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HPA axis responses to psychological challenge linking stress and disease: What do we know on sources of intra- and interindividual variability?

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ABSTRACT

Stress is an ubiquitous phenomenon with significant impact on human physiology when it lasts too long, when it is too intense, or when it hits vulnerable individuals. Examining the mechanisms linking stress exposure with health and disease is an important endeavor in psychoneuroendocrine research. Empirical evidence so far revealed large intra- as well as inter-individual variability in hypothalamic-pituitary-adrenal (HPA) axis responses to acute psychosocial stress, showing that the HPA axis is a highly adaptive system. Thus, the characterization of intra- und inter-individual patterns of HPA axis reactivity is of high scientific interest and forms the basis on which mechanistic links between stress response (dys)regulation and health impairments can be examined. To date, basic knowledge has been, and still is, accumulated on demographic, biological (including genetic and epigenetic) factors, lifestyle behavioral variables, consumption of substances and medication, psychological and personality factors, as well as on methodological aspects. Besides this, there is also very recent progress in respect to the development of laboratory stress paradigms that can be applied in virtual reality or inside an MRI-scanner. In sum, the present review updates our current knowledge on moderating and intervening factors as sources of intra- und inter-individual variability in human cortisol stress responses and offers recommendations for future research designs.

1. Introduction

Chronic stress is a major risk factor for several disorders, including highly prevalent diseases such as depression, anxiety disorders, cardiovascular diseases, and the metabolic syndrome (see 3.8 and 3.9). Since the finding of a disinhibited hypothalamic-pituitary-adrenal (HPA) axis in depressed patients (Carroll et al., 1980), it was assumed that alterations in the activity of this system may be a close correlate of stress-related pathology. The HPA axis is a core component of the neuroendocrine stress response. When encountering a challenge, neural stimulation of the paraventricular nucleus of the hypothalamus (PVN) leads to the release of the peptide corticotropin-releasing hormone (CRH). CRH initiates the cleavage of pro-opiomelanocortin (POMC) into adrenocorticotropin (ACTH), beta-endorphin, and other peptides and their subsequent release from the anterior pituitary gland into the blood stream. The primary target of ACTH is the adrenal cortex, where it triggers the secretion of glucocorticoids and adrenal androgens. The main glucocorticoid in humans is the steroid hormone cortisol, exerting its various metabolic, immunological, cardiovascular, affective, cognitive and behavioral effects as well as its effects on the HPA axis itself via the ubiquitous low affinity glucocorticoid receptor (GR) and the high affinity mineralocorticoid receptor (MR) (de Kloet et al., 2005).

In the past decades, major efforts have been made in psychoneuroendocrinology to develop a variety of methods allowing a detailed phenotyping of an individual's HPA axis regulation. One important line of progress was based on the assumption that, in addition to the assessment of basal HPA axis activity under chronic stress, the measurement of HPA axis responses to acute psychological stress would be of particular predictive value. It was assumed that a neuroendocrine system may appear fully functional under rest but it may show significant dysregulation under challenge. Basically, this is the main reason why investigating HPA axis responses to acute psychological challenge generated substantial interest over the last decades. Reviewing the literature as present today, it can be concluded that this approach was remarkably fruitful.

A striking and consistent feature of HPA axis responses to acute

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psychosocial stress is their distinct intra- as well as inter-individual variability. In order to understand the mechanisms linking HPA axis regulation and disease risk, it is of vital importance to identify the factors contributing to this variation. Which of the revealed variables are considered a confounder that should be controlled for and which factors are conceptualized as variables of interest completely depends on the given research question. The aim of the present paper is to provide a brief description of current standardized psychological stress paradigms (chapter 2) and to give an updated overview on important determinants of cortisol responses to psychological stress in humans (chapter 3). The current knowledge on the role of demographic and biological (including genetic as well as epigenetic) factors, lifestyle behavioral variables, consumption of substances and medication, psychological and personality factors, chronic stress and psychopathology as well as relevant methodological aspects will be summarized. While this review covers a relatively broad spectrum of factors, it is nevertheless not comprehensive. For example, the important influences of prenatal and early life experience on later HPA axis stress responses are not discussed as this topic is presented in the paper by Christine Heim, Claudia Buss and Sonja Entringer in this special issue of Psychoneuroendocrinology.

2. Standardized psychological stress paradigms

To further unveil psychoneuroendocrine pathways connecting altered stress regulation with ill health, HPA axis response variability constitutes a certain challenge that can best be controlled for in standardized experimental settings. Naturally occurring stress responses can certainly also be studied outside the laboratory with the potential advantage of an increased ecological validity. However, to allow for highly controlled stress exposures across subjects and to facilitate experimental study designs, laboratory stress paradigms have been established (for more details see Zänkert and Kudielka, 2018). Actually, most findings on intra- und inter-individual differences in human cortisol stress responses so far stem from laboratory studies and in this review, we mainly focus on these empirical studies. In respect to ecological settings, a recent review summarizes empirical examples for ambulatory psychobiological stress research (Rodrigues et al., 2015).

To date, several standardized psychological stress paradigms are available aiming at eliciting an emotional, cardiovascular, or endocrine stress response. These protocols can be differentiated regarding the psychological domain they are - at least predominantly - addressing, for example, cognitive stressors (e.g., mental arithmetic tasks), social stressors (e.g., social evaluation, peer rejection) and emotional stressors (e.g., presentation of emotional pictures or videos). In terms of this classification, the Trier Social Stress Test (TSST) is an example for a hybrid paradigm as cognitive load in combination with social evaluation are used as stress-eliciting components. The original TSST is composed of a free speech and a mental arithmetic task in front of an audience (Kirschbaum et al., 1993; for a detailed description see Kudielka et al., 2007). Meanwhile, a variety of modified versions of the TSST for different populations has been developed, like the TSST for children or elderly subjects (see Allen et al., 2017; Zänkert and Kudielka, 2018). Further, to overcome the resource-intensive protocol of the standard TSST and to be able to stress several subjects simultaneously, von Dawans et al. (2011) developed the TSST for groups for up to 6 participants (TSST-G). Moreover, a placebo version, a parallelized non-stress control condition that lacks the main stress-inducing components of the TSST (i.e., no committee, no video camera) was established (Het et al., 2009). Also, a 'friendly' version, the so-called f-TSST that can be used as control condition, was introduced. Subjects interact with a friendly committee as opposed to the neutral and reserved behavior shown in the original TSST (Wiemers et al., 2013). While for many psychobiological research questions a psychological stress protocol is preferable, HPA axis responses can certainly also be triggered by other means. For example, cortisol responses can be provoked in a highly standardized manner by physical stressors (intense physical exercise, physical pain, e.g. the Cold Pressor Test, CPT). Researchers should also be aware of the fact that meal intake can potentially elicit cortisol increases. Finally, pharmacological provocation tests systematically act at different levels of the HPA system and operate in a dose-dependent manner (see Kudielka et al., 2009; Seeman and Robbins, 1994). In this report, we will primarily focus on cortisol responses to standardized psychological stress paradigms.

