: the reactivity of the HPA-axis under psychosocial stress in panic disorder. Int J. Psychophysiol. 107, 9–15. https://doi.org/10.1016/j.ijpsycho.2016.06.008.

Wintermann, G.B., Noack, R., Steudte-Schmiedgen, S., Weidner, K., 2022. Cortisol response under low intensity exercise during cognitive-behavioral therapy is associated with therapeutic outcome in panic disorder-an exploratory study. PLoS One 17 (9), e0273413. https://doi.org/10.1371/journal.pone.0273413.

Woods, S.W., Charney, D.S., McPherson, C.A., Gradman, A.H., Heninger, G.R., 1987. Situational panic attacks. Behavioral, physiologic, and biochemical characterization. Arch. Gen. Psychiatry 44 (4), 365–375.

Yang, Y.L., Chao, P.K., Lu, K.T., 2006. Systemic and intra-amygdala administration of glucocorticoid agonist and antagonist modulate extinction of conditioned fear. Neuropsychopharmacology 31 (5), 912–924. https://doi.org/10.1038/sj.npp.1300899.

Zohar, J., Yahalom, H., Kozlovsky, N., Cwikel-Hamzany, S., Matar, M.A., Kaplan, Z., Yehuda, R., Cohen, H., 2011. High dose hydrocortisone immediately after trauma may alter the trajectory of PTSD: interplay between clinical and animal studies. Eur. Neuropsychopharmacol. 21 (11), 796–809. https://doi.org/10.1016/j.euroneuro.2011.06.001.

Appendix B – Publication II: DNA Methylation signatures in panic disorder

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"DNA Methylation signatures in panic disorder"

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Abstract

Panic disorder (PD) affects about four million Europeans, with women affected twice as likely as men, causing substantial suffering and high economic costs. The etiopathogenesis of PD remains largely unknown, but both genetic and environmental factors contribute to risk. An epigenome-wide association study (EWAS) was conducted to compare medication-free PD patients ($n = 89$) with healthy controls ($n = 76$) stratified by gender. Replication was sought in an independent sample (131 cases, 169 controls) and functional analyses were conducted in a third sample ($N = 71$). DNA methylation was assessed in whole blood using the Infinium HumanMethylation450 BeadChip. One genome-wide association surviving FDR of 5% (cg07308824, $P = 1.094 \times 10\text{-}7$, P-adj = 0.046) was identified in female PD patients ($N = 49$) compared to controls ($N = 48$). The same locus, located in an enhancer region of the *HECA* gene, was also hypermethylated in female PD patients in the replication sample ($P = 0.035$) and the significance of the association improved in the meta-analysis (P-adj = 0.004). Methylation at this CpG site was associated with *HECA* mRNA expression in another independent female sample ($N = 71$) both at baseline ($P = 0.046$) and after induction by dexamethasone ($P = 0.029$). Of 15 candidates, 5 previously reported as associated with PD or anxiety traits also showed differences in DNA methylation after gene-wise correction and included *SGK1*, *FHIT*, *ADCYAP1*, *HTR1A*, *HTR2A*. Our study examines epigenome-wide differences in peripheral blood for PD patients. Our results point to possible sex-specific methylation changes in the *HECA* gene for PD but overall highlight that this disorder is not associated with extensive changes in DNA methylation in peripheral blood.

Introduction

Panic disorder (PD) is the most disabling anxiety disorder, causing substantial suffering, and high economic and social costs. It affects about four million Europeans (12-month prevalence estimate is about 2%) with women being twice as likely to be affected as men[1]. PD is characterized by sudden episodes of acute anxiety (panic attacks) occurring without any apparent reason. It can be accompanied by a persistent concern of having additional attacks or worry about the possible consequences of the attacks (e.g., suffering of a heart attack, dying, losing control) and significant behavioral changes to avoid future panic attacks[2]. First onset for PD is in adolescence and early adulthood[2] and it is highly co-morbid with other mental disorders, especially agoraphobia[3].

Despite the substantial long-term disability, PD appears to be underdiagnosed and undertreated in mental health settings. The overall heritability of PD is substantial with heritability estimates up to 48%[4]. Genetic studies (genome-wide as well as candidate gene approaches) have identified some loci possibly contributing to disease risk such as variants in the *TMEM132D* locus[5, 6], *COMT*[7], *CRHR1*[8], *SLC6A4*, *MAOA*, *HTR1A*[9], but overall genetic linkage or association signals are usually not consistently replicated.

Exposure to negative life events are the complementing risk factors to genetics so that both genetic and environmental components are likely important mechanisms

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influencing disease risk[10]. The differential contribution of environmental and genetic factors in risk for anxiety disorders, personality disorders, alcohol use disorders has been previously supported by epidemiologic and twin studies[11–13]. Adverse life events have been shown to associate with specific epigenetic modifications, such as DNA methylation, which may mediate the lasting cellular consequences of these exposures in psychiatric disorders[14], also in the context of gene–environment interactions[15]. Exploring DNA methylation may thus be able to give an integrated view of both environmental and genetic risk factors. While epigenetic changes including DNA methylation are mainly tissue specific, some sites show cross tissue relevance[16, 17] and furthermore changes in peripheral tissues such as blood could serve as potential biomarker for disease risk.

To date, a few studies have investigated differences in DNA methylation in candidate genes in PD[5, 18–21]. Recently, one epigenome-wide study of DNA methylation in a relatively small Japanese PD case-control sample (48 patients vs. 48 controls) detected significant associations at 40 sites with overall small methylation differences[22]. So far, studies in the European population are lacking. To perform such a study in two independent samples of patients with PD vs. control was the aim of our study. To reduce confounding due to effects of drug treatment, both patients and controls were free of psychotropic medication. Given that both the prevalence of PD as well as DNA methylation pattern show large gender differences[23], a gender-stratified analysis was undertaken and complemented by a meta-analysis.

Previous studies report that hits identified in genome-wide association studies (GWAS) show changes in DNA methylation in peripheral blood, e.g., in schizophrenia[24] or bipolar disorder[25]. For this reason, in addition to an unbiased approach, we also investigated DNA methylation changes in candidate genes that have emerged from genome-wide or candidate gene studies for PD, anxiety disorders or anxiety related phenotypes either in humans or animals[5,26, 27].

PD has a high comorbidity rate not only with other psychiatric disorders like agoraphobia and depression, but also with other medical conditions, e.g., cardiovascular disorders, asthma and epilepsy[1]. DNA methylation age has been previously correlated with morbidity and mortality[28–31]. Therefore, we also investigated whether age acceleration was occurring in PD patients compared to controls.

Materials and Methods

Max Planck Institute of Psychiatry PD cohort

PD patients included in the discovery and replication sample were recruited in the anxiety disorders outpatient unit at the MPIP in Munich[5]. PD was the primary diagnosis; mild secondary depression was allowed (Table 1). The diagnosis was ascertained by trained psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria. All patients underwent the Structured Clinical Interviews for DSM-IV (SCID I and II). PD due to a medical or neurological condition or the presence of a comorbid Axis II disorder was an exclusion criterion. All patients underwent a

Table 1 Characteristics of the participants included in the study

Variable	Controls	Cases	Total
Discovery			
Participants, *N* (%)	76 (46%)	89 (54%)	165
Male, *N* (%)	28	40	68 (41%)
Female, *N* (%)	48	49	97 (59%)
Age, years (SD)	37 (7.5)	36 (10.4)	
Diagnosis	None	PDA 72%	
		PD 28%	
		Comorbidity:MDD 13.5%	
Replication			
Participants, *N* (%)	169 (56%)	131 (44%)	300
Male, *N* (%)	48	48	96 (32%)
Female, *N* (%)	121	83	204 (68%)
Age, years (SD)	38 (7.2)	38 (11.6)	
Diagnosis	None	PDA 61%	
		PD 39%	
		Comorbidity:MDD 13%	
Meta-analysis			
Participants, *N* (%)	245 (55%)	220 (45%)	465
Male, *N* (%)	76	88	164 (34%)
Female, *N* (%)	169	132	301 (66%)
Age, years (SD)	38 (7.3)	38 (11)	
Diagnosis	None	PDA 65%	
		PD 35%	
		Comorbidity:MDD 13%	
Dex-treatment study			
Participants, *N* (%)	29 (41%)	42 (59%)	71
Male, *N* (%)	0	0	0
Female, *N* (%)	29	42	71
Age, years (SD)	44 (11.4)	44 (13.7)	
Diagnosis	None	MDD	

PDA panic disorder with agoraphobia, *PD* panic disorder without agoraphobia, *MDD* major depressive disorder thorough medical examination including EEG, ECG and detailed hormone laboratory assessment.

Control subjects were recruited from a Munich-based community sample and screened for the absence of axis I psychiatric disorders using the Munich version of the Composite International Diagnostic Interview[32]. Controls were age-matched and sex-matched with patients.

To reduce confounding due to effects of drug treatment, both patients and controls were free of psychotropic medication for at least 4 weeks before the blood draw. All subjects were Caucasian and provided written informed consent. The Ethics Committee of the Ludwig Maximilians University, Munich, Germany, in accordance with the Declaration of Helsinki approved all procedures.

Microarray processing and quality control in the MPIP PD cohort

Genomic DNA was extracted from peripheral blood using the Gentra Puregene Blood Kit (Qiagen). DNA quality and quantity was assessed using NanoDrop 2000 Spectrophotometer (Thermo Scientific) and Quant-iT Picogreen (Invitrogen). To minimize batch effects, samples were randomized with respect to case-control status, sex and age.

Genomic DNA was bisulfite converted using the Zymo EZ-96 DNA Methylation Kit (Zymo Research) and DNA methylation levels were assessed for >480,000 CpG sites using the Illumina HumanMethylation450 BeadChip array. Hybridization and processing were performed according to the instructions of the manufacturer.

The Bioconductor R package *minfi* (version 1.10.2) was used for the quality control of methylation data including intensity read outs, normalization, cell type composition estimation, β-value and M-value calculation. Outliers, i.e., samples whose behavior deviated from that of others in terms of median intensity, were excluded from the analysis ($N = 3$ in the discovery sample, $N = 5$ in the replication sample) as well as samples with a discordant methylation-predicted vs. reported sex ($N = 1$ in the replication sample).

Failed probes were excluded based on a detection P-value larger than 0.01 in >50% of the samples. X and Y chromosome were removed to avoid a possible gender effect and also non-specific binding probes[33]. We also excluded probes if single nucleotide polymorphisms (SNPs) were documented in the interval for which the Illumina probe is designed to hybridize. Probes located close (10 bp from query site) to a SNP which had a minor allele frequency of ≥0.05, as reported in the 1000 Genomes Project, were also removed. This yielded a total of around 425,000 CpG sites in the discovery and replication sample for further analysis.

The data were then normalized with functional normalization (FunNorm)[34], an extension of quantile normalization included in the R package *minfi*.

Batch effects were identified by inspecting the association of principal components of the methylation levels with possible technical batches using linear regressions and visual inspection of principal component analysis plots using the Bioconductor R package *shinyMethyl* (version 0.99.3). Identified batch effects (i.e., bisulfite conversion plate and plate position) were removed using the Empirical Bayes' method *ComBat*[35]. Batch corrected M-values after *ComBat* were used for all further statistical analyses.

Epigenome-wide association analysis

Linear regression models were fit for each probe to test for a case vs. control difference within the R package MatrixEQTL (version 2.1.1)[36]. Sex, age and imputed white blood cell distribution from the Houseman projection[37] were included as covariates. Population stratification was investigated using multidimensional scaling and could not be observed (Supplementary Fig. S1). Significance after multiple testing was adjusted using false discovery rate (FDR) of 5%. As a first step all the samples were analyzed together (Table 1) but, given the higher prevalence of PD in females, we performed a sex-stratified analysis, first in the discovery and then in the replication sample. A fixed-effect meta-analysis across both samples was performed in Plink v1.9[38] following identification of hits in the individual analyses.

In order to investigate whether we can find clusters of association in the epigenome-wide analysis, we performed the differentially methylated region (DMR) analysis on the combined results from both samples based on the input of individual P-values of at least 5e-05 and within 500 bp using Comb-P[39].

Targeted gene analysis

A high number of studies showed mostly single SNP associations in different genes with PD, however, the replicability of these findings was low. Therefore, we used three lines of approaches to select candidate genes for the targeted methylome analysis: (1) candidate genes from human genetic studies confirmed in the recent meta-analysis of different international PD cohorts (*TMEM132D*, *COMT*, *NPSR1* and *HTR2A*)[7], (2) and/or having additional evidence from translational studies for anxiety and stress related-phenotypes (*CRH*, *CRHR1*, *ADCYAP1*, *ADCYAP1R1*, *FKBP5*, *SGK1*, *BDNF*, *HTR1A*)[8, 40–46] and lastly, (3) genes containing loci with previous evidence for differential methylation in PD and anxiety disorders (*GAD1*, *OXTR*)[20, 47]. All the genes examined ($N = 15$) showed previous evidence of association with stress-related phenotypes not only in clinical (human) studies but also in preclinical (animal) studies[5,26,27, 48–52].

The CpGs lying within the target genes were selected from the meta-analysis results of the epigenome-wide association study (EWAS) and FDR correction of 5% was applied for the number of CpGs included in the gene.

Disease association analysis

To investigate a possible enrichment for specific pathways, we conducted a disease association analysis using Web Gestalt[53, 54], DAVID[55, 56] and the R-package DOSE[57].

Tested genes for a disease enrichment were annotated from CpG sites with P-value < 0.001 in the meta-analysis results of the cases vs. controls EWAS in the whole sample ($N_{genes} = 312$), in the female subset ($N_{genes} = 428$) and in the male subset ($N_{genes} = 379$). The analysis was background corrected for the Illumina HumanMethylation450 BeadChip array annotated genes.

DNA Methylation age calculation

DNA methylation age was calculated from peripheral blood of patients and controls included in the discovery ($N = 165$) and replication sample ($N = 300$). DNA methylation-based age prediction was performed using the R code and statistical pipeline developed by Horvath[58]. This predictor was developed using 82 Illumina DNA methylation array datasets ($n = 7{,}844$) involving 51 healthy tissues and cell types[58]. The raw data were normalized using BMIQ normalization method[59] implemented in the Horvath DNA methylation-based age predictor R script[58]. We then tested whether epigenetic age acceleration (Δ-age), calculated by subtracting the actual chronological age from DNA methylation age[58], was associated with case-control status. Since DNA methylation age is calculated from raw beta values, technical batches identified for discovery and replication sample (96-well plate) were included as covariates in the linear regression model together with age, sex and cell counts (Houseman and Horvath cell counts, specifically: PlasmaBlast, CD8pCD28nCD45Ran, CD8.naive, CD4T, NK, Mono, Gran).

MPIP dexamethasone treatment study

Glucocorticoid-induced methylation and gene expression changes were examined in an independent sample of 71 Caucasian female subjects (29 healthy probands and 42 depressed) recruited at the MPIP. Recruitment strategies and characterization of participants have been previously described[60]. Baseline whole blood samples were obtained at 6 pm after 2 h of fasting and abstention from coffee and physical activity (baseline). Subjects then received 1.5 mg oral dexamethasone (DEX) and a second blood draw was performed at 9 pm 3 h after DEX ingestion (post-DEX). The study was approved by the local ethics committee and all individuals gave written informed consent.

DNA methylation and gene expression arrays in the MPIP dexamethasone treatment study

Genomic DNA was extracted from whole blood using the Gentra Puregene Blood Kit (QIAGEN) and processed as for the MPIP PD cohort. DNA methylation levels were assessed for >480,000 CpG sites using the Illumina HumanMethylation450 BeadChip arrays. Whole blood RNA was collected using PAXgene Blood RNA Tubes (PreAnalytiX), processed as described previously[60]. Blood RNA was hybridized to Illumina HumanHT-12 v3 and v4 Expression BeadChips arrays. All methylation and gene expression array probes have been subjected to an extensive quality control including filtering by low p-detection value, normalization (FunNorm for methylation and VSN for gene expression data) and batch correction with ComBat as previously described in ref. [61]. Cellular composition was estimated by using CellCode[62].

Statistical analysis in the MPIP dexamethasone treatment study

Methylation levels of cg07308824 were tested for association with gene expression levels of the *HECA* mRNA (ILMN_1770667) using a linear mixed effects model within the lme4 package[63].

Results

Genome-wide methylation differences in discovery and replication samples

Genome-wide associations were performed in the discovery sample, combined as well as stratified by gender. While no association survived correction for multiple testing in the overall samples and the male subset, one genome-wide association, cg07308824, surviving FDR of 5% ($P = 1.094 \times 10$-7, P-adj = 0.046) was observed in the female-only discovery sample. QQ plots for each of the analyses are presented in the Supplementary Figs. S2–S3. cg07308824 is located in the promoter of the *HECA* gene and was hypermethylated in female PD patients ($N = 49$) compared to controls ($N = 48$). The association and the direction of the association could be replicated in the second sample ($P = 0.035$) and yielded a combined P-value of 1.651e-08 in the meta-analysis, that would again survive correction for multiple testing (P-adj = 0.004) (Figs. 1, 2).

The DMR analysis using Comb-P revealed no significantly associated regions.

Targeted gene analysis

The targeted gene analysis (see Supplementary Tables S1–S15 for the complete list of genes tested) using the meta-analysis results, yielded in females one significant

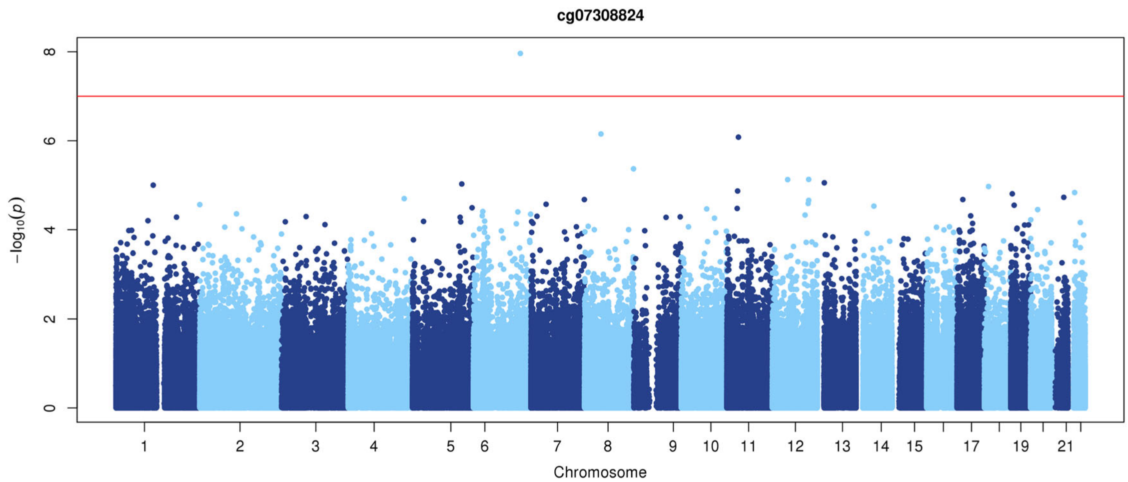

Fig. 1 Manhattan plot of the Panic Disorder EWAS in females (meta-analysis results): the *x*-axis shows chromosomal position and the *y*-axis shows −log10 (P). The red line represents the multiple test threshold ($P < 1.09 \times 10^{-7}$)

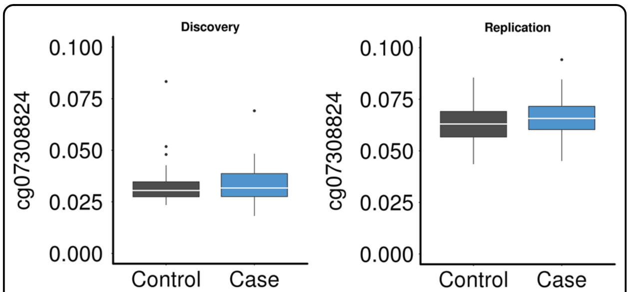

Fig. 2 Box plot of DNA methylation levels for the genome-wide significant CpG in the discovery (P-adj = 0.046) and replication sample (*P* = 0.035)

Table 2 Targeted gene analysis results for the significant CpGs

Sample	Gene	CpG	P-adj
Meta-analysis			
Whole	*SGK1*	cg00959636	0.035
Males	*FHIT*	cg07351758	0.010
	HTR2A	cg09361691	0.015
		cg06476131	0.029
Females	*ADCYAP1*(PACAP)	cg13940693	0.010
	HTR1A	cg16280141	0.041

CpG each (surviving 5% FDR correction over the CpGs in the gene) in *ADCYAP1* (P-adj = 0.010) and *HTR1A* (P-adj = 0.041) (Supplementary Fig. S4). The same analysis yielded one significant CpG in *SGK1* (P-adj = 0.035) (Supplementary Fig. S5) in the whole sample and in males in fragile histidine triad (*FHIT*, P-adj = 0.010) and two significant CpGs in *HTR2A* (P-adj = 0.015 and P-adj = 0.029) (Supplementary Fig. S6) (Table 2). Single nominal associations have been found in the genes *ADCY1P1R1*, *BDNF*, *COMT*, *CRH*, *CRHR1*, *GAD1*, *OXTR* and *TMEM132D*. No differential methylation was detected for *NPSR1* between cases and controls.

Disease association analysis

An enrichment for psychiatric disorders could be found in the whole sample (bipolar disorder, *P* = 1.9e-2; mental disorders, *P* = 2.5e-2) and in females (response to antipsychotic treatment, *P* = 5.3e-2; ADHD, *P* = 9.5e-2) using DAVID[55, 56].

Functional characterization of significant results

To assess the functionality of the significant CpG methylation site, association of methylation levels with gene expression of the *HECA* mRNA was tested. Methylation at this CpG site was associated with mRNA expression of *HECA* (ILMN_1770667) both at baseline (*P* = 0.046) and after induction by dexamethasone (*P* = 0.029) (Fig. 3). Gene expression was significantly altered in the sample following dexamethasone induction (*P* = 8.78e-05) but not DNA methylation (*P* = 0.796), indicating that the significant association between gene expression and DNA methylation is specific and not due to dexamethasone.

DNA Methylation age and case-control status

PD is a strong stressor for the people affected and so far no studies have been carried out to determine whether patients affected by PD also develop age acceleration. To answer this question, we compared the Δ-age of PD patients with healthy controls in the whole discovery ($N = 165$, $P = 0.980$) and replication sample ($N = 300$, $P = 0.282$) and found no significant differences. We then stratified for gender and found no significant results in males (discovery: $N = 68$, $P = 0.835$; replication: $N = 95$, $P = 0.467$) as well as in females (discovery: $N = 97$, $P = 0.964$; replication: $N = 204$, $P = 0.402$) (Fig. 4, Supplementary Fig. S7).

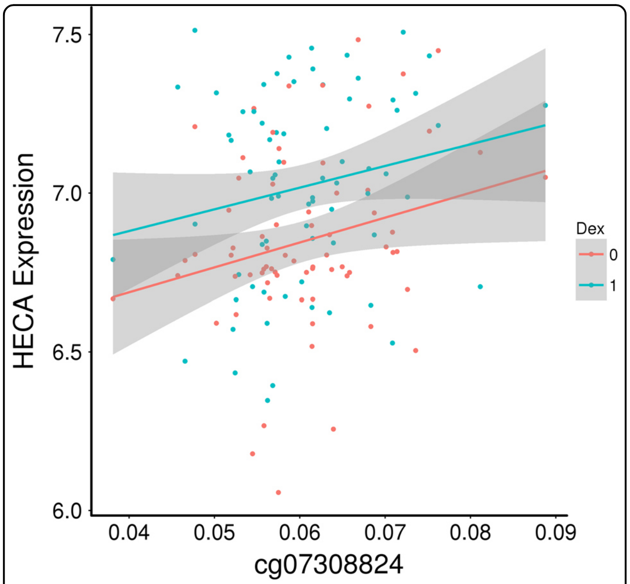

Fig. 3 Scatterplot showing the association between DNA methylation (x-axis, beta values) and gene-expression (y-axis, VSN normalized array probe intensity) in an independent female sample at baseline ($P = 0.046$) and after induction by dexamethasone ($P = 0.029$)

Discussion

Genome-wide association of whole blood DNA methylation with PD cases and matched controls identified a locus (cg07308824), which was hypermethylated in female PD patients compared to healthy controls. This locus was also associated with case-control status in females in another independent sample and results were further confirmed with a meta-analysis ($N = 301$). No methylation differences were identified at genome-wide level taking both genders together. This is the first and biggest EWAS for PD in a population with European background.

The methylation locus that we identified in females is located in the intragenic and enhancer region[64] of the Homo sapiens headcase homolog (Drosophila) (*HECA*) gene on Chromosome 6. The *HECA* gene is a cell cycle regulator and may play an important role in human cancers, e.g., hepatocellular carcinoma[65]; however, only a few publications about this gene are available to date. The potential functional relevance of cg07308824 was further investigated in the UCSC Genome Browser[64]. An overlap was observed between the location of cg07308824 probe and histone 3 lysine 27 acetylation (H3K27Ac) on seven cell lines from ENCODE[66] (Fig. 5). *H3K27Ac* was previously found near to active regulatory elements suggesting that the sequence where the probe is located is functional[67]. The *HECA* gene is expressed in brain at lower levels compared to blood (Supplementary Fig. S8) but we could not see any correlation between the methylation levels of the significant CpG found in blood and brain, which could indicate that the relevance of these results might be limited to blood. No significant correlations were found between cg07308824 methylation levels and four different brain regions (i.e., prefrontal cortex, superior temporal gyrus, entorhinal cortex and

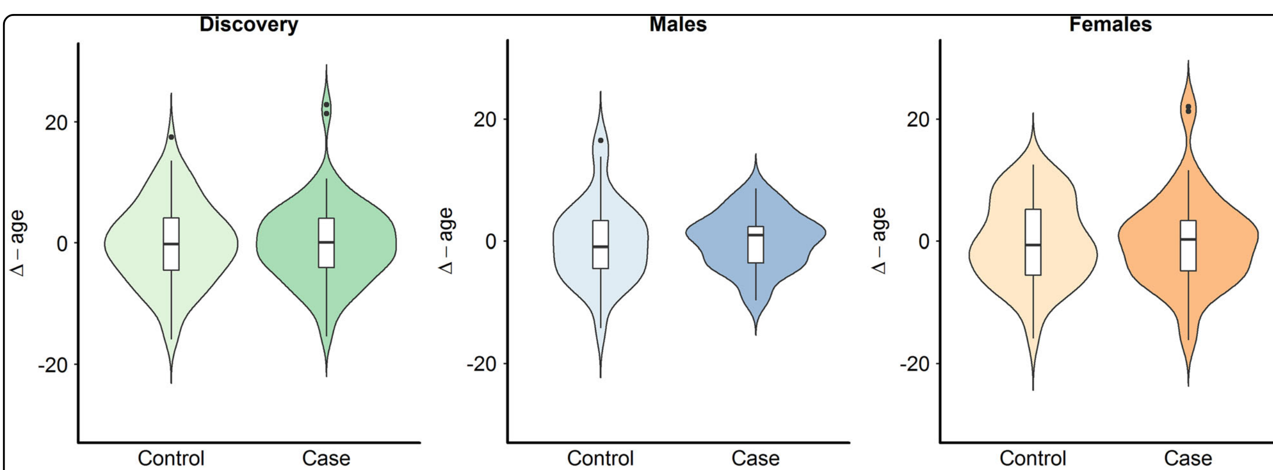

Fig. 4 Violin plots of Δ-age by case-control status in the discovery sample. From the left: whole sample ($P = 0.980$), males only ($P = 0.835$), and females only ($P = 0.964$)

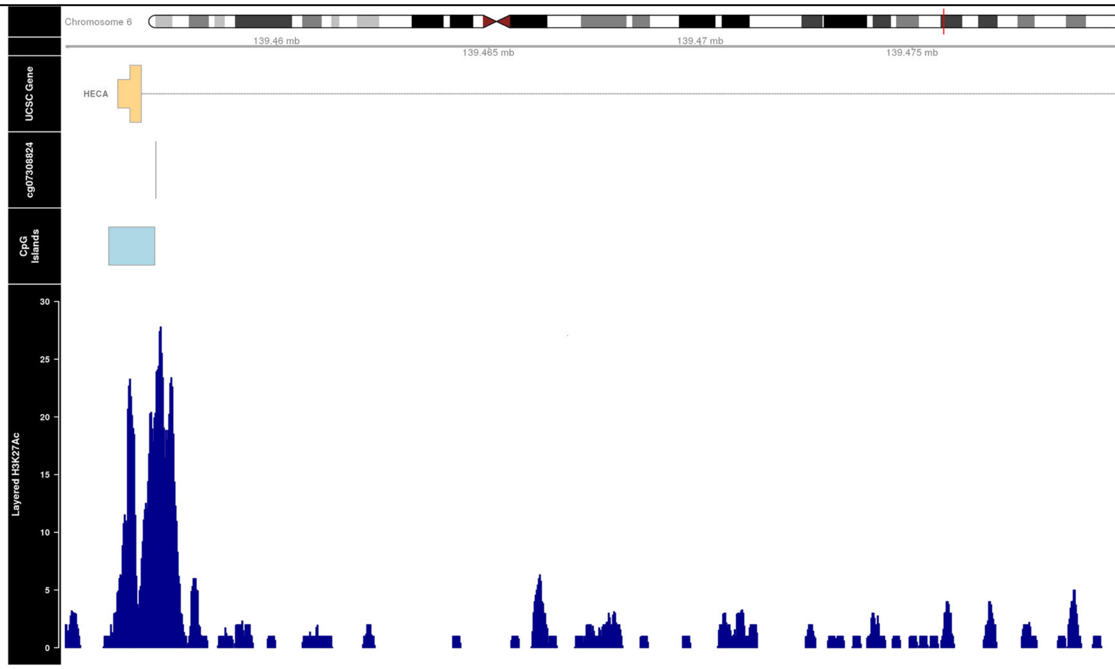

Fig. 5 Annotation of the genome-wide significant CpG located in the *HECA* gene. The top panel contains the *HECA* gene model, located on Chr 6. The other two panels show the genome-wide significant CpG and the CpG island where the CpG is located. The bottom panel shows the levels of enrichment of the H3K27Ac mark in the *HECA* gene. Data were obtained from UCSC Genome Browser and plotted using the R package Gviz[87]

cerebellum) in a linear regression model using a publicly available data set[16](Supplementary Fig. S9).

