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### Psychobiological adaptation to childhood adversity

*The role of contextual and individual factors*

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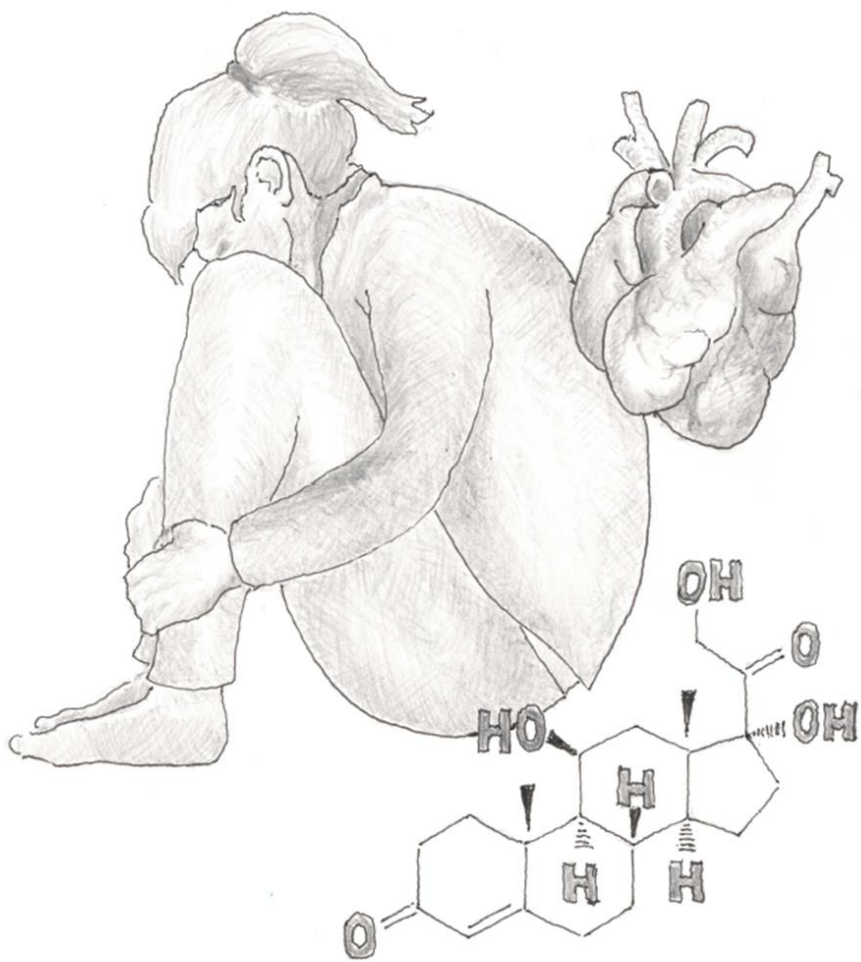
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## General discussion

The overarching aim of this dissertation was to broaden scientific knowledge on pathways leading from childhood adversity to psychopathology. The first aim was to review evidence on whether and how biological stress systems adapt after childhood adversity, focusing on the HPA axis (Chapter 2) and vagal functioning (Chapter 3). The second aim was to examine whether characteristics of childhood adversity explain different patterns of psychobiological adaptation (Chapters 2 and 3). The third aim was to investigate whether individual characteristics, both at the demographic, genotypical and phenotypical level, are associated with differences in psychobiological adaptation (Chapters 3, 4 and 6). Secondary aims of this dissertation were to broaden recommendations on cortisol sampling protocols to early childhood studies (Chapter 5), and to synthesize knowledge on the development and socialization of self-regulatory capacities, goals and motivation from infancy to adolescence (Chapter 7). In this chapter, a summary and overarching discussion of the research findings is provided, followed by strengths and limitations of this dissertation.

### **Section 1: Summarizing research on psychobiological correlates of childhood adversity**

The first aim was addressed by a narrative review on HPA axis functioning in adversity-exposed children younger than age 5 (Chapter 2) and a meta-analysis on vagal functioning in adversity-exposed individuals of all ages (Chapter 3). In Chapter 2, we demonstrated that both heightened and lowered levels of diurnal cortisol have previously been found in children younger than age 5 who were exposed to adversity. This finding mirrors former observations in adversity-exposed school-aged children, adolescents and adults (Doom et al., 2014; C. L. Kessler et al., 2021; Nicolson, 2004; E. Robson et al., 2021). According to the “attenuation hypothesis” (Fries et al., 2005; Susman, 2006), stress is expected to initially elevate cortisol levels, which in case of chronic stress likely rebound to below normal concentrations over time to protect the organism from the detrimental effects of long-term exposure to stress hormones. While former longitudinal work has suggested a transition from HPA axis hyper- to hypoactivity to occur in childhood (Doom et al., 2014) or adolescence (Trickett et al., 2010), Chapter 2 highlights that hypoactivity of the HPA axis can occur very early in development, with studies showing lower cortisol levels in adversity-exposed one- and two-year olds (M. Carlson & Earls, 1997; Cicchetti et al., 2011; Kroupina et al., 2012).