Laboratory psychological stress tasks have different potencies in their ability to reliably evoke salivary cortisol responses (Biondi and Picardi, 1999). In a meta-analysis covering 208 laboratory stress studies, Dickerson and Kemeny (2004) investigated conditions capable of eliciting HPA axis stress responses. They concluded that motivated performance tasks reliably elicit ACTH and cortisol responses if they were uncontrollable or characterized by social-evaluative threat. Tasks containing both elements, like the TSST, were associated with the largest hormonal changes and the longest recovery times. Recently, Skoluda et al. (2015) investigated whether there exists a 'stimulus-response specificity' comparing the TSST with other commonly used laboratory stressors (namely the Stroop task, CPT, bicycle ergometry) and a resting control condition. All paradigms provoked increases in self-reported stress, reaching the highest scores in the TSST, followed by ergometry, Stroop, and CPT. The highest HPA axis response was found in the TSST, followed by ergometry, CPT, and Stroop. Finally, there are other established psychosocial stress paradigms such as public speech tasks like the Leiden Public Speaking Task (Westenberg et al., 2009), which demands a speech prepared at home and given in front of a pre-recorded audience, or combined tasks like the Maastricht Acute Stress Test (MAST) (Smeets et al., 2012), which recombines the mental arithmetic elements of the TSST with the physical aspects of the CPT under a condition of social evaluation. The Yale Interpersonal Stressor (YIPS) represents an interpersonal social rejection paradigm that targets the stimulation of negative mood and the activation of the HPA axis (Stroud et al., 2002). A less resource-intensive alternative might be the Socially-Evaluated Cold Pressor Test (SECPT), combining the thermal pain component of the classical CPT with a social-evaluative component. Evidence shows that the SECPT, indeed, has the potential to provoke significant subjective as well as HPA axis stress responses (Schwabe and Schächinger, 2018). In respect to responder rates, the original TSST robustly induces a two-to-three fold increase in cortisol levels in approximately 70–80% of the participants with a reported average effect size of d' = .93 (Dickerson and Kemeny, 2004; Goodman et al., 2017; Kirschbaum et al., 1993; Skoluda et al., 2015). While comparably high responder rates are reported for the MAST (Smeets et al., 2012), medium responder rates were observed for the Leiden Public Speaking Task and the YIPS (Stroud et al., 2017; Westenberg et al., 2009). While relatively low responder rates were reported for the classical CPT (Skoluda et al., 2015), medium responder rates are reachable using the SECPT (Schwabe and Schächinger, 2018).

In psychoneuroendocrine stress research, two recent methodological developments deserve special attention in respect to psychological stress paradigms. These concern stress exposures in virtual reality (VR) and adaptations of stress paradigms for the application inside an MRI-scanner.

Firstly, during the last decade there have been several attempts to transfer the TSST into virtual reality environments (VR-TSST). This methodological development promises a reduction of required manpower while increasing experimental control for stress-evoking elements of the task like evaluator characteristics (e.g., by standardization/adjustment of sex, race, age, and physical attractiveness of the avatar), control of between-participant session replicability, and location of the task. For example, Fich et al. (2014) reported a difference of cortisol responses depending on the design of the virtual environment. Still, the type of employed VR-technology seems to be a critical issue in the capability to induce a robust stress response (Allen et al., 2017). For instance, the level of immersion did not elicit differences in HPA axis

activation (Montero-López et al., 2016) whereas adding a virtual competitor more likely leads to a robust cortisol response (Shiban et al., 2016). So far, VR-TSSTs have been conducted via monitor or projection (Fallon et al., 2016), head mounted displays (Kelly et al., 2007; Shiban et al., 2016), or inside a VR-CAVE system, an immersive virtual reality environment with projectors being directed to the walls of a room-sized cube (Jönsson et al., 2015; Wallergård et al., 2011). So far, paradigms in a VR-environment seem to be less capable than classical laboratory stress paradigms to elicit cortisol increases. Thus, important issues for future research on VR paradigms are the testing of their respective stress-eliciting potency and ecological validity (e.g., Shiban et al., 2016).

Secondly, progress in neuroimaging techniques now offers insight into regulatory networks and processing of the central nervous system during acute stress. Several methods have been applied to elicit stress inside an MRI-scanner. Yet, first approaches hardly induced robust cortisol responses (see Dedovic et al., 2009). In order to enable an investigation of central nervous HPA axis regulation, a prerequisite however is, that an applied neuroimaging stress paradigm reliably elicits HPA axis responses to acute stress. Likewise, significant cortisol responses are essential as they validate that the observed brain activation changes are a response to psychological stress and do not merely reflect the processing of the task itself, for example cognitive load. Thus, Pruessner and colleagues established the Montreal Imaging Stress Task (MIST) which contains a sequence of computerized challenging mental arithmetic tasks under an experimental condition of induced failure (Dedovic et al., 2005). By default, subjects receive negative feedback regarding their performance by the test jury after each run. Meanwhile, the MIST has been employed in several fMRI as well as PET (positron emission tomography) studies, and has also been adapted successfully by other laboratories (Kogler et al., 2015; Lederbogen et al., 2011). This paradigm appears to be less capable than classical laboratory stress paradigms to elicit cortisol increases, with about 50%–100% elevations relative to baseline in about 50% of subjects. The MIST uses a typical fMRI block design and it appears plausible that the frequent interruption of the stress inducing task by control blocks without stress contributes to this difference in mean cortisol responses.

A more recently developed paradigm for the induction of acute stress inside the scanner is the Scan*STRESS* paradigm which is conceptually closely linked to the original TSST. Scan*STRESS* particularly translates the 'Mason factors' uncontrollability and ego-involvement as well as a component of social-evaluative threat. These factors are operationalized by pressure to perform a given forced-failure task (with adaptive difficulty) communicated by an observer panel. This jury is presented to the participant via a live video stream during the scanning procedure. Like the TSST, Scan*STRESS* combines two different tasks, starting with a serial subtraction task followed by a mental rotation task (Streit et al., 2014). Meanwhile, it could be empirically confirmed that the Scan*STRESS* protocol has the potential to induce robust neuronal, heart rate, and cortisol stress responses (Dahm et al., 2017; Streit et al., 2017, 2014). Although Scan*STRESS* uses a block design like the MIST, first studies suggest that cortisol responder may be somewhat higher, with up to two-third of subjects showing increases > 1.5 nmol/L (Dahm et al., 2017; Streit et al., 2017).