It is noteworthy considering that methylation levels of the identified locus showed a significant correlation with gene expression levels of the *HECA* gene in another independent female sample, which points to the functional relevance of the observed methylation change. The fact that the direction of the association is positive (higher gene expression correlated with higher methylation) may be explained by the intragenic location of the significant locus. It has indeed previously been shown[68] that a positive correlation with gene expression is expected for CpG probes located in the body of the gene and a negative correlation is expected for CpG probes located close to a gene's TSS. The authors also report that however this is only partially verified, with one-third of the latter type showing a positive correlation and nearly half of the former type showing a negative correlation.

Notably, the significant changes in DNA methylation presented here are small with less than 1% difference (0.08%) but replicable. While the functional relevance of such small changes is debatable, these effect sizes are in line with other EWAS in psychiatry, including the recent small Japanese EWAS in PD[22], where 40 significant CpGs with overall low methylation (mostly under 0.05%) differences have been detected. Sex-specific associations were not reported, most likely because of the small sample size. Furthermore, similar effect sizes were observed in other methylome studies of psychiatric disorders (schizophrenia[24]) and other complex diseases (rheumatoid arthritis[69], multiple sclerosis[70] and Alzheimer's disease[71]). The EWAS in schizophrenia is largest EWAS in the psychiatric field to date, with 689 schizophrenia patients and 645 controls included in the analysis and shows methylation differences lower than 0.02% that replicate in an independent cohort. More work needs to be performed to understand what contributes to these small differences (e.g., slight differences in cell composition, genotype differences or differences in environmental exposures) and if they are informative beyond serving as biomarker.

Female-specific DNA methylation changes in PD have been previously shown both in mice[72] and in humans. A female-specific association and/or correlation of negative life events with decreased overall methylation levels has been shown for *GAD1* and *MAOA*[20, 73]. In contrast, female-specific effects in terms of increased methylation levels of promoter region were observed in the *FOXP3* gene for PD[18]. Sex-specific findings regarding the methylation pattern have been also detected in depression, which is highly comorbid with PD, and psychosis[74, 75].

Interestingly, the disease association analysis shows an enrichment for psychiatric disorders in the whole sample and in females, but not in males.

In our analysis with Comb-P, no evidence for clusters of differential methylation per gene could be detected, indicating that associated CpGs do not have adjacent signals. The genome-wide findings are thus not supported by DMR analysis. However, the structure of the 450k Illumina array has many loci that are only interrogated with single CpGs, especially in enhancer regions, so that single significant CpGs could reflect a differentially methylated region, but since no adjacent CpGs are probed, this cannot be assessed using only array data. Here bisulfite sequencing approaches are needed.

One of the limitation of the study is that we could not correct for the smoking status of the subjects, due to lack of information. We verified though that our significant genome-wide hit was not one of the top-associated CpGs in the biggest EWAS for cigarette smoking[76]. Another limitation is the lower number of males compared to females, which is due to the higher prevalence of the disease in the latter. This might also explain why no significant results were found in the male subset but only in females. For this reason a bigger study with a higher number of subjects, with possibly the same ratio between genders, is necessary in order to confirm the sex-specificity of our findings.

We have to point out that we observe increased lambdas in the QQ plots of the replication sample despite correction for population stratification and methodological issues, such as DNA extraction and batch effects. It is noteworthy that female subjects of the replication sample seem to contribute to the higher inflation in our study. Other previously published EWAS in psychiatric disorders have shown similar or even higher inflation[24,25, 77–79]. The different sample size of discovery and replication sample as well as between female and male subjects might be one reason for these effects. The sample size of female subjects is doubled in the replication sample (97 vs. 204). If we extrapolate the inflation factor from discovery to replication sample[80], we see an increase of lambda to 1.11. In our study, we saw the association in the *HECA* gene first in the discovery sample which showed only moderate inflation and could then confirm the finding in the second sample and meta-analysis, suggesting that this finding cannot be attributed just to the inflation effects in the replication sample. Additionally, if we correct for population stratification using the inflation factor qualitatively results are not altered.

A targeted gene approach was subsequently applied, with the aim of investigating if genes previously associated with PD or anxiety-related phenotypes are affected at the methylation level. Five of the genes we analyzed, i.e., *HTR1A*, *HTR2A*, *ADCYAP1* (pituitary adenylate cyclase-activating polypeptide (PACAP)), *FHIT* and *SGK1* showed different methylation patterns in PD patients compared to controls. For these genes, the evidence for a correlation with anxiety disorders at the genetic level could be confirmed in our data at the epigenetic level. SGK1 (serum/glucocorticoid regulated kinase 1) is one of the key player in the mediation of fast and chronic stress response and, therefore, could be implicated in the transition of the environmental stress influences via methylation[43]. It seems to play a role in the expression of conditioned fear in the animal model[27] and is one target of miRNAs in the glucocorticoid pathway affecting neurogenesis and leading to anxiogenic and depressiogenic behavior in mice[81]. Additional evidence from human studies point to the implication of this gene in the pathophysiology of traumatic stress, e.g., PTSD[82] and our results point to a possible involvement in PD as well. The second gene which is implicated in the stress response regulation is *PACAP*. Ressler et al. could demonstrate a female-specific significant correlation of the PACAP38 peptide concentration in blood with PTSD symptoms and diagnosis[83]. In line with these previous findings, we could also demonstrate a female-specific significant methylation difference in one locus between cases and controls, suggesting that this gene may play an important role in long-lasting stress dependent pathophysiology in PD or other anxiety disorders. Similarly, a female-specific methylation difference could be shown for the gene *HTR1A*. This serotonin receptor is the most abundant of all serotonin receptors in the brain and *HTR1A* variants have been shown to be associated with depression and defensive behavior in PD patients[41]. So far, there is no evidence for gender-specific implication of *HTR1A* gene in PD or other mental disorders. There are instead already previous studies showing an association of *HTR2A* and PD[7, 84], and supporting evidence comes from our results in males. Similarly, variants in the gene *FHIT* (fragile histidine triad) were nominally associated in GWAS studies with anxiety[85] and PD[5], and were not gender-specific. In a recent huge meta-analysis for broad depression phenotype, several variants in *FHIT* were among the most significant hits[86]. However, there was no difference in the burden of depressive symptoms or depression diagnosis between the male and female group in our study, therefore, we cannot refer our finding to depression as bias. For *HTR1A*, *HTR2A* and *FHIT*, gender-specific effects presented here need to be replicated and elucidated in further studies.

In the targeted gene analysis we observe five significant results, which is more than expected by chance (2–3 expected by chance). We corrected gene-wide because each gene was regarded as separate analysis driven by the positive evidence for association with anxiety phenotypes. The results might be biased by the selection of the candidate gene itself as one limitation of the gene-targeted approach. Therefore, these findings, while supporting previous results, have to be taken with caution and replicated elsewhere.

Another aim of the study was to determine the effect of PD on epigenetic aging, as measured with the epigenetic clock[58]. We found no differences between PD patients and healthy controls in the discovery and replication sample, also when separated by gender. This might be explained by the heterogeneity of the samples in terms of age. It was showed by Zannas et al.[61] that the effect of personal life stress on Δ-age is stronger in older as compared to younger people. This suggests that the effects in our study, if presents, might be diluted by the age-range. Additionally, the effect sizes of PD on epigenetic age might be low and higher sample sizes are needed to detect significant changes. As cumulative lifetime stress has been shown to contribute to faster epigenetic aging[61], analysis of other phenotypes, such as number of panic attacks or duration of the disorder, could be more effective in uncovering epigenetic aging effects of PD. In summary, our study examines epigenome-wide differences in peripheral blood for PD patients. Our results point to possible sex-specific methylation changes in the *HECA* gene for PD but overall highlight that this disorder is not associated with extensive changes in DNA methylation pattern in peripheral blood.

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Competing interests
The authors declare that they have no competing interests.

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References

1. American Psychiatric Association, *Diagnostic and Statistical Manual of Mental Disorders (DSM-5®)* (American Psychiatric Publishing, Arlington, VA, 2013).
2. Goodwin, R. D. & Rosi, S. et al. The epidemiology of panic disorder and agoraphobia in Europe. *Eur. Neuropsychopharmacol.* **15**, 435–443 (2005).
3. Noyes, R. Jr et al. Relationship between panic disorder and agoraphobia. A family study. *Arch. Gen. Psychiatry* **43**, 227–232 (1986).
4. Hettema, J. M., Neale, M. C. & Kendler, K. S. A. Review and meta-analysis of the genetic epidemiology of anxiety disorders. *Am. J. Psychiatry* **158**, 1568–1578 (2001).
5. Erhardt, A. et al. TMEM132D, a new candidate for anxiety phenotypes: evidence from human and mouse studies. *Mol. Psychiatry* **16**, 647–663 (2011).
6. Erhardt, A. et al. Replication and meta-analysis of TMEM132D gene variants in panic disorder. *Transl. Psychiatry* **2**, e156 (2012).
7. Howe, A. S. et al. Candidate genes in panic disorder: meta-analyses of 23 common variants in major anxiogenic pathways. *Mol. Psychiatry* **21**, 665–679 (2016).
8. Weber, H. et al. Allelic variation in CRHR1 predisposes to panic disorder: evidence for biased fear processing. *Mol. Psychiatry* **21**, 813–822 (2016).
9. Gottschalk, M. G. & Domschke, K. Novel developments in genetic and epigenetic mechanisms of anxiety. *Curr. Opin. Psychiatry* **29**, 32–38 (2016).
10. Teh, A. L. et al. The effect of genotype and in utero environment on interindividual variation in neonate DNA methylomes. *Genome Res.* **24**, 1064–1074 (2014).
11. Torvik, F. A. et al. Longitudinal associations between social anxiety disorder and avoidant personality disorder: a twin study. *J. Abnorm. Psychol.* **125**, 114–124 (2016).
12. South, S. C. et al A population based twin study of DSM-5 maladaptive personality domains. *Personal Disord.* **8**, 366–375 (2017).
13. Kendler, K. S. et al A National Swedish Twin-Sibling Study of alcohol use disorders. *Twin Res. Hum. Genet.* **19**, 430–437 (2016).
14. Slatkin, M. Epigenetic inheritance and the missing heritability problem. *Genetics* **182**, 845–850 (2009).
15. Klengel, T. & Binder, E. B. Epigenetics of stress-related psychiatric disorders and gene × environment interactions. *Neuron* **86**, 1343–1357 (2015).
16. Hannon, E., Lunnon, K., Schalkwyk, L. & Mill, J. Interindividual methylomic variation across blood, cortex, and cerebellum: implications for epigenetic studies of neurological and neuropsychiatric phenotypes. *Epigenetics* **10**, 1024–1032 (2015).
17. Farre, P., Jones, M. J., Meaney, M. J., Emberly, E., Turecki, G. & Kobor, M. S. Concordant and discordant DNA methylation signatures of aging in human blood and brain. *Epigenetics Chromatin* **8**, 19 (2015).
18. Prelog, M. et al. Hypermethylation of FOXP3 promoter and premature aging of the immune system in female patients with panic disorder? *PLoS. One* **11**, e0157930 (2016).
19. Ziegler, C. et al. MAOA gene hypomethylation in panic disorder-reversibility of an epigenetic risk pattern by psychotherapy. *Transl. Psychiatry* **6**, e773 (2016).
20. Domschke, K. et al. Epigenetic signature of panic disorder: a role of glutamate decarboxylase 1 (GAD1) DNA hypomethylation? *Prog. Neuropsychopharmacol. Biol. Psychiatry* **46**, 189–196 (2013).
21. Bayles, R. et al. Methylation of the SLC6a2 gene promoter in major depression and panic disorder. *PLoS. One* **8**, e83223 (2013).
22. Shimada-Sugimoto, M. et al. Epigenome-wide association study of DNA methylation in panic disorder. *Clin. Epigenetics* **9**, 6 (2017).
23. Yousefi, P. et al. Sex differences in DNA methylation assessed by 450 K BeadChip in newborns. *BMC Genom.* **16**, 911 (2015).
24. Montano, C. et al. Association of DNA methylation differences with schizophrenia in an Epigenome-Wide Association Study. *JAMA Psychiatry* **73**, 506–514 (2016).
25. Houtepen, L. C., van Bergen, A. H., Vinkers, C. H. & Boks, M. P. DNA methylation signatures of mood stabilizers and antipsychotics in bipolar disorder. *Epigenomics* **8**, 197–208 (2016).
26. Nieto, S. J., Patriquin, M. A., Nielsen, D. A. & Kosten, T. A. Don't worry; be informed about the epigenetics of anxiety. *Pharmacol. Biochem. Behav.* **146-147**, 60–72 (2016).
27. Knoll, A. T., Halladay, L. R., Holmes, A. J. & Levitt, P. Quantitative trait loci and a novel genetic candidate for fear learning. *J. Neurosci.* **36**, 6258–6268 (2016).
28. Horvath, S. et al. Decreased epigenetic age of PBMCs from Italian semi-supercentenarians and their offspring. *Aging (Albany NY)* **7**, 1159–1170 (2015).
29. Marioni, R. E. et al. DNA methylation age of blood predicts all-cause mortality in later life. *Genome Biol.* **16**, 25 (2015).
30. Chen, B. H. et al. DNA methylation-based measures of biological age: meta-analysis predicting time to death. *Aging (Albany NY)* **8**, 1844–1859 (2016).
31. Christiansen, L. et al. DNA methylation age is associated with mortality in a longitudinal Danish twin study. *Aging Cell.* **15**, 149–154 (2016).
32. Wittchen, H. U. & Pfister, H. DIA-X-Interviews: Manual für Screening-Verfahren und Interview (Swets & Zeitlinger, Frankfurt, 1997).
33. Chen, Y. A. et al. Discovery of cross-reactive probes and polymorphic CpGs in the Illumina Infinium HumanMethylation450 microarray. *Epigenetics* **8**, 203–209 (2013).
34. Fortin, J. P. et al. Functional normalization of 450k methylation array data improves replication in large cancer studies. *Genome Biol.* **15**, 503 (2014).
35. Johnson, W. E., Li, C. & Rabinovic, A. Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostatistics* **8**, 118–127 (2007).
36. Shabalin, A. A. Matrix eQTL: ultra fast eQTL analysis via large matrix operations. *Bioinformatics* **28**, 1353–1358 (2012).
37. Houseman, E. A. et al. DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinforma.* **13**, 86 (2012).
38. Purcell, S. et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559–575 (2007).

39. Pedersen, B. S., Schwartz, D. A. & Yang, KechrisK. J. IV Comb-p: software for combining, analyzing, grouping and correcting spatially correlated P-values. *Bioinformatics* **28**, 2986–2988 (2012).
40. Blaya, C. et al. Panic disorder and serotonergic genes (SLC6A4, HTR1A and HTR2A): association and interaction with childhood trauma and parenting. *Neurosci. Lett.* **485**, 11–15 (2010).
41. Straube, B. et al. The functional −1019C/G HTR1A polymorphism and mechanisms of fear. *Transl. Psychiatry* **4**, e490 (2014).
42. Ressler, K. J. et al. Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. *Nature* **470**, 492–497 (2011).
43. Cattaneo, A. & Riva, M. A. Stress-induced mechanisms in mental illness: a role for glucocorticoid signalling. *J. Steroid Biochem. Mol. Biol.* **160**, 169–174 (2016).
44. Zannas, A. S., Wiechmann, T., Gassen, N. C. & Binder, E. B. Gene-stress-epigenetic regulation of fkbp5: clinical and translational implications. *Neuropsychopharmacology* **41**, 261–274 (2016).
45. Han, E. J. et al. Evidence for association between the brain-derived neurotrophic factor gene and panic disorder: a novel haplotype analysis. *Psychiatry Investig.* **12**, 112–117 (2015).
46. Konishi, Y. et al. Genexgenexgender interaction of BDNF and COMT genotypes associated with panic disorder. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **51**, 119–125 (2014).
47. Ziegler, C. et al. Oxytocin receptor gene methylation: converging multilevel evidence for a role in social anxiety. *Neuropsychopharmacology* **40**, 1528–1538 (2015).
48. Desbonnet, L. et al. Physiological and behavioural responsivity to stress and anxiogenic stimuli in COMT-deficient mice. *Behav. Brain Res.* **228**, 351–358 (2012).
49. Leonard, S. K. et al. Pharmacology of neuropeptide S in mice: therapeutic relevance to anxiety disorders. *Psychopharmacol. (Berl.).* **197**, 601–611 (2008).
50. Benekareddy, M., Vadodaria, K. C., Nair, A. R. & Vaidya, V. A. Postnatal serotonin type 2 receptor blockade prevents the emergence of anxiety behavior, dysregulated stress-induced immediate early gene responses, and specific transcriptional changes that arise following early life stress. *Biol. Psychiatry* **70**, 1024–1032 (2011).
51. Mustafa, T., Jiang, S. Z., Eiden, A. M., Weihe, E., Thistlethwaite, I. & Eiden, L. E. Impact of PACAP and PAC1 receptor deficiency on the neurochemical and behavioral effects of acute and chronic restraint stress in male C57BL/6 mice. *Stress* **18**, 408–418 (2015).
52. Bahi, A., Al Mansouri, S. & Al Maamari, E. Nucleus accumbens lentiviral-mediated gain of function of the oxytocin receptor regulates anxiety- and ethanol-related behaviors in adult mice. *Physiol. Behav.* **164**, 249–258 (2016).
53. Wang, J., Duncan, D., Shi, Z. & Zhang, B. WEB-based GEne SeT AnaLysis Toolkit (WebGestalt). *Nucleic Acids Res.* **41**, W77–W83 (2013).
54. Zhang, B., Kirov, S. & Snoddy, J. WebGestalt: an integrated system for exploring gene sets in various biological contexts. *Nucleic Acids Res.* **33**, W741–W748 (2005).
55. Huang da, W., Sherman, B. T. & Lempicki, R. A. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res.* **37**, 1–13 (2009).
56. Huang da, W., Sherman, B. T. & Lempicki, R. A. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.* **4**, 44–57 (2009).
57. Yu, G., Wang, L. G., Yan, G. R. & He, Q. Y. DOSE: an R/Bioconductor package for disease ontology semantic and enrichment analysis. *Bioinformatics* **31**, 608–609 (2015).
58. Horvath, S. DNA methylation age of human tissues and cell types. *Genome Biol.* **14**, R115 (2013).
59. Teschendorff, A. E. et al. A beta-mixture quantile normalization method for correcting probe design bias in Illumina Infinium 450 k DNA methylation data. *Bioinformatics* **29**, 189–196 (2013).
60. Arloth, J. et al. Genetic differences in the immediate transcriptome response to stress predict risk-related brain function and psychiatric disorders. *Neuron* **86**, 1189–1202 (2015).
61. Zannas, A. S. et al. Lifetime stress accelerates epigenetic aging in an urban, African American cohort: relevance of glucocorticoid signaling. *Genome Biol.* **16**, 266 (2015).
62. Chikina, M., Zaslavsky, E. & Sealfon, S. C. CellCODE: a robust latent variable approach to differential expression analysis for heterogeneous cell populations. *Bioinformatics* **31**, 1584–1591 (2015).
63. Bates, D., Mächler, M., Bolker, B. & Walker, S. Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* **67**, 10.18637/jss.v067.i01 (2015).
64. Kent, W., Sugnet, C., Furey, T., Roskin, K., Pringle, T. & Zahler, A. et al. The human genome browser at UCSC. *Genome Res.* **12**, 996–1006 (2002).
65. Wang, J. et al. The human homolog of drosophila headcase acts as a tumor suppressor through its blocking effect on the cell cycle in hepatocellular carcinoma. *PLoS. One* **10**, e0137579 (2015).
66. Rosenbloom, K. R. et al ENCODE Data in the UCGC Genome Browser. *Nucleic Acids Res.* **41**, D56–D63 (2013).
67. Creyghton, M. P. et al. Histone H3K27ac separates active from poised enhancers and predicts developmental state. *Proc. Natl. Acad. Sci. USA* **107**, 21931–21936 (2010).
68. Wagner, J. R., Busche, S., Ge, B., Kwan, T., Pastinen, T. & Blanchette, M. The relationship between DNA methylation, genetic and expression inter-individual variation in untransformed human fibroblasts. *Genome Biol.* **15**, R37 (2014).
69. Liu, Y. et al. Epigenome-wide association data implicate DNA methylation as an intermediary of genetic risk in rheumatoid arthritis. *Nat. Biotechnol.* **31**, 142–147 (2013).
70. Huynh, J. L. et al. Epigenome-wide differences in pathology-free regions of multiple sclerosis-affected brains. *Nat. Neurosci.* **17**, 121–130 (2014).
71. Lunnon, K. et al. Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease. *Nat. Neurosci.* **17**, 1164–1170 (2014).
72. Papale, L. A. et al. Sex-specific hippocampal 5-hydroxymethylcytosine is disrupted in response to acute stress. *Neurobiol. Dis.* **96**, 54–66 (2016).
73. Domschke, K. et al. Monoamine oxidase A gene DNA hypomethylation—a risk factor for panic disorder? *Int. J. Neuropsychopharmacol.* **15**, 1217–1228 (2012).
74. Byrne, E. M. et al. Monozygotic twins affected with major depressive disorder have greater variance in methylation than their unaffected co-twin. *Transl. Psychiatry* **3**, e269 (2013).
75. Melas, P. A. et al. Genetic and epigenetic associations of MAOA and NR3C1 with depression and childhood adversities. *Int. J. Neuropsychopharmacol.* **16**, 1513–1528 (2013).
76. Joehanes, R. et al. Epigenetic signatures of cigarette smoking. *Circ. Cardiovasc. Genet.* **9**, 436–447 (2016).
77. Cecil, C. A. et al. Epigenetic signatures of childhood abuse and neglect: implications for psychiatric vulnerability. *J. Psychiatr. Res.* **83**, 184–194 (2016).
78. Houtepen, L. C. et al. Genome-wide DNA methylation levels and altered cortisol stress reactivity following childhood trauma in humans. *Nat. Commun.* **7**, 10967 (2016).
79. Viana, J. et al. Schizophrenia-associated methylomic variation: molecular signatures of disease and polygenic risk burden across multiple brain regions. *Hum. Mol. Genet.* **26**, 210–225 (2017).
80. Freedman, M. L. et al. Assessing the impact of population stratification on genetic association studies. *Nat. Genet.* **36**, 388–393 (2004).
81. Jin, J. et al. miR-17-92 cluster regulates adult hippocampal neurogenesis, anxiety, and depression. *Cell. Rep.* **16**, 1653–1663 (2016).
82. Licznerski, P. et al Decreased SGK1 expression and function contributes to behavioral deficits induced by traumatic stress. *PLoS. Biol.* **13**, e1002282 (2015).
83. Dias, B. G. & Ressler, K. J. PACAP and the PAC1 receptor in post-traumatic stress disorder. *Neuropsychopharmacology* **38**, 245–246 (2013).
84. Unschuld, P. G. et al. Polymorphisms in the serotonin receptor gene HTR2A are associated with quantitative traits in panic disorder. *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* **144b**, 424–429 (2007).
85. Luciano, M. et al. Genome-wide association uncovers shared genetic effects among personality traits and mood states. *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* **159b**, 684–695 (2012).
86. Direk, N. et al An analysis of two genome-wide association meta-analyses identifies a new locus for broad depression phenotype. *Biol. Psychiatry* **82**, 322–329 (2017).
87. Hahne, F. & Ivanek, R. Visualizing genomic data using Gviz and bioconductor. *Methods Mol. Biol.* **1418**, 335–351 (2016).

Appendix C – Publication III: Exposure-induced changes of plasma metabolome and gene expression in patients with panic disorder

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RESEARCH ARTICLE

Exposure-induced changes of plasma metabolome and gene expression in patients with panic disorder

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Abstract

Background: Anxiety disorders including panic disorder (PD) are the most prevalent psychiatric diseases leading to high disability and burden in the general population. Acute panic attacks are distinctive for PD but also frequent in other anxiety disorders. The neurobiology or specific molecular changes leading to and present during panic attacks are insufficiently known so far.

Methods: In the present pilot study, we investigated dynamic metabolomic and gene expression changes in peripheral blood of patients with PD ($n = 25$) during two exposure-induced acute panic attacks.

Results: The results show that the metabolite glyoxylate was dynamically regulated in peripheral blood. Additionally, glyoxylate levels were associated with basal anxiety levels and showed gender-related differences at baseline. As glyoxylate is part of the degradation circuit of cholecystokinin, this suggests that this neuropeptide might be directly involved in exposure-induced panic attacks. Only gene expression changes of very small magnitude were observed in this experimental setting.

Conclusions: From this first metabolome and gene expression study in exposure-induced acute panic attacks in PD we conclude that metabolites can potentially serve as dynamic markers for different anxiety states. However, these findings have to be replicated in cohorts with greater sample sizes.

KEYWORDS
cognitive behavioral therapy, exposure, gene expression, metabolomics, panic attack, panic disorder

1 | INTRODUCTION

Anxiety disorders including generalized anxiety disorder, panic disorder (PD) with or without agoraphobia (PDA, PD; due to high comorbidity we use PD for both disorders throughout the paper) and specific phobias are the most prevalent mental disorders. They affect up to a third of the general population during their lifetime (Kessler et al., 2006). PD is one of the most disabling anxiety disorders characterized by recurrent panic attacks often accompanied by agoraphobia, where patients avoid situations in which they might feel trapped or are unable to escape. The lifetime prevalence of PD is up to 4% and females are affected twice as often compared with men (McLean, Asnaani, Litz, & Hofmann, 2011). The underlying neurobiology of PD is highly complex and implicates various genetic, neurobiological and environmental factors (Binelli et al., 2015). Panic attacks are frequent in all anxiety disorders and are distinctive for PD. They appear suddenly with symptoms of intense fear and bodily sensations followed by self-perceived lack of control and anticipatory anxiety. The neurobiological circuits and molecular systems responsible for and activated by panic attacks within anxiety and PDs are poorly understood. In recent years, studies investigating the dynamic functional response within the panic emotions used panic-inducing compounds, such as sodium lactate and cholecystokinin-4 (CCK-4; Plag et al., 2012; Toru et al., 2013). However, these compounds might activate pathways which differ from the characteristic of a panic attack within an anxiety disorder. To exclude this, we chose to use exposure intervention within cognitive behavioral therapy (CBT) to induce and measure acute anxiety- and panic-related molecular changes in the blood.

Exposure is a core technique within CBT to treat phobia- and anxiety-related symptoms (Abramowitz, 2013). As part of this intervention, the patient is exposed to highly feared and mostly avoided situations. The main goal is to induce the highest possible anxiety levels to apply cognitive and behavioral strategies for dealing with anxiety feelings. The intervention is completed when the anxiety levels decrease significantly (habituation) without using avoidance or security strategies. Exposure has been suggested to alter the pathological fear structure by initially activating it and then updating the pathological, unrealistic associations in these structures (Kaczkurkin & Foa, 2015).

Despite the improvements in treatment, numerous patients with PD do not respond to current therapies (White et al., 2013). Currently no molecular biomarker exists for any anxiety trait, especially for disease-related acute anxiety states. Biomarkers will be essential for early diagnosis, accurate subtype classification, and treatment efficacy.

High-throughput-omics approaches offer the opportunity to identify novel candidate biomarkers and affected pathways (Bragazzi, 2013; Higdon et al., 2015). Recently, genetic and epigenetic markers have received great attention. However, robust associations in anxiety disorders are rather the exception than the rule, partly due to limited sample size (Andersen, Dogan, Beach, & Philibert, 2015; Ng et al., 2012). Other types of biomarkers resulting from neuroimaging (Schnack & Kahn, 2016), proteomics, and metabolomics(Filiou et al., 2011), studies have great potential for improved understanding of anxiety-related circuits. Omics-based technologies may identify early biochemical disease- or treatment-related changes and identify predictive biomarkers that can be used to reveal pathological abnormalities before the development of any clinical symptoms or to monitor dynamic changes during a therapeutic intervention (Sethi & Brietzke, 2016). The metabolome represents molecular pathway activities and can serve as a reflection of the disease phenotype (Peng, Li, & Peng, 2015). Studies have shown altered metabolite levels in schizophrenia (Liu et al., 2016; Xiao et al., 2011), depression (Abdallah et al., 2014; Ali-Sisto et al., 2016), and autism (Chugani, Sundram, Behen, Lee, & Moore, 1999; Lim et al., 2016), suggesting a distinct metabolic profile associated with disease state. Metabolomics and proteomics studies in animal models have identified differences in neurotransmission and energy metabolism (Filiou et al., 2011), mitochondrial functions, and oxidative stress (Hollis et al., 2015; Krolow, Arcego, Noschang, Weis, & Dalmaz, 2014) for anxiety-related behavior. Recently, blood biomarkers have been identified that predict the development of a subsequent depressive episode for the social disorder (Gottschalk et al., 2015). However, specific metabolites indicating disease-related states in anxiety disorders and predictive biomarkers are yet to be identified.

The primary goal of the present pilot study was to trace specific exposure-/panic-related plasma metabolite and gene expression changes of individuals ($n = 25$) with PD at three time points to identify biomarkers for acute anxiety states. In addition, dynamic changes of significantly or highly regulated metabolites in response to exposure were tested for association with the clinical response following CBT.

2 | METHODS

2.1 | Study participants and exposure procedure

A total of 25 patients were included in the study (males $n = 8$, females $n = 17$, mean age of 32 ± 9 standard deviation). The recruitment of patients was conducted between January 2014 and December 2015 in the Outpatient Clinic for Anxiety Disorders at the Max Planck Institute of Psychiatry. Inclusion criteria consisted of a current primary DSM-IV-TR diagnosis of PD with/without agoraphobia (PD/AG) and a clinical interview score >14 on the Hamilton Anxiety Scale (HAMA; Hamilton, Schutte, & Malouff, 1976). Exclusion criteria were somatic disorders, pregnancy, personality disorder, current suicidal intent, and other psychiatric disorders except other anxiety disorders (AD) or secondary mild or moderate depression. All patients included in the analysis were free of psychotropic medication for at least 4 weeks before the exposure.

The exposure treatment was applied within a highly structured and research approved short-term CBT (Lang, Helbig-Lang, Westphal, Gloster, & Wittchen, 2018). The therapy course consisted of 12 sessions of CBT (1–2 sessions per week, 6–8 weeks) and two booster sessions after 2 and 4 months. Therapy included two accompanied in vivo exposures (Sessions 6–11). Therapists were trained in exposure-based CBT. Exposure sessions were conducted outside the clinic, depending on the feared situation (e.g., subway, supermarket, and tower) and specific concern (e.g., fainting, asphyxiation, and losing control). The type of exposure was determined by the patient, with the goal of high fear provocation. Benzodiazepines were not allowed during the course of therapy.

