The effects of early life adversity on hypothalamic-pituitary-adrenal axis, sympathetic, and parasympathetic responses to the Montreal Imaging Stress Task

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Abstract

Early life adversity (ELA) has been shown to be associated with emergences of psycho- and other pathologies later in life. These physiological and psychological effects are hypothesized to be mediated by changes in stress physiology, namely the hypothalamic-pituitary-adrenal (HPA) axis, and the autonomic nervous system (ANS). Numerous studies have shown effects of ELA on HPA-axis response. However, findings have been mixed with some researchers reporting potentiating effects of ELA on HPA-reactivity, while others have found blunting effects of ELA. Importantly, the majority of studies so far have focused on HPA-reactivity, neglecting to incorporate measures of the independent branches of the ANS. This thesis hypothesized that inter-individual differences in the relationship between HPA-reactivity and ELA could be explained via a moderating effect of ANS reactivity. The thesis incorporated heart rate variability (HRV), specifically respiratory sinus arrhythmia (RSA) — an index of parasympathetic nervous system (PSNS) activity — as well as salivary α-amylase (sAA) — a marker of the ANS but with prominent sympathetic nervous system (SNS) components — to obtain a more thorough and complete picture of stress reactivity. Results found no effects of ELA on stress reactivity in the PSNS and HPA-axis. However, when controlling for the PSNS we found a difference in reactivity of sAA between those high in ELA and those low in ELA, providing initial evidence of observable effects of ELA on the SNS in sAA measures.

Résumé

L'exposition à un milieu défavorable au début du développement (early life adversity, ELA) est associé avec l'apparition des pathologies psychologique ainsi que d'autres formes de pathologie plus tard dans la vie. Une hypothèse indique que ces effets physiologique et psychologique sont médiatisés par des changements dans les structures physiologiques de stress, particulièrement dans l'axe hypothalamo-hypophyso-surrénalien (HHS) et le système

nerveux autonome (SNA). Plusieurs études ont démontrés un effet de l'ELA sur la réponse de l'axe HHS. Par contre, les résultats de ces études sont variable; quelques études ont reporté un effet multiplicateur de l'ELA sur la réaction de l'axe HHS, tandis que d'autres on trouvés un effet émoussant. La majorité des études au sujet de l'ELA ont eu un focus sur la réaction de l'axe HHS, sans inclure des mesures des deux branches du SNA. Cette thèse propose que les différences entre-individus dans la relation de l'ELA et la réaction de l'axe HHS peuvent être expliqués par l'effet modérateur de la réaction du SNA. Nous avons incorporé une mesure de variabilité du rythme cardiaque, l'arythmie des sinus respiratoires (ASR) — une indexe de l'activité du système nerveux parasympathique (SNPS) — ainsi qu'une mesure d'alpha-amylase salivaire (AAS) – un marqueur de l'activité du SNA, plus précisément l'activité du système nerveux sympathique (SNS) — pour obtenir une compréhension complète de la réactivité au stress. Nos résultats ne montrent aucun effet de l'ELA sur le SNPS ni l'axe HHS. Cependant, en contrôlant pour la variabilité du SNPS, nous avons trouvé une différence dans la réaction de l'AAS entre ceux qui démontrent plus et moins d'ELA. Cela fournis une preuve initiale des effets observables de l'ELA dans le SNS, particulièrement dans les mesures de AAS.

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1 Background & Review

1.1 Stress

Biological conceptions of stress are relatively recent in the history of science with the consensus view being that work by Hans Seyle in the 1930s — into what he termed the Generalized Adaptation Syndrome — marked the first investigations into what we now understand as stress (*c.f.* Jackson for an historical analysis[1]). Since then researchers have come to broadly define stress as the state in which there is a perceived or actual threat that challenges the organism's homeostatic and metabolic balance[2, 3]. In responding to stress the organism aims to redirect resources and energy from long-term goals (*e.g.* acquiring food, reproductive success) towards responding to the immediate threat of the stressors, a process referred to as allostasis in the literature[4]. Stressors, the perceived or actual threats evoking the stress, can be purely physiological, psychological, or, more commonly, a combination of both. Importantly, both physiological and psychological stressors evoke the same physiological response from the body's stress systems[5], and largely activate the same regions of the brain[6, 7] thus providing evidence of the unified nature of the stress systems and response.