3. Sources of intra- and interindividual variability in HPA axis responses to acute pyschological stress

3.1. Sex and sex steroid-related factors

A very recent meta-analysis based on 34 studies encompassing 1350 participants (710 men and 640 women) corroborates the view that men show higher salivary cortisol responses to the TSST than women at peak times and during recovery (Liu et al., 2017). This effect could be observed in younger as well as older adults (see Kudielka et al., 2009; Lopez-Duran et al., 2009). Already earlier reviews and meta-analytic reports concluded that sex is a prominent source of variability for HPA axis stress responses (Kajantie and Phillips, 2006; Kudielka et al., 2009, 2004a; Kudielka and Kirschbaum, 2005; Otte et al., 2005). Regarding underlying mechanisms, there has been a discussion whether, in particular, the psychological nature of the TSST promotes such sex effects since the cortisol response to the YIPS (see above) on the contrary has been reported to be greater in females than males. This raised the assumption that women might be more biologically reactive to interpersonal stress such as social rejection challenges while men might be more responsive to achievement stressors such as the TSST (Stroud et al., 2017, 2002). However, this finding was not consistently confirmed in other studies.

Another line of evidence indicates that intake of oral contraceptives in females and the menstrual cycle might be crucial. It appears to be a relatively consistent finding that women medicated with oral contraceptives (OC) show reduced free salivary cortisol responses to acute stress (see Kudielka et al., 2009). This effect might, at least in part, be explained by a moderating role of corticosteroid-binding-globulin (CBG) since oral contraceptives containing an ethinyl-estradiol component can alter endogenous steroid-binding-globulins in the blood, including CBG concentrations (Wiegratz et al., 2003). This view is supported by own data from Kumsta et al. (2007), reporting a significant negative correlation between CBG and salivary cortisol levels after TSST exposure in 115 women taking OC while no such correlation was found in 93 men. To further elucidate the role of the female menstrual cycle phase, we applied the TSST in a total study sample of 81 composed of men, women in the follicular phase, women in the luteal phase, and women using oral contraceptives in equal parts (Kirschbaum et al., 1999). While no sex differences emerged for total plasma cortisol, salivary cortisol responses differed significantly between groups. Women in the luteal phase had saliva cortisol stress responses comparable to those of men whereas women in the follicular phase or women taking oral contraceptives showed significantly lower salivary cortisol responses. Other studies replicated the finding of comparably high salivary cortisol stress responses in men and women during the luteal phase (see Kudielka et al., 2009) and recent data by Stephens et al. (2016) corroborated a more robust activation of the HPA axis in men compared to women tested during the follicular phase. However, there also exist other recent studies that failed to detect HPA axis response differences across the follicular, ovulatory, or luteal phase (Duchesne and Pruessner, 2013; Herbison et al., 2016). In this context, it should also be of note that sex-steroid supplementation may alter cortisol reactivity to acute stress and should be considered as potential source of HPA axis response variability (see Kudielka et al., 2009). However, available evidence on the impact of hormonal replacement therapy in humans is still fragmentary and heterogeneous.

In sum, we strongly suggest to control for the use of oral contraceptives and menstrual cycle phases in female participants when investigating HPA axis reactivity. Also, any sex steroid treatment in men and women should be excluded or at least reported. Finally, as a note of caution, it also cannot be ruled out that the premenstrual syndrome in cycling women as well as postmenopausal status in elderly women may impact on HPA axis responses to acute stress. Therefore, these issues should guide a substantiated composition of study samples.

3.2. Age

It was proposed that aging might come along with changes in HPA axis resiliency (see Seeman and Robbins, 1994). However, studies applying psychosocial stress paradigms showed no or only somewhat higher responses in older adults, primarily in men (Almela et al., 2011; Kudielka et al., 2009; Otte et al., 2005). For example, in a reanalysis of five independent studies with a total of 102 children, younger as well as older adults who were exposed to the TSST (Kudielka et al., 2004a), we found elevated salivary cortisol responses in the group of elderly men (for sex difference see also above).

From a mechanistic point of view, two opposing pathways have been proposed to explain age-related alterations in HPA axis regulation. First, the so-called 'glucocorticoid cascade hypothesis', that mainly stems from animal research, attributes age-related changes in HPA axis functioning to a decrease in the ability of hippocampal neurons to maintain sufficient negative feedback, leading to a vicious cycle of continuously increasing HPA axis responses (Sapolsky et al., 1986). Acknowledging contradicting evidence, the 'corticosteroid receptor balance theory' proposes a similar endocrine response to stress in younger and older adults. Within this theory, it is argued that even with older age homeostatic control could be maintained by a new balance between glucocorticoid (GR) and mineralocorticoid receptors (MR), leading to a propensity for unchanged HPA axis responses (de Kloet et al., 1998). Empirical results are rather heterogeneous and it remains still unclear if old age is characterized by a 'cascade of events' or a new 'compensatory receptor balance'. With this, we recommend to either assess the participants age and to control for it when it is statistically associated with the outcome, or to use predefined age restrictions for participant recruitment.

3.3. Factors related to pregnancy

It is well-known that pregnancy is accompanied by increases in CRH, ACTH, cortisol, and CBG levels. While basal cortisol levels appear to be increased, salivary cortisol responses to the TSST are dampened (Entringer et al., 2010). There is also some indication for a progressive attenuation of psychobiological stress responses with advancing gestation (see La Marca-Ghaemmaghami and Ehlert, 2015). From a mechanistic point of view, it is assumed that heightened circulating CRH or glucocorticoid levels during pregnancy act by negative feedback to blunt HPA axis responses to challenge, corticotrophic cells in the pituitary might be desensitized or, at least in part, the presence of CRH-binding proteins in maternal plasma reduces the concentration of circulating potentially bioactive CRH.

After giving birth, lactation has been associated with dampened hormonal responses to different stressors in rodents (Carter and Altemus, 1997). On the contrary, in humans there does not seem to be a difference in cortisol responses to psychosocial stress in lactating versus non-lactating mothers (Altemus et al., 2001). However breast-feeding directly before confrontation with the TSST reduces the salivary cortisol stress response (Heinrichs et al., 2001). As underlying mechanisms, a potential inhibitory impact of the lactogenic peptides oxytocin and prolactin on different levels of HPA axis regulation are discussed (see Heinrichs et al., 2002). In sum, lactation in women (in contrast to rodents) does not result in generally suppressed HPA axis responsivity to acute psychosocial stress (see Kudielka et al., 2009). Rather, breast feeding seems to exert a short-term suppression of the cortisol response to psychosocial stress in women.