The findings of Chapter 2 are important for at least two reasons. First, they raise important questions that call for additional studies: For those children whose

HPA axis is hypoactive, was this pattern preceded by hyperactivation? And for those children whose HPA axis is hyperactive, will a transition to hypoactivation occur later on? While longitudinal studies have tested children from school-age onwards (Doom et al., 2014; Trickett et al., 2011; Shenk et al., 2022), more longitudinal work is needed that also includes early childhood. As cortisol has been shown to mediate the adversity-related effects on psychopathology (Busso et al., 2017), investigating trajectories of psychobiological alterations in the first years of life may provide a deeper understanding of the mechanisms of how adversity shapes long-term mental health outcomes. This knowledge can inform approaches to counteract, reverse or compensate biological alterations early on, with the idea to treat the mechanism instead of the clinical symptoms that may manifest over time (Heim et al., 2019). As highlighted in Chapter 2, interventions that target parenting behaviours have been shown successful in remediating adversity-related cortisol alterations in childhood (see also Slopen et al., 2014).

Second, the findings of Chapter 2 hold important implications for meta-analytic work. An aggregation of study findings, with some studies showing heightened and others lowered cortisol levels, may result in a non-significant overall association which obscures adversity-related alterations. Next to between-study differences, it is possible that associations in the included primary studies were non-significant, as individual variability in psychobiological adaptation was not considered. With our findings of Chapter 2 in mind, meta-analyses reporting no effect of childhood adversity on HPA axis functioning should be interpreted with caution (e.g., Fogelman & Canli, 2018). For future studies, we recommend to consider between-individual differences in adversity-related cortisol alterations (see e.g., Suor et al., 2015). More research testing for moderators is needed to broaden our understanding of when we will observe heightened or lowered levels of cortisol in the context of adversity. While meta-analytic work commonly focuses on the role of single moderators (e.g., Bernard et al., 2017; Bunea et al., 2017; Fogelman & Canli, 2018), empirical work may inspect potentially additive and interactive effects of moderators (see e.g., Oresta et al., 2021; Shirtcliff et al., 2021).

Chapter 3 followed up on the previous chapter by quantitatively aggregating findings on the association between childhood adversity and vagal functioning. In both meta-analyses on baseline vagal activity and vagal reactivity to challenge, we did not observe a significant overall association with childhood adversity. Hence, we concluded that childhood adversity does not lead to alterations in vagal regulation per se, which indicates that this physiological system generally maintains its

functional integrity in the context of adversity. However, our moderator findings demonstrated that under specific circumstances—such as in the presence of severe adversity—childhood adversity is related to decreased levels of vagal activity and vagal reactivity to challenge (further discussed in Sections 2 and 3). Both lower vagal activity and reactivity have been proposed as physiological indicators of lower self-regulation (T. W. Smith et al., 2020). Thus, our findings support a part of the pathway that we proposed in Chapter 2, in which childhood adversity increases risk for psychopathology through affecting an individual’s self-regulatory abilities.

Alterations in stress system functioning have been proposed to act as mechanisms in the adversity-psychopathology link (Chapter 2; Raymond et al., 2018). Such a mediating role has been demonstrated by several cross-sectional and longitudinal studies for HPA axis functioning (Bernard, Zwerling, & Dozier, 2015; Busso et al., 2017; Conradt, Abar, Lester, et al., 2014; Davies et al., 2007; Duprey et al., 2020; Koss et al., 2016; L. O. White et al., 2017), but not for vagal functioning (Busso et al., 2017; Duprey et al., 2020; Fagan et al., 2017; Jankovic et al., 2021). Our findings of Chapter 3 may help to understand the lack of evidence for a mediating role of vagal functioning. While overall associations between childhood adversity and vagal functioning were non-significant, moderator analyses revealed only very small associations under specific circumstances. Hence, larger samples than the ones previously used to test for mediation are needed to account for the very small association between the predictor and mediator (for recommendations, see M. S. Fritz & MacKinnon, 2007). Future work may profit from our findings of Chapter 3 by focusing on those circumstances that were identified as more likely to lead to alterations in vagal functioning in the context of adversity.