On the day of the exposure, patients were instructed to eat a standardized breakfast before the exposure session (roll with jelly), not to smoke, not to exercise, not to take any medication and not to consume caffeine. The first blood sample (in the following referred to baseline b1 in the first exposure and b2 for the second exposure, for the overview of blood sampling procedure see Figure S1) was drawn between 8:30 and 9 a.m.

The exposure was started immediately after the basal blood draw by approaching the specific agoraphobic situations which was previously defined with the patient. As intended, all patients reached high subjective anxiety levels within the exposure intervention

TABLE 1 Psychometric measures for exposure sessions and therapy course

Exposure-related psychometry			
SUD	peak anxiety (0–10)		
Exposure 1	9.16 ± 1.03		
Exposure 2	8.08 ± 2.44		
	Baseline	Postexposure	*p*-Level
ACQ			
Exposure 1	1.58 ± 0.58	1.06 ± 0.11	5.1×10^{-4}
Exposure 2	1.58 ± 0.63	1.08 ± 0.13	6.9×10^{-3}
BSQ			
Exposure 1	2.12 ± 0.79	1.26 ± 0.28	8.9×10^{-5}
Exposure 2	1.96 ± 0.93	1.26 ± 0.29	9.8×10^{-3}
Therapy-related psychometry			
	Baseline	Therapy end	*p*-Level
HAMA	34.95 ± 6.85	13.11 ± 10.97	6.3×10^{-8}

Abbreviations: ACQ, Anxiety Cognition Questionnaire; BSQ, Body Sensation Questionnaire; HAMA, Hamilton Anxiety Rating Scale; SUD, subjective units of disturbance for anxiety.

(Table 1). A second blood draw was conducted 1 hr after the highest anxiety peak within exposure (between 11:00 and 12:00 a.m., postexposure, pe1 resp. pe2). A third blood sample was collected 24 hr after the onset of the exposure (referred to as 24-hr postexposure, p24, 8:30–9 a.m.) with the same dietary instructions as the day before. Each patient received two therapist-guided exposure sessions at an interval of 1 week between the sessions. Additionally, patients' anxiety levels were assessed before, during and after the exposure using the following questionnaires: the Anxiety Cognition Questionnaire (ACQ; Ehlers, Margraf, & Chambless, 2001) and Body Sensations Questionnaire (BSQ; Chambless, Caputo, Bright, & Gallagher, 1984) as well as an exposure protocol (Lang et al., 2018), including Subjective Units of Disturbance (SUD; 0–10) for anxiety and expectations about the exposure.

The clinical severity was assessed at three time points during the therapy: baseline, after 4th session before the exposure and at the end of therapy (12th session). Therapy remission was reached if symptom ratings were below the cut-off 14 in HAMA, the threshold for clinical relevance. Remission was assessed after the end of therapy (12th session). The study was approved by the ethics committee of Ludwig Maximilian University in Munich, Project number 318/00. Written informed consent was obtained from all subjects.

2.2 | Metabolomics analysis

Isolation of polar plasma metabolites. Human plasma was extracted with a fourfold excess (vol/vol) of 100% cold methanol. After vortexing for 2 min, samples were incubated on dry ice for 2 hr and centrifuged (2,053 *g*, 100 min, 4°C). Supernatants were filtered using a 0.22-μm ultrafiltration tube (1,105*g*, 2 min, 4°C) and the filtrates were lyophilized. The lyophilized metabolites were stored at −80°C until further use (see Supporting Information S1).

Targeted metabolomics analysis. Plasma extracts were resuspended in 20 μl liquid chromatography-mass spectrometry grade water. Ten microliters were injected and analyzed using a 5500 QTRAP triple quadrupole mass spectrometer (AB/SCIEX, Framingham, MA) coupled to a Prominence UFLC high-performance liquid chromatography system (Shimadzu, Columbia, MD) via SRM of a total of 293 endogenous water-soluble metabolites for steady-state analyses of samples.

Overall 309 metabolites were measured across three time points (baseline, 1 hr postexposure, and 24 hr postexposure). The first exposure included 25 patients of which 8 were males and 17 were females. The second exposure included 23 patients (8 males and 15 females).

2.3 | Identification of single metabolite alterations

Metabolite intensities were median-normalized and auto-scaled (mean-centering and dividing by the respective standard deviation) for statistical analysis. Metabolites with significant changes defined with a *p*-value below .05 in concentration over all three time points (baseline, 1 hr postexposure, and 24 hr postexposure) were identified via two-way analysis of variance for repeated measures correcting for age, sex, and all significant surrogate variables estimated via sva (Leek, Johnson, Parker, Jaffe, & Storey, 2012). Metabolites were excluded from the analysis if more than two subjects had missing values in the trajectory. Immediate effects of highest anxiety level were investigated by exclusively taking the baseline and the first postexposure metabolite concentrations into account using a paired *t* test. For both analyses, the *p*-values were false discovery rate (FDR)-corrected (FDR ≤ 0.10). All statistical analyses were performed in R version 3.4.3 (Team, 2018).

2.4 | Gene expression

For all three time points, baseline as well as 1-hr postexposure and 24-hr postexposure blood RNA were collected using PAXgene Blood RNA Tubes (PreAnalytiX), processed as previously described (Menke, Rex-Haffner, Klengel, Binder, & Mehta, 2012). Blood RNA was hybridized to Illumina HumanHT-12 v4 Expression BeadChips (Illumina, San Diego, CA). Raw probe intensities were exported with Illumina's GenomeStudio. The raw expression values were normalized using the variance-stabilizing normalization method from the vsn package (Huber, von Heydebreck, Sultmann, Poustka, & Vingron, 2002). Batches that were significantly associated with the first 10 principal components were removed using the ComBat function from the sva package (Leek et al., 2012). Two rounds of ComBat were necessary to remove associations with the Bead Chip ID and the Chip Barcode. Cell composition was estimated using CellCODE (Chikina, Zaslavsky, & Sealfon, 2015). Significantly altered genes overexposure were detected by using a linear mixed effect model (lme4 package;

Bates, Maechler, Bolker, & Walker, 2014). We corrected for age, sex, cell composition as well as random effects (De Boeck et al., 2011).

2.5 | Identification of significantly enriched pathways

We included all metabolites showing significant changes over two or three time points (FDR 0.1) in a pathway analysis using MetaboAnalyst (Xia, Mandal, Sinelnikov, Broadhurst, & Wishart, 2012). These were considered significantly enriched for Reactome (Joshi-Tope et al., 2005) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (Kanehisa & Goto, 2000) if they presented with an enrichment *p*-value of .05. A second pathway analysis was performed using the 227 genes which were significantly altered over time.

2.6 | Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

3 | RESULTS

Anxiety levels measured with ACQ, BSQ, and SUD showed a significant reduction across each exposure. The patients reached high subjective anxiety levels in both exposures (Table 1). We included two exposure measurements per patient into the analysis to capture stable anxiety-induced metabolomics and gene expression effects. In general, we observed a significant reduction of all therapy-related measures at the end of therapy and an overall reduction of all exposure-related measures in the second exposure (Table 1).

3.1 | Altered metabolites over time

Short-term effects of the exposure were captured by only considering the differences between baseline and the first postexposure time point (1 hr after anxiety peak). We identified 14 metabolites with nominally significantly altered levels between these time points for the first exposure. In the second exposure the changes for 17 metabolites were nominal significant (see Tables S1 and S2). However, there was no overlap between the list of altered metabolites from the two exposure sessions.

In the next step, metabolite levels were analyzed across all three time points to capture prolonged exposure-related effects. Of the 309 metabolites measured, 160 (Measurement 1) and 163 (Measurement 2), respectively, passed the filter for missing values (see Section 2).

The metabolite glyoxylate showed significant changes across all three time points of the first exposure ($p = 6.30 \times 10^{-4}$, FDR corrected $p = 7.49 \times 10^{-3}$). For the second exposure, no changes in metabolite levels for all three time points withstood correction for multiple testing. However, the change in glyoxylate levels was nominal significant in the second measurement ($p = 1.27 \times 10^{-3}$). Metabolites with a nominal significant *p*-value included

TABLE 2 Long-term metabolic profile

Metabolite	KEGG compound ID	Exposure 1	Exposure 2
Glyoxylate	C00048	$\mathbf{6.30 \times 10^{-4}}$	1.27×10^{-3}
Ascorbic acid	C00072	2.28×10^{-2}	1.63×10^{-3}
2-Oxobutanoate	C00109	3.03×10^{-2}	3.44×10^{-2}
Homocysteine	C00155	3.25×10^{-2}	2.9×10^{-2}
Glutamine	C00064	3.76×10^{-2}	3.75×10^{-2}
O8P-O1P	Not available	NA[a]	8.50×10^{-3}

Note: Nominal significant metabolite level changes across three time points. Bold: significant after correction for multiple testing.
Abbreviation: KEGG, Kyoto Encyclopedia of Genes and Genomes.
[a]Missing values in trajectories.

oxobutanoate, ascorbic acid and homocystein in both exposures (see Table 2). The course of glyoxylate levels in the first exposure (see Figure 1) showed at least two different dynamics: One cluster of eight patients with mostly high baseline glyoxylate levels which decreased toward the first postexposure time point, the other cluster with 17 patients where glyoxylate levels were mostly stable across the exposure. However, we found no difference across time between patients in the two subgroups with regard to age, anxiety levels during exposure, depression scores or response to CBT.

3.2 | Glyoxylate and peak anxiety

To assess if metabolite level dynamics correlated with phenotype, glyoxylate levels were mapped to the SUD peak anxiety level that each individual experienced during the exposure (Figure 2).

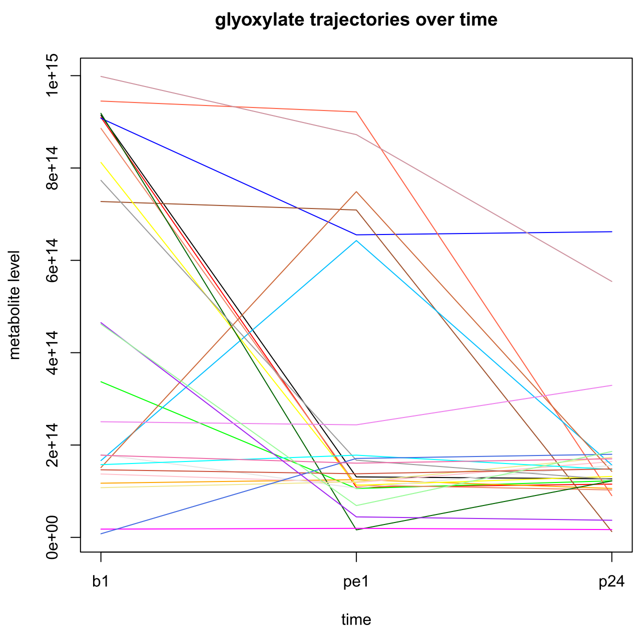

FIGURE 1 Progression of glyoxylate levels during exposure. For each individual the course of blood glyoxylate levels (*y*-axis) is shown at baseline (time point 0), 1 hr postexposure (time point 1), and 24 hr later (time point 2)

The baseline glyoxylate levels correlated highest with the peak anxiety level experienced by the individuals at the basal time point b1 ($p = .03$, adjusted $R^2 = 28\%$). Glyoxylate levels for the other time points showed no significant correlation with the peak anxiety (postexposure: $p = 0.26$, adj. $R^2 = 27\%$; 24 hr postexposure: $p = 0.83$, adj. $R^2 = 12\%$).

3.3 | Glyoxylate, clinical response, and gender-related differences

Glyoxylate levels in remitting ($n = 9$) and nonremitting ($n = 12$) individuals were compared for all three time points to assess correlation with clinical response at the end of the therapy. Although we observed some differences in glyoxylate levels between the remitters and nonremitters (e.g., stable glyoxylate overexposure in nonremitters compared with initially high levels pre-exposure and a drop after exposure in remitters), the changes were not significant at any time point, $p(b1) = 0.59$, $p(pe1) = 0.19$, $p(p24) = 0.83$.

However, the baseline time point showed a significant difference in glyoxylate levels between genders in the first exposure with higher levels in male patients ($p = .006$, see Figure 3). The difference in glyoxylate levels between genders decreased during exposure and was not significant for both postexposure time points.

3.4 | The metabolome as proxy for baseline anxiety levels

To investigate whether baseline anxiety levels could be projected onto metabolite levels which were nominally significantly altered over two or three time points, we tested if their baseline levels were significantly associated with baseline ACQ and BSQ. After multiple testing correction, only 2-hydroxy-2-methylbutanedioic acid was significantly positively associated with both ACQ ($r = 0.71$, $p = 3.6 \times 10^{-4}$, FDR-corrected p-value $= 1.15 \times 10^{-2}$) as well as BSQ, $r = 0.68$, $p(BSQ) = 6.1 \times 10^{-4}$, FDR-corrected p-value $= 1.95 \times 10^{-2}$.

Further, we tested the correlation between the nominal significant metabolites levels over three time points (Table 2) and anxiety values during the therapy using the Hamilton Anxiety Rating Scale (HAMA). The most consistent and significant correlation was found for postexposure levels of ascorbic acid with HAMA one session before the exposure (HAMA exp; Exposure 1: $r = -0.44$, $p = .02$; Exposure 2: $r = -0.46$, $p = .03$). Additionally, postexposure glyoxylate levels were significantly correlated with HAMA exp in the first exposure ($r = 0.43$, $p = .04$). In the second exposure, 24 hr postexposure was correlated with anxiety levels at the end of therapy ($r = 0.61$, $p = .001$).

3.5 | Pathway analysis

Pathway analysis was performed using all metabolites which were nominal significantly associated with exposure in the analysis of the first two time points to delineate exposure-related effects. We used hits from both exposures to capture a broader signal. The top enriched pathway (see Table 3) was pyrimidine metabolism.

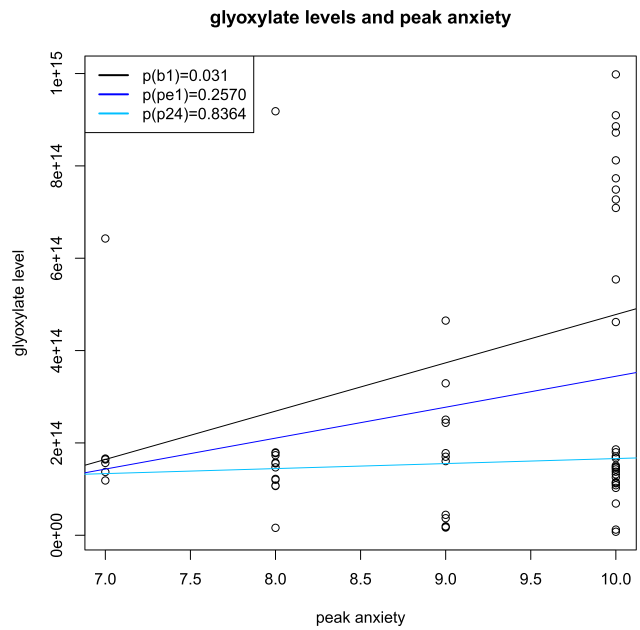

FIGURE 2 Correlation of glyoxylate levels and peak anxiety level (Subjective Units of Disturbances). Glyoxylate levels (y-axis) and anxiety (x-axis) at baseline (shown in black), at 1 hr postexposure (dark blue) and 24 hr after exposure (light blue)

3.6 | Gene expression

With regard to the changes in gene expression between two time points (baseline –1 hr), there were no significantly differentially expressed genes (all log-fold changes < 0.1, see Figure S2). To test for changes across the exposure we included all three time points, by using a liner mixed model to detected genes that were significantly altered (FDR < 0.05) which

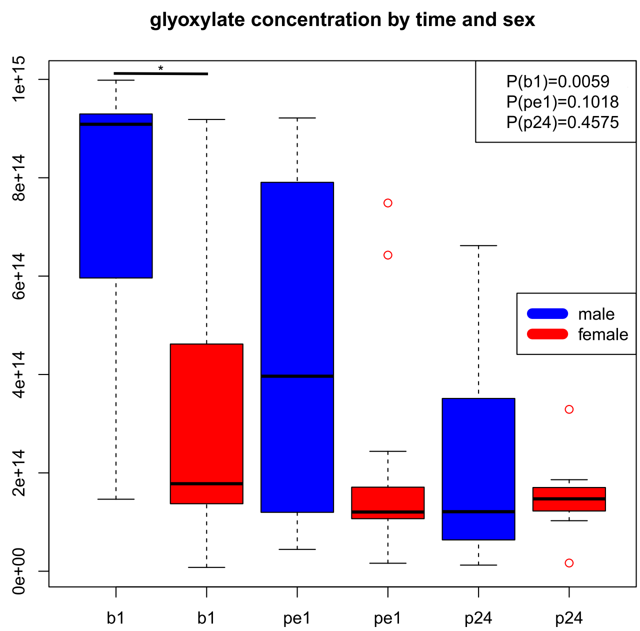

FIGURE 3 Glyoxylate levels by time and gender. Depicted are glyoxylate levels by gender at each time point. The boxes are the quartiles. The line with the box denotes the median and the whiskers represent the upper/lower quartiles

TABLE 3 Enrichment of altered metabolites

Pathway	Raw *p*	FDR-corrected *p*
Pyrimidine metabolism	$\mathbf{1.21 \times 10^{-4}}$	$\mathbf{9.70 \times 10^{-3}}$
Nitrogen metabolism	4.30×10^{-3}	1.71×10^{-1}
Alanine, aspartate, glutamate metabolism	1.78×10^{-2}	4.75×10^{-1}
Arginine and proline metabolism	2.76×10^{-2}	5.53×10^{-1}
Purine metabolism	4.36×10^{-2}	6.99×10^{-1}

Note: Pathways with nominal significant raw *p*-values for enrichment are shown, only the pyrimidine pathway holds after correction for multiple testing. Bold: significant after correction for multiple testing.
Abbreviation: FDR, false discovery rate.

TABLE 4 Enrichment of altered genes

Pathway	*p*	FDR-corrected *p*
BH3-only associated proteins	9.26×10^{-5}	2.54×10^{-2}
PCE/CE pathway	1.83×10^{-4}	3.65×10^{-2}
RHO-GTPases	2.96×10^{-5}	1.08×10^{-2}
Mitotic anaphase	9.52×10^{-6}	1.04×10^{-2}
Immune system	3.5×10^{-5}	1.10×10^{-2}

Note: Only pathways with nominal significant *p*-values for enrichment are shown.
Abbreviation: FDR, false discovery rate.

resulted in 227 altered genes (Table S3). These genes were used to perform overrepresentation analysis within reactome/KEGG pathways. This yielded five pathways which were significantly enriched after FDR correction (Table 4). However, these pathways did not overlap with the pathways obtained in the analysis of metabolites (Table 3). As glyoxylate is a by-product in the biosynthesis of CCK, we looked for differential CCK gene expression over all time points and remission status which resulted in significantly higher CCK gene expression only at the baseline in remitters ($p = .03$, Figure S3).

4 | DISCUSSION

In this study, we analyzed plasma metabolome and blood cell gene expression data during anxiety exposure in PD patients. The primary goal of the study was to identify metabolites and gene expression levels which correlate with different acute anxiety states during the exposure, such as anticipatory anxiety (b), anxiety provocation (*p*) and 1 day following the exposure markers for stable anxiety phenotype and could serve as biomarker for anxiety phenotypes. When taking basal and postexposure time points into account, several metabolites were nominal significantly altered in the course of the first and second exposure. However, as there was no overlap between the first and second exposure, these metabolites thus do not seem to depict robust anxiety-related changes. In contrast, the metabolite glyoxylate showed significant dynamic changes across all three time points of the experiment. Interestingly, baseline glyoxylate levels but not the first postexposure levels correlated with peak anxiety and showed gender-dependent differences. We conclude from our preliminary results that baseline glyoxylate levels of an individual reflect the anxiety level that will be experienced during exposure.

We could not detect an association with CBT response of glyoxylate or any other metabolites from the exposure experiment. The pathway analysis of all regulated metabolites across the exposure revealed an enrichment of the pyrimidine pathway which was shown to be involved in the clinical response in other psychiatric diseases, for example, depression (Park et al., 2016).

Generally, gene expression showed minimal fold changes over the exposure experiment (<0.09). The small magnitude in regulation suggests limited usability of this tool in the clinical context. So far only one study has investigated genome-wide gene expression in anxiety disorder patients over CBT course ($n = 102$; Hudson et al., 2013) and yielded no significant results (suggestive outcome for 20 genes). We conclude from this preliminary study with a small number of individuals, that gene expression in the blood might not be the best tool to capture acute anxiety-related states. Therefore, how we can use gene expression results in the clinical context needs further research.

Glyoxylate levels showed significant changes across all three time points of the first exposure. Glyoxylate is mainly a product of enzymatic glycolate oxidation in peroxisomes (Kunze & Hartig, 2013). Another source of glyoxylate is the 4-hydroxyproline metabolism in mitochondria (Salido, Pey, Rodriguez, & Lorenzo, 2012). Nikiforova et al. (2014) attempted to link glyoxylate to diabetes type 2. However, the lack of strong correlations between plasma and liver tissue suggests that these pathways do not directly contribute to changes in plasma glyoxylate levels.

Interestingly, glyoxylate is also a by-product in the biosynthesis of CCK. Bioactive CCKs are synthesized by two enzymatic reactions from the immediate precursors, the glycine-extended CCKs (Bradwejn & Vasar, 2013). CCK has a function in the alimentary tract and the central nervous system (CNS), where it seems to be the most abundant neuropeptide in the CNS (Lindefors, Linden, Brene, Sedvall, & Persson, 1993). Its role in anxiety is well established in the literature where the acute anxiogenic effects of CCK appear to be the result of *CCK*-B receptor activation in the basolateral amygdala (Ravard & Dourish, 1990). Different CCK-8 fragments have been reported to have a degradation half-life of less than 1 hr in human plasma (sulphated: 5–17 min: unsulphated CCK-8: 50 min). CCK-9 analogs were rapidly converted into their corresponding octapeptides (half-life of 2.7 min in human plasma). CCK-4 showed a half-life of 13 min (Koulischer, Moroder, & Deschodt-Lanckman, 1982) and is therefore difficult to capture in our experimental setting. Metabolic degradation of glyoxylate in mammals takes place exclusively in the liver (Knight & Holmes, 2005; Salido et al., 2012) and therefore is longer present in the plasma.

The fact that glyoxylate is a by-product of and therefore an indicator of CCK levels is supported by our finding that baseline glyoxylate levels significantly correlate with the peak anxiety level during exposure (see Figure 2). Genetic association of CCK-B receptor repeat polymorphism and PD has been previously shown (Hösing et al., 2004). CCK-4 peptide has been used to induce panic attacks in healthy controls and PD patients for research purposes. Nevertheless, there is a debate whether this peptide's activities adequately mimic the natural appearance of panic attacks. Our preliminary metabolomic results suggest that intrinsic CCK circuits are activated during an exposure-induced anxiety provocation. According to the glyoxylate levels over the course of our study, CCK might be involved in anticipatory anxiety thereby influencing the severity of the anxiety within the exposure. To gain more information about the regulation of CCK in our experiment we analyzed the trajectory of CCK gene expression. CCK gene expression was significantly higher before exposure in patients with remitter status after therapy, again suggesting that CCK is involved in anticipatory anxiety and activation of brain circuits important for successful learning in the exposure intervention. Further studies with larger sample size and direct measurements of CCK-related circuits are needed to prove this hypothesis.

Functional and physiological parameters are known to differ between men and women and affect gene expression and metabolite levels. Previous studies have reported sex-dependent gene expression in liver (Kwekel, Desai, Moland, Branham, & Fuscoe, 2010) and in stem cells (Ronen & Benvenisty, 2014). Plasma and urine metabolite profiles are able to classify samples by sex with >90% accuracy. Main features used for the classification included creatinine, sarcosine and branched chain amino acids (Rist et al., 2017). For the top hits, we find a significant gender-specific difference for glyoxylate at baseline ($p = 1.46 \times 10^{-4}$). Interestingly, there are animal data showing a reduction of CCK in the limbic structures including amygdala in response to estrogen injection (Holm, Liang, Thorsell, Hilke, & Behavior, 2014). Given the link between CCK metabolism and glyoxylate, this effect might be one explanation for the sex-difference at the basal time point. Furthermore, we observed differences for eight other metabolites which have been previously reported to vary by gender (Table S2). However, due to the relatively small sample size ($n = 25$) the identified metabolite levels differences need to be validated with a larger set of samples.

2-Hydroxy-2-methylbunaneioic acid was significantly associated with anxiety cognition and body sensation levels at the basal time point. This and another four metabolites (nominal effects over three time points in both measurements: ascorbic acid, 2-oxobutanoate, homocysteine, and glutamine) might be candidates for further studies. Interestingly, ascorbic acid is implicated in oxidative metabolism and was the only metabolite significantly associated with anxiety measures during the therapy (Uluata, McClements, & Decker, 2015). A previous study reported an association between oxidative stress and certain anxiety disorders (obsessive-compulsive disorder and PD), implicating that oxidative metabolism may affect anxiety (Kuloglu et al., 2002). Furthermore, reduced ascorbic acid levels in the hippocampus of mice with high anxiety-related behavior has been associated with anxiolytic effects of MitoQ treatment (Nussbaumer et al., 2016). Thus, ascorbic acid levels may reflect anxiety-related changes in the periphery and the brain.

Using the metabolites with altered levels between the first and the second time point, we performed overrepresentation analysis which highlighted significantly the pyrimidine and nominally the nitrogen, amino acids, and purine pathways. Pyrimidine and purine pathways have been previously linked to MDD (Park et al., 2016) and proteins of these pathways are significantly correlated with clinical response in MDD patients. In our sample, the burden of depression was low (HAMD = 11.3 ± 6.1). This raises the question whether these pathways, and especially pyrimidine pathway in our experiment, comprise general markers of symptom burden in PD and depression and are linked to clinical response in a disease-unrelated manner.

One limitation of the present study is the small sample size. Given the exploratory nature of the study, namely to investigate the magnitude of metabolite and gene expression changes as well as to explore their potential as biomarker usability in the prediction of acute anxiety states, the results have to be replicated in further studies. This includes the investigation of how acute anxiety metabolome during exposure might be related to clinical response. We tried to minimize external bias related to metabolome before exposure and the day after (standardized breakfast, no coffee, no exercise). However, a comprehensive standardization was not possible as we applied exposure in real-world situations. For future studies, standardized exposure using virtual reality might reduce the number of possible bias. Additionally, there is no previous evidence for the best time point to measure metabolite level or gene expression changes in the context of acute anxiety. In our study, we decided to use the time point 1 hr after the peak of anxiety to capture more stable metabolic and gene expression changes. However, other time points might be more suitable to gain the maximum effect. Although we think that the identified metabolite changes are mainly dependent on the underlying anxiety pathology, we cannot exclude that they are also due to the general distress response during the exposure. A comparison with a control group is not feasible, because anxiety induction during the exposure works on the basis of phobic fear, which is normally absent in healthy probands. Studies on dynamic metabolome analyses during stress exposure in nonanxiety settings are lacking. Furthermore, significantly associated metabolites in our study do not overlap with exercise-induced metabolites described by Lewis et al. (2010). Future studies are needed to disentangle the specific anxiety pathology related markers from general stress-induced metabolome changes.

5 | CONCLUSIONS

To our knowledge, this is the first study investigating metabolite level changes and gene expression during acute anxiety induced by exposure intervention. We could detect glyoxylate as the metabolite dynamically regulated during the course of acute exposure and 24 hr later. This metabolite was indicative for the severity of experienced anxiety during the exposure and showed gender-specific differences with higher baseline values in man. As glyoxylate is a metabolite of CCK, we hypothesize, that this system is intrinsically involved in exposure-induced anxiety. We conclude from this exploratory study that metabolomics has the potential to predict anxiety states. Metabolite profiles might reflect the phenotype more closely than gene expression data, which in our study showed low fold changes. Further studies with greater sample sizes are needed to confirm these conclusions.

ACKNOWLEDGMENTS

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DATA ACCESSIBILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Abdallah, C. G., Jiang, L., De Feyter, H. M., Fasula, M., Krystal, J. H., Rothman, D. L., … Sanacora, G. (2014). Glutamate metabolism in major depressive disorder. *American Journal of Psychiatry*, *171*(12), 1320–1327. https://doi.org/10.1176/appi.ajp.2014.14010067

Abramowitz, J. S. (2013). The practice of exposure therapy: Relevance of cognitive-behavioral theory and extinction theory. *Behavior Therapy*, *44*(4), 548–558. https://doi.org/10.1016/j.beth.2013.03.003

Ali-Sisto, T., Tolmunen, T., Toffol, E., Viinamaki, H., Mantyselka, P., Valkonen-Korhonen, M., … Lehto, S. M. (2016). Purine metabolism is dysregulated in patients with major depressive disorder. *Psychoneuroendocrinology*, *70*, 25–32. https://doi.org/10.1016/j.psyneuen.2016.04.017

Andersen, A. M., Dogan, M. V., Beach, S. R., & Philibert, R. A. (2015). Current and future prospects for epigenetic biomarkers of substance use disorders. *Genes*, *6*(4), 991–1022. https://doi.org/10.3390/genes6040991

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2014). lme4: Linear mixed-effects models using Eigen and S4. *R package version*, *1*(7), 1–23.

Binelli, C., Muniz, A., Sanches, S., Ortiz, A., Navines, R., Egmond, E., … Martin-Santos, R. (2015). New evidence of heterogeneity in social anxiety disorder: Defining two qualitatively different personality profiles taking into account clinical, environmental and genetic factors. *European Psychiatry*, *30*(1), 160–165. https://doi.org/10.1016/j.eurpsy.2014.09.418

Bradwejn, J., & Vasar, E. (2013). *Cholecystokinin and anxiety: From neuron to behavior*. Heidelberg: Springer Science & Business Media.

Bragazzi, N. L. (2013). Rethinking psychiatry with OMICS science in the age of personalized P5 medicine: Ready for psychiatome? *Philosophy, Ethics, and Humanities in Medicine: PEHM*, *8*(1), 4. https://doi.org/10.1186/1747-5341-8-4

Chambless, D. L., Caputo, G. C., Bright, P., & Gallagher, R. (1984). Assessment of fear of fear in agoraphobics: The body sensations questionnaire and the agoraphobic cognitions questionnaire. *Journal of Consulting and Clinical Psychology*, *52*(6), 1090–1097.