Long-lasting changes in HPA axis functioning, including cortisol responses to acute stress in adulthood, can also be ascribed to factors that date back to childbearing. Indicators of prenatal development (like birth weight and length of gestation), but also pre- and early postnatal environmental adversity (like prenatal substance exposure or psychosocial adversity during early childhood), have been shown to be related to potentially lifelong alterations of HPA axis responses to stress (Bunea et al., 2017; Hunter et al., 2011; Kajantie and Röikkönen, 2010). As indicated earlier, the impact of prenatal and early life experiences on later HPA axis stress responses are discussed elsewhere in this issue (see Heim et al.).

From a methodological point of view, we advise not to admit pregnant and lactating/breast feeding women as study participants unless it is the central topic of the study. In addition, it might be informative to inquire if study volunteers had been exposed to severe pre- or postnatal childhood adversity.

3.4. Genetic and epigenetic factors

A significant influence of genetic factors on different markers of basal HPA axis activity was repeatedly shown in twin studies as well as in genome-wide association studies (Bartels et al., 2003; Bolton et al., 2014; Rietschel et al., 2017; Velders et al., 2011; Wöst et al., 2000). A few twin studies do also exist on the heritability of HPA axis responses to acute psychological stress, suggesting a significant, though moderate, heritability of cortisol stress responses (Federenko et al., 2004; Ouellet-Morin et al., 2008; Steptoe et al., 2009).

Another approach to identify potential sources of interindividual variability in HPA axis stress responses are candidate gene studies. Significant associations between cortisol regulation and sequence variation in candidate genes belonging to the HPA axis pathway in a narrow sense as well as with variation in more 'distant' genes have repeatedly been reported. For example, we found *GR* and *MR* variants to be related to ACTH and cortisol responses to the TSST, partly in a sex-specific manner (see Kudielka et al., 2009; Kumsta et al., 2013). Variation in the gene coding for FK506 binding protein (*FKBP5*), an important GR regulator, was repeatedly shown to be associated with altered cortisol stress reactivity (e.g., Ising et al., 2008; Luijk et al., 2010) and cortisol responses to the TSST were also found to be associated with variation in the CRH receptor gene (*CRHR1*) (Mahon et al., 2013). Genetic variation in other neurotransmitter systems, like the serotonergic system (e.g., 5 H T transporter-linked polymorphic region, *5-HTTLPR*) and the dopaminergic system (e.g., catechol-O-methyltransferase gene, dopamine D4 receptor gene) was also shown to be associated with psychosocial stress responses (see Allen et al., 2017; Foley and Kirschbaum, 2010; Miller et al., 2013). Moreover, associations with variation in genes coding for neuropeptides or their receptors like brain-derived neurotrophic factor, alpha-2B adrenergic receptor and monoamine oxidase A were found (Allen et al., 2017). For example, own studies point to an association between sequence variants in the neuropeptide S receptor gene, a novel candidate gene for anxiety disorders, and the cortisol stress response to psychosocial challenge in the laboratory and MRI-scanner environment (Kumsta et al., 2013; Streit et al., 2017). Overall, it is well known that effects of single gene variants are inherently small, a phenomenon that, for example, has been demonstrated recently for the association between *5-HTTLPR* and cortisol stress reactivity (Miller et al., 2013). Nevertheless, there is no doubt that studying variability within a single gene or a circumscribed gene system can be of substantial relevance for psychological stress research. However, genetic effects of interest in our field cannot be adequately explained by a single gene variant in a single gene. Therefore, a sufficient number of sequence variants across the gene (or the gene system) has to be genotyped in order to achieve an adequate coverage and to capture the genetic variability that can be ascribed to the gene (system) of interest. Meanwhile, several biostatistical tools for the processing and integration of information from larger numbers of genetic variants are available. A promising example for a strategy that already has been successfully applied in stress research are multi-locus approaches like biologically informed multi-locus scores or polygenic scores derived from genome-wide association studies (Di Iorio et al., 2017; Utge et al., 2018).

Of particular interest for psychobiological stress research is the joint analysis of genetic and environmental factors. Gene-environment (GxE) interactions are presumed to be highly relevant for the understanding of mechanisms linking stress and disease as they are proposed to contribute significantly to the 'missing heritability' (Manolio et al., 2009; Uher, 2014). Furthermore, they can guide the search for epigenetic modifications. For instance, in subjects with a significant history of stressful life events who were homozygous for the s-allele of the *5-HTTLPR*, an elevated cortisol secretion in response to the TSST was observed (Alexander et al., 2009). Further, research on *FKBP5* also points to an interaction between childhood trauma and sequence variants in this gene on cortisol reactivity in different age groups using

different stress paradigms (Buchmann et al., 2014; Luijk et al., 2010; Zannas and Binder, 2014).

Altogether, an individual's HPA axis regulation is a highly complex phenotype that is only in part directly accessible and measurable. In order to adequately describe relevant domains of this complex phenotype, several methods have been developed in psychoneuroendocrine research, including paradigms to assess HPA axis responses to acute psychosocial stress. The findings from candidate gene and candidate GxE studies presented in this chapter suggest that the assessment of HPA axis stress responses indeed can serve as a valuable intermediate phenotype for HPA axis regulation *in toto*, which, in turn, is a close correlate of stress-related pathology. However, to tap the full potential of this approach, future candidate studies may be advised to focus not only on a thorough assessment of the target phenotype but also of relevant environmental variables. An even more sophisticated option would be to plan study designs allowing a systematic variation of environmental variables in GxE experiments (van Ijzendoorn et al., 2011) and to use longitudinal designs. Overall, modern candidate (GxE) studies are a fruitful approach complementing genome-wide strategies and they offer great potential to reveal stress-related disease mechanisms in humans.

Initial findings suggest that also epigenetic mechanisms might be important modulators of the neurobiological stress response. Epigenetic processes modify gene activity and expression by influencing the accessibility of the DNA without changing its sequence. To date, mechanisms that have mainly been studied in behavioral science are DNA methylation and histone modification. These epigenetic modifications can be altered by environmental influences including psychological factors such as prenatal maternal stress and early life adversity (Allen et al., 2017; Isles, 2015; Serpeloni et al., 2016; Turecki, 2016). For example, findings on *5-HTTLPR* by environment interactions have been extended through epigenetic research, investigating methylation and gene expression profiles (Alexander et al., 2014; Duman and Canli, 2015). *GR* methylation was shown to be associated with the cortisol recovery slope after stress exposure (van der Knaap et al., 2015) and a moderating role of *GR* methylation on the association between childhood trauma and cortisol stress reactivity was reported (Alexander et al., 2018). Consistent with the development in genetics, it also became quickly evident in epigenetic research that studying single genes can be informative but that research on a genome-wide level is additionally required. For example, a recent study on the association between genome-wide DNA methylation profiles and HPA axis regulation identified a novel and presumably relevant pathway. Methylation at the locus of the Kit ligand gene significantly mediated the relationship between childhood trauma and cortisol stress reactivity later in life (Houtepen et al., 2016). While it is known from animal research that stress can induce epigenetic marks in a variety of brain regions, including hippocampus, amygdala, and prefrontal cortex, it is not yet well understood whether these alterations are maladaptive or whether they rather contribute to a proper dynamic regulation (Hunter et al., 2015).