Experts have recently rated both HRV and salivary cortisol as “possible future candidates” to measure stress activation in children in primary care practice (Shonkoff et al., 2022). The use of such biomarkers can circumvent problems that come along with questionnaire measures of adversity, including that young children may not be able to self-report on adversity and that parental reports may be biased (Oh et al., 2018). While our findings of Chapter 2 and 3 generally support the suggestion of including biomarkers of stress system functioning in clinical practice, there are also reasons for caution. First, as demonstrated in Chapter 3, overall associations between childhood adversity and HRV were rather small, and only observable under specific circumstances. Second, biological measures of stress system functioning come along with high within-person variability (see Chapters 2, 5 and 6, but also Segerstrom et al., 2014, 2017), which may require repeated

measurement over multiple days to achieve adequate reliability. Nevertheless, it may be useful to include HRV and cortisol in a composite that further comprises other biomarkers of stress activation (e.g., interleukins as a measure of inflammation). Before being implemented in clinical practice, validation studies need to establish normative values and clinically-relevant cut-off points to identify children at risk with high sensitivity and specificity (Shonkoff et al., 2022). With our findings of Chapter 2 in mind, cut-off points related to cortisol ought to consider that both heightened and lowered levels of diurnal cortisol may indicate increased stress exposure.

## **Section 2: Do characteristics of childhood adversity explain different patterns of psychobiological adaptation?**

In Section 2 (Chapters 2 and 3), we examined whether characteristics of childhood adversity explain different patterns of psychobiological adaptation. First, we analyzed in Chapter 2 whether different adversity types would differentially relate to HPA axis hyper- or hypoactivity. Perhaps the clearest links were observed for severe deprivation, which was related to lower levels of cortisol (M. Carlson & Earls, 1997; Kroupina et al., 2012), and for emotional unavailability, which was related to heightened levels of cortisol across studies (Bugental et al., 2003; Suor et al., 2015). However, as we performed a narrative instead of a systematic review, we cannot rule out that there are also reports on heightened cortisol related to deprivation, and reports on lowered cortisol related to emotional unavailability. With regard to maltreatment, while some of the included studies pointed at lower cortisol levels (Cicchetti et al., 2011; Bernard et al., 2010), others reported on both lower and heightened cortisol levels within the same study (Bruce et al., 2009; Dozier, Manni, et al., 2006). Further, both directions of change in HPA axis functioning were previously observed in the context of poverty (Blair et al., 2005; Zalewski, Lengua, Kiff, & Fisher, 2012) and family instability (Suor et al., 2015). Hence, based on Chapter 2, we cannot conclude that specific adversity types are clearly linked to HPA axis hyper- or hypoactivity.

In Chapter 2, we further raised the question whether abuse and neglect—two different forms of maltreatment—have different effects on HPA axis functioning in early childhood. This hypothesis seemed plausible, given that different dimensions of adversity such as threat (which relates to abuse) and deprivation (which relates to neglect) have been differentially linked to brain structure and function (Banihashemi et al., 2021; Hein et al., 2020), cognitive ability (Usacheva et al., 2022), and emotion regulation (Milojevich et al., 2019). In our review, we identified one study by Bruce

and colleagues (2009) who found that foster children who experienced more severe physical neglect had low cortisol levels, whereas foster children who experienced more severe emotional maltreatment (a combination of emotional abuse and emotional neglect) had high cortisol levels. However, broadening the scope to studies in school-aged children and adolescents, neglect and abuse have been associated with both higher *and* lower cortisol levels (Cicchetti & Rogosch, 2001; C. L. Kessler et al., 2021; Sullivan et al., 2013). In Chapter 3, we also addressed the question of whether deprivation and threat would differentially relate to vagal functioning. Based on analyses controlling for the inter-relatedness of moderators, the dimension of adversity was not a significant moderator influencing an individual's vagal adaptation after adversity. Integrating these meta-analytic findings of Chapter 3 with those of our review of Chapter 2, it seems that there is limited evidence that deprivation and threat affect the functioning of the stress systems in distinct ways. However, it needs to be acknowledged that the methodology employed in both of our works—reviewing former studies—may have concealed differential associations that might be observable in studies specifically designed to disentangle experiences of threat from those of deprivation (see Busso et al., 2017; Lambert et al., 2017). This is not an easy undertaking, as deprivation and threat often co-occur (McLaughlin et al., 2012) and may not always be separable, as for instance being neglected portrays a major threat to an infant's life.

Intriguingly, findings of Chapter 2 suggest that the severity of adversity may contribute to explaining the direction of change in HPA axis functioning, as variations in the severity of neglect were related to different cortisol outcomes: Within studies on children younger than age 5, maternal unresponsiveness (a 'mild' form of neglect) was associated with heightened cortisol levels, whereas severe neglect experienced by institutionalized children was associated with lowered cortisol levels. Unfortunately, we did not identify studies that would enable the comparison of different degrees of severity within threat-related adversity types. However, recent evidence focusing on a composite of deprivation- and threat-related adversities supports our hypothesis, showing that minor adversities were associated with greater average daily cortisol, whereas major adversities were associated with a flatter daily slope in adolescence (C. L. Kessler et al., 2021). Integrating these and our findings of Chapter 2 with the attenuation hypothesis (Fries et al., 2005), it seems possible that experiences of severe adversity accelerate the transition from stress-induced initial HPA axis hyperactivity to subsequent hypoactivity. Further studies are needed to test whether this hypothesis gains support in early childhood.