1.1.1 Parasympathetic nervous system

The PSNS is the first system to respond to a stressor. The primary efferents of the PSNS important to the stress response originate in the nucleus ambiguus (NA) and the dorsal motor nucleus (DMN) of the X^{th} cranial nerve, also referred to as the vagus nerve. Projections from the nuclei synapse directly onto end organs such as the heart and lungs. At rest these inhibitory descending fibers are highly active and lead to a lower resting heart rate, slower breathing rate, and increased blood flow to the digestive system. In the literature, this tonic activity is referred to as the "vagal brake"[8], particularly with respect to its ability to slow the activity of the heart. Upon encountering a stressor the prelimbic cortex and PVN inhibit

the NA and DMN, respectively, removing the slowing, tonic activity these systems provide. This provides a near instantaneous response — on the order of 1 second[9] — allowing for an immediate increase in heart rate and breathing rate, and removing the vagal brake that normally inhibits the SNS from activating (see Jänig for more detail[10]).

1.1.2 Sympathetic nervous system

The SNS is activated several seconds after the PSNS has withdrawn. Compared to the more distributed control systems of the PSNS, the SNS is anatomically highly localized. All efferents of the SNS originate in the preganglionic neurons of the intermediolateral (IML) cell column in the spinal cord. Upon encountering a stressor descending inputs from the ventrolateral medulla (VLM), locus coeruleus (LC), and the PVN activate preganglionic cells found in the IML. The ganglia of the IML synpase onto the second set of ganglia located along the external length of the spinal cord. These intermediary ganglia send their efferents to various end organs including the heart, lungs, and sweat glands. The SNS acts at these sites through the release of catecholamines, primarily norepinephrine, and causes an increase in heart rate, breathing rate, dilation of the pupils, and the other physiological changes characteristic of the classic "fight-or-flight" response first described by Walter Cannon in 1927[11]. Additionally, post-ganglionic efferents are sent to the medulla of the adrenal glands where they stimulate the release of more catecholamines — primarily epinephrine though also some norepinephrine — into the bloodstream, further stimulating an increase in metabolism (see Jänig for more detail[10]). Additional release of catecholamines occurs in the CNS[12] which play an important role in modulating behavioural responses to stress, such as anxiety responses in rats[13]. However, it is important to note that these catecholamines have their origin in the CNS itself. The peripheral catecholamines released by the SNS are unable to cross the blood brain barrier.

Upstream of the separate brain stem centres controlling the PSNS and SNS, the two branches of the ANS share common regulatory centres in higher order regions in the frontal lobes[14]. Lesion studies in humans have demonstrated that damage to the frontal lobes prevents patients from producing adequate ANS responses to stimuli[15]. This reinforces that though the PSNS and SNS are anatomically distinct, functionally they share important regulatory areas that facilitate a coordinated response to stress.

1.1.3 Hypothalamic-pituitary-adrenal axis

The HPA-axis plays a critical role in mounting a slower, more sustained response to stressors. The endocrinological cascade begins in the paraventricular nucleus (PVN) of the hypothalamus which secretes corticotropin releasing hormone (CRH) into the anterior pituitary. This stimulates the release of adrenocorticotropic hormone (ACTH) by the anterior pituitary to circulate through the bloodstream. Upon reaching the cortex of the adrenal glands ACTH causes the release of glucocorticoids (GC) into the body — cortisol in humans and some primates, corticosterone in most other mammals — which acts as the final hormone in the cascade. GCs work to mobilize energy reserves in the body increasing general metabolism and promoting glyconeogenesis. GCs are integral in regulating their own production feeding back onto the adrenal cortex, anterior pituitary, and PVN acting on GC receptors (GRs) in these regions in order to suppress their output. GCs also bind to GRs in higher order cortical areas further regulating their own response and suppressing the ANS stress response[16]. The entire cascade takes approximately 15 to 25 minutes to peak, and remains at elevated activity levels for around 30 minutes before returning to baseline.