3.5. Lifestyle and behavioral variables

Meanwhile, (quasi-)experimental studies have been accumulated, elucidating the potential influence of some lifestyle and behavioral variables on HPA axis responses to stress, ranging from the consumption of alcohol, nicotine, coffee or dietary energy supplies, and intake of medication to physical exercise, body composition and sleep habits. In the following, existing evidence will be briefly discussed.

Acute as well as chronic alcohol consumption, alcohol dependency and even a positive family history of alcohol dependency is potentially related to altered HPA axis responses to psychosocial stress (Foley and Kirschbaum, 2010; Kudielka et al., 2009; Van Hedger et al., 2017). However, existing evidence is not unanimous with reports on dampened as well as unaltered HPA axis stress responses (Bibbey et al., 2015). Heterogeneous findings might, at least in part, be attributed to different ethanol dosages in some studies or insufficient statistical power in others. Based on this evidence, we recommend to exclude heavy alcohol users from basic research. Also, subjects should be instructed to refrain from acute alcohol intake on study days and the day before their laboratory appointment. It might also be advisable to assess regular and recent alcohol consumption in study participants.

Smoking, either acute or habitual, can significantly modulate HPA axis responses to acute stress. Smoking itself acutely activates free cortisol increases since nicotine acts as a potent stimulator of the HPA axis through induction of CRH release after binding to cholinergic receptors. After the consumption of only two cigarettes, significant salivary cortisol increases were observed (see Kudielka et al., 2009). Thus, we would advise to strictly prevent acute smoking before and during stress testing. Importantly, habitual nicotine consumption could lead to chronically elevated ACTH and cortisol levels and, in consequence, dampened HPA axis responsiveness to acute psychosocial stress (see Kudielka et al., 2009; Herbison et al., 2016; Rohleder and Kirschbaum, 2006; Van Hedger et al., 2017). As a further note of caution, although some empirical findings support the view that nicotine abstinence does not alter salivary cortisol responses to psychosocial stress, it is still possible that acute nicotine craving affects cortisol stress responses in smokers. Thus, we recommend to exclude habitual smokers or, at least, to control (statistically) for smoking status.

There exist some empirical evidence that caffeine consumption potentially stimulates basal cortisol levels. However, its pure stimulatory potency for HPA axis activation is not unequivocal because several other studies did not report such enhancing effects. Experimental evidence raised the idea that there might at least be a combined stimulatory effect of acute coffee consumption and psychosocial stress exposure (see Kudielka et al., 2009; Van Hedger et al., 2017). Recently, habitual caffeine consumption was reported to be associated with a greater cortisol stress reactivity to the TSST (Vargas and Lopez-Duran, 2017). With this, we advise to instruct study participants to refrain from coffee consumption before and during a laboratory testing and to potentially exclude volunteers with heavy habitual coffee consumption.

In respect to nutritional state, cortisol does not only affect energy metabolism but is itself influenced by energy intake. For example, low endogenous glucose levels have been associated with blunted free cortisol stress responses whereas in glucose-treated subjects stress exposure triggered larger salivary cortisol responses (with unchanged basal cortisol levels) (see Kudielka et al., 2009). At first glance, this empirical evidence seems to speak against the classical view that glucocorticoids function to provide the individual with energy in stress situations. Presently, it is assumed that a central mechanism may be responsible for regulation of energy balance and HPA axis activation rather than peripheral mechanisms (see Rohleder and Kirschbaum, 2006). Based on these empirical findings, it appears reasonable to standardize blood glucose levels when studying salivary cortisol in response to stress, for example by providing a standardized meal or administration of a glucose-containing standard beverage about 45 min before stress exposition (see Kudielka et al., 2009).

Further, it is important to acknowledge that chronic as well as short-term medication, vaccines, or intake of dietary supplements (irrespective of route of administration) potentially impact on salivary cortisol responses to psychosocial stress. This applies to patient groups as well as healthy controls. Highly relevant substances are, for example, synthetic glucocorticoids and psychotropic drugs (see Strahler et al., 2017; Houtepen et al., 2015; Zorn et al., 2017). Very recently, Van Hedger et al. (2017) summarized evidence on the effects of single doses of typical pharmacological agents on subjective as well as HPA axis stress responses including anxiolytics, antidepressants and sedatives, analgesics, and beta blockers. Obviously, the spectrum of relevant pharmaceutical ingredients is widespread, and thus, underlying chemical pathways are manifold (see Granger et al., 2009; Holsboer and Barden, 1996; Pariante and Miller, 2001). Considering the high number of available active substances, typically prescribed dosages as well as

interactions with other drugs, it cannot come as a surprise that our knowledge on their respective effects on HPA axis regulation is fragmentary and selective. Since various pharmaceutical agents interact with HPA axis regulation, it is advisable to exclude subjects with long-term as well as acute medication intake and recent inoculation/vaccination. If medication cannot be precluded, we recommend to inquire on subjects' medication intake. However, it might be impossible to statistically control for such effects if the study population is heterogeneous. At least, researchers should be aware of the half-life of the substance as indicated in the package insert.

Body composition and physical fitness have repeatedly been discussed as potential sources of HPA axis alterations. While several empirical studies points to an increased cortisol stress responsivity to psychosocial stress in obesity, at least in abdominal obesity (Rodriguez et al., 2015), there are also reports on normal salivary cortisol responses to stress in samples composed of only slightly overweight but otherwise healthy participants (Herbison et al., 2016; Jayasinghe et al., 2014). As shown in between-subjects designs (group comparisons) as well as in within-subject designs (interventional studies), physical fitness and intense physical exercise potentially comes along with a blunted cortisol response to acute psychosocial stress (Klaperski et al., 2013; Rimmele et al., 2009; Strahler et al., 2016). Such evidence points to the necessity to make a reasonable decision about the targeted study population, e.g., researchers should decide if obesity qualifies as exclusion criteria. Further, study participants should be instructed to refrain from heavy physical exercise before study participation (even the day before testing) and researchers might want to assess and (statistically) control for physical fitness.