While the severity of adversity may influence the direction of change in HPA axis functioning (Chapter 2), it may further influence whether alterations in vagal functioning emerge in the context of childhood adversity. In Chapter 3, we found that maltreatment as a proxy for severe adversity was related to lower baseline vagal activity, whereas less severe adversity types were non-significantly associated with vagal activity. When we controlled for the interrelatedness of various significant moderators in one statistical model, only the proximity of adversity remained a significant moderator. We found that direct types of adversity (a larger category comprising maltreatment but also peer victimization and adverse parenting) were negatively associated with vagal activity, whereas no significant association was present with indirect types of adversity (e.g., poverty). Given that direct adversities are likely perceived as more severe as indirect adversities, an interrelation of the severity and proximity of adversity may have rendered moderator findings on the severity of adversity insignificant. Hence, despite the lack of evidence from multivariate moderator analyses, we conclude that the severity of adversity likely influences whether an individual develops vagal alterations after adversity.

### **Section 3: Are individual characteristics associated with differences in psychobiological adaptation?**

In Section 3, we investigated whether individual characteristics influence psychobiological adaptation in the context of childhood adversity. We tested for a moderating role of age, gender, ethnicity, and psychopathology in two meta-analyses (Chapter 3), and for a moderating role of genetic constitution (Chapter 4) and effortful control (Chapter 6) in two empirical studies. In Chapter 3, we found across both meta-analyses that females were not more likely than men to develop alterations in vagal (re)activity in the context of adversity. Likewise, ethnic minorities were not more likely than ethnic majorities to develop vagal alterations in the context of adversity. Considering the non-significant overall associations between adversity exposure and vagal functioning, our results suggest that gender and ethnicity may not be informative to identify those individuals who are most likely to develop psychobiological alterations in the broad context of childhood adversity. As we tested for a moderating role of gender and ethnicity across all adversity types, future (meta-analytic) work may examine whether gender or ethnic differences exist in psychobiological adaptation to specific adversity types.

In Chapter 3, we found that childhood adversity was associated with lower baseline vagal activity in samples in which part of the participants were diagnosed with a psychiatric disorder. In the discussion of this chapter, we proposed several

possible interpretations of this finding, which raised the central question whether vagal functioning acts as a *mediator* or *moderator* in the adversity-psychopathology link. The literature rather provides evidence for a moderating role of vagal functioning, demonstrating that low resting vagal activity acts as a risk factor, and high resting vagal activity as a protective factor in adverse environments (Carnevali et al., 2018; Koenig, 2020; Patron et al., 2021). However, considering the literature on self-regulation—for which vagal activity is a physiological indicator (T. W. Smith et al., 2020)—it is also reasonable to assume that vagal functioning acts a mechanism in the adversity-psychopathology link. In line with our proposed pathway emphasizing a mediating role of self-regulation (see Chapter 2), a recent meta-analysis has shown that childhood adversity was positively associated with difficulties in emotion regulation, which in turn were positively associated with psychopathology (Miu et al., 2022). Integrating these different lines of evidence, we conclude that both a moderating and a mediating role may apply to vagal functioning. More specifically, individuals with the high-risk endophenotype of lower resting vagal activity (and low self-regulation skills) at the time of adversity exposure may be the ones that more likely develop further reductions in vagal activity over time, which are preceded or paralleled by alterations in brain structure and function. Longitudinal studies spanning different developmental periods are needed to shed light on the role of vagal functioning in such a complex developmental cascade leading from adversity to psychopathology.

We further observed in Chapter 3 that childhood adversity was related to lower vagal reactivity to challenge in older individuals who had experienced adversity less recently. Along the same lines, another meta-analysis found blunted cortisol stress reactivity in adversity-exposed individuals, which was stronger in adults compared to children and adolescents (Bunea et al., 2017). These findings altogether suggest that childhood adversity can set in motion a detrimental developmental cascade in which alterations in stress system functioning become more apparent in the long run. This raises the question of what happens after childhood adversity has ended that leads to an intensification of psychobiological alterations? There are two theoretical frameworks that may apply to address this question. First, the Generalized Unsafety Theory of Stress (GUTS; Brosschot et al., 2018) posits that prolonged stress responses are due to generalized and largely unconsciously perceived unsafety rather than stressors. Applying this theory, experiences of severe childhood adversity may disrupt the child's safety learning, leading to a failure to recognize the signals of safety and consequently to a stress response that is always "on". Hence, perceptions of generalized unsafety may