1.2 Early life adversity

ELA, alternatively referred to in the literature as early life stress, early life trauma, or adverse childhood experiences, is(are) any highly stressful experience(s) that occurs early in development — either pre-, peri-, or early postnatally. Ambroise Tardieu is oft credited with conducting the first scientific work into the negative effects of ELA in 1860 (*c.f.* Knight for a brief history[17]) albiet with a focus on the immediate harmful effects of abuse rather than long term outcomes. It was not until Sigmund Freud and Josef Breuer that the hypothesis that experiences early in life can have long term effects on physical and mental health was first posed[18]. Future advances in our understanding of the neurobiology of the developing brain and the multitude of plastic changes it undergoes during development lent credence to the hypothesis that early life was a period of heightened susceptibility to stress-related pathologies[19, 20]. Decades of research have produced a large body of evidence consistently demonstrating links between ELA and psychopathology[21–25], overall worsened physical health[23, 24, 26–29], and poorer performance in areas of cognitive performance and affective regulation[30]. Research into the negative effects of ELA is highly relevant as prevalence rates reported in one study range from 11 percent (sexual abuse) to 45 percent (emotional neglect) amongst males and 14 percent (physical neglect) to 37 percent (emotional abuse and neglect) amongst females[31]. Though prevalence varies slightly across studies, these rates are comparable to those reported in another study[32].

Typical experiences considered to be indicative of ELA include, but are not limited to, emotional, physical, and sexual abuse[33, 34], parental bereavement[35], being raised in an orphanage[36], or poor quality maternal care[37]. As there exists a large diversity in experiences that constitute ELA, researchers have many different questionnaires to measure levels of ELA. The most common of these are the Childhood Trauma Questionnaire (CTQ)[38], the Parental Bonding Instrument (PBI)[39], and the Early Trauma Inventory (ETI)[40]. The CTQ and PBI were used for this thesis and are discussed in more detail below (see section

3.3).

1.3 Adaptive calibration model

The Adaptive Calibration Model (ACM)[41] of stress responsivity is a theoretical evolutionary model put forth to explain individual variation in stress reactivity across the stress systems. More specifically, the model provides an explanation for how different early life environments and conditions alter the endophenotype of the stress response in an adaptive manner (see Figure 1). Following from life history theory[42] — on which the ACM heavily relies — the ACM postulates that the developmental environment an organism is reared in encodes relevant information about the likely future environment the organism will attempt to reproduce in. This information is encoded by and into the stress systems, which undergo long-term, adaptive changes in order to maximize the fitness of the organism for the predicted environment. Under this model changes in stress reactivity observed in ELA are changes in the physiology of the organism that have been coordinated in order to maximize reproductive success later in life. Importantly, the ACM makes specific predictions on how these adaptive changes manifest in the baseline and reactivity levels of each of the distinct branches of the stress system. This provides a framework that lends itself well to making specific predictions on how increasing levels of ELA will effect patterns of stress reactivity.

It is important to note that the ACM is just one of several theories postulating how stress responsivity in late life is molded by early life. An alternative theory that predominates in the field of stress research is that of allostatic load[43]. Under stress, the organism is said to be under increased allostatic load which puts increased strain on the systems. An increased allostatic load over an extended period of time is harmful and leads to the stress related pathologies seen in ELA. In the theory of allostatic load, the authors conceptualize allostasis as a see-saw. At rest, the see-saw is level. Under increased allostatic load there are weights on

either end of the see-saw. The see-saw itself is still level, but is subject to wear-and-tear that is not present in the weightless case[43]. In the theory of allostatic load the stress response is advantageous in the short-term, but harmful over the long-term if repeatedly activated. Changes that occur in stress physiology reflect the breakdown of the stress machinery. From this perspective any ELA related changes in stress reactivity are necessarily dysfunctional and maladaptive.

The ACM, on the other hand, provides a more parsimonious interpretation of changes in stress physiology. As explicated above, changes in stress physiology due to ELA are not viewed as maladaptive wear-and-tear. Rather they are adaptations to the early environment meant to maximize reproductive fitness later in life. Under this framework the stress systems can be seen as engaging in a crude form of learning. They take in data on the early environment and adjust their physiology to accommodate for a future environment projected to bear similar characteristics. The difference in how the two theories understand changes in stress physiology is being emphasized as it provides a justification for preferring the ACM over the theory of allostatic load. The theory of allostatic load explains how negative health outcomes are caused by ELA, but cannot explain why there exist disparities in the research in how ELA effects stress physiology (see section 1.4). The ACM, however, provides a framework for exactly this. It predicts that the relationship between ELA and the stress physiology of each specific system changes contingent on differences in ELA. These changes occur because different profiles of stress baseline activity and reactivity are advantageous in different environments. The ACM goes further in answering the "why" of changes in the stress systems in response to ELA and, furthermore, provides a tool for making specific predictions for how each arm of the stress system will adapt and change.