Chikina, M., Zaslavsky, E., & Sealfon, S. C. (2015). CellCODE: A robust latent variable approach to differential expression analysis for heterogeneous cell populations. *Bioinformatics*, *31*(10), 1584–1591. https://doi.org/10.1093/bioinformatics/btv015

Chugani, D. C., Sundram, B. S., Behen, M., Lee, M. L., & Moore, G. J. (1999). Evidence of altered energy metabolism in autistic children. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *23*(4), 635–641.

De Boeck, P., Bakker, M., Zwitser, R., Nivard, M., Hofman, A., Tuerlinckx, F., & Partchev, I. (2011). The estimation of item response models with the lmer function from the lme4 package in R. *Journal of Statistical Software*, *39*(12), 1–28.

Ehlers, A., Margraf, J., & Chambless, D. (2001). *Fragebogen zu körperbezogenen Ängsten, Kognitionen und Vermeidung*. Göttingen: Beltz-Test.

Filiou, M. D., Zhang, Y., Teplytska, L., Reckow, S., Gormanns, P., Maccarrone, G., … Turck, C. W. (2011). Proteomics and metabolomics analysis of a trait anxiety mouse model reveals divergent mitochondrial pathways. *Biological Psychiatry*, *70*(11), 1074–1082. https://doi.org/10.1016/j.biopsych.2011.06.009

Gottschalk, M. G., Cooper, J. D., Chan, M. K., Bot, M., Penninx, B. W., & Bahn, S. (2015). Discovery of serum biomarkers predicting development of a subsequent depressive episode in social anxiety disorder. *Brain, Behavior, and Immunity*, *48*, 123–131. https://doi.org/10.1016/j.bbi.2015.04.011

Hamilton, M., Schutte, N., & Malouff, J. (1976). Hamilton Anxiety Scale (HAMA). Sourcebook of adult. Assessment: Applied clinical psychology. In Bellack, A. S., & Herson, M. (Eds.), *undefined* (pp. 154–157). Switzerland: Springer.

Higdon, R., Earl, R. K., Stanberry, L., Hudac, C. M., Montague, E., Stewart, E., … Kolker, E. (2015). The promise of multi-omics and clinical data integration to identify and target personalized healthcare approaches in autism spectrum disorders. *OMICS*, *19*(4), 197–208. https://doi.org/10.1089/omi.2015.0020

Hollis, F., van der Kooij, M. A., Zanoletti, O., Lozano, L., Canto, C., & Sandi, C. (2015). Mitochondrial function in the brain links anxiety with social subordination. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(50), 15486–15491. https://doi.org/10.1073/pnas.1512653112

Holm, L., Liang, W., Thorsell, A., Hilke, S. J. P. B., & Behavior (2014). Acute effects on brain cholecystokinin-like concentration and anxiety-like behaviour in the female rat upon a single injection of 17β-estradiol. *Pharmacology Biochemistry & Behavior*, *122*, 222–227.

Hösing, V. G., Schirmacher, A., Kuhlenbäumer, G., Freitag, C., Sand, P., Schlesiger, C., … Rietschel, M. (2004). In Müller, T., & Riederer, P. (Eds.), *Cholecystokinin-and cholecystokinin-B-receptor gene polymorphisms in panic disorder. Focus on extrapyramidal dysfunction* (pp. 147–156). Vienna, Austria: Springer.

Huber, W., von Heydebreck, A., Sultmann, H., Poustka, A., & Vingron, M. (2002). Variance stabilization applied to microarray data calibration and to the quantification of differential expression. *Bioinformatics*, *18*(Suppl 1), S96–S104.

Hudson, J. L., Lester, K. J., Lewis, C. M., Tropeano, M., Creswell, C., Collier, D. A., … Eley, T. C. (2013). Predicting outcomes following cognitive behaviour therapy in child anxiety disorders: The influence of genetic, demographic and clinical information. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, *54*(10), 1086–1094. https://doi.org/10.1111/jcpp.12092

Joshi-Tope, G., Gillespie, M., Vastrik, I., D'Eustachio, P., Schmidt, E., de Bono, B., … Stein, L. (2005). Reactome: A knowledgebase of biological pathways. *Nucleic Acids Research*, *33* (Database issue), D428–D432. https://doi.org/10.1093/nar/gki072

Kaczkurkin, A. N., & Foa, E. B. (2015). Cognitive-behavioral therapy for anxiety disorders: An update on the empirical evidence. *Dialogues in Clinical Neuroscience*, *17*(3), 337–346.

Kanehisa, M., & Goto, S. (2000). KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Research*, *28*(1), 27–30.

Kessler, R. C., Chiu, W. T., Jin, R., Ruscio, A. M., Shear, K., & Walters, E. E. (2006). The epidemiology of panic attacks, panic disorder, and agoraphobia in the National Comorbidity Survey Replication. *Archives of General Psychiatry*, *63*(4), 415–424. https://doi.org/10.1001/archpsyc.63.4.415

Knight, J., & Holmes, R. P. (2005). Mitochondrial hydroxyproline metabolism: Implications for primary hyperoxaluria. *American Journal of Nephrology*, *25*(2), 171–175. https://doi.org/10.1159/000085409

Koulischer, D., Moroder, L., & Deschodt-Lanckman, M. (1982). Degradation of cholecystokinin octapeptide, related fragments and analogs by human and rat plasma in vitro. *Regulatory Peptides*, *4*(3), 127–139.

Krolow, R., Arcego, D. M., Noschang, C., Weis, S. N., & Dalmaz, C. (2014). Oxidative imbalance and anxiety disorders. *Current Neuropharmacology*, *12*(2), 193–204. https://doi.org/10.2174/1570159x11666131120223530

Kuloglu, M., Atmaca, M., Tezcan, E., Gecici, O., Tunckol, H., & Ustundag, B. (2002). Antioxidant enzyme activities and malondialdehyde levels in patients with obsessive-compulsive disorder. *Neuropsychobiology*, *46*(1), 27–32. https://doi.org/10.1159/000063573

Kunze, M., & Hartig, A. (2013). Permeability of the peroxisomal membrane: Lessons from the glyoxylate cycle. *Frontiers in Physiology*, *4*, 204. https://doi.org/10.3389/fphys.2013.00204

Kwekel, J. C., Desai, V. G., Moland, C. L., Branham, W. S., & Fuscoe, J. C. (2010). Age and sex dependent changes in liver gene expression during the life cycle of the rat. *BMC Genomics*, *11*(1), 675. https://doi.org/10.1186/1471-2164-11-675

Lang, T., Helbig-Lang, S., Westphal, D., Gloster, A. T., & Wittchen, H.-U. (2018). *Expositionsbasierte Therapie der Panikstörung mit Agoraphobie*: Hogrefe Verlag, Göttingen.

Leek, J. T., Johnson, W. E., Parker, H. S., Jaffe, A. E., & Storey, J. D. (2012). The sva package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics*, *28*(6), 882–883. https://doi.org/10.1093/bioinformatics/bts034

Lewis, G. D., Farrell, L., Wood, M. J., Martinovic, M., Arany, Z., Rowe, G. C., … Yang, E. (2010). Metabolic signatures of exercise in human plasma. *Science Translational Medicine*, *2*(33), 33ra37.

Lim, C. K., Essa, M. M., de Paula Martins, R., Lovejoy, D. B., Bilgin, A. A., Waly, M. I., … Guillemin, G. J. (2016). Altered kynurenine pathway metabolism in autism: Implication for immune-induced glutamatergic activity. *Autism Research*, *9*(6), 621–631. https://doi.org/10.1002/aur.1565

Lindefors, N., Linden, A., Brene, S., Sedvall, G., & Persson, H. (1993). CCK peptides and mRNA in the human brain. Progress in Neurobiology, *40*(6), 671-690.

Liu, P., Jing, Y., Collie, N. D., Dean, B., Bilkey, D. K., & Zhang, H. (2016). Altered brain arginine metabolism in schizophrenia. *Translational Psychiatry*, *6*(8), e871–e871. https://doi.org/10.1038/tp.2016.144

McLean, C. P., Asnaani, A., Litz, B. T., & Hofmann, S. G. (2011). Gender differences in anxiety disorders: Prevalence, course of illness, comorbidity and burden of illness. *Journal of Psychiatric Research*, *45*(8), 1027–1035. https://doi.org/10.1016/j.jpsychires.2011.03.006

Menke, A., Rex-Haffner, M., Klengel, T., Binder, E. B., & Mehta, D. (2012). Peripheral blood gene expression: It all boils down to the RNA collection tubes. *BMC Research Notes*, *5*, 1. https://doi.org/10.1186/1756-0500-5-1

Ng, J. W., Barrett, L. M., Wong, A., Kuh, D., Smith, G. D., & Relton, C. L. (2012). The role of longitudinal cohort studies in epigenetic epidemiology: Challenges and opportunities. *Genome Biology*, *13*(6), 246. https://doi.org/10.1186/gb-2012-13-6-246

Nikiforova, V. J., Giesbertz, P., Wiemer, J., Bethan, B., Looser, R., Liebenberg, V., … Rein, D. (2014). Glyoxylate, a new marker metabolite of type 2 diabetes. *Journal of Diabetes Research*, *2014*, 685204–685209. https://doi.org/10.1155/2014/685204

Nussbaumer, M., Asara, J. M., Teplytska, L., Murphy, M. P., Logan, A., Turck, C. W., & Filiou, M. D. J. N. (2016). Selective mitochondrial targeting exerts anxiolytic effects in vivo. *Neuropsychopharmacology*, *41*(7), 1751–1758.

Park, D. I., Dournes, C., Sillaber, I., Uhr, M., Asara, J. M., Gassen, N. C., … Turck, C. W. (2016). Purine and pyrimidine metabolism: Convergent evidence on chronic antidepressant treatment response in mice and humans. *Scientific Reports*, *6*, 35317. https://doi.org/10.1038/srep35317

Peng, B., Li, H., & Peng, X. X. (2015). Functional metabolomics: From biomarker discovery to metabolome reprogramming. *Protein & Cell*, *6*(9), 628–637. https://doi.org/10.1007/s13238-015-0185-x

Plag, J., Gaudlitz, K., Zschucke, E., Yassouridis, A., Pyrkosch, L., Wittmann, A., … Strohle, A. (2012). Distinct panicogenic activity of sodium lactate and cholecystokinin tetrapeptide in patients with panic disorder. *Current Pharmaceutical Design*, *18*(35), 5619–5626.

Ravard, S., & Dourish, C. T. (1990). Cholecystokinin and anxiety. *Trends In Pharmacological Sciences*, *11*(7), 271–273.

Rist, M. J., Roth, A., Frommherz, L., Weinert, C. H., Kruger, R., Merz, B., …Watzl, B. (2017). Metabolite patterns predicting sex and age in participants of the Karlsruhe Metabolomics and Nutrition (KarMeN) study. *PLOS One*, *12*(8), e0183228. https://doi.org/10.1371/journal.pone.0183228

Ronen, D., & Benvenisty, N. (2014). Sex-dependent gene expression in human pluripotent stem cells. *Cell Reports*, *8*(4), 923–932. https://doi.org/10.1016/j.celrep.2014.07.013

Salido, E., Pey, A. L., Rodriguez, R., & Lorenzo, V. (2012). Primary hyperoxalurias: Disorders of glyoxylate detoxification. *Biochimica et Biophysica Acta/General Subjects*, *1822*(9), 1453–1464. https://doi.org/10.1016/j.bbadis.2012.03.004

Schnack, H. G., & Kahn, R. S. (2016). Detecting neuroimaging biomarkers for psychiatric disorders: Sample size matters. *Frontiers in Psychiatry*, *7*, 50. https://doi.org/10.3389/fpsyt.2016.00050

Sethi, S., & Brietzke, E. (2016). Omics-Based Biomarkers: Application of Metabolomics in Neuropsychiatric Disorders. *International Journal of Neuropsychopharmacology*, *19*(3), pyv096. https://doi.org/10.1093/ijnp/pyv096

R Core Team (2018). *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.

Toru, I., Maron, E., Raag, M., Vasar, V., Nutt, D. J., & Shlik, J. (2013). The effect of 6-week treatment with escitalopram on CCK-4 challenge: A placebo-controlled study in CCK-4-sensitive healthy volunteers. *European Neuropsychopharmacology*, *23*(7), 645–652. https://doi.org/10.1016/j.euroneuro.2012.08.011

Uluata, S., McClements, D., & Decker, E. (2015). How the multiple antioxidant properties of ascorbic acid affect lipid oxidation in oil-in-water emulsions. *Journal of Agricultural Food Chemistry*, *63*(6), 1819–1824.

White, K. S., Payne, L. A., Gorman, J. M., Shear, M. K., Woods, S. W., Saksa, J. R., & Barlow, D. H. (2013). Does maintenance CBT contribute to long-term treatment response of panic disorder with or without agoraphobia? A randomized controlled clinical trial. *Journal of Consulting and Clinical Psychology*, *81*(1), 47–57. https://doi.org/10.1037/a0030666

Xia, J., Mandal, R., Sinelnikov, I. V., Broadhurst, D., & Wishart, D. S. (2012). MetaboAnalyst 2.0—A comprehensive server for metabolomic data analysis. *Nucleic Acids Research*, *40*(Web Server issue), W127–W133. https://doi.org/10.1093/nar/gks374

Xiao, X. L., Dawson, N., MacIntyre, L., Morris, B. J., Pratt, J. A., Watson, D. G., & Higham, D. J. (2011). Exploring metabolic pathway disruption in the subchronic phencyclidine model of schizophrenia with the Generalized Singular Value Decomposition. *BMC Systems Biology*, *5*(1), 72.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Appendix D – Publication IV: DNA-methylation dynamics across short-term, exposure-containing CBT in patients with panic disorder

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Contributions: Jennifer Lange served in investigation, data acquisition and pre-processing, writing – original draft preparation (supporting), and revision of the manuscript. She took part in designing the study protocol, planned and executed the experiments, treated the majority of patients, was responsible for data entry, and contributed to parts of the methods section (study participants, treatment procedure, and blood sample collection) of the original draft of the manuscript.

ARTICLE OPEN

DNA-methylation dynamics across short-term, exposure-containing CBT in patients with panic disorder

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Interaction of genetic predispositions and environmental factors via epigenetic mechanisms have been hypothesized to play a central role in Panic Disorder (PD) aetiology and therapy. Cognitive Behavioral Therapy (CBT), including exposure interventions, belong to the most efficient treatments of PD although its biological mechanism of action remains unknown. For the first time, we explored the dynamics and magnitude of DNA-methylation and immune cell-type composition during CBT ($n = 38$) and the therapeutic exposure intervention ($n = 21$) to unravel their biological correlates and identify possible biomarkers of therapy success. We report transient regulation of the CD4 + T-Cells, Natural Killers cells, Granulocytes during exposure and a significant change in the proportions of CD4 + T cells, CD8 + T cells and B-Cells and Granulocytes during therapy. In an epigenome-wide association study we identified cg01586609 located in a CpG island and annotated to the serotonin receptor 3 A (HTR3A) to be differentially methylated during fear exposure and regulated at gene expression level with significant differences between remitters and non-remitters ($p = 0.028$). We moreover report cg01699630 annotated to ARG1 to undergo long lasting methylation changes during therapy (paired t test, genome-wide adj.p value = 0.02). This study reports the first data-driven biological candidates for epigenetically mediated effects of acute fear exposure and CBT in PD patients. Our results provide evidence of changes in the serotonin receptor 3 A methylation and expression during fear exposure associated with different long-term CBT trajectories and outcome, making it a possible candidate in the search of markers for therapy success. Finally, our results add to a growing body of evidence showing immune system changes associated with PD.

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INTRODUCTION

Panic disorder (PD) is a disabling psychiatric condition and characterized by repetitive and unpredictable panic attacks associated with psychological and somatic symptoms, such as fear of dying, feeling unreal, feel of being out of control, heart racing, shortness of breath sweating, feeling dizzy or trembling [1]. The majority of PD patients are affected by agoraphobia, where situations with limited escape or help possibilities are accompanied by intense fear, panic attacks, avoidance and anticipatory anxiety [2]. According to epidemiological studies, the lifetime prevalence ranges from 2.5 to 4% with women being affected twice as often as men [3, 4]. The appearance and course of panic symptomatology can be remitting-relapsing or chronic and comorbidity rates with other anxiety disorders and lifetime depression are high [5]. Age of onset for both PD and agoraphobia is in the adolescence and early adulthood impacting the individual personal and professional development at an early stage and causing high socioeconomic costs [6].

Anxiolytic antidepressants and cognitive behavioral therapy (CBT) including exposure interventions as treatment options show the best evidence for PD [7, 8]. However, the biological mechanisms underlying the effect of CBT and exposure interventions have not been elucidated yet and to date no biomarkers are available in the clinical routine to individually assign the best matching therapy to each patient or to supervise therapy-related progress.

Substantial evidence suggests a complex interplay between genetic and environmental factors in the etiology of PD and their role in the treatment-related outcomes [9]. The heritability is modest with 40–50% including frequent and rare variations of different effect sizes across the genome [10]. Thus, environmental influences are relevant for shaping biological processes which lead to PD pathology and recovery during therapy [11].

Epigenetics describes gene-regulatory mechanisms which are responsive to environmental influences, heritable, time-stable but also highly dynamic. This makes them interesting candidates to mediate effects of therapy in general and of exposure interventions in particular. Epigenetic changes include histone modifications, small/micro RNA-related gene silencing and DNA methylation (DNAm). The latter occurs at cytosines through addition of a methyl-group, mediated through DNA methyltransferases (DNMT). This process modulates gene expression by regulating accessibility of transcription factors to their binding sites [12]. DNAm in the promoter region often leads to reduced gene expression, whereas decreased or no methylation results in more active gene expression [13].

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Few case-control epigenome-wide association studies (EWAS) are available for PD or PD-related symptomatology showing significant association of CpG sites in genes involved in cell-cycle regulation and immune system with overall small effect sizes [14–16]. A meta-analysis of two published EWAS [17] identified 61 significantly differentially methylated CpGs on epigenome-wide level. Several of those CpGs were annotated to genes which play a role in immune system regulations, such as the T-lymphokine- activated killer cell-originated protein kinase (*TOPK*) or in brain development and stress-induced depressive behavior, such as SMARCA5. The authors also observed differences in immune phenotypes and reported a decreased proportion of CD8 + T cells and an increased neutrophil-to-lymphocytes ratio in PD cases. While several other studies have reported similar results [18, 19], other disagreed [20], thereby highlighting a probably complex inter-play between PD and the immune system.

Despite these previous studies providing important evidence for DNAm and immune phenotypes regulation in PD, temporal dynamics of these changes during CBT and exposure still remain unclear. Indeed, to the best of our knowledge, only one longitudinal study assessed the evolution of DNAm over the course of CBT in PD [21]. While this study did not report statistically significant changes, it presented suggestive evidence for differential DNAm in *IL1R1* between treatment responders and non-responders. Identification and confirmation of such biomarkers could prove very useful to stratify and improve treatment in clinical practice. In summary, to date few data is available on the magnitude of the DNAm changes over the therapy and no studies are available on epigenetic changes during CBT interventions known to drive the therapeutic effects.

Consequently, the present study aims to investigate changes in DNAm and immune cell-types proportion acutely during the exposure intervention and longitudinally over the course of short standardized CBT. As a proof-of concept study with a relatively small sample size ($n = 42$), the changes in DNAm are investigated both at epigenome-wide level, aiming for uncovering new biological knowledge at the cost of a lower power, and at a more powerful but restricted candidate gene-approach.

METHODS

Study participants

Fourty-two patients (males $n = 15$, females $n = 27$, mean age of 32 ± 10) (Supplementary Table 2) recruited in the Outpatient Clinic for Anxiety Disorders at the Max Planck Institute of Psychiatry between January 2014 and December 2015 were included in this study. As previously described in Martins et al. [22], all included individuals had a current primary DSM-IV-TR diagnosis of panic disorder (PD) with/without agoraphobia (PD/AG) and a clinical interview score >14 on the Hamilton Anxiety Scale (HAMA). Due to high comorbidity of the disorders, we use the term PD for the comorbid status throughout the text. Individuals with somatic disorders, pregnancy, personality disorders, current suicidal intent and other psychiatric disorders except other anxiety disorders or secondary mild or moderate depression were excluded from the study. Patients with anxiety disorders due to a medical or neurological condition or a comorbid Axis II disorder were also excluded. All patients included in the analysis were free of psychotropic medication and non-psychotropic drugs for at least 4 weeks before the therapy. Therefore, all medical conditions needing a pharmacological treatment as well as neurological conditions with putative damage of the central nervous system were exclusion criterion. The study was approved by the ethics committee of Ludwig-Maximilians-University in Munich, Project number 318/00. Written informed consent was obtained from all subjects.

Treatment procedure and blood sample collection

Treatment followed a structured and empirically validated manual by Lang [23], which consists of 12 sessions of CBT (1–2 sessions per week, 6–8 weeks) as well as two booster sessions after 2 and 4 months. Therapy included two accompanied in vivo exposures (sessions 5 and 7) with therapists trained in exposure-based CBT. Exposure sessions were conducted outside the clinic, depending on the feared situation (e.g., subway, supermarket, height) and specific concern (e.g., fainting, asphyxiation, losing control). The type of exposure was determined by the patient, with the goal of highest fear provocation (for detailed information on therapy and exposure intervention see [24]).

Therapy course related blood samples were collected before the treatment (T0), after the forth session (T4), at the end of therapy (E) and 2 months after the therapy (K) in 38 patients. Additionally, 3 blood samples were collected during the first exposure session (before the exposure (BE)), 1 h after the highest anxiety peak (post-exposure, P1h) and 24 h h after the exposure (P24h) (for detailed information see [22] and Fig. 1) in 21 patients. In total, 17 patients had their blood collected during both during the therapy and the exposure phase (Fig. 1). Blood collection, was performed at a fixed day time (9am) for every patient and every time point. Psychometric ratings of anxiety symptom severity were performed by a clinician not involved in the treatment using the German version of the clinician-rating Hamilton Anxiety Scale [25]. Patients were informed that their therapists would not have access to their responses on the measures. Therapy remission was reached if symptom ratings were below the cut-off 7. Remission was assessed after the end of therapy (12th session).

DNA methylation data processing and quality control

Genomic DNA was extracted using the Gentra Puregene Blood Kit (Qiagen) and bisulfite converted with the Zymo EZ-96 DNA Methylation Kit (Zymo Research) to assess the methylations levels at ca. 480,000 CpGs sites with the llumina HumanMethylation450 BeadChip array, as described in [16]. Further processing and quality control, including removal of failing or cross-reactive probes, functional normalization, batch correction, beta-value calculation and cell composition estimation were performed as described in [16]. Beta-values were inverse-normal transformed to satisfy the normality assumption of linear models. A total of ca 425,000 CpGs remained after QC.

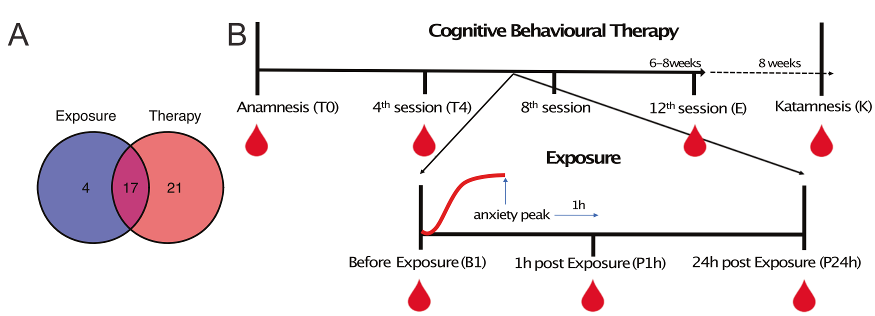

Fig. 1 Cohort, experimental procedure and data collection. A Cohort**:** Our study cohort comprised 42 patients. 21 were included in the therapy part only, 17 in both the exposure and therapy and 4 only in the exposure part. **B** Experimental procedure: Treatment consisted of 12 sessions of CBT (1–2 sessions per week, 6–8 weeks) as well as two booster sessions after 2 and 4 months. Therapy included two accompanied in vivo exposures (sessions 5,7) conducted outside the clinic in a feared situation.Therapy course related blood samples were collected before the treatment (T0), after the forth session (T4), at the end of therapy (E) and 2 months after the therapy (K). Additionally, 3 blood samples were collected during the first exposure session (before the exposure basal (BE)), 1 h after the highest anxiety peak (post-exposure, P1h) and 24 h h after the exposure (P24h).

Gene expression data processing and quality control

Blood RNA was collected at three timepoints (BE, P1h, P24h) and further processed as described in [26]. Blood RNA was hybridized to Illumina HumanHT-12 v4 Expression BeadChips and Illumina's GenomeStudio was used to export raw probe intensities. Further processing including normalization, batch correction and cell type composition estimation were performed as described in [22]. Expression values were inverse-normal transformed to satisfy the normality assumption of linear models.

Changes in immune cell types composition during CBT

The white blood cell types composition was estimated from the DNAm data using the Houseman deconvolution method [27]. Linear mixed models (LMM) including sex, age, and daily cigarettes consumption as covariates, the respective cell-types proportions as outcome and first or second degree polynomial of the time as predictor were used to assess if cell types proportion changed during the exposure phase or therapy course. FDR-corrected p values derived from the best model, identified by likelihood ratio tests (LRT) and Akaike Information Criterion (AIC) as described in Supplementary Methods, are reported and used to evaluate regulation of cell-type proportions during the exposure or therapy.

For the therapy outcome, a LMM with the first order polynomial of time remission status and their interaction as predictor was fitted to the first (T0) and last (E) timepoint to test for differences between beginning and end of therapy stratified by remission status.

Identification of differentially methylated CpGs during the course of exposure or therapy

Four LMMs including first- or second-degree polynomial of the time as predictor (see Supplementary Methods) were used to identify which CpGs were differentially methylated during the course of exposure or therapy and how they were regulated (i.e., in a linear or non-linear manner). These models were computed and compared using LRT and AIC as described in the Supplementary methods and included age, sex, number of cigarettes smoked per day immune cell types proportions and surrogate variables as covariates. The timepoints of therapy course and of the exposure phase were analyzed separately, as we hypothesized different types of regulation in these two time periods.

Paired-sample t tests comparing the first and last timepoints within the exposure or therapy phase were conducted to assess whether particular CpGs did return to their original levels after any kind of regulation. P values were FDR-corrected over the total number of test performed.

Residualization of the methylation and gene expression data

Given the reported immune cell types changes in the course of exposure and therapy, we used linear regression models to regress them out from the DNAm and gene expression for visualization purposes and statistical models for which the inclusion of covariates is not possible (e.g., Paired-sample t tests).

DMR analysis

We performed differentially methylated region (DMRs) analysis to group neighboring differentially methylated CpGs. Using the Comb-p software [28], we investigated regions containing at least 2 probes within a 750 base-pair window with a nominal p value for the best model smaller than 0.05. Because FDR-correction formally required by our model selection process would disturb the spatial correlation structure of the p values along the epigenome, which is needed in the Comb-p algorithm, we used uncorrected p values from the model in Eq. (2) (Supplementary Methods) for the exposure and Eq. (1) (Supplementary Methods) for the therapy as input for Comb-p. These models were selected as being the best model, as identified using LRT and AIC, for the majority of CpGs in that condition.

RESULTS

White blood cell types regulation

To investigate whether exposure or CBT drive changes in the immune system state of PD patients, we assessed the evolution of different white blood cells proportions, derived from methylation data, over 3 time points in the exposure and 4 timepoints covering the whole therapy duration. We hypothesized that exposure and therapy are two distinct processes which differ regarding both their amplitude and duration, the exposure being an extreme but short event and the therapy being a steady but long-time process. We therefore analyzed them separately in this study.

We identified 3 immune cell types, the CD4 + T-Cells, the Natural Killers cells (NK) and the Granulocytes which were significantly regulated during the exposure (Table 1). Consequently, the granulocytes-to-lymphocyte ratio (GLR) was significantly regulated as well. The LMM with random intercept and second degree polynomial of the time as predictor (Eq. (2) in

Table 1. Regulation of Immune Cell Type.

Cell type	*P* value time	Q value time	*P* value remission	Q value remission
Exposure				
CD8T	0.03	0.16	NA	NA
CD4T	$\mathbf{9.88 \times 10^{-5}}$	$\mathbf{7.37 \times 10^{-4}}$	NA	NA
Natural Killer cells	$\mathbf{1.00 \times 10^{-6}}$	$\mathbf{2.80 \times 10^{-5}}$	NA	NA
B-cells	0.40	0.73	NA	NA
Monocytes	0.76	0.81	NA	NA
Granulocytes	$\mathbf{3.09 \times 10^{-6}}$	$\mathbf{4.32 \times 10^{-5}}$	NA	NA
GLR	$\mathbf{1.00 \times 10^{-4}}$	$\mathbf{7.38 \times 10^{-4}}$	NA	NA
Therapy				
CD8T	$\mathbf{5.68 \times 10^{-3}}$	**0.013**	0.53	0.68
CD4T	$\mathbf{7.54 \times 10^{-4}}$	$\mathbf{2.64 \times 10^{-3}}$	0.86	0.86
Natural Killer cells	0.45	0.45	0.32	0.68
B-cells	0.02	**0.03**	0.49	0.68
Monocytes	0.32	0.37	0.32	0.68
Granulocytes	$\mathbf{5.80 \times 10^{-4}}$	$\mathbf{2.64 \times 10^{-3}}$	0.48	0.68
GLR	0.01	**0.02**	0.58	0.68

This table reports the results of the white blood cell types regulation analysis during the exposure and therapy respectively. The P value columns report the p value for the effect of time and of the remission status (for the therapy analysis) in the selected model The Q-value columns report the corresponding p value after multiple testing correction. LMMs were fitted on the 3 timepoints of the exposure and on the beginning and end of the therapy respectively. Statistically significant results are highlighted in bold.