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activate the stress systems in a similar way to conditions of ongoing adversity, leading to stronger psychobiological alterations over time, even after adversity has ended. Second, the neurocognitive social transactional model of psychiatric vulnerability (McCrorry et al., 2022) suggests that neurocognitive alterations induced by experiences of severe adversity can influence how individuals interact with, and shape, their social environment. Neurocognitive alterations can contribute to both increased stressful interactions (stress generation) and attenuated social support (social thinning), which in turn increase vulnerability for psychopathology. To conclude, at the core of both theories, ongoing experiences of stress after adversity has ended may continue to induce psychobiological alterations, which may explain stronger associations of childhood adversity with HPA axis and vagal functioning in older individuals (Chapter 3; Bunea et al., 2017).

Apart from meta-analytic investigation of a moderating role of participant characteristics commonly assessed across studies, we also conducted two empirical studies to broaden knowledge on specific individual moderators that were outside the scope of our meta-analysis. Chapter 4 presents a gene-environment interaction study in which we examined whether rs1360780, a single nucleotide polymorphism (SNP) within the FKBP5 gene, would moderate the association between child abuse and resting-state functional connectivity between the amygdala and other areas of the salience network. We addressed our research question from a “diathesis-stress” perspective (Monroe & Simons, 1991), according to which some individuals are affected more than others by the detrimental effects of adversity due to individual vulnerabilities such as genetic constitution. Based on former evidence that allelic variations of rs1360780 differentially relate to cortisol stress recovery (Ising et al., 2008), we considered the TT allele of rs1360780 as the “vulnerability” (or “risk”) allele. However, we found that as compared to CT/CC allele carriers with and without abuse, TT allele carriers with a history of abuse demonstrated strongest amygdala-insula resting-state functional connectivity, whereas those without a history of abuse showed lowest connectivity between these regions. Hence, our findings were rather in line with Differential Susceptibility Theory (Belsky et al., 2007; Ellis et al., 2011), according to which those individuals who are genetically more susceptible to negative environmental influences may also be the ones who more likely benefit from positive environmental influences. However, our interpretation that the TT allele of rs1360780 represents a susceptibility allele remains speculative given two reasons: First, we cannot infer that participants without experiences of abuse grew up in a positive environment; and second, we cannot infer that lowest functional connectivity represents a beneficial outcome.

While a differential susceptibility function of rs1360780 has aggregated support beyond our study (Binder et al., 2004; VanZomeren-Dohm et al., 2015), there is also evidence in support of rs1360780 as a vulnerability, but not susceptibility factor (Keijsers et al., 2021), or evidence against a moderating role of rs1360780 (Fuller et al., 2021). As these studies focused on different environmental influences (e.g., peer victimization, financial strain) and outcomes (e.g., depressive symptoms, treatment response), future work may aggregate findings to evaluate under which circumstances rs1360780 renders an individual differentially susceptible to the environment.

Given that the human genome contains about 24,000 protein-coding genes (Salzberg, 2018), it is reasonable to question the hypothesis that variations in one SNP of one specific gene—as was the case in our study presented in Chapter 4—can modulate effects of environmental experience on brain structure and function to a meaningful extent. A criticism of the candidate gene approach is the widely-observed lack of reproducibility and inconsistency in study findings, which may be a function of the small underpowered samples that have often been used to test for interactions and that increase the probability of false positive results (Assary et al., 2018; Martins et al., 2022). Another drawback relates to the need of a strong biological hypothesis to choose appropriate candidate genes, for which often only limited knowledge is available (Assary et al., 2018). This is why the field has started to employ hypotheses-free genome-wide gene-by-environment interaction studies (GEWIS), in which the entire genome is inspected for variants that moderate the effects of the environment on psychobiological outcomes (Bolhuis et al., 2022). However, GEWIS require even larger samples than candidate gene studies, and cannot pinpoint specific genetic variants that are responsible for modulating environmental effects, which would require extensive additional testing (Elbau et al., 2019; Khoury & Wacholder, 2009).

An alternative approach that may counterbalance the limitations of candidate gene studies and GEWIS are polygenic risk scores, which are calculated as a weighted count of thousands of risk variants that an individual carries (C. M. Lewis & Vassos, 2020; Martins et al., 2022). Given that several other SNPs next to rs1360780 have been shown to modulate HPA axis functioning (see e.g., Alexander et al., 2011; Sumner et al., 2014; Weeger et al., 2020), we could extend the research in Chapter 6 in the future by employing a polygenic risk score. This may yield more accurate identification of those adversity-exposed individuals that develop alterations in functional brain connectivity. It is interesting to note that polygenic risk

scores have recently been rated as a promising candidate measure “ready for consideration” to identify individuals at heightened risk in the context of adversity (Shonkoff et al., 2022). Nevertheless, the application of polygenetic risk scores comes along with several limitations, including complex bioinformatical processing and potentially limited acceptability by parents (Shonkoff et al., 2022; Wray et al., 2021). Hence, more feasible markers of vulnerability are needed that can easily be implemented in clinical practice.