1.4 ELA & Stress Reactivity

1.4.1 ELA & the HPA axis

Early research in rat pups provided the first direct evidence of early life experiences causing alterations in HPA-reactivity in later life. To model early life stress in rat pups researchers used a handling model involving periods in early post-natal days wherein rat pups would be handled by researchers for periods of 15 minutes. Both the handling and the time spent separated from their mother are known to be stressful for the pups. Studies using this model consistently found blunting of HPA-axis reactivity (corticosterone response in the case of rats) later in life in handled pups compared to non-handled controls[44–46]. Expanding on the handling paradigm other researchers used a model of ELA termed maternal separation (MS). MS involves a similar intervention by the researcher in handling the pups, but handling periods last for at least 1 hour rather than 15 minutes. Use of these models resulted in a hyper-responsive corticosterone response later in life[47]. These studies demonstrated the highly plastic nature of the HPA-axis in early life, as well as highlighting the adaptive nature of the response (*i.e.* it can become hypo- or hyper-responsive).

For several decades now researchers have been looking to replicate the rat findings described above in humans. The plurality of studies, following from the rat models emphasizing the importance of maternal care, use the quality of early maternal care as a measure of ELA[37, 48–52]. Additional components of early life such as parental interactions[53, 54], parental bereavement[35], being raised in foster care[36], and experiencing abuse[33, 34, 55, 56] have also been used as measures of ELA. Similar to the rat studies above results have been mixed in all of these. Perhaps significantly, the majority of these studies found a blunting effect of increased ELA on HPA-reactivity[33–37, 49–54], though there are also many studies that report a potentiating effect[48, 55], and a few which report null results[57, 58].

It has been argued that looking at the HPA-axis in isolation is uninformative and misses the complete picture of ELA-related changes[59]. Additionally, both the highly inter-linked and coordinated nature of the stress systems (see section 2) and predictions stemming from the ACM support this idea. Though it is becoming increasingly common to look at multiple stress systems, many of the earlier studies in this field neglected to incorporate measures outside of the HPA-axis. Following this trend, I propose that the additional information contained in the other stress systems will be able to clarify the above ambiguities in the relationship between ELA and the HPA-axis.

1.4.2 ELA & the SNS

Research linking ELA with SNS reactivity is sparse, and studies specifically using sAA as a marker are even fewer in number. Looking at pre-ejection period (PEP), another, more pure marker of SNS activity[60], one study found a blunting effect of ELA on SNS reactivity to stress[36]. Another study similarly using PEP found a potentiating effect of ELA on SNS reactivity at moderate levels, a relationship which switched to a blunting at higher levels of ELA[61]. Replicating this finding, but using electrodermal response as a measure of SNS activity, a third study found that moderate levels of ELA potentiated SNS reactivity while high levels blunted it[62]. These findings, though far fewer in number, are far more consistent than those on HPA-reactivity. They suggest an inverted-u relationship between SNS reactivity and ELA, findings that are consistent with predictions of the ACM.

Extending the SNS literature to include sAA as a stress marker yields less convincing results. One study by Ali and colleagues found no difference in sAA reactivity to stress between high and low ELA groups[63]. However, the same study did find a difference between high and low ELA groups in the sAA over cortisol ratio suggesting an important interaction effect between the two stress systems. A second study from a year later similarly found no

effect of ELA on sAA reactivity[64]. Unlike the previous study, however, the latter study failed to show a significant stress response in any physiological markers in relation to the employed stress paradigm. This would perhaps suggest that the employed stressor was insufficiently stressful for any existing differences in reactivity to be observable.

Taken together, the results from these studies suggest that there is an effect of ELA on SNS reactivity, but not one that is observable using sAA as a marker. Importantly, sAA is not considered a pure measure of SNS activity[65], though it correlates strongly with changes in sympathetically mediated release of catecholamines into the blood in response to stress[66]. This suggests that potential SNS mediated ELA differences in sAA may be confounded by other autonomic influences. This thesis will look to see if, by controlling for PSNS levels (*i.e.* looking at RSA measures as a mediator) the same differences as those observed in more pure measures of the SNS can be observed in sAA.