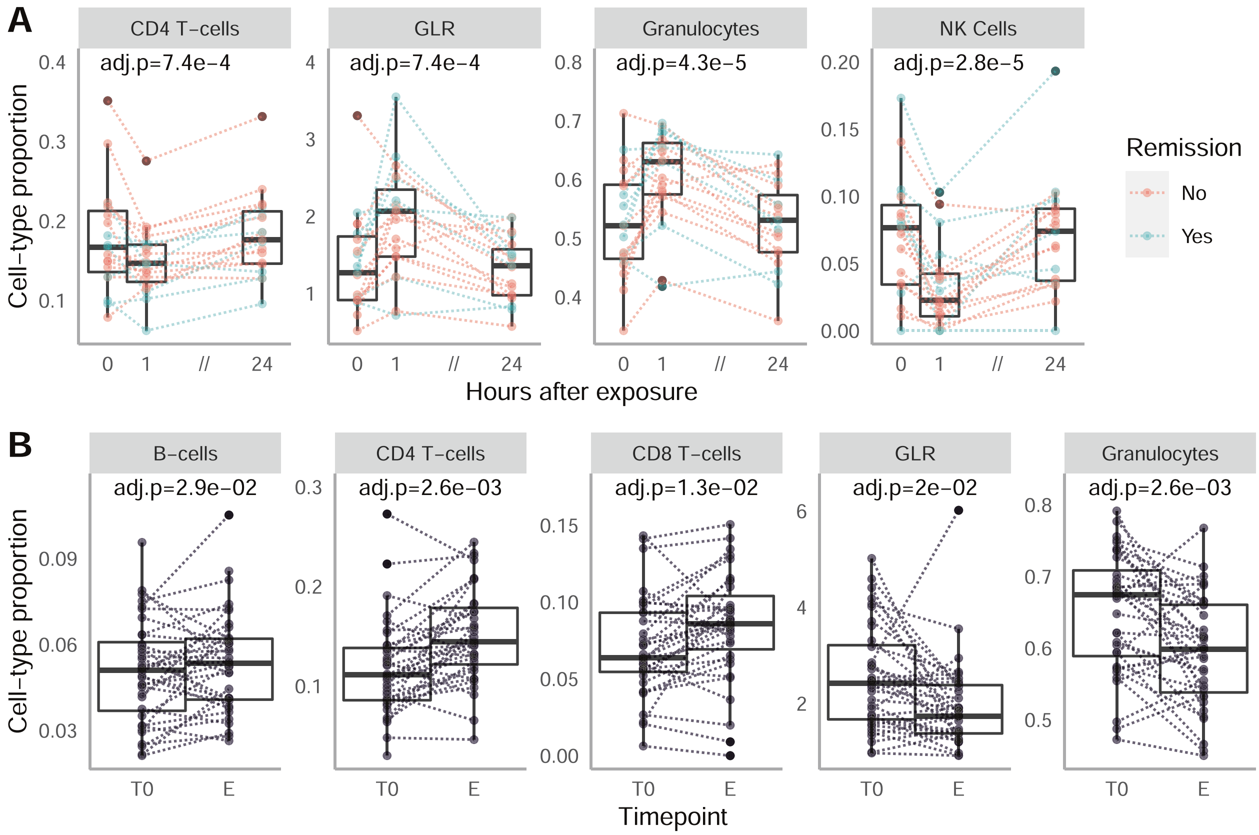

Fig. 2 Changes in White blood cells during exposure and therapy. A. Regulation of CD4 + T-cells (left), Granulocytes (middle) and Natural Killer cells (right) during the exposure phase. Thicker lines represent the mean for the remitters (blue) and non-remitters (red). The adjusted *p* values are reported for the effect of Time in the selected LMM (see "Methods"). **B**. Regulation of CD4 + T-cells, CD8 + T-cells, B-cells, Granulocytes and of the granulocytes-to-lymphocyte ratio (GLR) during the course of therapy. Cell-types proportions were directly estimated from the methylation data. The adjusted *p* values are reported for the effect of Time in a LMM fitted on the first and last time point of the therapy.

Supplementary Methods) was selected for these 3 cell types and the GLR, suggesting a non-linear regulation during the exposure, coherent with an acute event. Indeed, per individual cell-types proportions trajectories show a strong decrease (CD4T, NK cells, Fig. 2A) or increase (Granulocytes, GLR, Fig. 2A) one hour after the peak of anxiety before returning to pre-exposure value after 24 h. The magnitude of this effect, which happens over the course of one hour, is similar or greater than the changes observed in a time-scale of weeks during the therapy.

For the course of the CBT, we report a significant change in cell-type proportion for Granulocytes, CD4T + and CD8 + T-cells, B-cells and for the GLR between the begin and the end of therapy (Table 1 and Fig. 2B). As expected for a gradual process such as therapy, the best selected model for all cell-types included the first-degree polynomial of the time as predictor (Eq. (1) in Supplementary Methods) and indicates a linear regulation. This is confirmed by the observation of the per-individual trajectories (Supplementary Fig. 1), which moreover show a higher inter-individual variance than that in the exposure, coherent with a weaker effect.

In the search of a biological marker for therapy outcome, we also investigated if individual differences in immune cell-types trajectories during exposure or therapy could be predictive of remission. However, no significant difference could be observed between remitters and non-remitters during the exposure (Fig. 2A red vs blue and Supplementary Fig. 1) or therapy course (Table 1, Supplementary Fig. 2).

Identification of CpGs regulated during the course of exposure or therapy

With the aim of identifying possible biomarkers for therapy outcome as well as new mechanistical insights into the brain mechanisms triggered by exposure and therapy, we performed a methylome wide search for CpGs differentially methylated during exposure and therapy. We used LMMs for two reasons. Firstly, they allow to account for potential confounders, which was needed given the significant changes observed in the different white blood cell types proportions. Secondly, they allow to distinguish between linear and non-linear regulation, which might change the mechanistic interpretation of the findings.

No CpG was significantly regulated after multiple testing correction during the exposure or therapy in the LMMs analyses. Observations of the Quantile-Quantile diagnostics Plots (Supplementary Fig. 3), however, showed evidence of statistical signal and no signs of *p* value inflation. Based on this, we decided to rank the CpGs according to the strength of their statistical evidence. Similarly as described in [21], we combined this statistical ranking with a biological ranking based on the maximum absolute methylation difference between timepoints.

Exposure. We selected the top 100 CpGs (Table 2) in that sum ranking. These CpGs display both the highest statistical evidence for being regulated as well as potential for causal biological effects. The non-linear LMM was selected in more than 70% of this CpGs (Fig. 3A), which is coherent with the acute nature of the exposure and confirms that our approach is able to select

Table 2. Best-ranked CpGs for the exposure intervention and therapy.

CpG	*P* value	Q value	Bio rank	Stat rank	Sum rank	Gene
Exposure						
cg01586609	4.3×10^{-5}	0.83	53	60	113	HTR3A
cg02279108	2.95×10^{-5}	0.85	419	214	633	MNX1-AS1
cg27120833	1.63×10^{-4}	0.89	35	620	655	ARFGAP3
cg13927247	2.2×10^{-4}	0.87	448	290	738	DTWD2
cg03002526	1.9×10^{-4}	0.87	673	305	978	HACE1
cg09855140	2.38×10^{-4}	0.91	205	1044	1249	FKSG29
cg20611272	2×10^{-4}	0.86	1068	251	1319	ODF1
cg07428959	9.7×10^{-5}	0.90	445	885	1330	JADE1
cg13857354	5.7×10^{-5}	0.91	172	1219	1391	RNPEPL1
cg13054007	2.4×10^{-4}	0.87	1180	271	1451	SPRY2
Therapy						
cg01848660	1.00×10^{-4}	1	124	42	166	C1D
cg23839680	3.03×10^{-4}	1	180	153	333	PSPH
cg07205203	3.43×10^{-4}	1	211	175	386	PPP1CB
cg03308839	2.34×10^{-4}	1	584	107	691	NDE1
cg09160681	6.62×10^{-4}	1	553	323	876	PARM1
cg12594803	1.74×10^{-3}	1	28	873	901	DLG1
cg19676182	1.26×10^{-3}	1	471	624	1095	CCDC149
cg20956594	8.78×10^{-4}	1	701	425	1126	POMP
cg15210526	2.27×10^{-4}	1	1054	103	1157	MPZL1
cg25853622	1.19×10^{-3}	1	699	590	1289	LPP

This Table displays the 10 best CpGs ranked according to the cumulative, statistical and biological ranking (see "Methods") for the exposure intervention and therapy respectively. The *p* value column reports the *p* value for the effect of time in the selected model. The Q value columns reports the corresponding *p* value after multiple testing correction. The Gene Column reports the gene annotated to the CpG.

regulated CpGs. Furthermore, we used paired sample t test on the residuals of the methylation (see methods) to test which of these 100 CpGs were regulated in a long-lasting manner (i.e. different between timepoint BE and P24h), as these CpGs are good candidates to participate in the effect of exposure on remission.

The first CpG in both the overall and long-lasting CpGs ranking was annotated to the serotonin receptor 3 A gene, *HTR3A*. Methylation at cg01586609 showed a slow decrease between the 4th therapy time point towards the beginning of the exposure followed by an abrupt increase after the peak of anxiety to reach a summit 24 h thereafter (Fig. 3). We next investigated the DNAm dynamics of this CpG during exposure in remitters and non-remitters. When looking at the difference in DNAm compared to the first exposure time point, remitters show a stronger decrease of DNAm towards the peak of exposure, which is not fully recovered until the end of therapy. Given the low sample size and number of remitters ($n_{total} = 21$, $n_{remitters} = 7$), this trend is not statistically significant.

We next investigated if these DNAm changes had functional consequences at gene expression levels and looked at mRNA levels changes during exposure for 3 different probes annotated to the *HTR3A* gene. While the ILMN_1681492 probe associated with the first, longer transcript of the HTR3A gene did not show strong evidence of regulation (Supplementary Fig. 4), the ILMN_1662070 probe associated to the second transcript, coding for the canonical isoform of HTR3A, did. When looking at the residuals of gene expression after regressing out the immune cell-types proportions, expression drops drastically during the first hour of exposure only in remitters (Fig. 3B left). Afterwards, expression rises sharply in remitters towards the third time point while it decreases in non-remitters. Both, the regulation over-time and the difference in regulation between remitters and non-remitters, were statistically nominally significant (Linear mixed model; *p* value for $Time^2 = 0.022$, *p* value for interaction of $Time^2$ and remission status: 0.028).

The second probe annotated to the canonical isoform and located more distally at the very end of exon 9, did however not show the same evidence of regulation (Fig. 3B middle). Nevertheless, a clear trend is still observable when averaging the expression values of the two probes from the second transcript (Fig. 3C left) even if the regulation between timepoints and its interaction with the remission status are not significant anymore with this low sample size ($n = 21$ Linear mixed model; *p* value for $Time^2 = 0.087$, *p* value for interaction of $Time^2$ and remission status: 0.166).

Therapy. In contrary to the exposure, the first order LMM was selected for majority of the best 100 ranked CpGs for therapy which is coherent with the linear nature of the therapy process (Fig. 3A). Only 1 CpG was significantly differently methylated at the end of therapy as compared to the beginning in the paired-t test after multiple testing correction (cg01699630, ARG1; paired *t* test FDR corrected *p* value = 0.02). Additionally, the first and fourth CpGs in the sum ranking (Table 2) were annotated to the *C1D* and *NDE1* genes which have been respectively associated with Schizophrenia and epilepsy [29, 30]. Cg01848660, annotated to *C1D*, showed a linear increase during therapy in almost all patients but was also downregulated from the end of the exposure toward the end of therapy (Supplementary Fig. 6). Cg03308839, annotated to *NDE1*, showed an increase between the beginning (T0) and the fourth session of therapy (T4) followed by a decrease before the exposure and a stabilization in the subsequent timepoints (Supplementary Fig. 6).

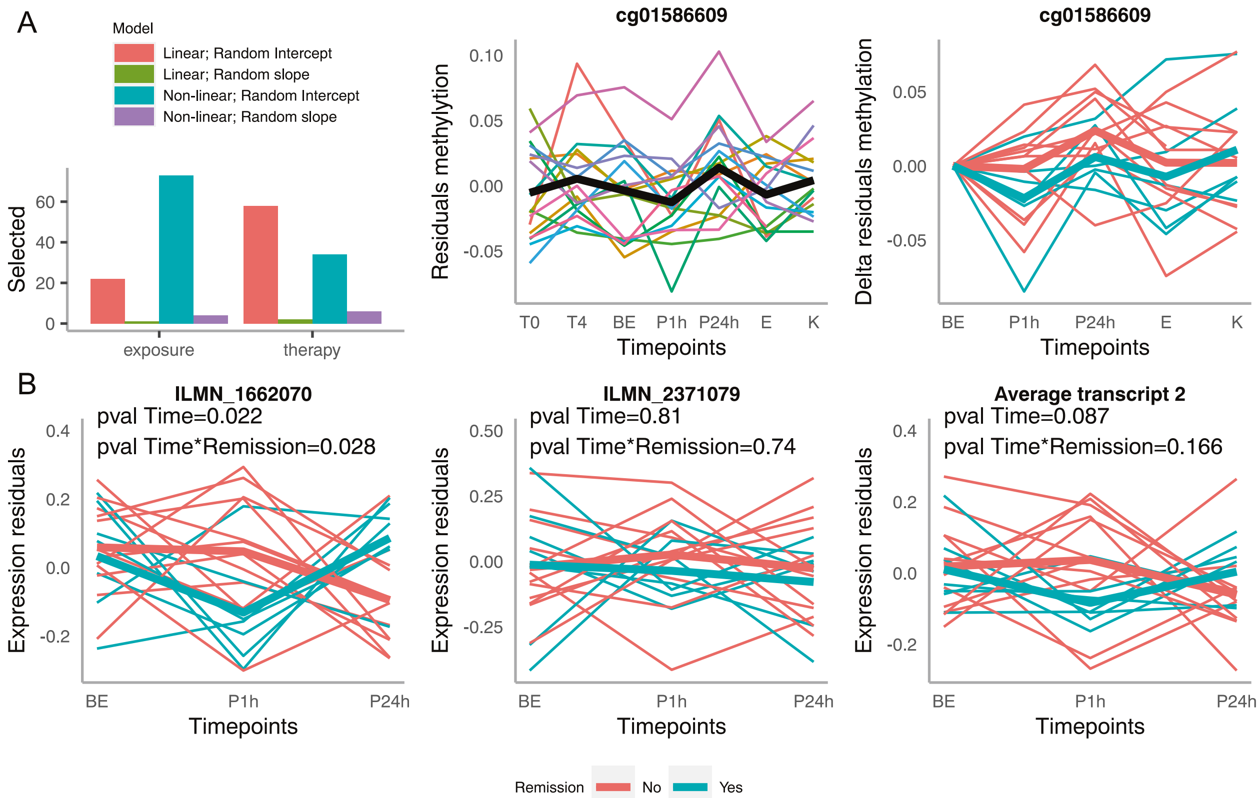

Fig. 3 Regulation of HTR3A methylation and gene expression during the exposure phase. A Methylation: Left: Distribution of the selected models among the 100 best sum-ranked CpGs in the exposure and therapy phase. Middle: Evolution over time of cg cg01586609's methylation residuals after regressing out the immune cell-type composition (referred as methylation residuals later) during the therapy (T0, T4, E, K) and exposure (BE, P1h, P24h) timepoints. The thick black line represents the mean over all samples. Right: Evolution over the exposure timepoints and subsequent therapy timepoints of the methylation residuals, centered at the value observed 1h before exposure. This graph represents the absolute changes in methylation during exposure, independent of the actual methylation levels. Thicker lines represent the mean for the remitters (blue) and non-remitters (red). **B** Gene Expression: Evolution over the therapy time points of the residuals of the gene expression data after regressing out the immune cell-types proportions of the ILMN_1662070 (left) and ILMN_2371079 (middle) probes annotated to the HTR3A gene and their average (right). Thicker lines represent the mean for the remitters (blue) and non-remitters (red).

Candidate gene analysis

We also conducted a targeted analysis for CpGs associated with genes which expression we had found to be altered during exposure in a previous study [22] (Supplementary Table 1). No CpG withstood multiple testing correction. However, 2 CpGs annotated to the *MAL1D1* gene ranked among the top 10 best ranked CpGs for the therapy (Supplementary Table 2). Further analysis of the DNAm dynamics of these 2 CpGs over exposure and therapy timepoints showed a shared and highly consistent pattern across individuals (Supplementary Fig. 5), i.e. first decrease of DNAm during the beginning of therapy, followed by increase in anticipation of the fear exposure which continues during the exposure itself before a gradual decrease during the following therapy timepoints. This regulation is nominally significant for cg24577389 (LMM; *p* value fixed effect of time: 0.004, *p* value fixed effect of time2: 0.007269) and almost nominally significant for cg10418812 (LMM; *p* value fixed effect of time: 0.05, *p* value fixed effect of time^2: 0.08).

Differentially methylated regions analysis

It has been suggested that groups of spatially close co-regulated CpG probes, Differentially Methylated Regions (DMR), could be of particular biological relevance for several phenotypes. We therefore assessed if such clusters of co-regulated CpGs could be detected during therapy or exposure. Using the Comb-P software (Methods) we found six DMRs for exposure and one DMR for therapy (Supplementary Table 3). Of particular interest are the *SLC6A12* gene coding for a GABA transporter previously associated with negative symptoms in schizophrenia [31] and the insulin receptor substrate 2 which has been proposed to regulate dopamine cell morphology [32].

DISCUSSION

Blood cell count

In the present study, we found a dynamic change of specific immune cell types during exposure and therapy. While some findings in regard to platelet reactivity indicators and red cell distribution width in PD compared to healthy controls suggest their diagnostic and predictive capacity [33, 34], investigations of immune markers in PD are still at the early stage. Petersen et al. analyzed immune cell proportions from two previous case-control DNAm studies in PD and observed significantly lower proportions of CD8 + T-lymphocytes, higher proportion of neutrophil as well as increased neutrophil-to-lymphocyte ratio in PD cases, however, after adjustment for age and sex, only CD8 + reduction was still significant [17]. The authors suggest that reduced CD8 + T cells are consistent with lower levels of IFN-gamma previously detected in a case-control study in PD [35]. Coherently, we report a significant regulation of the CD8 + T cells during therapy phase, with the proportion at the end of therapy being significantly higher than at the beginning. We also observe a significant decrease in Granulocytes and in the Granulocytes-to-lymphocytes ratio during therapy, in agreement with the trend described by

Petersen and colleagues. As a whole, it seems that the immune cell-type composition changes over the course of therapy from a "PD-case-like" status towards a more "control-like" status.

In addition, we observe an increase of the CD4 + T cells proportion and decrease of the Granulocytes proportions during the therapy course. These changes are the opposite of the dynamics observed during the exposure phase. Indeed, CD4 + T cells shown an abrupt decrease one hour after the peak of anxiety, whereas the Granulocytes proportion increased dramatically. These variations in proportion are of greater magnitude than during therapy and happen in a very short time scale, pointing towards an acute response to the strong stress induced by exposure. Comfortingly, similar changes have been observed in response to acute stress in rodents [36, 37]. Taken together, our results suggest that exposure induces an acute stress response of the immune system whereas the therapy might lead to a gradual transition towards a "less-stressed" state of the immune system.

Interestingly, we did not observe any difference in any of the cell-types regulations between responders and non-responders. Whether this means that these regulations are physiological responses to stress, which are not malfunctioning in PD patients, or that they are part of the disease etiology but not affecting the therapy outcome, cannot be hypothesized from this study. Further studies, with higher sample size and inclusions of healthy control during the exposure phase are needed to clarify this question.

Methylation regulation

In the present study, we conducted an epigenome-wide and a targeted candidate analysis of DNAm changes over the course of exposure and therapy. These two types of analysis have the potential to complement each other. The former being able to generate new hypotheses at the cost of a lower power, and the later allowing a higher power for a set of genes of interest.

We acknowledge that the sample size of our cohort of 42 patients provides only limited power for an epi- genome-wide analysis using LMMs. However, as this is the first study of this kind, and very little is known about therapy-related methylation in PD, we suggested that a hypothesis free approach could allow generating new hypotheses about possibly involved biological mechanisms and explore the magnitude of DNAm dynamics. Indeed, even if no CpGs reached epigenome-wide significance, the *p* values obtained from the LMMs are still indicative of the strength of the statistical evidence and allow to rank CpGs from the more to the less likely to be regulated. Combined with a ranking based on the magnitude of the observed changes, our statistical analysis allowed to uncover CpGs with a strong potential to be regulated. The validity of this approach is moreover confirmed by the very different distributions of selected LMMs for the best ranked CpGs during therapy versus exposure. Indeed, non-linear models were widely predominant in the exposure phase, consistent with an acute event, and linear models were predominant in the therapy phase, consistent with a long lasting process. This suggests that our models could indeed find meaningful biological changes.

We included several covariates, such as sex and age and smoking in our models, and used LMM which are able to account for unspecified variations among samples in order to correct for possible confounders in our study population. However, we cannot exclude that remaining confounders, such as diet, physical activity or stressful life events might have influenced our results. In particular if these variables were heterogeneously distributed between remitters and non-remitters. Moreover, the inclusion of healthy control group and randomized controlled experimental design with patients undergoing therapy and CBT free patients would be necessary to show that the DNAm and expression changes reported here are solely caused by the exposure or CBT. Nonetheless, we argue that the strict exclusion criteria of the study, such as the psychotrop-medication-free condition, the very homogenous study sample and very similar DNAm and expression trajectories observed across patients, plead in favor of a genuine effect of the interventions.

In the epigenome-wide association analysis of the exposure phase, the first CpG in both the overall and long-lasting CpGs ranking was annotated to the serotonin receptor 3 A gene (*HTR3A)* (product: $5HT_3A$ receptor, 5HT3AR), which has been associated with numerous psychiatric disorders in human and related behavior in mice [38–42]. The $5\text{-}HT_3$ receptor is a ligand-gated ion channel composed of five subnits, in which 5-HT binding occurs to the extracellular N-terminus of the $5\text{-}HT_{3A}$ subunit [43]. 5HT3AR subtype is localized in limbic brain regions, such as the amygdala, hippocampus and throughout the cortex, closely involved in the regulation of panic states [44, 45]. The antagonism of the HT3 receptors display anxiolytic effects and blunted response to acute stress in rodents and primates, e.g., 5HT3AR null mice exhibit anxiolytic behavioral phenotype [46]. Furthermore, around 30% of GABAergic interneurons contain 5HT3AR and were suggested to influence cortical circuits during specific behavioral contexts [47]. A subset of these interneurons also co-expresses cholecystokinin (CCK) [48], a neuropeptide system used for panic induction via CCK4 and recently linked to metabolomic response during exposure [22, 49].

To investigate if these DNAm changes during exposure had functional consequences, we assessed the evolution of *HTR3A* gene expression in peripheral blood during exposure.

We report a strong decrease of *HTR3A* expression one hour after the peak of anxiety, compatible with a regulation through DNAm. Although both DNAm and gene expression changes are transient, with levels going back to initial values at the end of exposure, they could have long lasting implication during therapy and even predict therapy outcome. Indeed, transient changes in gene expression driven by DNAm changes have for example been showed to allow memory formation [50]. We therefore assessed whether *HTR3A* methylation and gene expression were differentially regulated in remitters vs non-remitters. And indeed, we observed a different dynamic of *HTR3A* expression with a nominally significant effect of the interaction between the remission indicator variable and the time in the LMM suggesting a different effect of the acute exposure on this serotonin receptor in remitters vs non-remitters. Decreased *HTR3AR* production in remitters suggest a pronounced anxiolytic effect in compare to non-remitted group after the exposure. From that point, antagonism of 5HT3AR might be a candidate mechanism to booster exposure effects by enhancing the anxiolysis and diminishing stress-induced deleterious effects in AD. In fact, there is evidence from pharmacological studies of improved antidepressive efficacy of Serotonine-Reuptake-Inhibitors, the first line treatment agents in most AD, after blockade of 5HT3AR [51]. Interestingly, the significant expression difference between remitters and non-remitters was found in a probe annotated to the canonical, functional, isoform of HTR3A, whereas the probe associated to the long isoform did not show significant regulation. This long isoform has been shown to be unable to form functional homomeric receptors but to be able to modify the response of heteromeric HTR3A receptors [52]. This suggest that the effect of HTR3A regulation on treatment outcome might be underpinned by a decrease of the canonical-homomeric form of HTR3A rather than by a modulation the serotonin response by heteromeric receptors.

In addition to *HTR3A*, a previous case-control study from our group identified methylation differences at CpGs annotated to two other serotonin receptors, namely *HTR1A* and *HTR2A* [16]. Taken together, these results suggest a shared involvement of the serotonin system in the etiology of PD and in response to acute fear during the exposure phase and further studies are needed to clarify a potential clinical application of 5HT3AR in therapeutic exposure and pharmacological treatment in AD.

Follow-up studies, with higher sample size would therefore be needed to replicate this preliminary finding and in vivo experiments would in addition be needed to assess whether these changes play a mechanistic role in therapy and exposure effects. In particular, the present study assessed the DNAm and gene expression in the blood as a proxy for brain DNA and expression, which are not readily accessible in humans. Several studies have shown that shown brain and blood transcriptomics to be very similar [53], and especially for receptors in general and for HTR2Ain particular [54], another members subtypes of serotonin receptors. Concordantly, the human protein atlas [55] reports similar HTR3A expression levels for the average of Peripheral Blood Mononuclear Cells (PBMC) and for several brain regions, including the cortex, thalamus and midbrain (https://www.proteinatlas.org/ENSG00000166736-HTR3A, accessed on 2021-10-20). However, it remains to be directly shown that the observed in the blood during the exposure and therapy phase are reflecting changes in the brain.

In the candidate analysis, we found two CpGs in the *MAD1L1* gene to be nominally regulated during the therapy. *MAD1L1* (mitotic arrest deficient 1like 1) dysfunction is associated with chromosomal instability and risk variations in this genes were linked to anxiety-related psychopathology [56], such as broad anxiety symptomatology [57] and neuroticism [58], but also further major psychiatric phenotypes, such as depression [59], bipolar disorder [60] and schizophrenia [61]. Furthermore, DNAm markers have been identified as associated with higher risk for PTSD in military male subjects in a longitudinal set up [62]. Snijders and colleagues reported decreased methylation at cg12169700, post trauma associated with decrease gene expression. On the contrary, we report decreasing methylation at cg10418812 and cg24577389 during the first phase of therapy followed by an increase during the exposure phase. These differences could be due to different effects of the initial trauma, therapy and exposure on the regulation of *MAD1L1* regulation. Nevertheless, these apparently opposite effect could participate in the same regulation process, given that cg12169700 is positioned inside the 16th exon and cg10418812 is located respectively 5 kb before transcription start and in the 12th exon. Indeed, whereas hyper-methylation of CpG in the promoter region is well understood and associated with gene expression suppression, the consequence of gene-body CpG methylation are still under study. Notably, several studies have shown gene-body CpG methylation to correlate with gene expression [63, 64]. Further studies, assessing gene expression during the therapy would be needed to conclude on the regulation of *MAD1L1* at the expression level during CBT.

In a complementary approach, we used paired t test to test for difference between the end and the beginning of the therapy only, ignoring the dynamics of the methylation in the intermediate time-points but looking for therapy-driven long-lasting changes. In the paired-t test, we obtained one CpG significantly differently methylated at the end of therapy as compared to the beginning in the gene *ARG1*. This gene is involved in the urea cycle and missense mutations in this gene cause serious developmental and neurological syndroms [65]. *ARG1* is important for macrophages specification and their effector functions [66] again highlighting that immunometabolism might be of importance in therapy-related effects.

CONCLUSION

This is the first longitudinal study of DNAm and immune cell-type composition combining CBT course and acute fear exposure in PD patients. We firstly demonstrate that CBT and acute fear exposure do have measurable biological correlates, a critical argument in favor of their efficacy. Our results moreover provide evidence of the involvement of *HTR3A* in CBT success, calling for further experiment to dissect its mechanism of action and clarify potential clinical application. In addition, we also identified several genes with high potential of regulation during therapy to be investigated in further candidate studies. Finally, our study adds to the growing body of evidences linking PD and regulation of the immune system state.

CODE AVAILABILITY

The code written to perform the analysis is available at https://github.com/sylvain-moser/CBT_DNAm.