A potential vulnerability factor easy to assess via questionnaire is the temperamental dimension of effortful control, which we studied in Chapter 6. Former research has demonstrated that parenting stress may affect children’s HPA axis functioning (Saridjan et al., 2010), and that children low in effortful control are more likely to develop behavior problems in the context of adversity than children high in effortful control (Lengua et al., 2008; M. J. Thompson et al., 2020). We aimed to extend these findings in two ways: First, by investigating whether effortful control would already render a child more or less vulnerable to the effects of parenting stress in toddlerhood. Second, by focusing on diurnal cortisol levels as a psychobiological outcome that may precede behavior problems. In contrast to our expectations, parenting stress was not associated with toddler’s morning or evening cortisol levels, and toddler’s effortful control did not moderate these hypothesized associations.

In the discussion of Chapter 6, we brought forward different interpretations of our findings: On the one hand, parenting stress may have not constituted significant adversity for the child, which would imply that there was no reason for psychobiological adaptation. On the other hand, parenting stress may have constituted adversity for the child, which was not revealed in toddlers’ diurnal cortisol levels but might have been observable in other outcomes not reported in the study (e.g., cortisol stress reactivity, problem behavior). It is also possible that our diurnal cortisol measures were not reliable enough to reveal adversity-related alterations (further discussed under Secondary aims). Another interpretation of our findings could be that individual differences in effortful control at 15 months of age were not yet meaningful to influence child outcomes, as effortful control skills were just about to develop. In the future, we could extend the research of Chapter 6 by repeatedly assessing parenting stress along with toddlers’ effortful control, cortisol levels and problem behavior, spanning entire toddlerhood (age 12–36 months). Longitudinal associations could then reveal at what age an assessment of effortful control may be useful in pediatric practice to identify those children who are at

heightened risk to develop psychobiological alterations and problem behavior in the context of adversity.

An interesting outcome of Chapter 6 was that higher levels of parenting stress were related to lower levels of toddlers' effortful control, which resonates with previous reports by studies in infants and toddlers (Gartstein et al., 2013; Oddi et al., 2013; Khalsa et al., 2022). As we measured effortful control by questionnaire, this raises the question whether ratings of toddlers' effortful control were biased by parental experiences of parenting stress. Behavioral measures of effortful control may have served to address this question, which we actually had incorporated in the study design. However, due to restrictions related to the Covid-19 pandemic, we were only able to collect behavioral effortful control data from a subsample of 13 participants, which was too small to be included in the analyses of Chapter 6. As behavioral measures of self-regulation come along with high within-person variability (Enkavi et al., 2019), a more comprehensive picture on the relation between parenting stress and effortful control may be gained by adding measures of kindergarten teachers' reports on toddlers' effortful control to accommodate the limitations of parental reports and behavioral paradigms. However, for clinical practice, parental reports are probably the easiest to implement. Given that former studies have demonstrated a moderating role of children's effortful control in the association between adversity and behavior problems by using behavioral measures of effortful control (Lengua et al., 2008; M. J. Thompson et al., 2020), it remains to be elucidated if and at what time in development parental reports of effortful control could serve as a measure of risk.

### **Section 4: Addressing secondary aims of this thesis**

#### ***Secondary aim 1: Developing recommendations on cortisol sampling protocols for early childhood studies***

In the study of Chapter 5, we investigated what times of day would be most reliable in revealing between-individual differences in young children's cortisol levels. To this end, we instructed parents to take ten saliva samples from their toddler over two days, yielding five samples per day. Comparing intra-class correlation coefficients of each sampling time, we found that saliva samples taken by parents in the morning between 30 and 80 min after wake-up and bedtime samples were more reliable in revealing between-individual differences in toddlers' basal cortisol levels as compared to samples taken within the first 30 min after wake-up, in the noon or in the afternoon. Hence, we decided on the more reliable morning and evening

samples for the intervention study of Chapter 6. However, while reliability of morning cortisol levels was good in our methodological study of Chapter 5, it was poor in our study of Chapter 6. We can only speculate that parental non-adherence to the sampling protocol may have led to low reliability, as there was considerable variability in the sampling times within families across both collection days. In fact, it is difficult to compare reliability findings across studies, given that the focus of the methodological study was solely on cortisol as compared to several assessments conducted in the study of Chapter 6. Hence, parents in the methodological study may have paid more attention to the timing of saliva sampling. Together, our findings demonstrate that more than two samples are needed to achieve adequate reliability in the measurement of basal cortisol levels. The exact number of sampling days needs to be determined in future studies based on larger samples representative of the general population (for study design recommendations, see Segerstrom et al., 2014, 2017). Nevertheless, our methodological study made a valuable first step in demonstrating which times of day are more suitable to measure young children's cortisol levels, if researchers are restricted to a few samples only.