1.4.3 ELA & the PSNS

Much like the SNS, studies examining the effects of ELA on the PSNS have been far fewer in number, with results being mixed. One study found a blunting effect of ELA on RSA at baseline, though this result had disappeared at the two year follow-up measurement[67]. Another study similarly found a negative relationship between scales measuring abuse and RSA, but only in subjects with pre-existing psychopathology[68]. Along a similar line a study by McLaughlin and colleagues found that RSA interacted with childhood abuse to predict internalizing problems[69]. More specifically, high baseline RSA had a protective effect on the occurrence of internalizing problems associated with childhood abuse. However, a subsequent study by the same group found no effects of ELA — in this case growing up in foster care — and RSA reactivity to stress[36]. Finally, an earlier study by Del Giudice and colleagues found no effect of ELA on both RSA at baseline and RSA reactivity

to stress[62]. Overall the sparse research examining the relationship between ELA and the PSNS, not to mention the greater dearth of studies looking at PSNS reactivity, tend to show no effects of ELA on the PSNS. However, strong evidence exists showing that the PSNS, as measured through RSA, is strongly linked to pathologies found in increased incidence in ELA[70–73]. as well as the PSNS playing an important regulatory role in the HPA-axis response to stress[74]. Following this line of reasoning I expect that though there may be no effects of ELA on PSNS reactivity, the PSNS will play an important moderating role in the reactivity of the other two stress systems.

1.5 The Montreal Imaging Stress Task

The Montreal Imaging Stress Task (MIST) is a standardized psychosocial stress task designed for the scanner environment. Its design was inspired by the arithmetic portion of the Trier Social Stress Task (TSST)[75] in order to be able to study the effects of psychosocial stress in the brain. Similar to the TSST the MIST involves a component of psychosocial feedback paired with demanding mental arithmetic. The bulk of the MIST involves answering timed mental arithmetic questions on a screen using a rotary dial (see Figure 2 for an example screen of the experimental condition). Participants are presented with randomly generated math questions and are provided a limited time to answer correctly. The difficulty of the task is automatically adjusted according to each participant's performance in an attempt to keep performance levels at a rate of 40 - 45% correct. Difficulty of the task is adjusted by changing the time allotted to answer the questions as well as changing the frequency of presentation of more difficult questions. The difficulty is additionally modified by varying the upper limit of integers that can be present in a question. To add further stress, the MIST features intrusive auditory cues for the timer and when participants answer questions incorrectly.

Separate from the computer interface is the actual psychosocial feedback participants receive. Before beginning, participants are informed that performance on the task in their peers is usually around 85% correct. To reinforce this participants are instructed in the function of the performance bar (at the top of the image in Figure 2). This bar provides participants with the (fabricated) performance level of their peers in direct contrast to their poor performance. During the task, the bar indicating peer performance is high on the meter (in the green), whereas participants tend to be in the yellow - red regions of the bar. They are also instructed that it is critical for the experimental design that they maintain performance near that of their peers. After completing the first trial of the MIST participants are asked leading questions concerning their current mental acuity and competency in relevant areas. Following the initial trial participants are also under direct observation by an experimenter reinforcing the social-evaluative component.

Though the TSST is the standard psychosocial stressor used in the majority of stress studies — due to it being reliable and highly standardized — it was not used in this study due to some limiting factors. Chief amongst them is that the TSST introduces difficulties and confounds in measurements of HRV. As a measure HRV is sensitive to changes in posture, breathing, and even whether a subject is seated or standing[76]. All of these parameters change over the course of a standard TSST paradigm. For one, subjects are typically seated during baseline measurements preceding the TSST, but are necessarily standing during the actual procedure. This makes meaningful comparisons between measurements obtained during the TSST and those surrounding it difficult. Further complicating matters subjects are required to speak throughout the TSST. This typically introduces unnecessary motion artefacts into the EKG, as well as causing potentially significant and unpredictable changes in breathing patterns. Bearing these in mind the MIST was used in place of the TSST. The MIST has been shown to elicit moderate cortisol responses[77]. It is valuable in that subjects are seated throughout the task and are instructed to minimize speaking. Additionally,

effects of ELA on cortisol reactivity were recently demonstrated in the MIST[78].

For this thesis I developed a new version of the MIST written in JavaFX and FXML. The new version features an updated UI and algorithms that are more responsive to participants' performances. During early piloting of the study using the old version of the MIST it was noted that the longer durations employed in this study (without intermittent control conditions) would resulted in the task becoming too difficult near its conclusion. This lead to performance in the 20 - 30% correct range and frequently resulted in participants seeing through the deception. In other words participants did not believe that it was possible for anyone to perform at the 80% level and would report suspicions that we were lying to them. The new version of the MIST was developed to remedy this by behaving in a manner that made the stated average performance level of the task seem theoretically achievable.