Sleep variables have been repeatedly proposed as influential factors for HPA axis functioning. Although this reasoning appears intuitively plausible, empirical evidence remains surprisingly contradictory. For example, studies investigating the effects of sleep length the night before testing on cortisol stress responses do not support strong effects (for review see van Dalfsen and Markus, 2018). At least, for low sleep quality and excessive daytime sleepiness (van Dalfsen and Markus, 2018) as well as sleep deprivation (Vargas and Lopez-Duran, 2017; but see also Schwarz et al., 2018) there is some indication for potentially altered acute cortisol responsivity the day after. Thus, the assessment of sleep habits could be reasonable. Related to this topic and as a final note of caution, researchers should be reminded to ensure that laboratory appointments do not interfere with the cortisol awakening response (CAR), day-time napping, shift work, or jet-lag (see Stalder et al., 2016).

3.6. Psychological factors and interventions

It is plausible to assume that the social environment exerts modulating effects on endocrine stress responses. Indeed, psychosocial stress paradigms like the TSST heavily rely on contextual factors to trigger HPA axis activation. Empirical evidence supports this view. For example, social support, at least in men, dampened the HPA axis response to the TSST (Ditzen et al., 2008; Kirschbaum et al., 1995; Kudielka et al., 2009). In order to unveil underlying mechanisms, the neuropeptide oxytocin (OT) has been scrutinized as one biological causal link (see Hostinar et al., 2014). Indeed, OT administration enhanced the buffering effect of social support on salivary cortisol stress responsiveness in young men (Heinrichs et al., 2003). In accordance, young women who obtained a massage before they performed the TSST (presumably increasing endogenous OT levels) showed significantly reduced cortisol responses compared to women who received social support without positive physical partner contact or had no social interaction (Ditzen et al., 2007). Also, other social factors such as the position in the social hierarchy appears to be relevant as shown in an earlier study in army recruits by Hellhammer et al. (1997).

Beside this, psychological interventions like group-based cognitive-behavioral stress management, mind-body exercises including progressive muscle relaxation, Taiji practice and some forms of meditation, or even relaxing music have been shown to have the potency to reduce endocrine stress responses to subsequent acute psychological stress exposure (see Kudielka et al., 2009). Thus, in order to avoid any unintended social interactional effects between subjects and investigators or environmental influences that might (un)systematically alter subjects' stress responses, we recommend to adhere to given standardized instructions as strictly as possible.

3.7. Personality

The psychoendocrine response to psychological stress can be viewed as a close interaction between person and situation variables within a given context. Thus, it was repeatedly assumed that stable personality traits are closely related to salivary cortisol stress responses (see Kudielka et al., 2009). However, a meta-analysis by Chida and Hamer (2008) could not find any evidence for an association between negative psychological states or traits (e.g., negative affect, neuroticism, hostility, anxiety, aggression, etc.) and acute HPA axis stress responses whereas some evidence for associations between decreased HPA axis reactivity and positive psychological states or traits (e.g., happiness, positive mood, internal locus of control, self-esteem, empathy, spirituality, active coping, etc.) was reported. Only personality traits that have traditionally been associated with greater psychopathology (like high neuroticism or lower extraversion) do occasionally show an effect on HPA axis regulation after onetime stress exposure (Oswald et al., 2006). Also, in respect to trait rumination empirical evidence is inconsistent. While some studies have shown that rumination can prolong the salivary cortisol response to stress (Shull et al., 2016; Stewart et al., 2013; Zoccola et al., 2010), other studies do not support this view (Young and Nolen-Hoeksema, 2001; Zoccola and Dickerson, 2015). How can such results be explained? The novelty of a stress situation appears to cover the impact of personality on HPA axis regulation on first time exposure. This idea is supported by studies showing that the relationship between salivary cortisol responses and personality factors became apparent after repeated stress exposures. A given cortisol response to acute stress is certainly determined by both trait and state factors, with the latter changing over repeated exposures (e.g., novelty, predictability, uncontrollability, learning, memory, etc.). Data aggregation over repeated stress sessions appears to enhance the chance to uncover otherwise or initially masked relationships between personality traits as assessed by self-report and salivary cortisol stress responses. For example, Pruessner et al. (1997) reported a considerable increase in the correlation between psychological trait measures and mean cortisol stress responses when aggregating over several test days. Consistently, the heritability of HPA axis responses to the TSST increased over repeated exposures, probably due to a relative decrease of state effects (Federenko et al., 2004). Therefore, the pattern of habituation in itself may be an important marker worth investigating (see also Rohleder, in this issue).

Thus, we advise to apply multiple stress exposure sessions if researchers are interested in studying associations between stable personality traits and acute cortisol stress responses. As a further note, there might meanwhile be other promising approaches, like the use of implicit measures instead of self-reports (e.g., Schultheiss et al., 2014), to gain valuable insight into associations between personality factors and endocrine stress responses.

3.8. Chronic stress and burnout

As stated above, the aim of the present paper is to provide an updated overview on moderating and intervening factors as sources of intra- und inter-individual variability in human cortisol responses to psychological stress. From this perspective, chronic stress and stress-related psychopathology (chapter 3.9) can, of course, be listed as significant modulators. However, it should be pointed out that basically,

the interest of psychoendocrinological research in HPA axis responses to acute stress is founded on the assumption that studying these responses is a fruitful approach contributing to our understanding of the development of chronic stress and stress-related pathology. From that angle, it would in fact be more appropriate to conceptualize stress and stress-related pathology as major outcome variables and not as modulators. However, it is self-evident that it depends on the specific research question which of the two perspectives appears more suitable.

Either way, it is reasonable to assume that continuing stress leads, in the long run, to enduring changes in HPA axis regulation (for meta-analysis see Miller et al., 2007). There is also empirical evidence for altered HPA axis stress reactivity in individuals suffering from chronic stress, states of exhaustion, and burnout (for overviews and meta-analysis see Chida and Hamer, 2008; Eddy et al., 2018; Heim et al., 2000; Kudielka et al., 2006b). In this field of research, one major challenge is that conceptualizations of chronic stress (and related assessment tools) differ substantially, ranging for example from family caregiving to effort-reward imbalance, job strain, unemployment (including financial strain), etc. This might, at least in part, explain the great variability in reported results ranging from HPA axis hyper- to hyporesponsivity in chronically stressed individuals. In their seminal review, Heim et al. (2000) reported evidence for hypocortisolism in individuals living under conditions of chronic stress. As potential mechanistic pathways, they discuss a reduced biosynthesis or depletion of CRH, ACTH, and cortisol, CRH hypersecretion and an adaptive down-regulation of pituitary CRH receptors or changes in receptor sensitivity, increased feedback sensitivity of the HPA axis, or morphological changes. Studies on chronic work stress and burnout are somewhat mixed, reporting either hyper- or hyporesponsivity (for review see Kudielka et al., 2006a). This might not come as a surprise, considering that firstly, there still exists no consistent definition of burnout, and secondly, the burnout syndrome shows a large symptom overlap with different forms of depression. At least, the few studies that assessed chronic work stress according to the effort-reward-imbalance model appear to be more consistent, merely pointing to HPA axis hyporeactivity to acute stress (for review see Bellingrath and Kudielka, 2016; but see also Eddy et al., 2018). In accordance, more recent studies on severe burnout, clinical cases of burnout or insufficient long-term rescovery after exhaustion disorder also merely point to blunted salivary cortisol responses to psychosocial stress (Bellingrath and Kudielka, 2016; de Vente et al., 2015; Eddy et al., 2018; Jönsson et al., 2015; Lennartsson et al., 2015).