***Secondary aim 2: Providing an overarching summary of the development and socialization of self-regulation***

As outlined in previous chapters, effective self-regulatory abilities may protect an individual from developing negative mental health outcomes in the context of childhood adversity. To address one of the secondary aims of this thesis, Chapter 7 presents a meta-review on the development and socialization of self-regulation from infancy to adolescence. Based on narrative reviews, systematic reviews and meta-analyses, we disentangled developmental and socialization processes of self-regulatory capacities from those of goals and motivation that underlie the willingness to self-regulate. We found that the development in complex capacities (e.g., planning) is preceded and paralleled by development in simpler capacities (e.g., working memory). Over time, coordination between these capacities improves, and goals and motivation shift from exploring the immediate environment to thriving in multiple domains. Focusing on socialization processes, we further observed that parents, peers, and teachers all have a major impact on the development of self-regulation, with self-regulation transitioning from being a co-regulated process to an increasingly internally regulated process (see e.g., Eisenberg & Sulik, 2012). While important processes in the socialization of self-regulatory capacities are co-regulation and modelling (e.g., A. L. Miller et al., 2020), the socialization of goals and motivation is influenced by visible parental aspirations and support (e.g., Massey et al., 2008), positive teacher-student relationships (e.g.,

Garner, 2010), and peer norms (e.g., Massey et al., 2008). Given these findings, we emphasized that self-regulation development should be studied in light of the social environment with specific attention to the distinction between self-regulatory capacities, goals and motivation.

The findings of Chapter 7 bear important methodological implications by demonstrating that what is considered as adaptive self-regulation is relative to the broader context. A toddler who is growing up under conditions of adversity may be less motivated to delay gratification if asked to do so, as the toddler's former experiences suggest reduced likelihood of a future payoff when waiting (Sturge-Apple et al., 2016). If this toddler does not resist the immediate temptation of a reward in an experimental task, would that imply that this toddler has low self-regulatory capacities? The answer based on our review is a clear 'no, not necessarily'. Further work is required to develop experimental paradigms that enable valid assessment of children's self-regulatory capacities by taking the child's goals into consideration (see Chapter 7), particularly in the context of childhood adversity.

Several studies have shown that a lack of self-regulation sets a child at heightened risk to develop negative mental health outcomes, in both adverse and non-adverse contexts (Chapter 2; Hamby et al., 2017; Moffitt et al., 2011). Hence, fostering the effective development of self-regulatory abilities may place children in a better position to cope with future adversities. Our finding of Chapter 7 that parents, teachers and peers all have considerable influences on the development of self-regulation offers several targets for intervention. An effective development of self-regulatory capacities can be fostered for instance by promoting sensitive and responsive parenting (Chapter 2), the teaching of self-regulation skills in the school context, and the establishment of pro-social norms influencing peer behavior (see Chapter 7, but also Morawska et al., 2019; Pahigiannis & Glos, 2020; Schunk, 2005).

### **Strengths and limitations**

The present thesis yields several contributions to the field of childhood adversity research by combining qualitative and quantitative review work with empirical studies. First, we summarized and integrated knowledge on adversity-related alterations in diurnal HPA axis functioning by focusing on early childhood. We thereby extended former review work on adversity-related cortisol stress reactivity in early childhood (Hunter et al., 2011). Further, we expanded meta-analytic work on the association between child maltreatment and resting vagal activity by broadening the scope to multiple adversity types and by adding vagal



reactivity to challenge as an outcome. In addition, we widened knowledge on the role of individual factors related to genetic constitution and temperament in influencing psychobiological adaptation in empirical studies. Beyond that, we made a valuable first step in filling the gap of methodological research on cortisol sampling protocols for early childhood studies. Finally, we provided an overarching summary of the review literature on the development and socialization of self-regulation—a field that has up to now received so much attention that a meta-review appeared necessary and timely to provide a broader but condensed picture of the field.

Nevertheless, the research presented in this doctoral thesis has limitations. First, while we studied the functioning of the HPA axis and the ANS in the context of childhood adversity, we did so in separate studies. Although the study of Chapter 6 was conceptualized to include heart rate data from toddlers, we only reported on cortisol as an outcome, as heart rate data was missing for the first 18 out of 31 children who needed to be tested online due to governmental restrictions released during the Covid-19 pandemic. Given that the HPA axis and the ANS are physically interconnected and highly coordinated in responding to a stressful environment (Ulrich-Lai & Herman, 2009), investigating the interrelation between both biological stress systems may have provided a deeper understanding of the biological embedding of childhood adversity. This would have further allowed to determine whether alterations in the functioning of both stress systems are correlated, or whether one stress system is more likely than the other to become altered in the context of childhood adversity. On a similar note, we did not combine measures of stress system functioning with neuroimaging data (as available in Chapter 4), which would have enabled insights in the relation between central and peripheral effects of adversity.