2 Rationale & Objectives

The putative interdependence of the arms of the stress system is supported by evidence demonstrating that upstream all of the systems share a common regulatory centre. Regulatory centres appear to culminate in the medial prefrontal cortex (mPFC)[79, 80] which acts to coordinate the HPA, ANS, and behavioural responses to stress. Research in rats has refined the role of the mPFC illuminating the subregions involved. Prelimbic mPFC (PL) was found to be necessary to inhibit the HPA and ANS stress responses. Contrarily, infralimbic mPFC (IL) is essential in for initiating a suitable HPA and ANS response to stress. These regions roughly correspond to Brodmann areas 32 (PL) and 25 (IL) in humans[81] and have a high density of GRs[82]. As discussed above GRs play a critical role in the self-regulation — more precisely the negative-feedback — of the HPA-axis' stress response[83]. Long-term changes in HPA-axis reactivity found in rat pups in models of ELA map onto changes in GR density in the mPFC[84]; that is rats with blunting reactivity show an increase in GRs (relative to controls) while hyperreactive rats are found to have an accompanying down-regulation of GRs. Furthermore the very regions that are the site of changes in GR concentration are integral in regulation of the ANS stress reponse[14].

In spite of these demonstrated links few studies have incorporated measures of the autonomic nervous system in investigating the effects of ELA. Those that have tend to use crude measures (*e.g.* heart rate, blood pressure) that reflect a mix of SNS and PSNS activity. This is in spite of evidence reporting changes in SNS reactivity associated with ELA, and predictions about PSNS changes due to ELA. Futhermore, as all the stress systems share common regulatory regions in the mPFC it would be reasonable to assume that alterations in HPA-axis-reactivity to stress would not occur independently of the other stress systems. Supporting this are studies showing links between SNS and HPA-reactivity[63], as well as an important role of the PSNS in regulating diurnal cortisol[85, 86] and HPA-reactivity[74]. Additionally, research reporting extensively on the links between PSNS basal activity and

psychopathology[70–73] and the potential protective effect of RSA on the the psychopathological effects of ELA[69] suggests that there would be a link between ELA and the PSNS.

Stemming from the above considerations I wanted to test 4 main hypotheses for this thesis:

I The MIST will induce vagal withdrawal (*i.e.* a drop in RSA measures)

II There will be a main effect of ELA on cortisol reactivity

III There will be an effect of ELA on sAA reactivity when controlling for the PSNS (RSA)

IV Subjects can be differentiated into categories similar to those in the ACM based on physiological data (cortisol, sAA, RSA). These groups will be defined by differences in ELA measures.

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3 Materials & Methods

3.1 Participants

Participants were recruited via advertisements posted on online university and community classifieds. A total of 1,933 individuals responded to the ads. Respondents were then pre-screened for early life experiences based on cut-off scores for the maternal care (MC) subscale of the PBI[39] in order to establish two ELA groups; one of high MC and one of low MC. Participants that passed the exclusion criteria (see below) were then contacted via phone (127 potential participants) for a pre-screening interview in order to confirm their eligibility and, if applicable, schedule participation. A final total of 49 participants were tested in the lab.

All participants were male in order to control for sex effects on HPA-axis activity[87] and RSA[88–90]. Participants were pre-screened for existing physical or mental health conditions, as well as family history of mental illness. Additionally, participants were screened for factors known to affect HPA-axis function including alcohol consumption[85] (no greater than 20 drinks per week), smoking[91] (exclusion for smoking greater than 2 cigarettes per week), and drug use[92] (no recreational or prescription drugs) for the period 6 months preceding participation. To control for potential effects of age[89, 93] and BMI[94–96], all participants were between the ages of 18 - 30, and had BMI's of 17.5 - 26.5. Participants were pre-screened for depression using the Patient Health Question[97] (PHQ-9) and anxiety using the Generalized Anxiety Disorder[98] (GAD-7) scale. Those scoring greater than a 9 or 7, respectively, were excluded.

3.2 Experimental procedure

Participants were informed to refrain from consuming alcohol, nicotine, or other drugs 24 hours before participation. Participants were also asked to avoid exercise and caffeine consumption the day of testing. To control for circadian rhythms in cortisol all testing took place between 11h00 - 15h30.