Taken together, the results picture would be in accordance with a time-course or two-stage model as proposed earlier (Hellhammer and Wade, 1993; see also Bellingrath and Kudielka, 2016): An early state of chronic stress (characterized by hyperactivity of the HPA axis) could, in the long run, lead to a hyporeactive state as result of a functional adaptation to excessive exposure to stress hormones. In consequence, such changes over time could then blur results pattern. Thus, in group analysis hyper- and hypocortisolemic effects in different individuals could cancel each other out. In sum, long-term dysregulations in HPA axis functioning due to chronic stress and states of exhaustion or burnout, in turn, affect acute psychosocial stress responses. Thus, researchers might want to inquire into the subjects' experience of not only acute but also chronic stress.

3.9. Psychopathology

Stress plays a crucial role in the pathogenesis, onset, and progression of various illnesses. In turn, numerous somatic as well as psychiatric diseases come along with altered HPA axis responses to acute stress (Chrousos, 2009). Detailed reviews and meta-analysis can be found elsewhere, focusing for example in particular on somatic illnesses (Strahler et al., 2017), autoimmune disorders (Buske-Kirschbaum et al., 2002; Tsigos and Chrousos, 1994), psychiatric diseases (Bradley and Dinan, 2010; Ciufolini et al., 2014; Knorr et al., 2010; Zorn et al., 2017), or pathophysiological conditions in children (Jessop and Turner-Cobb, 2008). Empirical findings on particular diseases are usually not unambiguous regarding the direction of HPA axis dysregulation. For example, depression was reported to be related to either hyperresponsiveness as well as hyporesponsiveness, depending on depression subtype (e.g., major depression or melancholic depression versus atypical or seasonal depression), comorbidity with other diseases like anxiety, or depending on sex. However, so far, available evidence predominantly suggests HPA axis hyporesponsiveness in, for example, schizophrenia, adult PTSD, chronic fatigue syndrome, fibromyalgia and atopic dermatitis and, possibly, in anxiety disorders. Hyperresponsiveness was, for example, observed in anorexia nervosa, panic disorder and PTSD in children. Mechanistically, it is not always clear, whether a disease leads to altered HPA axis alterations, or if HPA axis alterations contribute to health impairments, or both. In general, we advise to screen subjects carefully for disease states and to define eligibility clearly by health-related exclusion criteria. Irrespective of the heterogeneity of findings and evident methodological differences across studies, results support the view that differences between patients and healthy controls are more likely to be observed when the system is challenged.

3.10. Methodological aspects

In the following, we summarize evidence regarding some methodological aspects like time of testing, the (complementary) collection of blood samples, habituation to repeated testing, anticipation effects, and the assessment of stress appraisal. Finally, we will briefly discuss the issue of inter-laboratory variations.

It is well-known that the secretion of cortisol follows a typical circadian rhythm. To account for regular diurnal changes, and at the same time, to avoid any interference with the CAR (Stalder et al., 2016), we advise to schedule acute stress sessions in the afternoon. In their recent comprehensive meta-analysis, Goodman et al. (2017) did not observe pronounced differences in effects sizes regarding cortisol responses at different times of day, but cortisol responses were slightly lower and more variable during morning sessions. In a reanalysis of own data based on five independent studies, we analysed cortisol stress responses to the TSST in the morning versus afternoon (Kudielka et al., 2004b). Data showed that net salivary cortisol stress responses could be assessed with comparable reliability in morning and afternoon sessions, taking into account that pre-stress cortisol levels are systematically higher during the morning. However, we found that higher basal cortisol levels were slightly (but significantly) associated with lower acute stress responses pointing to the presumption that higher baseline levels might, to some degree, reduce a superimposed net stress response. Thus, to increase the likelihood of stronger cortisol responses, stress sessions should ideally be scheduled during the afternoon. However, other time windows may still present feasible alternatives, at least if avoiding meal times and interference with the CAR. Of course, experimenters should ensure that all test sessions of a study are performed during the same time window to avoid any bias caused by circadian rhythm effects.

If a researcher intends to take saliva and blood samples concomitantly, it should be acknowledged that a venepuncture elicits a cortisol response in more than one-third of subjects. Thus, cannula insertion should be followed by an extended relaxation period in order to allow cortisol levels to return back to baseline levels before experimental blood samples are drawn (Weckesser et al., 2014).

In case of repeated acute stress exposures, mean HPA axis stress responses typically show a rapid habituation across sessions. With this, the HPA axis is different from the sympathetic nervous, immune and blood coagulation system as well as indices of hemoconcentration which all show rather uniform activation patterns after repeated acute stress exposures (see Kudielka et al., 2009). In an own study, we set out to scrutinize this phenomenon in more detail (Wöst et al., 2005). Data showed that there is substantial variability of salivary cortisol response habituation patterns in healthy young individuals. The majority of our

participants (52%) exhibited the well-known response habituation across three TSST test sessions, about a third (30%) did not show obvious response alterations and almost 16% of participants even showed a response sensitization. It can be speculated that habituation of the HPA axis to psychosocial stress by many subjects is due to decreasing experience of task novelty, unpredictability and uncontrollability, leading to a reduction in context variables across sessions. The phenomenon of habituation should influence the researchers' choice for an adequate study design. For example, a simple within-subjects design with repeated pre- and post-interventional TSST exposures would not qualify as valid proof of stress-reducing effects of a given treatment (e.g., psychotherapy, stress management training, etc.) on acute HPA axis stress regulation. In such a design, it would remain unclear whether the treatment or the familiarization with the TSST exposure had caused a potential reduction of the stress response. It was argued, however, that pure habituation effects in studies with repeated stress exposition might potentially be prevented, or at least reduced to a considerable extent, by large enough between trial intervals and/or changing test settings (see Foley and Kirschbaum, 2010). As already discussed above, habituation to a repeated stressor can, on the other hand, also help to unveil associations with factors that might otherwise be masked at first stress exposure, such as personality traits, genetic factors or work-related exhaustion (see Kudielka et al., 2009). In this case, a within-subjects design might be the study design of choice.