REFERENCES

1. Ninan PT, Dunlop BW. Neurobiology and etiology of panic disorder. J Clin Psychiatry. 2005;66:3–7.
2. Goodwin RD, Faravelli C, Rosi S, Cosci F, Truglia E, De Graaf R, et al. The epidemiology of panic disorder and agoraphobia in Europe. Eur Neuropsychopharmacol. 2005;15:435–43.
3. Wittchen HU, Jacobi F, Rehm J, Gustavsson A, Svensson M, Jönsson B, et al. The size and burden of mental disorders and other disorders of the brain in Europe 2010. Eur Neuropsychopharmacol. 2011;21:655–79.
4. Kessler RC, Petukhova M, Sampson NA, Zaslavsky AM, Wittchen HU. Twelve-month and lifetime prevalence and lifetime morbid risk of anxiety and mood disorders in the United States. Int J Methods Psychiatr Res. 2012;21:169–84.
5. Batelaan NM, De Graaf R, Penninx BWJH, Van Balkom AJLM, Vollebergh WAM, Beekman ATF. The 2-year prognosis of panic episodes in the general population. Psychol Med 2010;40:147–57.
6. Hendriks SM, Spijker J, Licht CMM, Hardeveld F, de Graaf R, Batelaan NM, et al. Long-term disability in anxiety disorders. BMC Psychiatry. 2016;16:248.
7. Carpenter JK, Andrews LA, Witcraft SM, Powers MB, Smits JAJ, Hofmann SG. Cognitive behavioral therapy for anxiety and related disorders: a meta-analysis of randomized placebo-controlled trials. Depress Anxiety. 2018;35:502–14.
8. Bandelow B, Sher L, Bunevicius R, Hollander E, Kasper S, Zohar J, et al. Guidelines for the pharmacological treatment of anxiety disorders, obsessive-compulsive disorder and posttraumatic stress disorder in primary care. Int J Psychiatry Clin Pr Int J Psychiatry Clin Pr. 2012;16:77–84.
9. Deckert J, Erhardt A Predicting treatment outcome for anxiety disorders with or without comorbid depression using clinical, imaging and (epi)genetic data. Curr Opin Psychiatry. 2019;32:1–6.
10. Meier SM, Trontti K, Purves KL, Als TD, Grove J, Laine M, et al. Genetic Variants Associated With Anxiety and Stress-Related Disorders. JAMA Psychiatry. 2019;76 (Sep):924.
11. Hettema JM, Prescott CA, Myers JM, Neale MC, Kendler KS The structure of genetic and environmental risk factors for anxiety disorders in men and women. Arch Gen Psychiatry. 2005;62:182–9.
12. Kribelbauer JF, Lu XJ, Rohs R, Mann RS, Bussemaker HJ. Toward a mechanistic understanding of DNA methylation readout by transcription factors. J Mol Biol. 2020;432:1801–15. https://doi.org/10.1016/j.jmb.2019.10.021.
13. Jackson-grusby L, Beard C, Possemato R, Tudor M, Fambrough D, Csankovszki G, et al. Loss of genomic methylation causes p53- dependent apoptosis and epigenetic deregulation. Nat Genet. 2001;27:31–9.
14. Shimada-Sugimoto M, Otowa T, Miyagawa T, Umekage T, Kawamura Y, Bundo M, et al. Epigenome-wide association study of DNA methylation in panic disorder. Clin Epigenetics. 2017;9:1–11. https://doi.org/10.1186/s13148-016-0307-1.
15. Emeny RT, Baumert J, Zannas AS, Kunze S, Wahl S, Iurato S, et al. Anxiety associated increased CpG methylation in the promoter of Asb1: a translational approach evidenced by epidemiological and clinical studies and a murine model. Neuropsychopharmacology. 2018;43:342–53.
16. Iurato S, Carrillo-Roa T, Arloth J, Czamara D, Diener-Hölzl L, Lange J, et al. DNA methylation signatures in panic disorder. Transl Psychiatry. 2017;7:1–10. https://doi.org/10.1038/s41398-017-0026-1.
17. Petersen CL, Chen JQ, Salas LA, Christensen BC. Altered immune phenotype and DNA methylation in panic disorder. Clin Epigenetics. 2020;12:1–10. https://doi.org/10.1186/s13148-020-00972-9.
18. Perini GI, Zara M, Carraro C, Tosin C, Gava F, Santucci MG, et al. Psychoimmunoendocrine aspects of panic disorder. Hum Psychopharmacol Clin Exp. 1995;10:461–5.
19. Rapaport MH. Circulating lymphocyte phenotypic surface markers in anxiety disorder patients and normal volunteers. Biol Psychiatry. 1998;43:458–63.
20. Schleifer SJ, Keller SE, Bartlett JA. Panic disorder and immunity: Few effects on circulating lymphocytes, mitogen response, and NK cell activity. Brain Behav Immun. 2002;16:698–705. https://doi.org/10.1016/S0889-1591(02)00022-3.
21. Ziegler C, Grundner-Culemann F, Schiele MA, Schlosser P, Kollert L, Mahr M, et al. The DNA methylome in panic disorder: a case-control and longitudinal

psychotherapy-epigenetic study. Transl Psychiatry. 2019;9:314. Available from: https://doi.org/10.1038/s41398-019-0648-6.
22. Martins J, Czamara D, Lange J, Dethloff F, Binder EB, Turck CW, et al. Exposure-induced changes of plasma metabolome and gene expression in patients with panic disorder. Depress Anxiety. 2019;36:1173–81.
23. Lang T, Helbig-Lang S, Westphal D, Gloster AT, Wittchen H-U Expositionsbasierte Therapie der Panikstörung mit Agoraphobie. Expositionsbasierte Therapie der Panikstörung mit Agoraphobie. Hogrefe Verlag; 2012.
24. Lange J, Goerigk S, Nowak K, Rosner R, Erhardt A. Attachment style change and working alliance in panic disorder patients treated with cognitive behavioral therapy. Psychotherapy. 2021;58:206–18.
25. Hamilton M. The assessment of anxiety states by rating. Br J Med Psychol. 1959;32:50–5.
26. Menke A, Rex-Haffner M, Klengel T, Binder EB, Mehta D. Peripheral blood gene expression: It all boils down to the RNA collection tubes. BMC Res Notes. 2012;5:1–8.
27. Houseman EA, Accomando WP, Koestler DC, Christensen BC, Marsit CJ, Nelson HH, et al. DNA methylation arrays as surrogate measures of cell mixture distribution. BMC Bioinformatics. 2012;13:86.
28. Pedersen BS, Schwartz DA, Yang IV, Kechris KJ. Comb-p: Software for combining, analyzing, grouping and correcting spatially correlated P-values. Bioinformatics. 2012;28:2986–8.
29. Kimura H, Tsuboi D, Wang C, Kushima I, Koide T, Ikeda M, et al. Identification of rare, single-nucleotide mutations in NDE1 and their contributions to schizophrenia susceptibility. Schizophr Bull. 2015;41:744–53.
30. Gavrilovici C, Jiang Y, Kiroski I, Sterley T-L, Vandal M, Bains J, et al. Behavioral deficits in mice with postnatal disruption of Ndel1 in forebrain excitatory neurons: implications for epilepsy and neuropsychiatric disorders. Cereb Cortex Commun. 2021;2:1–18.
31. Park HJ, Kim JW, Lee SK, Kim SK, Park JK, Cho AR, et al. Association between the SLC6A12 gene and negative symptoms of schizophrenia in a Korean population. Psychiatry Res. 2011;189:478–9. https://doi.org/10.1016/j.psychres.2011.01.023.
32. Russo SJ, Bolanos CA, Theobald DE, DeCarolis NA, Renthal W, Kumar A, et al. IRS2-Akt pathway in midbrain dopamine neurons regulates behavioral and cellular responses to opiates. Nat Neurosci. 2007;10:93–9.
33. Asoglu M, Aslan M, Imre O, Kivrak Y, Akil O, Savik E, et al. Mean platelet volume and red cell distribution width levels in initial evaluation of panic disorder. Neuropsychiatr Dis Treat. 2016;12:2435–38.
34. Ransing RS, Gupta N, Agrawal G, Mahapatro N. Platelet and red blood cell indices in patients with panic disorder: a receiver operating characteristic analysis. J Neurosci Rural Pract. 2020;11:261–6.
35. Tükel R, Arslan BA, Ertekin BA, Ertekin E, Oflaz S, Ergen A, et al. Decreased IFN-γ and IL-12 levels in panic disorder. J Psychosom Res. 2012;73:63–7.
36. Dhabhar FS, Malarkey WB, Neri E, McEwen BS. Stress-induced redistribution of immune cells-From barracks to boulevards to battlefields: a tale of three hormones - Curt Richter Award Winner. Psychoneuroendocrinology [Internet]. 2012;37:1345–68. Available from: https://doi.org/10.1016/j.psyneuen.2012.05.008.
37. Dhabhar FS, Miller AH, McEwen BS, Spencer RL. Effects of stress on immune cell distribution. Dynamics and hormonal mechanisms. J Immunol. 1995;154:5511–27. http://www.ncbi.nlm.nih.gov/pubmed/7730652.
38. Hammer C, Cichon S, Mühleisen TW, Haenisch B, Degenhardt F, Mattheisen M, et al. Replication of functional serotonin receptor type 3A and B variants in bipolar affective disorder: a European multicenter study. Transl Psychiatry. 2012;2:e103.
39. Mizuta N, Akiyoshi J, Sato A, Hanada H, Tanaka Y, Tsuru J, et al. Serotonin receptor 3A (HTR3A) gene is associated with personality traits, but not panic disorder. Psychiatr Genet. 2008;18:44.
40. Martin V, Riffaud A, Marday T, Brouillard C, Franc B, Tassin JP, et al. Response of Htr3a knockout mice to antidepressant treatment and chronic stress. Br J Pharmacol. 2017;174:2471–83.
41. Gatt JM, Williams LM, Schofield PR, Dobson-Stone C, Paul RH, Grieve SM, et al. Impact of the HTR3A gene with early life trauma on emotional brain networks and depressed mood. Depress Anxiety. 2010;27:752–9.
42. Perroud N, Zewdie S, Stenz L, Adouan W, Bavamian S, Prada P, et al. Methylation of serotonin receptor 3A in ADHD, borderline personality, and bipolar disorders: link with severity of the disorders and childhood maltreatment. Depress Anxiety. 2016;33:45–55.
43. Derkach V, Surprenant A, North RA. 5-HT3 receptors are membrane ion channels. Nature. 1989;339:706–9.
44. Gorman JM, Kent JM, Sullivan GM, Coplan JD. Neuroanatomical hypothesis of panic disorder, revised. Am J Psychiatry. 2000;157:493–505.
45. Tecott LH, Julius D. A new wave of serotonin receptors. Curr Opin Neurobiol. 1993;3:310–5.
46. Kelley SP, Bratt AM, Hodge CW. Targeted gene deletion of the 5-HT3A receptor subunit produces an anxiolytic phenotype in mice. Eur J Pharmacol. 2003;461:19–25.
47. Rudy B, Fishell G, Lee S, Hjerling-Leffler J. Three groups of interneurons account for nearly 100% of neocortial GABAergic neurons. Dev Neurobiol. 2011;71:45–61.
48. Férézou I, Cauli B, Hill EL, Rossier J, Hamel E, Lambolez B. 5-HT3 receptors mediate serotonergic fast synaptic excitation of neocortical vasoactive intestinal peptide/cholecystokinin interneurons. J Neurosci. 2002;22:7389–97.
49. Kellner M. Experimental panic provocation in healthy man-a translational role in anti-panic drug development? Dialogues Clin Neurosci. 2011;13:485–93.
50. Miller CA, Sweatt JD. Covalent modification of DNA regulates memory formation. Neuron. 2007;53:857–69.
51. Martin SL, Power A, Boyle Y, Anderson IM, Silverdale MA, Jones AKP. 5-HT modulation of pain perception in humans. Psychopharmacol (Berl). 2017;234:2929–39.
52. Brüss M, Barann M, Hayer-Zillgen M, Eucker T, Göthert M, Bönisch H. Modified 5-HT (3A) receptor function by co-expression of alternatively spliced human 5-HT(3A) receptor isoforms. Naunyn Schmiedebergs Arch Pharmacol. 2000;362:392–401.
53. Liew CC, Ma J, Tang HC, Zheng R, Dempsey AA. The peripheral blood transcriptome dynamically reflects system wide biology: a potential diagnostic tool. J Lab Clin Med. 2006;147:126–32.
54. Sullivan PF, Fan C, Perou CM. Evaluating the comparability of gene expression in blood and brain. Am J Med Genet - Neuropsychiatr Genet. 2006;141 B:261–8.
55. Sjöstedt E, Zhong W, Fagerberg L, Karlsson M, Mitsios N, Adori C, et al. An atlas of the protein-coding genes in the human, pig, and mouse brain. Science (80-) [Internet]. 2020 Mar;367. Available from: https://www.science.org/doi/10.1126/science.aay5947.
56. Ask H, Cheesman R, Jami ES, Levey DF, Purves KL, Weber H. Genetic contributions to anxiety disorders: where we are and where we are heading. Psychol Med. 2021;9:1–16.
57. Levey DF, Gelernter J, Polimanti R, Zhou H, Cheng Z, Aslan M, et al. Reproducible Genetic Risk Loci for Anxiety: Results From ~200,000 Participants in the Million Veteran Program. Am J Psychiatry. 2020;:appi.ajp.2019.1.
58. Nagel M, Jansen PR, Stringer S, Watanabe K, De Leeuw CA, Bryois J, et al. Meta-analysis of genome-wide association studies for neuroticism in 449,484 individuals identifies novel genetic loci and pathways. Nat Genet. 2018;50:920–7.
59. Howard DM, Adams MJ, Shirali M, Clarke TK, Marioni RE, Davies G, et al. Genome-wide association study of depression phenotypes in UK Biobank identifies variants in excitatory synaptic pathways. Nat Commun. 2018;9:1–10.
60. Ikeda M, Takahashi A, Kamatani Y, Okahisa Y, Kunugi H, Mori N, et al. A genome-wide association study identifies two novel susceptibility loci and trans population polygenicity associated with bipolar disorder. Mol Psychiatry. 2018;23:639–47. https://doi.org/10.1038/mp.2016.259.
61. Ripke S, Neale BM, Corvin A, Walters JTR, Farh KH, Holmans PA, et al. Biological insights from 108 schizophrenia-associated genetic loci. Nature. 2014;511:421–7.
62. Snijders C, Maihofer AX, Ratanatharathorn A, Baker DG, Boks MP, Geuze E, et al. Longitudinal epigenome-wide association studies of three male military cohorts reveal multiple CpG sites associated with post-traumatic stress disorder. Clin Epigenetics. 2020;12:1–13.
63. Yang X, Han H, DeCarvalho DD, Lay FD, Jones PA, Liang G. Gene body methylation can alter gene expression and is a therapeutic target in cancer. Cancer Cell [Internet]. 2014;26:577–90. Available from: https://doi.org/10.1016/j.ccr.2014.07.028.
64. Arechederra M, Daian F, Yim A, Bazai SK, Richelme S, Dono R, et al. Hypermethylation of gene body CpG islands predicts high dosage of functional oncogenes in liver cancer. Nat Commun. 2018;9. Available from: https://doi.org/10.1038/s41467-018-05550-5.
65. Diez-Fernandez C, Rüfenacht V, Gemperle C, Fingerhut R, Häberle J. Mutations and common variants in the human arginase 1 (ARG1) gene: Impact on patients, diagnostics, and protein structure considerations. Hum Mutat. 2018;39:1029–50.
66. Kieler M, Hofmann M, Schabbauer G. More than just protein building blocks: How amino acids and related metabolic pathways fuel macrophage polarization. FEBS J. 2021;288:3694–714.

AUTHOR CONTRIBUTIONS

AE, SM, and BMM contributed to conception and design. JL, DC and JD contributed to data acquisition and pre-processing. SM analysed the data. SM, AE, and BMM drafted the manuscript. All authors participated in revising it critically for important intellectual content.

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Appendix E – Publication V: Attachment style change and working alliance in panic disorder patients treated with cognitive behavioral therapy

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Attachment Style Change and Working Alliance in Panic Disorder Patients Treated With Cognitive Behavioral Therapy

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An insecure attachment style (AS), described as being highly anxious and/or avoidant, is often assumed to be stable over time, yet some studies show that AS can change. To the extent that AS may be malleable over shorter time periods, it potentially impacts key therapy processes and outcomes. In the present study, we first investigated the stability of AS in patients with panic disorder ($N = 49$) treated with short-term cognitive behavioral therapy (CBT) including follow-up. Second, we tested whether time-specific change of AS predicted subsequent symptom severity, interpersonal distress (ID), and alliance, or vice versa. Third, we investigated if anxious attachment and ID average levels impact the alliance–outcome relation. Analyses were conducted at within- and between-patient levels with 5 measurements (baseline, intermediate, end, and follow-up after 4 and 8 months) over the course of CBT (12 sessions/8 weeks, 2 booster sessions) using linear mixed-effects models. A strong decrease in anxious attachment was found that was stable until 8 months after therapy. At the within-patient level, a reciprocal relationship of reduction in anxious attachment with less symptom severity, stronger alliance, and a prediction of less ID was found. ID decrease preceded less avoidance. At the between-patient level, anxious attachment and ID moderated the relation of alliance change and subsequent depressive symptoms. The prolonged improvement in interpersonal patterns suggests that short-term CBT positively modifies attachment working models in panic disorder patients. Effects on therapy process indicate that anxiously attached and interpersonally distressed patients seem to benefit more from alliance increase.

Clinical Impact Statement

Question: (a) Does attachment style (AS) change over short-term treatment and follow-up? (b) Does such AS change predict subsequent outcome, interpersonal distress (ID), and alliance or vice versa? and (c) Is an effect of improvement in alliance on subsequent outcome impacted by AS and ID? ***Findings:*** (a) Standardized short-term cognitive behavioral therapy (CBT) may effectively reduce anxious attachment, symptom severity, and ID in patients with panic disorder beyond therapy. (b) AS improvement seems to be reciprocally related to symptom severity, alliance change, and interpersonal difficulties. (c) Furthermore, AS and ID appear to moderate the relation of the therapeutic alliance and subsequent depressive symptom severity, with more anxiously attached and interpersonal distressed patients benefitting more from an improvement of alliance. ***Meaning:*** Short-term CBT for panic disorder consists of standardized, exposure-based interventions and is not aimed to influence the quality of the relationship. Hence, it seems not necessary to target AS directly, but it may be sufficient to target overcoming one's fear to promote enduring improvement in anxious attachment, or the "inner working model of self". However, it seems important to foster the alliance in patients with anxious attachment and interpersonal difficulties regarding symptom reduction. ***Next Steps:*** It is recommended to

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Jennifer Lange served as lead for conceptualization, data curation, formal analysis, investigation, and writing – original draft. Stephan Goerigk served in a supporting role for formal analysis. Katja Nowak served in a supporting role for investigation. Rita Rosner contributed equally to conceptualization and supervision and served in a supporting role for writing – review and editing. Angelika Erhardt served as lead for funding acquisition, project administration, and supervision and served in a supporting for writing – review and editing.

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monitor AS and ID as risk factors for symptom severity and to target the alliance accordingly outside the CBT protocol.

Keywords: attachment style, working alliance, cognitive behavioral therapy, psychotherapy process, anxiety disorders

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The attachment style (AS) of a person is a concept derived from John Bowlby's (1969) theory that describes the way someone behaves in a close reciprocal relationship and is conceptualized as a systematic pattern of relational expectations and emotions. Based on early experiences of a child with caring persons, the theory describes the relevance of internalized models for the development of relationships later in life. Early experiences interact with heritable personality factors of individuals that effect relationships, which in turn leads to a stabilization of the attachment orientation as well as the environment (Waters et al., 2000). Children who experience an inadequate response by the caregiver tend to develop secondary strategies to cope with threat and to preserve proximity and protection of the attachment figure (Nolte et al., 2011), which leads to either deactivation (attachment avoidance) or hyperactivation of the attachment system (attachment anxiety; Mikulincer & Shaver, 2012). In Bartholomew and Horowitz's (1991) model, the four categories of adult AS—*secure*, *preoccupied* with relationships, *dismissing*, and *fearful* of intimacy—can be described as positions along two orthogonal dimensions of insecurity: *avoidance* and *anxiety*. These are parallelized by negative or positive working models of self and others (Griffin & Bartholomew, 1994).[1] Secure attachment is conceptualized as relatively low anxiety and avoidance.

Previous evidence supports Bowlby's core assumption that AS is relatively stable over time across significant portions of the life span and, therefore, is "trait-like," but results have been inconsistent (Fraley et al., 2011). At the same time, AS seems to remain open to revision in face of ongoing or new relationships and attachment-related (e.g., traumatic) life events (Davila et al., 1997; Horesh et al., 2014; Levy et al., 2018; Waters et al., 2000). However, some studies show that despite temporary variations, there seems to be an underlying enduring construct in the AS, which is called the *prototype hypothesis* (Fraley et al., 2011; Jones et al., 2018). As proposed by this, current working models may be revised across the life span by deviating attachment-relevant experiences, whereas the prototype working models developed during early childhood continue to exist as a stable core and shape attachment patterns throughout life by unconsciously influencing a person's expectations, fears, defenses, and behavior and thereby recreating interpersonal experiences (Fraley et al., 2011; Mikulincer & Shaver, 2007). Hence, even after a shift in AS security due to intense experiences, it might gravitate back to a robust prototype value. Recent longitudinal research reveals that a wide array of life events (e.g., related to work, relationships, family, and health) seems to lead to changes in attachment, but whether these changes are enduring is depending on how positive or negative the events are construed (Fraley et al., 2020). Fraley and colleagues (2011) suggested that to achieve a prolonged change in the prototype, it might be useful to target another enduring latent factor that opposes the working models, for example, automatic specific patterns of thought, feeling, and behavior. This may be done with cognitive behavioral therapy (CBT).

A growing body of research suggests that AS change can occur through psychotherapy in various disorders using different, mainly psychodynamic approaches (for a review, see Levy et al., 2018; Mikulincer et al., 2013; Taylor et al., 2015), and even long-term change (Kirchmann et al., 2012). An insecure AS appears to be a predisposition for the development of anxiety disorders (AD) as well as a maintaining factor (Colonnesi et al., 2011; Esbjorn et al., 2012; Levy et al., 2011; Manning et al., 2016) and vice versa (Davila et al., 1997; Horesh et al., 2014). The constant sense of threat in AD probably leads to frequent activation of the attachment system (Zalaznik et al., 2019). Earlier studies show that AD patients often have an insecure and mainly preoccupied/anxious AS (de Ruiter & Van Ijzendoorn, 1992; Manning et al., 2016; Marazziti et al., 2007; Nielsen et al., 2017). Some studies investigated changes of AS in AD with mixed results: Research in patients with posttraumatic stress disorder (PTSD), treated with various forms of psychotherapy, suggests improvement in AS and maintenance over 6 to 12 months (Müller & Rosenkranz, 2009; Murphy et al., 2016). Other studies, using CBT, revealed an improvement of AS in PTSD patients to posttreatment (Stovall-McClough & Cloitre, 2003) and to 3-month follow-up (Rimane et al., 2020) or no effect (Madigan et al., 2015). An effect on the improvement of anxious and avoidant attachment was also found in patients with social anxiety disorder enduring until follow-up after 12 months (Strauß et al., 2018). One study found no effect of CBT plus communication and problem-solving training on AS in panic disorder (PD) patients (Belanger et al., 2011). In summary, results regarding malleability of AS in patients with AD are inconsistent. Following this, we first investigate the change of a global AS in response to a disorder-specific, manualized, and hence highly structured short-term CBT for PD including follow-up measurements.

Furthermore, an insecure AS has a detrimental effect on psychotherapy outcome (for a meta-analysis, see Levy et al., 2018). Findings indicate that attachment anxiety seems to predict worse treatment outcome, whereas avoidant attachment might be less related to therapy outcome (Levy et al., 2011). Improvements in attachment security coincide with improved therapy outcome during treatment and beyond (Levy et al., 2018; Müller & Rosenkranz, 2009). However, the assumption that change in AS is

[1] Using discrete types of AS have limited statistical power compared with dimensional measures. Even if we use categorical descriptions in this work for descriptive purposes, we are referring to a two-dimensional space in which individuals are continuously distributed (Mikulincer & Shaver, 2007).

meaningful in itself and not merely a by-product of changes in symptoms needs to be tested by establishing temporal precedence. Results of only one study using CBT for PD patients showed a decrease of an avoidant and anxious attachment measured by self-report *preceded* by improved anxiety sensitivity, avoidance behavior, and emotion regulation, but without follow-up assessments (Zalaznik et al., 2019). The latter is recommended to disentangle the direction of the AS–outcome relation (Levy et al., 2018). There is a lack in studies examining longitudinal AS change in AD using CBT, in particular exposure therapy (ET), and the effect on subsequent symptom level. Whether attachment improvement *causes* better outcomes is unclear and further research is required (Levy et al., 2018). The present study aims to answer the dynamic of AS and outcome with follow-up assessments.

Additionally, a person's attachment orientation at a particular time may not simply be state or a trait but a combination of effects from contextual as well as enduring factors (Fraley et al., 2011). Thus, it is important to differentiate between patients' general ability to form satisfying relationships ("trait-like" component) and the dynamic of changes within patients in that ability through social interaction ("state-like" component). On the interpersonal level, the dysfunctional strategies of insecure attached patients lead to conflicts of autonomy and dependence, having a disadvantageous influence on relationships. Previous findings show that an anxious AS is associated with more interpersonal problems (Bartholomew & Horowitz, 1991). In PD with agoraphobia (AG), interpersonal relationships appear to be problematic and can decrease treatment efficacy (Carter et al., 1994). It stands to reason that if AS improves, the ability to form stable and satisfying relationships is promoted, and hence interpersonal problems are reduced. Research shows that greater decrease in attachment avoidance is increasingly associated with continuing improvement of interpersonal difficulties (Maxwell et al., 2014). Furthermore, change of "trait-like" interpersonal patterns—AS as well as related interpersonal problems—within the timeline of a limited therapy challenges the developmental perspective of continuity and slow meaningful change over a long time span. Opposing the "state-artifact" hypothesis, aggregated findings support the "cause-correction" hypothesis and the inference that therapy can lead to lasting personality trait change (for a meta-analysis, see Roberts et al., 2017). There is a gap in the literature with regard to therapeutic techniques that lead to change, how fast the change occurs, and if the change remains. To determine stability, the pace, dynamic and directionality of change, multiple assessments over a longer period of time are required. Following this, we examine second, if improvement in AS precedes decrease in symptom severity and ID beyond therapy, disaggregated for between-patient (BP) effects, that are likely to be partly indicative of the stable person-level characteristic, for example, about how much anxious or avoidant attachment patients experience on average during therapy relative to one another, and the within-patient (WP) effect, which is concerned with changes from assessment-to-assessment, provides information about the temporal relationship and may imply corrective experiences (Zilcha-Mano, 2017).

As noted before, an efficacious change mechanism for interpersonal patterns might be social interaction, specifically the therapeutic alliance, which is an effective and critical common factor for outcome across therapy methods (Horvath, 2018; Levy et al., 2018; Wampold, 2015). From Bowlby's (1969) attachment theory perspective, the client–therapist relationship can be conceptualized as containing an attachment bond that provides a reliable source of security and support with serving the function of a physical and emotional safe haven as well as a secure base for exploration (Mikulincer et al., 2013). The alliance is a part of this relationship by representing its interactive and collaborative aspects (e.g., common goals and task agreement) based on trust and an appreciative and empathetic contact (Castonguay et al., 2006). It was shown to be a general moderate but robust therapeutic and process-based factor for treatment outcome (aggregated correlation of alliance and response: $r = .28$; Flückiger et al., 2018; Horvath et al., 2011) and to contribute to that substantially and consistently independent of therapy method (Norcross & Lambert, 2018), patient or therapist intake factors (e.g., demographics and/or pretreatment symptomatic severity, personally traits, interpersonal distress, and treatment expectancy), and treatment processes (e.g., adherence and competence; Flückiger, Del Re, et al., 2020). For AD, there is a limited amount of research on the relationship between working alliance and treatment outcome in CBT for AD, and the findings showed evidence for a significant alliance–outcome relation (Buchholz & Abramowitz, 2020). Alliance has been shown to be associated with outcome (Weiss et al., 2014) and to be a predictor in exposure-based CBT for PD of symptom severity (Huppert et al., 2014) and change in agoraphobic avoidance behavior (Weck et al., 2016). However, one study with PD patients found no significant association with treatment outcome in ET (Maiwald et al., 2019). To utilize alliance, components again must be differentiated that are "trait-like" (BP effects) and can moderate treatment outcome as precondition for therapeutic work (e.g., patient's ability to make progress), and "state-like" components (WP effects) that may be curative in itself, have the potential to be changed, and consequently facilitate symptom improvement. Several studies showed that the "state-like" aspect of alliance precedes symptom reduction, which supports the notion that alliance may be therapeutic in itself (for a review, see Zilcha-Mano, 2017). WP meta-analytic analysis session-by-session shows that symptoms and alliance are reciprocally related to one another *early* in therapy, with higher alliance and lower symptom severity positively impacting the relation between alliance and symptoms in the subsequent session (Flückiger, Rubel, et al., 2020). There are a few studies with AD patients investigating WP effects: Findings show that the alliance seems to be a facilitative factor for change in CBT in generalized anxiety disorder, irrespective of therapists adherence (Rubel et al., 2019) and for subsequent PTSD symptom reduction (Hoffart et al., 2013). Though both WP and BP effects seem to move in the same direction in the case of alliance, with general better capability to create a stronger alliance likely impacting outcome, and WP improvement in alliance improving subsequent treatment outcome (Zilcha-Mano, 2017), WP effects may be independent of the respective BP effects and induced by different therapeutic factors (Flückiger, Rubel, et al., 2020).

The capability to form satisfying relationships, understood to be impacted by AS, affects the therapeutic relationship. Patients with a secure AS and the ability of forming a strong and satisfying relationship with others, likely form a strong alliance with their therapist, and thus benefit from a better treatment outcome. Research shows that a secure AS seems to promote positive attitudes toward therapy and constructive therapeutic behavior, like self-disclosure and inner exploration (Mikulincer et al., 2013). Though findings are inconsistent, they overall suggest a small influence on alliance by the developed AS, whereby a secure AS is correlated with a stronger alliance (for a meta-analysis, see Bernecker et al., 2014; weighted correlation: avoidance: $r = -.14$, anxiety: $r = .12$; for a meta-analysis, see Diener & Monroe, 2011; weighted correlation: $r = .17$; for an overview, see Mikulincer et al., 2013; Smith et al., 2010). Alliance has also been shown to mediate the relationship between client attachment and psychotherapy outcome (subjective distress, interpersonal relations, and social role performance) in clients presenting with mixed pathology (e.g., mood and anxiety disorders; Byrd et al., 2010). One study in PD patients showed that changes in anxious attachment *preceded* improvement in alliance across CBT (Zalaznik et al., 2019).

On the other hand, it is likely that the alliance–outcome relation is impacted by patients' interpersonal characteristics, and a probable candidate is attachment (Constantino et al., 2017). Recent research shows a moderating role of AS in patients with substance use disorder (Gidhagen et al., 2020; Zack et al., 2015), but studies in other disorders are lacking. Improvement in the ability to form a satisfactory relationship with the therapist seems to affect general interpersonal ability, resulting in a reduction in symptom severity (Zilcha-Mano, 2017). Empirical findings support the notion that the WP effect of alliance on subsequent outcome is mediated by changes in interpersonal distress (ID; Constantino et al., 2016; Coyne et al., 2019). Other research suggests that patients suffering from low interpersonal agency and reporting problems with submissiveness, which impact depressive symptoms, benefit from an improved therapeutic alliance that facilitates changes in interpersonal problems (Gómez Penedo et al., 2020). In summary, improvement in interpersonal patterns seems to be a consequence of change in alliance and in turn predict symptom severity. Taken together, whether a secure bond—alliance—is the catalyst for improvement in AS or the other way round and whether AS impacts the alliance–outcome relation is unclear and needs to be tested over a longer period of time, which is our third goal.

In summary, we want to answer the following questions with our study: (a) does AS change over short-term treatment and follow-up? (b) does such AS change predict subsequent outcome, ID, and alliance, or vice versa? and (c) is an effect of improvement in alliance on subsequent outcome impacted by AS and/or ID? The following hypotheses were examined:

> *Hypothesis 1:* An insecure AS improves over the course of therapy, enduring after the therapy.

> *Hypothesis 2:* Improvement in AS predicts subsequent improvement in outcome and interpersonal patterns and is preceded by alliance change.

> *Hypothesis 3:* The effect of change in alliance on subsequent outcome is moderated by AS and ID.