Participants arrived and were given 30 minutes to habituate to the test settings (see Figure 3 for a timeline of the protocol). During habituation, participants answered questionnaires followed by colouring in an adult colouring book. After habituation, participants were given the first saliva sample. With the exception of the sample acquired after the first round of the MIST — taken 15 minutes after the preceding samples — all other samples were taken at 10 minutes intervals for a total of 9 samples. As the sampling procedure is still novel for the first sample, and participants are still habituating to the test settings during it, the first sample for all participants was discarded leaving the final number of samples at 8 per participant. Following a second baseline sample, participants were moved to the testing room and informed that they would be completing a challenging mental task. The MIST was explained to participants and they were given a practice round of 75 seconds of the control condition (questions presented in the absence of a time limit or performance feedback) to familiarize themselves with the interface and controls. After this, participants completed the first experimental phase of the MIST lasting 9 minutes. Upon completion, participants were given psycho-social feedback from the experimenter. The experimenter then left the room to go "talk" to an unseen supervisor. Following this, participants were asked more leading questions (*e.g.* "Have you had difficulty with arithmetic in the past?") and informed that they would be performing another round of the task to attempt to salvage results. Another 9-minute trial of the MIST was completed. After the second trial, participants received additional psycho-social feedback and were then asked to sit quietly and wait. After 10 minutes participants were moved from the testing room back to the habituation room where

they stayed for the remainder of the study (30 minutes). Upon concluding the procedure, participants were debriefed and compensated with 40$.

3.3 ELA measures

ELA was measured with two retrospective questionnaires: the PBI[39] and the short form of the CTQ[38]. The PBI is a 50 item questionnaire (25 for maternal and paternal rearing each) that probes for parental care and over-protection in the first 16 years of life. It asks subjects to rate on a 4 point Likert scale how similar or dissimilar their parents were to various statements. For example, "Spoke to me in a warm voice," or "Made me feel I wasn't wanted." The CTQ consists of 28 items asking about experiences of sexual, physical, and emotional abuse, and physical and emotional neglect in the first 18 years of life. Each subscale consists of 5 questions scored from 1 to 5, with 3 questions serving as validation measures. Additionally, participants completed the Mini-K, a substantially shorter form of the Arizona Life History Battery[99]. The Mini-K does not measure ELA per se, but rather life history strategy (*i.e.* how impulsive someone is, or how likely they are to engage in risky sexual behaviours). However, following from the ACM[41] life history should be a predictor of both ELA and the stress response. Thus I included the Mini-K as an exploratory measure.

3.4 Psychological assessment

Additional questionnaires being administered included an English language version of the Trier Inventory of Chronic Stress[100] (TICS). High levels of current stress have been show to affect RSA measures[101]. Therefore, the TICS was used to ensure that any effects of ELA on RSA measures were not contaminated by current chronic stress. Additional measures for depression (the Beck Depression Inventory - II[102, 103], [BDI-II]), and anxiety (the State and Trait Anxiety Inventory[104] [STAI]) were included. Effects of ELA on measures

of depression and anxiety have been found in sub-clinical samples[105] therefore these measures were included for later comparisons between MC groups. The Rosenberg Self-Esteem Scale[106] (RSES) was included as a measure of self-esteem as it has been found that self-esteem can affect cortisol reactivity[107, 108]. Finally, the State Self-Esteem Survey[109] (SSES) was included immediately before the MIST was administered and immediately after the second trial was completed to further validate the subjective stress effects of the MIST.

Subjective stress was measured using visual analog scales (VAS). A line was presented with each saliva sample asking participants, "How stressed do you feel at this moment?" Participants were instructed to place a mark on a line between two poles (left most — "Not at all stressed"; right most — "Extremely stressed") corresponding to how they felt in that moment. The VAS probing stress was presented alongside 3 other VASs probing fatigue, confidence, and relaxation in order to mask the main interest in stress. All scales were 10 centimetres in length and scored on an 100 point scale from 0.0 - 10.0 cm. In order to ensure that participants were answering the VAS attentively with each presentation, the order that the 4 scales were presented in varied with each sample.