As already known, cortisol stress responses can be evoked, at least in men, by the sole annunciation of an upcoming psychosocial stress task, pointing to a pure anticipation effect (Kirschbaum et al., 1992). Thus, researchers should have in mind that anticipation might impact on reactive cortisol stress responses (see Engert et al., 2013). This underlines the importance of standardized study instructions as well as adequate relaxation periods in which subjects should not ruminate about the upcoming stress task (see also Goodman et al., 2017).

Related to this, Gaab et al. (2005) reported that anticipatory stress appraisals, but not retrospective appraisals, of a psychosocial challenge explain up to 35% of the variance of the salivary cortisol stress response. With this, we recommend to psychometrically assess not only the participants' subjective stress experience after stress exposition but also directly before stress exposure. In line with this, a close correspondence between subjective emotional and biological stress responses has been rarely reported (see Campbell and Ehlert, 2012). This seems to speak for a lack of covariance between subjective and physiological stress response markers. At first glance, this appears to be surprising. Acute stress elicits multiple psychological as well as physiological responses in humans. Theoretically, such different responses to a given stressor represent indicators of the same construct. Therefore, clear associations between acute psychological and physiological responses, and thus a high psychoendocrine covariance, should be expected. However, empirically this does not seem to be the case. Obviously, reduced correspondence could be explained by the effects of multiple confounding variables (as reviewed in this chapter) and, at least in part, by measurement error but it also reflects imperfect coupling of the different stress response systems. Schlotz et al. (2008) indeed showed that lowered interrelations can be ascribed to the different dynamics of these systems. Acute subjective psychological stress responses occur within seconds and may change dynamically during a prolonged stress situation whereas cortisol responses reach their peak approximately 15–20 min after the onset of stress exposure and change less dynamically. According to the hypothesis that associations between an acute psychological and endocrine stress response should be higher when response correlations are computed at similar system-specific stages relative to the onset of the stressor, it was observed that subjective psychological responses precede HPA axis responses and that high levels of cortisol are associated with lower later levels of anxiety and activation using a cross-correlational analytic approach (see Kudielka et al., 2009). In sum, it could be shown that psychoendocrine responses are indeed coupled with cortisol levels if time-lagged correlations are applied. Results indicate that the so-far described lack of covariance might be, at least in part, explained by the different time courses of psychological and endocrine responses to stress, with subjective psychological responses preceding HPA axis responses. Therefore, if we want to draw valid conclusions about psychoendocrine covariance in response to acute stress, the different time courses of psychological and biological responses need to be accounted for.

Although stress paradigms like the TSST are principally highly standardized protocols, there is variation between laboratories. Some researchers decide to apply modified versions of the TSST, other deviations are caused by practical considerations. Goodman et al. (2017) recently analysed various protocol modifications of the original TSST and provided a list of recommendations that ensure a robust activation of the HPA axis. Fortunately, the effectiveness of the TSST appears to be relatively robust to (some) methodological variability. Nevertheless, we recommend adhering to the original protocol specifications to ensure maximized cortisol responses and to enable better comparability across studies.

4. Conclusions

Identifying determinants of inter- and intra-individual variability in cortisol regulation as well as understanding the mechanisms underlying pathologically relevant dysregulation of cortisol activity are key topics in psychobiological stress research. However, the phenotyping of markers of HPA axis reactivity in humans is a challenging, laborious, and time-consuming task.

To date, we know that numerous moderating and intervening factors, carefully described in different laboratories and summarized in the present paper, can have an impact on cortisol responses. Knowing relevant modulators increases the chance to detect true effects and to improve, technically spoken, the signal to noise ratio. This is crucial as the effects that can be expected in psychobiological stress research are usually of modest size (although they can well be of psychobiological or clinical relevance). This is certainly a somewhat challenging situation as it is not possible to control for countless potential confounders in each study and this holds particularly true when sample sizes are relatively modest. Therefore, in this review we aim at giving specific recommendations on how researchers might handle the respective variables. Of course, researchers should be aware of the fact that this list is still selective and new insight is accumulating continuously.

The presented knowledge might be helpful at different stages of a research project (see Kudielka et al., 2009). First, when planning an experiment, it might guide the researchers' decision on exclusion criteria, eligibility and selection of subjects (depending on the study question), further information that should preferably be provided by participants (e.g., in accompanying demographic or psychometric assessments), factors that could be held constant across subjects and issues that are relevant for the instruction of subjects before and during the assessment period. For example, smoking or the intake of oral contraceptives can be defined as exclusion criteria, can be held constant across different study groups, can afterwards be used as covariate, or defined as the experimental manipulation in a (quasi-) experimental study design. Such decisions consequently influence the theoretically optimal sample size of a study, apart from considerations of feasibility.

Second, when it comes to data analysis, knowledge about moderating and intervening factors can help to select potentially relevant control variables to be used, for example as covariates in statistical models. However, researchers should be aware of the fact that the appropriate number of covariates depends on the sample size since model overfitting might lead to spurious results (Babyak, 2004). Furthermore, Miller and Plessow (2013) offer a discussion on transformation techniques of cortisol data to meet distributional criteria for use in general linear model-based analysis.

Third, the acknowledgement of potential sources of variance is finally essential when it comes to data interpretation. This might be

important, for example, for studies based on small sample sizes, quasi-experimental designs, studies with limitations in randomization, or studies conducted under ambulatory settings and field conditions, etc. A discussion of potential sources of variance might contribute to the explanation of contradictory or conflicting results across different studies and might trigger the exploration of further yet unknown sources of variance. Another important aspect is the issue of generalizability of given results. Finally, in case of secondary analyses and reanalyses taking advantage of preexisting samples or data sets, researchers should be particularly aware of potential differences between samples, for example due to the specific study aim, design characteristics or due to possible variance in cortisol concentrations obtained with different biochemical assays. However, we should also bear in mind that even in highly controlled studies results might be sample-specific for unknown reasons. Therefore, replications in diverse study samples are always a necessary requirement.

To conclude, with the present review we aim to provide an updated overview on moderating and intervening factors as sources of intra- und inter-individual variability in human cortisol responses to psychological stress. We hope that this knowledge will be helpful for further research investigating pathways leading from individual psychobiological stress regulation to health and disease. So far, laboratory stress paradigms have proven useful tools in the field of experimental basic, applied, and clinical stress research. However, there is still a pressing need of studies investigating the ecological validity of psychological stress protocols by comparing real-life stress responses to those observed in the laboratory (see Henze et al., 2017). Similarly, the predictive validity of laboratory stress paradigms should be further evaluated by using them in prospective longitudinal studies on chronic stress or stress-related clinical outcomes. A major future task will be to translate more and more our basic knowledge to clinical application, for example, in order to predict disease susceptibility, symptom severity and/or to develop therapeutic approaches and monitor the efficacy of practical interventions.

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Conflict of interest

All authors declare no conflict of interest related to this review.

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