Method

Participants

Inclusion criteria consisted of (a) a current primary *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision,* diagnosis of PD with/without AG and a clinical interview score >14 on the Hamilton Anxiety Scale (Hamilton, 1996). Exclusion criteria were major somatic disorders, pregnancy, personality disorder, current suicidal intent, and other psychiatric disorders except other AD ($n = 7$; 14%), comorbid OCD ($n = 5$; 10%), or secondary mild or moderate depression ($n = 7$; 14%). In total, 49 patients (33 woman, 17 men; $M_{age} = 32.2$ years; age range: 18–61 years; ethnicity: Caucasian) with PD with ($n = 42$; 86%) or without AG ($n = 7$; 14%) were included with 88% ($n = 43$) being free of psychotropic medication. Medicated patients ($n = 6$) were several months on medication (SSRIs) before the study and continued with the same dosage during the CBT and the follow-up period. Benzodiazepines were not allowed during the course of therapy. Possible attachment-related events were assessed in the follow-up period. A total of 14 patients reported stressors after the therapy: death of a relative or friend ($n = 5$), ending an intimate relationship ($n = 6$), abortion ($n = 1$), and severe sickness of a relative ($n = 2$).

Treatment

Treatment followed a highly structured and empirically supported manual by Lang et al. (2011), which consists of 12 sessions of CBT (one to two sessions per week, 6–8 weeks) as well as two booster sessions after 2 and 4 months. The manual includes precise instructions and worksheets for every session and consists of the following modules: establishing therapeutic rapport, psychoeducation (e.g., self-monitoring, functional analysis; Sessions 1–3), interoceptive exposure (Sessions 4–5), at least one accompanied exposure in vivo (Sessions 6–11), as well as relapse prevention (Session 12 and two booster sessions). Exposure sessions were conducted outside the clinic, depending on the feared situation (e.g., subway, supermarket, height) and specific concern (e.g., fainting, asphyxiation, losing control). Treatment was conducted by two trainees for CBT in advanced years of training. Both had received introduction to the theory, case formulation, and technique of CBT for AD and were under individual supervision including videotaped sessions for feedback by senior supervisors with extensive experience in CBT. Study protocol included three psychiatric consults for patients during and after therapy as well as 1-hr weekly group supervision for therapists by a senior psychiatrist administrating the study.

Procedure

The study was conducted in the outpatient clinic for anxiety disorders of the Max Planck Institute for Psychiatry. The study was approved by the ethics committee of the Ludwig-Maximilians-University. Participants were recruited from the pool of anxiety patients seeking treatment in the clinic and meeting inclusion criteria. After describing the study protocol and informing of limitation of sessions, written informed consent of the patients was obtained. Self-report as well as clinician-ratings were administered at five time points: baseline, fourth session, end (after main therapy), follow-up 1 (after 4 months), and follow-up 2 (after 8 months; see also the study design in Figure S1 in the online supplemental materials). If deviant, administration schedule for the specific measure is found in the materials section. Clinician ratings of symptom severity were performed by a clinician not involved in the treatment. Patients were informed that their therapists would not have access to their responses on the measures.

We defined dropout as failure to complete the 12-session treatment and follow-up assessment protocol. Based on this, 31% ($n =$

15) of patients were lost either during therapy (n = 8) or after the main therapy (n = 7) for the following reasons: early symptom remission (n = 5), worsening of symptoms with need for inpatient treatment (n = 3), comorbid psychiatric disorder more relevant (n = 2), acute somatic disease (n = 2), interrupted contact (n = 1), pregnancy (n = 1), and other reasons (n = 1). In total, 34 patients completed the treatment as well as follow-up assessments. Patients who needed further treatment after the follow-up were either treated thoroughly or forwarded to other therapists. Of the completers, two patients reported that they sought further therapy elsewhere after end of main therapy, and seven patients reported additional therapy after the first follow-up.

Materials

Attachment Style

AS is measured as self-report by the validated German version of the Relationship Scales Questionnaire (RSQ; Griffin & Bartholomew, 1994; Steffanowski et al., 2001) in close (not necessarily intimate or particular) relationships with 30 items (internal consistency: Cronbach's α = .72 to .81). For the formation of attachment orientation, the two scales Anxiety (e.g., "I worry about being abandoned.") and Avoidance of Intimacy (e.g., "I worry about others getting too close to me.") are used. An insecure AS is defined as either high anxious (cutoff: >2.89; i.e., preoccupied) and/or avoidant attachment (cutoff: >2.76; i.e., dismissing). Individuals who score high in both dimensions are classified as fearful; individuals underneath both cutoffs are classified as securely attached (see Footnote 1). Psychometric evaluation is adequate and the assessed AS robust (Kirchmann et al., 2012; Steffanowski et al., 2001). AS was measured at four time points: baseline (before contact with the therapists), end (after main therapy), follow-up 1 (second booster session) and follow-up 2.

Working Alliance

Alliance was measured as self-report by the short German version of the Working Alliance Inventory (WAI) for patients (Wilmers et al., 2008) at five time points: after the first, the fourth, the eights, the 12th session (end), and at follow-up 1 (second booster session). It assesses the scales Bond, Task, and Goals with 12 items (internal consistency: Cronbach's α = .82 to .93). Psychometric validity of the measure is well established.

Interpersonal Problems

The German short version of the Inventory of Interpersonal Problems (IIP; Horowitz et al., 2000) is a self-report instrument with 64 items that identifies a person's most salient interpersonal difficulties in eight scales: Domineering, Vindictive, Cold, Socially Avoidant, Nonassertive, Exploitable, Overly Nurturant, and Intrusive (retest reliability: r_{tt} = .81 to .90; internal consistency: Cronbach's α = .36 to .64). We only used the IIP total score, which is a measure of patients' general distress derived from interpersonal problems. Factorial, differential, prognostic, and criterion validity of the measure is well established. It was measured at the same four time points as AS.

Symptom Severity

Anxiety and depression were measured by the German versions of the clinician-rating Hamilton Anxiety Scale (HAMA; Hamilton, 1996; internal consistency: Cronbach's α = .82 to .87; interrater reliability: r = .89), the Agoraphobic Cognitions Questionnaire (ACQ; Ehlers & Margraf, 2001; internal consistency: Cronbach's α = .79), and the Beck Depression Inventory (BDI; Hautzinger et al., 2000; internal consistency: Cronbach's α = .88). Psychometric validity of the measures is well established.

Data Analysis

The stability of the AS (Hypothesis 1) as well as the change of symptomatology, ID, and alliance were examined with linear mixed-effects models (LMM) to test for significant changes over time as well as to adjust for the interdependence of the repeated observations within individuals, providing a robust strategy to handle missing data. All analyses contain all patients (including drop-outs), and models are run as intent-to-treat analyses. Coefficients were estimated using restricted (residual) maximum likelihood estimation. Analyses were conducted in RStudio Version 1.2.5001 (RStudioTeam, 2020) using the "lme4" package (Bates et al., 2015). A continuous linear time factor with five or four repeated assessments (baseline, intermediate, end, and follow-up after 4 and 8 months) depending on the scale was included as a fixed effect, patient intercepts and slopes as random effects. Preliminary analyses to test for therapist effects on the study variables revealed no systematic differences between the therapists in variables during treatment. Nevertheless, variance attributed to therapists was controlled for by including it as a covariate. Significance was calculated using the "lmerTest" package (Kuznetsova et al., 2017); which applies Satterthwaite's method to estimate degrees of freedom to calculate p values for mixed models. Effect sizes (Cohen's d) for changes from baseline to follow-up 2 were calculated by multiplying the estimate of the time slope with the number of measurements and dividing it through the baseline standard deviation (Feingold, 2009). Bonferroni-corrected post hoc analyses were computed to identify differences in change between the time points (baseline to all as well as end to all measurements).

We further examined associations between change in AS, symptom severity, ID, and alliance (Hypothesis 2) parsed in WP and BP effects. The "within" part consists of the relative attachment on a particular occasion compared with other occasions of the particular patient, whereas the "between" part consists of the average attachment of one patient compared with the other patients. Following recommendations of Wang and Maxwell (2015), we centered within the individual patient's mean. For the BP effects, we used the individual patient's mean. As changes over time are expected during treatment in the current study and are a constitutive part of the therapy process, no detrending for time was performed, as it might result in suppression of effect sizes, could lead to overcorrection of WP associations, and masking of effects (Falkenström et al., 2017; Wang & Maxwell, 2015). To assess temporal precedence between variables, lagged models per Falkenström et al. (2017) were used and dependent variables were lagged (t + 1). To test for directionality, reverse models were implemented. To avoid "endogeneity," we did not control for the lagged dependent variable in our analyses (Falkenström et al., 2017).

To test for moderation (Hypothesis 3) of WP change of alliance (t) on subsequent outcome scores ($t + 1$), we included mean anxious AS and ID scores as moderators in the model. BP moderators were grand mean centered to facilitate interpretation. To further understand the effect of AS on the WP alliance effect, we calculated regions of significance as a follow-up analysis.

Results

Efficacy Analysis

Symptom Severity

The results of the LMM analysis show a highly significant reduction for anxiety (HAMA and ACQ) as well as depressive (BDI) symptoms with large effect sizes (see Table 1).

Relationship Scales Questionnaire

In our sample of anxiety patients, 35% ($n = 17$) showed a secure AS and 65% ($n = 32$) displayed an insecure AS, with high scores on the Anxiety dimension in 47% (preoccupied: $n = 23$), on Avoidance in 10% (dismissing: $n = 5$), and to a minor part on both Anxiety and Avoidance (fearful: $n = 4$; 8%), (see Footnote 1). LMM analyses showed a significant reduction in anxiety, $B = -.16$, $t(41.18) = -3.94$, $p < .001$; $d = -.74$, 95% confidence interval (CI) [−.86, −.62], as well as a trend result for avoidance, $B = -.06$, $t(39.77) = -1.84$, $p = .073$; $d = -.25$, 95% CI [−.33, −.17]; see Table 1. Significant Bonferroni-corrected post hoc analyses (comparisons of all measurements) indicate that patients were less anxiously attached directly after therapy ($p < .01$). Importantly, this effect endured after therapy throughout follow-up after 4 and 8 months. The trend effect for avoidance was not seen until follow-up 2. Detailed information about model parameters and post hoc analyses of the primary outcomes are summarized in Tables S1 and S2 in the online supplemental materials.

Working Alliance Inventory

Over the course of therapy, a significant increase in alliance from baseline to follow-up 1 (final booster session) was detected, $B = .17$, $t(47.48) = 7.91$, $p < .001$; see $d = 1.05$, 95% CI [.99, 1.11]; see Table 1.

Inventory of Interpersonal Problems

A significant reduction of ID until follow-up 2 was detected, $B = -1.33$, $t(37.47) = -8.17$, $p < .001$; $d = -.86$, 95% CI [−.92, −.79]; see Table 1. Post hoc analyses showed significant changes between all measurements ($p < .001$; see Table S2 in the online supplemental material). Hence, the improvement in ID was observed directly after therapy and sustained until follow-up 2.

Lagged Effects

LMM analyses allowed disaggregation into WP and BP effects. Significant BP effects are reflective of "trait-like" differences in

Table 1
Symptom Severity, Attachment, Interpersonal Distress, and Alliance Over the Course of Therapy

Measure	Baseline	4th session		End	Follow-up 1	Follow-up 2	LMM df_1/df_2	LMM F	LMM d	LMM p
N	49	49		41	38	34				
HAMA										
M	33.92	21.24		12.76	10.87	8.88	1	168.00	−3.27	<.001
SD	7.64	10.22		10.04	10.41	8.36	43.65			
BDI										
M	14.78	10.22		7.62	5.26	5.00	1	46.88	−1.06	<.001
SD	9.34	8.05		9.01	5.63	5.59	33.96			
ACQ										
M	2.03	1.82		1.54	1.33	1.28	1	81.60	−1.43	<.001
SD	0.56	0.61		0.52	0.38	0.28	30.43			
RSQ										
Anxiety										
M	2.87	—		2.69	2.59	2.46	1	15.50	−0.74	<.001
SD	0.65			0.66	0.74	0.67	41.18			
Avoidance										
M	2.13	—		2.16	2.04	1.97	1	3.40	−0.25	.07
SD	0.73			0.76	0.85	0.79	39.77			
IIP										
M	10.01	—		8.81	6.84	6.19	1	66.76	−0.86	<.001
SD	4.66			5.25	4.50	4.12	37.47			
		Session 4th	Session 8th							
WAI										
M	4.08	4.30	4.57	4.69	4.73	—	1	62.52	1.05	<.001
SD	0.65	0.56	0.47	0.43	0.40		47.48			

Note. HAMA = Hamilton Anxiety Scale; BDI = Beck Depression Inventory; ACQ = Agoraphobic Cognitions Questionnaire; RSQ = Relationship Scales Questionnaire; IIP = Inventory of Interpersonal Problems; WAI = Working Alliance Inventory – Patient; LMM = linear mixed effects models.

patient characteristics. Significant WP effects reflect differences due to varying patient state, divergence from the subject's specific mean. Dependent variables are lagged ($t + 1$).

Anxious Attachment Effects

The analysis of RSQ-Anxiety WP yielded significant result in preceding ACQ, IIP, and WAI. BP effects were found for RSQ-Anxiety on symptom severity (HAMA and BDI) and IIP (see Table 2). Hence, patients who generally reported less anxious attachment across treatment tended to report less anxiety and depressive symptoms as well as less ID. When patients reported time-specific decrease of anxious attachment compared with their usual level, they reported fewer agoraphobic cognitions, less ID, and stronger alliance at the next measurement.

Avoidant Attachment Effects

The analysis of RSQ-Avoidance showed a significant result only on the BP level preceding IIP (see Table 2). Thus, patients' higher average levels of avoidant attachment were related to more ID.

Alliance Effects

Lagged WP effects of alliance on symptom severity (HAMA, ACQ, and BDI), IIP, and RSQ-Anxiety, as well as a BP effect on BDI, were significant (see Table 2). Thus, reported increase from usual alliance preceded less symptom severity as well as less ID and anxious attachment later. Generally stronger alliance predicted less depressive symptoms.

Interpersonal Distress Effects

Results showed lagged WP effects of IIP on ACQ and RSQ-Avoidance. BP effects were found for symptom severity (HAMA, ACQ, and BDI) and both RSQ-Anxiety and RSQ-Avoidance (see Table 2). When patients reported time-specific decrease of ID from their mean, they reported less agoraphobic cognitions and avoidant attachment at the next measurement. Patients' higher average levels of ID predicted more symptom severity, anxious and avoidant attachment.

Symptom Severity Effects

Lagged WP and BP effects of symptom severity (HAMA, ACQ, and BDI) on IIP were found. Effects on alliance were found on the WP and BP level only for anxiety symptoms (HAMA and ACQ). Further, BP effects of symptom severity (HAMA, ACQ, and BDI) on RSQ-Anxiety were found (see Table 2). Hence, reported decrease from usual symptom levels preceded less ID and a stronger alliance later. Higher average levels of symptom severity predicted more anxious attachment.

Moderation Analysis

Building on our results, we tested for moderation of WP change (t) of alliance on subsequent outcome scores ($t + 1$). Including anxious attachment and ID average scores (person mean) as moderators of the WP effect of alliance on outcome showed a significant effect: A greater mean score of RSQ-anxiety and IIP across treatment was related to an increase in the association between fluctuation in the alliance and the subsequent BDI scores, but not the HAMA or ACQ scores (see Table 3). For the significant BDI models, the interaction was probed by testing the conditional effects of alliance at each level of RSQ-Anxiety and IIP scores: Increase of WAI from one's usual levels was significantly related to less subsequent depressive symptom levels if individuals mean anxious attachment was deviating from the group mean above $-.09$ (possible range of $-.92$ to 1.80; $p < .05$), and mean IIP deviating above -1.48 (possible range -6.78 to 10.88, $p < .05$) points (see also Figures S2 and S3 in the online supplemental materials). Thus, patients with generally higher attachment anxiety and ID did benefit more from increase in alliance relating to less depressive symptoms later.

Discussion

In the present study, our first goal was to test the stability of AS over the course of time-limited CBT for AD and follow-up assessments. Our study results suggest that short-term CBT leads to an effective positive modification of AS, specifically anxious attachment, as well as reduction in ID and symptom severity, and growth in alliance. Importantly, these effects were enduring after therapy and until follow-up after 8 months. Second, we investigated the relationship, particularly the temporal precedence, of modifications in AS with interpersonal patterns and symptom severity, disaggregated for BP differences in the average levels and WP shifts. On the BP level, we found as expected that patients reporting on average more anxious attachment and ID also reported higher symptom severity, which indicates a risk and maintaining factor for less effective therapy and higher psychopathology. Further, patients who compared with others generally reported more anxious and avoidant attachment reported more interpersonal difficulties. A generally stronger alliance was related to less symptom severity. On the WP level, time-specific improvement in anxious attachment and ID, as well as higher than average levels of alliance, predicted subsequent less symptom severity and vice versa. Further, less anxious attachment compared with average levels predicted better alliance later and vice versa. Increase in alliance and decrease in anxious attachment from average levels preceded less subsequent ID. Furthermore, decrease of ID compared with average levels predicted less subsequent avoidant attachment. Third, we tested the impact of a person's average attachment anxiety and ID across treatment on the WP alliance–outcome association: Patients with higher anxious attachment and interpersonal difficulties were found to benefit more from increase in alliance from their average levels relating to lower subsequent depressive symptom levels.

One of our main findings is a more secure AS, particularly less anxious attachment, after short-term CBT enduring until 8 months later. Similarly to previous studies and in line with attachment models of AD, at the beginning, the majority showed high anxiety and had an insecure, specifically preoccupied/anxious AS and only a few were high in avoidance, particularly fearful (de Ruiter & Van Ijzendoorn, 1992; Marazziti et al., 2007; see Footnote 1). The trend in avoidance reduction was mainly observed after 4 or 8 months after therapy, which suggests that change in internal models of others is lagged. Though, it must be noted that avoidance was already not high in our participants at baseline. The prompt and enduring change in anxious attachment and probably the internal model of self, measured directly after therapy and beyond, indicates a lasting beneficial effect of the interventions used independent of continuing contact with the therapist. The alleged effect of therapy on AS did not wear off with time and scores did not return to their baseline; thus, we may conclude that the improved

Table 2

Within- and Between-Patient Lagged and Reverse Effects of Attachment, Alliance, Interpersonal Distress, and Outcome From Baseline to Follow-Up 2

	$HAMA_{t+1}$			BDI_{t+1}			ACQ_{t+1}		
Predictor	*B* (*SE*)	*t* (*df*)	*p*	*B* (*SE*)	*t* (*df*)	*p*	*B* (*SE*)	*t* (*df*)	*p*
RSQ-Anxiety									
$Within_t$	−1.76 (2.34)	−0.75 (59.91)	.45	−0.11 (1.64)	−0.07 (17.17)	.95	0.33 (0.09)	3.77 (22.80)	<.001
Between	4.03 (2.00)	2.01 (36.30)	<.05	4.39 (1.67)	2.63 (32.88)	<.01	0.07 (0.09)	0.73 (19.42)	.48
RSQ-Avoidance									
$Within_t$	−1.61 (2.21)	−0.73 (55.99)	.47	−0.43 (1.68)	−0.26 (21.15)	.80	0.13 (0.08)	1.74 (56.22)	.09
Between	1.99 (1.75)	1.13 (35.51)	.26	1.76 (1.40)	1.25 (33.06)	.22	0.12 (0.09)	1.29 (35.03)	.20
WAI									
$Within_t$	−6.58 (2.21)	−2.98 (7.64)	<.05[a]	−3.52 (1.00)	−3.51 (61.74)	<.001[a]	−0.46 (0.08)	−5.65 (19.74)	<.001[a]
Between	−3.59 (2.67)	−1.34 (42.57)	.19[a]	−4.30 (2.13)	−2.01 (44.75)	<.05[a]	−0.28 (0.15)	−1.89 (34.74)	.07[a]
IIP									
$Within_t$	0.29 (0.43)	0.69 (18.89)	.50	−0.04 (0.23)	−0.17 (10.52)	.87	0.04 (0.01)	3.17 (31.77)	<.001
Between	0.58 (0.27)	2.17 (36.25)	<.05	0.87 (0.20)	4.29 (34.09)	<.001	0.03 (0.01)	2.73 (38.15)	<.01
HAMA									
$Within_t$	—	—	—	—	—	—	—	—	—
Between	—	—	—	—	—	—	—	—	—
BDI									
$Within_t$	—	—	—	—	—	—	—	—	—
Between	—	—	—	—	—	—	—	—	—
ACQ									
$Within_t$	—	—	—	—	—	—	—	—	—
Between	—	—	—	—	—	—	—	—	—

Note. *B* = unstandardized coefficient; *SE* = standard error; RSQ = Relationship Scales Questionnaire; WAI = Working Alliance Inventory – Patient; IIP = Inventory of Interpersonal Problems; HAMA = Hamilton Anxiety Scale; BDI = Beck Depression Inventory; ACQ = Agoraphobic Cognitions Questionnaire. Statistic for the effect in linear mixed random-effects models including preassessments over the course of therapy (four time points: baseline, end, follow-up 1 and 2) is indicated. Predictors are person mean centered.
[a] Analysis from baseline to follow-up 2 with five time points: baseline, fourth session, end, follow-up 1 and 2.

AS did not gravitate back, like the prototype hypothesis suggests. We conclude from this preliminary study that even short-term interventions can induce prolonged changes in AS.

As Bowlby (1969) postulated, tasks that are hypothesized to be essential to the revision of mental representations (Mikulincer et al., 2013) are met with ET and may strengthen a positive self-perception. Beliefs and expectations about oneself and the body (e.g., I am weak, I am not able to . . . , I cannot endure . . . , etc.) as well as maladaptive behaviors are questioned and transformed through exposure exercises and training of alternative ways of coping—in progressing therapy increasingly unaccompanied—, subsequently finding strength and self-confidence. Thus, it makes sense that a decrease in symptom severity was found to impact subsequent levels of anxious attachment and ID. Overcoming one's fear, the main goal of ET, probably increases the individual's self-efficacy, braking the vicious cycle of relying on a "safe person" to cope with anxiety symptoms, and thereby reducing anxious attachment and interpersonal problems (Zalaznik et al., 2019). Interestingly, decrease in anxious attachment was found to precede particularly less agoraphobic cognitions: With a more positive model of self, it is likely that in return the fear of fear is reduced and perception of control and trust in one's body is facilitated. It seems plausible that ET targets mainly beliefs about oneself, which is connected to anxious attachment, but probably not so much the beliefs about others, which are related to avoidant attachment.

As expected, both dimensions of AS, anxiety and avoidance, were strongly intertwined with interpersonal difficulties on several levels. Our results of decrease in anxious attachment preceding levels of ID suggest that change in anxious AS may function as a change agent for interpersonal difficulties. Furthermore, following our results, reduction in ID may lead to less avoidant attachment, which suggests a complex bidirectional process for AS and ID. The scales of the IIP resemble pathological forms of low extraversion and low agreeableness (Roberts et al., 2017). The relatively fast reduction during main therapy (8 weeks) with continuation until 8 months later emphasizes the change in "trait-like" interpersonal patterns. Roberts and colleagues (2017) argued that if trait change, achieved in a relatively short amount of time, persists over a long period of time, then this suggests a therapy shifting change in "trait variance" rather than "state variance."

Concerning the question if change in the therapeutic relationship impacts subsequent AS or vice versa, results indicate that there is a reciprocal temporal relationship between change in alliance and anxious, but not avoidant attachment. The WP effects suggest on the one hand that improvement in anxious attachment may have facilitated the ability to form a relationship with the therapist, concurring with Zalaznik and colleagues (2019), and with an increasing therapeutic bond probably enabling treatment to be effective. This is in line with general findings that a secure AS is associated with a stronger therapeutic relationship (Bernecker et al., 2014; Diener & Monroe, 2011; Mikulincer et al., 2013; Smith et al., 2010). On the other hand, findings suggest that change in alliance was a fostering component for subsequent change in anxious AS. This means that enhancing the therapeutic relationship may improve preoccupation with relationships and probably the "positive model of self" and underscores the

Table 2 (*continued*)

IIP_{t+1}			WAI_{t+1}			RSQ-$Anxiety_{t+1}$			RSQ-$Avoidance_{t+1}$		
B (*SE*)	*t* (*df*)	*p*	*B* (*SE*)	*t* (*df*)	*p*	*B* (*SE*)	*t* (*df*)	*p*	*B* (*SE*)	*t* (*df*)	*p*
1.88 (0.62)	3.01 (49.87)	<.001	−0.28 (0.11)	−2.62 (46.36)	<.01	—	—	—	—	—	—
3.16 (1.07)	2.94 (39.35)	<.01	−0.08 (0.11)	−0.79 (38.71)	.44	—	—	—	—	—	—
−0.11 (0.58)	−0.19 (71.17)	.85	0.13 (0.11)	1.22 (48.30)	.23	—	—	—	—	—	—
4.21 (0.82)	5.15 (39.28)	<.001	0.05 (0.09)	0.53 (40.11)	.60	—	—	—	—	—	—
−1.78 (0.44)	−4.06 (61.74)	<.001	—	—	—	−0.23 (0.08)	−2.95 (68.12)	<.001	−0.11 (0.09)	−1.26 (67.96)	.21
−1.43 (1.72)	−0.83 (44.76)	.41	—	—	—	−0.41 (0.23)	−1.78 (37.91)	.08	−0.00 (0.28)	−0.01 (37.93)	.99
—	—	—	−0.02 (0.02)	−1.11 (44.67)	.27	0.02 (0.02)	1.40 (26.98)	.17	0.04 (0.02)	2.30 (70.04)	<.05
—	—	—	−0.00 (0.01)	−0.28 (40.61)	.78	0.07 (0.02)	3.52 (40.52)	<.001	0.10 (0.02)	4.51 (40.88)	<.001
0.07 (0.01)	4.92 (29.17)	<.001	−0.02 (0.00)	−6.02 (29.09)	<.001	0.01 (0.00)	2.17 (68.86)	<.05	0.00 (0.00)	0.94 (29.16)	.35
0.21 (0.09)	2.30 (40.28)	<.05	−0.02 (0.01)	−2.04 (50.77)	<.05	0.03 (0.01)	2.48 (40.20)	<.05	0.01 (0.02)	0.90 (40.27)	.37
0.16 (0.03)	5.25 (29.19)	<.001	−0.01 (0.01)	−1.72 (69.19)	.09	0.01 (0.01)	1.77 (71.11)	.08	0.00 (0.01)	0.52 (68.53)	.60
0.48 (0.09)	5.60 (31.07)	<.001	−0.01 (0.01)	−1.07 (43.25)	.29	0.05 (0.01)	4.30 (39.67)	<.001	0.02 (0.02)	0.95 (38.79)	.35
2.42 (0.48)	5.04 (23.05)	<.001	−0.30 (0.09)	−3.36 (28.07)	<.001	0.22 (0.08)	2.79 (69.52)	<.01	−0.04 (0.10)	−0.38 (16.18)	.71
5.63 (1.74)	3.23 (38.27)	<.001	−0.28 (0.13)	−2.15 (38.76)	<.05	0.40 (0.26)	1.58 (39.18)	.12	0.41 (0.29)	1.39 (37.44)	.17

importance of fostering the working alliance in therapy as a possible mechanism of action in enhancing AS. It makes sense that through the therapeutic relationship trust is facilitated. To face up against one's anxiety and expectations requires courage and trust in the therapist, which in ET is often quickly rewarded with overcoming one's fears, and hence assured. The therapist is implicitly serving the function of providing comfort, encouragement, and a safe base for exploration (Levy et al., 2018; Mikulincer et al., 2013), in our case for aversive emotions and their regulation. Subsequently, an unconscious generalization to other relationships may take place and might explain the trend in later reduction of avoidance here. The increase of alliance, the state aspect, predicting less subsequent ID enforces this assumption and is in line with previous research (Coyne et al., 2019).

Furthermore, our results are in line with findings that improvement in alliance is reciprocally associated with therapy outcome, such that each influences the other subsequently, probably resulting in a positive upward spiral of higher alliance/lower symptoms that

Table 3

Results of Conditional Effects Model With Attachment Anxiety and Interpersonal Distress as Moderators of the Alliance–Outcome Effect

	$HAMA_{t+1}$			ACQ_{t+1}			BDI_{t+1}		
Conditional effects model	ß (*SE*)	95% CI	*p*	ß (*SE*)	95% CI	*p*	ß (*SE*)	95% CI	*p*
RSQ-Anxiety									
Intercept	0.06 (0.10)	[−0.14, 0.27]	<.001	0.08 (0.14)	[−0.19, 0.35]	<.001	0.08 (0.12)	[−0.16, 0.32]	<.001
WAI Between	−0.11 (0.10)	[−0.31, 0.08]	.25	−0.20 (0.13)	[−0.47, 0.06]	.14	−0.20 (0.12)	[−0.43, 0.03]	.09
WAI $Within_t$	−0.22 (0.07)	[−0.35, −0.08]	<.001	−0.25 (0.06)	[−0.36, −0.14]	<.001	−0.12 (0.05)	[−0.22, −0.02]	<.05
Anxiety Between	0.25 (0.10)	[0.05, 0.44]	<.01	0.13 (0.14)	[−0.14, 0.40]	.34	0.37 (0.12)	[0.14, 0.60]	<.001
WAI $Within_t$ × Anxiety Between	−0.06 (0.04)	[−0.14, 0.03]	.18	−0.04 (0.04)	[−0.11, 0.04]	.31	−0.11 (0.03)	[−0.17, −0.04]	<.001
Marginal R^2/Conditional R^2	0.154/0.403			0.116/0.672			0.249/0.697		
IIP									
Intercept	0.06 (0.11)	[−0.16, 0.28]	<.001	0.04 (0.14)	[−0.23, 0.32]	<.001	0.05 (0.12)	[−0.19, 0.28]	<.001
WAI Between	−0.07 (0.11)	[−0.29, 0.14]	.51	−0.24 (0.14)	[−0.51, 0.04]	.10	−0.16 (0.12)	[−0.39, 0.08]	.20
WAI $Within_t$	−0.23 (0.07)	[−0.36, −0.10]	<.001	−0.27 (0.05)	[−0.37, −0.16]	<.001	−0.15 (0.05)	[−0.25, −0.05]	<.001
IIP Between	0.26 (0.11)	[0.05, 0.47]	<.05	0.30 (0.13)	[0.04, 0.55]	<.05	0.43 (0.11)	[0.21, 0.65]	<.001
WAI $Within_t$ × IIP Between	−0.05 (0.06)	[−0.18, 0.07]	.43	−0.04 (0.05)	[−0.14, 0.06]	.43	−0.15 (0.05)	[−0.24, −0.06]	<.001
Marginal R^2/Conditional R^2	0.142/0.426			0.125/0.670			0.125/0.666		

Note. HAMA = Hamilton Anxiety Scale; ACQ = Agoraphobic Cognitions Questionnaire; BDI = Beck Depression Inventory; CI = confidence interval; RSQ = Relationship Scales Questionnaire; WAI = Working Alliance Inventory—Patient; IIP = Inventory of Interpersonal Problems. Between-patient moderators are grand mean centered.

predict higher alliances/lower symptoms later (Flückiger, Rubel, et al., 2020; Gidhagen et al., 2020). Moreover, in our study, it appears that this temporal relationship is found by investigating intervals of several months rather than weeks/sessions including follow-up assessments. The impact of the "state-like" aspect of alliance on therapy outcome suggests that alliance is therapeutic in itself, which is in line with previous studies (Zilcha-Mano, 2017). However, the ability to form a bond is probably not sufficient to induce change by itself but might enable the use of effective aspects of treatment (Zilcha-Mano, 2017). Findings show for example that the therapeutic relationship seems to be an essential tool for promoting treatment adherence in ET (Buchholz & Abramowitz, 2020).