Second, while we adopted a broad view on childhood adversity in our review works of Chapters 2 and 3, we investigated very different adversity types across empirical studies, focusing on child abuse in Chapter 4 and on parenting stress as a proxy for childhood adversity in Chapter 6. In addition, we studied the links of these different adversity types to different aspects of psychobiological adaptation in different developmental periods, impeding the comparison and integration of our empirical findings.

Third, despite the fact that we framed psychobiological alterations as mechanisms in the adversity-psychopathology link (see Chapter 2, but also Raymond et al., 2018), we did not include measures of psychopathology in our studies. With regard to our empirical studies, it would have been interesting to test

whether toddlers with low levels of effortful control were more likely to develop internalizing or externalizing behaviors in the context of parenting stress (Chapter 6), and whether young adult TT allele carriers with a history of abuse experienced increased symptoms of psychopathology (Chapter 4). With regard to our meta-analysis (Chapter 3), we could have applied meta-analytic structural equation modeling to identify whether alterations in vagal functioning mediate the association between childhood adversity and psychopathology (see e.g., Miu et al., 2022). This is an important avenue for future work, which may profit from our findings of Chapter 3 by focusing on those circumstances that were identified as more likely to lead to alterations in vagal functioning in the context of adversity.

Fourth, the empirical work in this dissertation was cross-sectional. By measuring exposure, potential mediator, and outcome concurrently, it becomes impossible to determine whether adversity has preceded or followed altered psychobiological functioning, and whether altered psychobiological functioning has preceded or followed psychopathology. As recommended by Preacher (2015), there should be at least three time points at which independent, mediating, and dependent variables are all measured, which will allow researchers to control for a priori variance and draw conclusions on the temporal sequence of observed effects.

Fifth, although research suggests that multiple rather than single factors influence psychobiological adaptation in the context of adversity (Chapter 3; see also Hales et al., 2022; J. Martini et al., 2022; Yule et al., 2019), we investigated several moderating factors separately. To determine whether different moderating factors have compensatory, mutually reinforcing or overlapping effects, it is relevant for future studies to investigate them in conjunction. However, this comes along with power issues, which can perhaps most effectively be addressed by establishing collaborations and making research data publicly available.

Finally, while we demonstrated in several chapters of this thesis that exposure to adversity can result in a change in psychobiological functioning, several questions remain unanswered. So far, we cannot precisely predict under which conditions adversity-related psychobiological alterations will emerge. With regard to HPA axis functioning, we are further not able to predict the direction of change that we will likely observe. While we know that a transition from HPA axis hyper- to hypoactivity can take place at some point, we don't know at what exact point the threshold is reached at which the HPA axis downregulates. We also have limited understanding of the mechanisms of how adversity becomes biologically embedded,

with epigenetic studies increasingly addressing this topic (Bush et al., 2018; Vaiserman & Koliada, 2017).

## **Conclusion**

The present dissertation summarizes a broad literature on alterations in HPA axis and vagal functioning that have been observed in the context of adversity. Our findings indicate that both heightened and lowered cortisol levels can be present in adversity-exposed children younger than age 5. While different adversity types or dimensions such as abuse and threat cannot clearly be linked to a pattern of HPA axis alteration in early childhood, we found severe adversity to more likely lead to lowered cortisol levels. Our results further demonstrate that the vagal system generally maintains its functional integrity in the broad context of adversity. However, exposure to severe adversity may lead to reductions in vagal activity, and also reductions in vagal reactivity to challenge may arise over time. Overall, our findings point to the complexity of predicting psychobiological adaptation, which is not only influenced by adversity characteristics, but also by individual characteristics such as genetic constitution. To illustrate, we demonstrated in this thesis that the TT allele of rs1360780 of the FKBP5 gene can render an individual more likely to develop alterations in the communication between brain regions of the salience network, which could underlie or increase the risk for psychopathology. In future clinical practice, the joint assessment of stress biomarkers such as cortisol and HRV may become routine to identify those individuals who are most in need of support. This is of particular relevance in young children who cannot self-report on their stress experiences. Yet more research is needed to identify clinically relevant cut-offs, and to develop guidelines for the implementation of interventions following the identification of children “at risk” for maladaptive health trajectories. While this thesis built upon previous work to broaden knowledge in the field of adversity research, there is still a great deal to learn. More longitudinal studies that begin their first assessment(s) in early childhood, incorporate multiple measures of stress system functioning, and consider multiple protective factors are now needed to trace diverse pathways from childhood adversity to psychopathology.