Importantly, the effect of alliance increase on subsequent depressive symptom levels was found to be moderated by individuals' general level of anxious attachment and ID, with patients less anxiously, hence more securely attached, and with lower interpersonal problems benefiting less from alliance increase, which is in line with previous research (Gómez Penedo et al., 2020). It is plausible that patients with negative self-representations and interpersonal distress, who likely rely more on others as a safe base, benefit more from a comforting and encouraging bond than self-sufficient and socially well-adjusted individuals. The effect particularly on depressive symptoms makes sense, as the BDI measures a lot of cognitive aspects relating to negative self-representations. This concurs with our hypothesis that in anxiously attached patients, who overcome their fear and probably gain a feeling of control and confidence, promoted by a growing therapeutic alliance, a reduction in symptom severity may be facilitated.

Limitations

One limitation in our study is the relatively small and, because of the dropouts, decreased sample size at follow-up assessments. Therefore, we had no potential to test for between-group or interaction effects (e.g., categorical AS). The results are limited in inferring causal associations, but temporality can be established and thus are indicative of possible causal pathways. Due to the lack of patients for a waiting control group and of a control group because of ethical reasons, conclusions that the results are exceptional to CBT or merely the effect of time or common process factors across therapies are limited. Furthermore, although therapists received frequent supervision from an expert, no adherence ratings were administered. Moreover, although we asked for important life events or additional therapy since the end of therapy, we could not fully control in the follow-up for subsequent psychotherapy or environmental influences (e.g., separation or death of a relative) on AS development. Nevertheless, as the change of AS occurred during main therapy and remained stable afterward, we may assume that this change was due to treatment and influences afterward had no significant effect. The question whether the disorder changed the AS just temporarily or if attachment status reflects merely current impaired functioning affected by symptoms cannot be answered with our study design because the follow-up was restricted to 8 months after therapy and we had no information about the AS before the onset of the AD. For that, longitudinal and controlled cohort studies are needed. Additionally, the measurement schedule precluded us from using other methods of disentangling BP and WP effects, such as testing prediction from session to session.

Conclusion and Future Directions

In conclusion, an insecure AS, specifically anxious attachment, and ID may be effectively and enduringly improved by a standardized exposure based short-term CBT, which is not aimed to influence the quality of the relationship directly. Hence, at least for anxious attachment, it seems not necessary to target the AS directly or overtly identify, clarify, and restructure the attachment orientation, but it seems to be sufficient to target overcoming one's fear, which is the focus of ET. For other affective and anxiety disorders, we assume that similar mechanisms of CBT may apply in improving attachment security. However, improvement in anxious attachment seems to be reciprocally related with alliance increase. Alliance also seems to be an important factor in improving ID. Importantly, generally more anxiously attached and interpersonally distressed patients seem to benefit more from a stronger alliance regarding depressive symptoms. Thus, recommendations for clinical practice would be to monitor AS and ID as risk factors for symptom severity and to specifically target the alliance in preoccupied and interpersonally despaired patients.

Probable mechanisms of action for improvement of AS and for the maintenance of treatment gains like enhancing self-efficacy and self-esteem through changes in behavior or modifying negative self-representations, reducing anxiety sensitivity, promoting emotion regulation and reflective function seem promising (Buchholz & Abramowitz, 2020; Mikulincer & Shaver, 2012, 2019; Nielsen et al., 2017; Zalaznik et al., 2019) and should be investigated in further studies. We would recommend assessing AS more frequently over the course of therapy and with longer follow-up intervals after therapy. It would be interesting to determine in what phase of ET the attachment representations change: for example, as early as after interoceptive exposure, after accompanied exposure in vivo, or after succeeding exposure alone. Our study is contributing to the very limited research on AS in AD treated with CBT as well as disaggregation of WP and BP effects and supports the independence and disentangling of the "state-like" and "trait-like" aspects in the future.

References

Bartholomew, K., & Horowitz, L. M. (1991). Attachment styles among young adults: A test of a four-category model. *Journal of Personality and Social Psychology*, *61*(2), 226–244. https://doi.org/10.1037/0022-3514.61.2.226

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Belanger, C., Marcaurelle, R., Marchand, A., El-Baalbaki, G., Guay, S., & Pecknold, J. (2011). Effect of a marital communication training on treatment outcome in panic disorder with agoraphobia. In A. M. Columbus (Ed.), *Advances in psychology research* (pp. 141–163). Nova Publishers.

Bernecker, S. L., Levy, K. N., & Ellison, W. D. (2014). A meta-analysis of the relation between patient adult attachment style and the working alliance. *Psychotherapy Research*, *24*(1), 12–24. https://doi.org/10.1080/10503307.2013.809561

Bowlby, J. (1969). *Attachment and loss*. Basic Books.

Buchholz, J. L., & Abramowitz, J. S. (2020). The therapeutic alliance in exposure therapy for anxiety-related disorders: A critical review. *Journal of Anxiety Disorders*, *70*, 102194. https://doi.org/10.1016/j.janxdis.2020.102194

Byrd, K. R., Patterson, C. L., & Turchik, J. A. (2010). Working alliance as a mediator of client attachment dimensions and psychotherapy outcome. *Psychotherapy*, *47*(4), 631–636. https://doi.org/10.1037/a0022080

Carter, M. M., Turovsky, J., & Barlow, D. H. (1994). Interpersonal relationships in panic disorder with agoraphobia: A review of empirical evidence. *Clinical Psychology: Science and Practice*, *1*(1), 25–34. https://doi.org/10.1111/j.1468-2850.1994.tb00004.x

Castonguay, L. G., Constantino, M. J., & Holtforth, M. G. (2006). The working alliance: Where are we and where should we go? *Psychotherapy*, *43*(3), 271–279. https://doi.org/10.1037/0033-3204.43.3.271

Colonnesi, C., Draijer, E. M., Jan, J. M. S. G., Van der Bruggen, C. O., Bogels, S. M., & Noom, M. J. (2011). The relation between insecure attachment and child anxiety: A meta-analytic review. *Journal of Clinical Child and Adolescent Psychology*, *40*(4), 630–645. https://doi.org/10.1080/15374416.2011.581623

Constantino, M. J., Coyne, A. E., Luukko, E. K., Newkirk, K., Bernecker, S. L., Ravitz, P., & McBride, C. (2017). Therapeutic alliance, subsequent change, and moderators of the alliance-outcome association in interpersonal psychotherapy for depression. *Psychotherapy*, *54*(2), 125–135. https://doi.org/10.1037/pst0000101

Constantino, M. J., Laws, H. B., Coyne, A. E., Greenberg, R. P., Klein, D. N., Manber, R., Rothbaum, B. O., & Arnow, B. A. (2016). Change in patients' interpersonal impacts as a mediator of the alliance-outcome association in treatment for chronic depression. *Journal of Consulting and Clinical Psychology*, *84*(12), 1135–1144. https://doi.org/10.1037/ccp0000149

Coyne, A. E., Constantino, M. J., Westra, H. A., & Antony, M. M. (2019). Interpersonal change as a mediator of the within- and between-patient alliance-outcome association in two treatments for generalized anxiety disorder. *Journal of Consulting and Clinical Psychology*, *87*(5), 472–483. https://doi.org/10.1037/ccp0000394

Davila, J., Burge, D., & Hammen, C. (1997). Why does attachment style change? *Journal of Personality and Social Psychology*, *73*(4), 826–838. https://doi.org/10.1037/0022-3514.73.4.826

de Ruiter, C., & Van Ijzendoorn, M. H. (1992). Agoraphobia and anxious-ambivalent attachment: An integrative review. *Journal of Anxiety Disorders*, *6*(4), 365–381. https://doi.org/10.1016/0887-6185(92)90006-S

Diener, M. J., & Monroe, J. M. (2011). The relationship between adult attachment style and therapeutic alliance in individual psychotherapy: A meta-analytic review. *Psychotherapy*, *48*(3), 237–248. https://doi.org/10.1037/a0022425

Ehlers, A., & Margraf, J. (2001). *Fragebogen zu körperbezogenen Ängsten, Kognitionen und Vermeidung (AKV) Manual* [Body-related fears, cognitions and avoidance questionnaire] (2nd ed.). Beltz Test GmbH.

Esbjorn, B. H., Bender, P. K., Reinholdt-Dunne, M. L., Munck, L. A., & Ollendick, T. H. (2012). The development of anxiety disorders: Considering the contributions of attachment and emotion regulation. *Clinical Child and Family Psychology Review*, *15*(2), 129–143. https://doi.org/10.1007/s10567-011-0105-4

Falkenström, F., Finkel, S., Sandell, R., Rubel, J. A., & Holmqvist, R. (2017). Dynamic models of individual change in psychotherapy process research. *Journal of Consulting and Clinical Psychology*, *85*(6), 537–549. https://doi.org/10.1037/ccp0000203

Feingold, A. (2009). Effect sizes for growth-modeling analysis for controlled clinical trials in the same metric as for classical analysis. *Psychological Methods*, *14*(1), 43–53. https://doi.org/10.1037/a0014699

Flückiger, C., Del Re, A. C., Wampold, B. E., & Horvath, A. O. (2018). The alliance in adult psychotherapy: A meta-analytic synthesis. *Psychotherapy*, *55*(4), 316–340. https://doi.org/10.1037/pst0000172

Flückiger, C., Del Re, A. C., Wlodasch, D., Horvath, A. O., Solomonov, N., & Wampold, B. E. (2020). Assessing the alliance-outcome association adjusted for patient characteristics and treatment processes: A meta-analytic summary of direct comparisons. *Journal of Counseling Psychology*, *67*(6), 706–711. https://doi.org/10.1037/cou0000424

Flückiger, C., Rubel, J., Del Re, A. C., Horvath, A. O., Wampold, B. E., Crits-Christoph, P., Atzil-Slonim, D., Compare, A., Falkenström, F., Ekeblad, A., Errázuriz, P., Fisher, H., Hoffart, A., Huppert, J. D., Kivity, Y., Kumar, M., Lutz, W., Muran, J. C., Strunk, D. R., . . . Barber, J. P. (2020). The reciprocal relationship between alliance and early treatment symptoms: A two-stage individual participant data meta-analysis. *Journal of Consulting and Clinical Psychology*, *88*(9), 829–843. https://doi.org/10.1037/ccp0000594

Fraley, R. C., Gillath, O., & Deboeck, P. R. (2020). Do life events lead to enduring changes in adult attachment styles? A naturalistic longitudinal investigation. *Journal of Personality and Social Psychology*. Advance online publication. https://doi.org/10.1037/pspi0000326

Fraley, R. C., Vicary, A. M., Brumbaugh, C. C., & Roisman, G. I. (2011). Patterns of stability in adult attachment: An empirical test of two models of continuity and change. *Journal of Personality and Social Psychology*, *101*(5), 974–992. https://doi.org/10.1037/a0024150

Gidhagen, Y., Holmqvist, R., Philips, B., & Falkenström, F. (2020). The role of the working alliance in psychological treatment of substance use disorder outpatients. *Psychotherapy Research*. Advance online publication. https://doi.org/10.1080/10503307.2020.1807639

Gómez Penedo, J. M., Babl, A., Krieger, T., Heinonen, E., Flückiger, C., & Grosse Holtforth, M. (2020). Interpersonal agency as predictor of the within-patient alliance effects on depression severity. *Journal of Consulting and Clinical Psychology*, *88*(4), 338–349. https://doi.org/10.1037/ccp0000475

Griffin, D. W., & Bartholomew, K. (1994). Models of the self and other: Fundamental dimensions underlying measures of adult attachment. *Journal of Personality and Social Psychology*, *67*(3), 430–445. https://doi.org/10.1037/0022-3514.67.3.430

Hamilton, M. (1996). Hamilton Anxiety Scale (HAMA). In CIPS (Ed.), *Internationale Skalen für Psychiatrie* [International scales for psychiatry] (pp. 19–21). Beltz Test GmbH.

Hautzinger, M., Bailer, M., Worrall, H., & Keller, F. (2000). *Beck-Depressions-Inventar (BDI) Testhandbuch* [Beck Depression Inventory (BDI) Test Manual] (3rd ed.). Huber.

Hoffart, A., Øktedalen, T., Langkaas, T. F., & Wampold, B. E. (2013). Alliance and outcome in varying imagery procedures for PTSD: A study of within-person processes. *Journal of Counseling Psychology*, *60*(4), 471–482. https://doi.org/10.1037/a0033604

Horesh, D., Cohen-Zrihen, A., Ein Dor, T., & Solomon, Z. (2014). Stressful life events across the life span and insecure attachment following combat trauma. *Clinical Social Work Journal*, *42*(4), 375–384. https://doi.org/10.1007/s10615-014-0477-2

Horowitz, L. M., Strauß, B., & Kordy, H. (2000). *Inventar zur Erfassung interpersonaler Probleme. Deutsche Version Manual* (IIP-D) [Inventory of Interpersonal Problems. German version] (2nd ed.). Beltz Test GmbH.

Horvath, A. O. (2018). Research on the alliance: Knowledge in search of a theory. *Psychotherapy Research*, *28*(4), 499–516. https://doi.org/10.1080/10503307.2017.1373204

Horvath, A. O., Del Re, A. C., Fluckiger, C., & Symonds, D. (2011). Alliance in individual psychotherapy. *Psychotherapy*, *48*(1), 9–16. https://doi.org/10.1037/a0022186

Huppert, J. D., Kivity, Y., Barlow, D. H., Shear, M. K., Gorman, J. M., & Woods, S. W. (2014). Therapist effects and the outcome-alliance correlation in cognitive behavioral therapy for panic disorder with agoraphobia. *Behaviour Research and Therapy*, *52*, 26–34. https://doi.org/10.1016/j.brat.2013.11.001

Jones, J. D., Fraley, R. C., Ehrlich, K. B., Stern, J. A., Lejuez, C. W., Shaver, P. R., & Cassidy, J. (2018). Stability of attachment style in adolescence: An empirical test of alternative developmental processes. *Child Development*, *89*(3), 871–880. https://doi.org/10.1111/cdev.12775

Kirchmann, H., Steyer, R., Mayer, A., Joraschky, P., Schreiber-Willnow, K., & Strauss, B. (2012). Effects of adult inpatient group psychotherapy on attachment characteristics: An observational study comparing routine care to an untreated comparison group. *Psychotherapy Research*, *22*(1), 95–114. https://doi.org/10.1080/10503307.2011.626807

Kuznetsova, A., Brockhoff, P., & Christensen, R. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, *82*(13), Article 26. https://doi.org/10.18637/jss.v082.i13

Lang, T., Helbig-Lang, S., Westphal, D., Gloster, A. T., & Wittchen, H.-U. (2011). *Expositionsbasierte Therapie der Panikstörung mit Agoraphobie: Ein Behandlungsmanual mit CD-ROM* [Exposure-based therapy for panic disorder with agoraphobia: A treatment manual with CD-ROM]. Hogrefe.

Levy, K. N., Ellison, W. D., Scott, L. N., & Bernecker, S. L. (2011). Attachment style. *Journal of Clinical Psychology*, *67*(2), 193–203. https://doi.org/10.1002/jclp.20756

Levy, K. N., Kivity, Y., Johnson, B. N., & Gooch, C. V. (2018). Adult attachment as a predictor and moderator of psychotherapy outcome: A meta-analysis. *Journal of Clinical Psychology*, *74*(11), 1996–2013. https://doi.org/10.1002/jclp.22685

Madigan, S., Vaillancourt, K., McKibbon, A., & Benoit, D. (2015). Trauma and traumatic loss in pregnant adolescents: The impact of trauma-focused cognitive behavior therapy on maternal unresolved states of mind and posttraumatic stress disorder. *Attachment and Human Development*, *17*(2), 175–198. https://doi.org/10.1080/14616734.2015.1006386

Maiwald, L. M., Junga, Y. M., Lang, T., Montini, R., Witthöft, M., Heider, J., Schröder, A., & Weck, F. (2019). The role of therapist and patient insession behavior for treatment outcome in exposure-based cognitive behavioral therapy for panic disorder with agoraphobia. *Journal of Clinical Psychology*, *75*(4), 614–626. https://doi.org/10.1002/jclp.22738

Manning, R. P., Dickson, J. M., Palmier-Claus, J., Cunliffe, A., & Taylor, P. J. (2016). A systematic review of adult attachment and social anxiety. *Journal of Affective Disorders*, *211*, 44–59. https://doi.org/10.1016/j.jad.2016.12.020

Marazziti, D., Dell'osso, B., Catena Dell'Osso, M., Dell'Osso, M. C., Consoli, G., Del Debbio, A., Mungai, F., Vivarelli, L., Albanese, F., Piccinni, A., Rucci, P., & Dell'Osso, L. (2007). Romantic attachment in patients with mood and anxiety disorders. *CNS Spectrums*, *12*(10), 751–756. https://doi.org/10.1017/s1092852900015431

Maxwell, H., Tasca, G. A., Ritchie, K., Balfour, L., & Bissada, H. (2014). Change in attachment insecurity is related to improved outcomes 1-year post group therapy in women with binge eating disorder. *Psychotherapy*, *51*(1), 57–65. https://doi.org/10.1037/a0031100

Mikulincer, M., & Shaver, P. R. (2007). *Attachment in adulthood: Structure, dynamics, and change*. Guilford Press.

Mikulincer, M., & Shaver, P. R. (2012). An attachment perspective on psychopathology. *World Psychiatry*, *11*(1), 11–15. https://doi.org/10.1016/j.wpsyc.2012.01.003

Mikulincer, M., & Shaver, P. R. (2019). Attachment orientations and emotion regulation. *Current Opinion in Psychology*, *25*, 6–10. https://doi.org/10.1016/j.copsyc.2018.02.006

Mikulincer, M., Shaver, P. R., & Berant, E. (2013). An attachment perspective on therapeutic processes and outcomes. *Journal of Personality*, *81*(6), 606–616. https://doi.org/10.1111/j.1467-6494.2012.00806.x

Müller, R. T., & Rosenkranz, S. E. (2009). Attachment and treatment response among adults in inpatient treatment for posttraumatic stress disorder. *Psychotherapy*, *46*(1), 82–96. https://doi.org/10.1037/a0015137

Murphy, S., Elklit, A., Hyland, P., & Shevlin, M. (2016). Insecure attachment orientations and posttraumatic stress in a female treatment-seeking sample of survivors of childhood sexual abuse: A cross-lagged panel study. *Traumatology*, *22*(1), 48–55. https://doi.org/10.1037/trm0000060

Nielsen, S. K. K., Lonfeldt, N., Wolitzky-Taylor, K. B., Hageman, I., Vangkilde, S., & Daniel, S. I. F. (2017). Adult attachment style and anxiety – The mediating role of emotion regulation. *Journal of Affective Disorders*, *218*, 253–259. https://doi.org/10.1016/j.jad.2017.04.047

Nolte, T., Guiney, J., Fonagy, P., Mayes, L. C., & Luyten, P. (2011). Interpersonal stress regulation and the development of anxiety disorders: An attachment-based developmental framework. *Frontiers in Behavioral Neuroscience*, *5*, 55. https://doi.org/10.3389/fnbeh.2011.00055

Norcross, J. C., & Lambert, M. J. (2018). Psychotherapy relationships that work III. *Psychotherapy*, *55*(4), 303–315. https://doi.org/10.1037/pst0000193

Rimane, E., Steil, R., Renneberg, B., & Rosner, R. (2020). Get secure soon: Attachment in abused adolescents and young adults before and after trauma-focused cognitive processing therapy. *European Child and Adolescent Psychiatry*. Advance online publication. https://doi.org/10.1007/s00787-020-01637-x

Roberts, B. W., Luo, J., Briley, D. A., Chow, P. I., Su, R., & Hill, P. L. (2017). A systematic review of personality trait change through intervention. *Psychological Bulletin*, *143*(2), 117–141. https://doi.org/10.1037/bul0000088

RstudioTeam. (2020). *Rstudio: Integrated Development for R*. Rstudio, PBC. http://www.rstudio.com/

Rubel, J. A., Hilpert, P., Wolfer, C., Held, J., Vîslă, A., & Flückiger, C. (2019). The working alliance in manualized CBT for generalized anxiety disorder: Does it lead to change and does the effect vary depending on manual implementation flexibility? *Journal of Consulting and Clinical Psychology*, *87*(11), 989–1002. https://doi.org/10.1037/ccp0000433

Smith, A. E., Msetfi, R. M., & Golding, L. (2010). Client self-rated adult attachment patterns and the therapeutic alliance: A systematic review. *Clinical Psychology Review*, *30*(3), 326–337. https://doi.org/10.1016/j.cpr.2009.12.007

Steffanowski, A., Oppl, M., Meyerberg, J., Schmidt, J., Wittmann, W. W., & Nübling, R. (2001). Psychometrische Überprüfung einer deutschsprachigen Version des Relationship Scales Questionaire (RSQ) [Psychometric evaluation of a German version of the Relationship Scales Questionaire]. In M. Bassler (Ed.), *Störungsspezifische Therapieansätze – Konzepte und Ergebnisse* [Disorder-specific therapy approaches - concepts and results] (pp. 320–342). Psychosozial-Verlag.

Stovall-McClough, K. C., & Cloitre, M. (2003). Reorganization of unresolved childhood traumatic memories following exposure therapy. *Annals of the New York Academy of Sciences*, *1008*, 297–299. https://doi.org/10.1196/annals.1301.036

Strauß, B., Altmann, U., Manes, S., Tholl, A., Koranyi, S., Nolte, T., Beutel, M. E., Wiltink, J., Herpertz, S., Hiller, W., Hoyer, J., Joraschky, P., Nolting, B., Ritter, V., Stangier, U., Willutzki, U., Salzer, S., Leibing, E., Leichsenring, F., & Kirchmann, H. (2018). Changes of attachment characteristics during psychotherapy of patients with social anxiety disorder: Results from the SOPHO-Net trial. *PLoS ONE*, *13*(3), Article e0192802. https://doi.org/10.1371/journal.pone.0192802

Taylor, P., Rietzschel, J., Danquah, A., & Berry, K. (2015). Changes in attachment representations during psychological therapy. *Psychotherapy Research*, *25*(2), 222–238. https://doi.org/10.1080/10503307.2014.886791

Wampold, B. E. (2015). How important are the common factors in psychotherapy? An update. *World Psychiatry*, *14*(3), 270–277. https://doi.org/10.1002/wps.20238

Wang, L. P., & Maxwell, S. E. (2015). On disaggregating between-person and within-person effects with longitudinal data using multilevel models. *Psychological Methods*, *20*(1), 63–83. https://doi.org/10.1037/met0000030

Waters, E., Weinfield, N. S., & Hamilton, C. E. (2000). The stability of attachment security from infancy to adolescence and early adulthood: General discussion. *Child Development*, *71*(3), 703–706. https://doi.org/10.1111/1467-8624.00179

Weck, F., Grikscheit, F., Hofling, V., Kordt, A., Hamm, A. O., Gerlach, A. L., Alpers, G. W., Arolt, V., Kircher, T., Pauli, P., Rief, W., & Lang, T. (2016). The role of treatment delivery factors in exposure-based cognitive behavioral therapy for panic disorder with agoraphobia. *Journal of Anxiety Disorders*, *42*, 10–18. https://doi.org/10.1016/j.janxdis.2016.05.007

Weiss, M., Kivity, Y., & Huppert, J. D. (2014). How does the therapeutic alliance develop throughout cognitive behavioral therapy for panic disorder? Sawtooth patterns, sudden gains, and stabilization.

Psychotherapy Research, *24*(3), 407–418. https://doi.org/10.1080/10503307.2013.868947

Wilmers, F., Munder, T., Leonhart, R., Herzog, T., Plassmann, R., Barth, J., & Linster, H. W. (2008). Die deutschsprachige Version des Working Alliance Inventory—Short revised (WAI-SR) – Ein schulenübergreifendes, ökonomisches und empirisch validiertes Instrument zur Erfassung der therapeutischen Allianz [The German version of the Working Alliance Inventory – Short Revised (WAI-SR) – A pantheoretic, economic, and empirically validated instrument to assess the therapeutic alliance]. *Zeitschrift Für Klinische Diagnostik Und Evaluation*, *1*(3), 343–358.

Zack, S. E., Castonguay, L. G., Boswell, J. F., McAleavey, A. A., Adelman, R., Kraus, D. R., & Pate, G. A. (2015). Attachment history as a moderator of the alliance outcome relationship in adolescents. *Psychotherapy*, *52*(2), 258–267. https://doi.org/10.1037/a0037727

Zalaznik, D., Weiss, M., & Huppert, J. D. (2019). Improvement in adult anxious and avoidant attachment during cognitive behavioral therapy for panic disorder. *Psychotherapy Research*, *29*(3), 337–353. https://doi.org/10.1080/10503307.2017.1365183

Zilcha-Mano, S. (2017). Is the alliance really therapeutic? Revisiting this question in light of recent methodological advances. *American Psychologist*, *72*(4), 311–325. https://doi.org/10.1037/a0040435

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Appendix F – Publications

Original research

(Publications marked with“*”are part of the cumulative dissertation)

Diehm, M., J. Kunz, S. Goerigk, S., Wolf, J., **Lange, J.**, Jobst, A., Padberg, F., & Reinhard, M. A. (2025). “Reciprocal Dynamics of Therapeutic Alliance and Depressive Symptoms in Inpatient Cognitive Behavioural Analysis System of Psychotherapy: The Role of Attachment Insecurity.” *Clinical Psychology & Psychotherapy* 32, no. 6: e70200. https://doi.org/10.1002/cpp.70200.

*__Lange, J.,__ & Erhardt-Lehmann, A. (2025). HPA system in anxiety disorder patients treated with cognitive behavioural therapy: A review. *Biomarkers in Neuropsychiatry, 12,* 100116. https://doi.org/10.1016/j.bionps.2024.100116

Sabaß, L., Buchenrieder, N., Rek, S. V., Nenov-Matt, T., **Lange, J**., Barton, B. B., Musil, R., Jobst, A., Padberg, F., & Reinhard, M. A. (2022). Attachment mediates the link between childhood maltreatment and loneliness in persistent depressive disorder. *Journal of Affective Disorders*, *312*, 61–68. https://doi.org/https://doi.org/10.1016/j.jad.2022.06.021

*Moser, S., Martins, J., Czamara, D., **Lange, J.**, Müller-Myhsok, B., & Erhardt, A. (2022). DNA-methylation dynamics across short-term, exposure-containing CBT in patients with panic disorder. *Translational Psychiatry*, *12*(1), 46. https://doi.org/10.1038/s41398-022-01802-7

*__Lange, J.__, Goerigk, S., Nowak, K., Rosner, R., & Erhardt, A. (2021). Attachment style change and working alliance in panic disorder patients treated with cognitive behavioral therapy. *Psychotherapy (Chic)*, *58*(2), 206-218. https://doi.org/10.1037/pst0000365

*Martins, J., Czamara, D., **Lange, J.**, Dethloff, F., Binder, E. B., Turck, C. W., & Erhardt, A. (2019). Exposure-induced changes of plasma metabolome and gene expression in patients with panic disorder. *Depress Anxiety*, *36*(12), 1173-1181. https://doi.org/10.1002/da.22946

*Iurato, S., Carrillo-Roa, T., Arloth, J., Czamara, D., Diener-Holzl, L., **Lange, J**., Muller-Myhsok, B., Binder, E. B., & Erhardt, A. (2017). "DNA Methylation signatures in

panic disorder". *Transl Psychiatry*, *7*(12), 1287. https://doi.org/10.1038/s41398-017-0026-1

Lincoln, T. M., **Lange, J.**, Burau, J., Exner, C., & Moritz, S. (2010). The effect of state anxiety on paranoid ideation and jumping to conclusions. An experimental investigation. *Schizophr Bull*, *36*(6), 1140–1148. https://doi.org/10.1093/schbul/sbp029

Conference contributions

Lange, J., Dewald-Kaufmann, J., Holl, A. T., Semm, A., Reinhard, M. A., Welker, F., Buchheim, A., Jobst, A. & Padberg, F. (2018, 2019). Attachment style and working alliance changes in patients with chronic depression treated with cognitive behavioral analysis system of psychotherapy (CBASP) [Poster presentation]. Kongress der Deutschen Gesellschaft für Psychiatrie und Psychotherapie (DGPPN) & 9th World Congress of Behavioural and Cognitive Therapy WCBCT, Berlin, Germany.

Lange, J., Rosner, R. & Erhardt, A. (2017). Attachment Style and Working Alliance Changes in Panic Disorder Patients treated with Cognitive Behavioral Therapy (CBT) [Poster presentation]. WPA XVII World Congress of Psychiatry , Berlin, Germany.

Lange, J., Meyer-Cirkel, V., Reinhard, M., Welker, F., Jobst, A., & Padberg, F. (2017). The effect of cognitive behavioral analysis system of psychotherapy (CBASP) on anxiety symptoms in chronic forms of major depression [Poster presentation]. CBASP-Netzwerktreffen, München, Germany.

Lange, J., Nowak, K., Binder, E., & Erhardt, A. (2015). Biological correlates and procedural factors of response to CBT in panic disorder [Poster presentation]. Anxiety and Depression Association of America (ADAA), Miami, United States.

Lange, J., Nowak, K., Iurato, S., Sämann, P., & Erhardt, A. (2014). Implication of TMEM132D gene variants in the response to CBT in patients with panic disorder [Poster presentation]. Anxiety and Depression Association of America (ADAA), Chicago, United States.