3.5 Physiological markers

Free salivary cortisol was sampled before, during, and after the MIST. Samples were taken once every 10 minutes, using cotton salivettes (Sarstedt Inc., Québec City, Canada). Participants were instructed to keep salivettes in their mouth for 90 seconds as timed by the experimenter. They were told to refrain from chewing on the salivettes as well as avoid actively trying to produce saliva. After the experiment was completed, salivettes were stored at $-20\,^{\circ}\mathrm{C}$ until analysis via time-resolved immunofluorescence assay[110]. The inter- and intra-assay variability are typically around 10% and 12%, respectively.

sAA was analysed using the same salivettes as cortisol. This was done using an enzyme kinetic method with a manual imprecision of 3.2%[111]. Both cortisol and sAA analyses were performed externally at the University of Trier, Germany.

RSA, the changes in heart rate tied to respiration, is an index of vagal, and hence PSNS, activity[112]. These changes occur in the duration of the inter-beat interval in heart beat. To extract this information, EKG was recorded at 256 Hz using an athletic undershirt called a Hexoskin (Carré Technologies, Montréal, QC). The EKG was then run through software developed in-house by Ellen Zakreski, another graduate student in the laboratory that extracts the R-peaks (the standard point counted as a heart beat) and creates RR-interval (RRI) series of heart period. The RRI series were then manually inspected for artefacts and errors, and corrected for arrhythmias according to Berntson and colleagues and the HRV Task Force[112, 113]. From the edited RRI series, 2-minutes segments of clean data from the middle of each sampling period were used to derive the root mean square of successive differences (RMSSD), a measure of RSA. RMSSD was primarily used as it requires no resampling of the time series, which can introduce artefacts[114], is less susceptible to respiratory influences[115, 116], and is more stable over repeated measures than other methods[76]. For comparison with RMSSD other spectral measures of RSA were derived. To obtain spectral measures — specifically high frequency (HF) HRV — RRI series were resampled at 7 Hz using a cubic spline interpolation. This was done in order to minimize resampling artefacts[114, 117]. A Lomb-Scargle (LS) periodogram[114] was also used as a measure of HF-HRV. The LS method, unlike the more traditional Fourier and auto-regressive (AR) methods, requires no resampling.

3.6 Statistical methods

Data were analyzed in a combination of SPSS 23 (IBM Corporation, Armonk, NY), Matlab R2016b (Mathworks, Natick, MA) and R (3.2.2). The α level was set to 0.05 for significance for all tests unless otherwise specified (*i.e.* when correcting for multiple comparisons).

Group comparisons were performed between the high and low MC groups for all outcome measures using two-tailed, independent samples t-tests (corrected for multiple comparisons using the Bonferroni-Holm correction).

Data were checked for the assumption of normality using the Anderson-Darling test. Where data violated this assumption tests were repeated using non-parametric methods (Mann-Whitney U test).

To confirm that stress measures exhibited significant responses to the procedure linear mixed models (LMEs) were used. LMEs allow for modelling the specific shape of the response curve (in this case parabolic/quadratic) and are better suited to analyses where variance in terms is correlated as in a repeated measures design. Furthermore, LMEs allow for missing pieces of data. In constructing the models time^2 was the fixed effect (with an accompanying linear term) and the intercepts were allowed to vary randomly for each participant. Models were estimated using maximum likelihood, and the model residuals were tested for normality using the Anderson-Darling test. Where residuals significantly differed from normality, data were transformed using the inverse hyperbolic sine function and re-run in the model. β-coefficients were tested to differ from zero using t-tests.

Following these validations a MANOVA was performed comparing baseline and reactivity measures of stress, with MC group as the factor, based on the Wilks' Λ score. Reactivity to stress was quantified using the area under the curve with respect to increase (AUC_i) derived

using the trapezoid method[118].

Principle component analysis (PCA) was performed using MC and the five subscales of the CTQ in order to extract a measure of ELA that most strongly represented the variance in the sample. Subscales were centered around their cut-off values and normalized before PCA was performed using built-in Matlab algorithms.

K-means clustering was performed on physiological data using built-in Matlab algorithms. Data were clustered into two, three and four groups. Grouping validity was visually determined by inspecting silhouette plots. Differences in ELA measures were tested in the resulting groups using a MANOVA with assigned group as the factor.

4 Results

4.1 Group differences

Independent, two-tailed t-tests were performed on all outcome measures between the high and low MC groups (see Table 1). Significant differences were found between scores on the Mini-K ($t(38) = 5.03$, $p = 0.000$), total CTQ scores ($t(38) = -5.07$, $p = 0.000$), father care ($t(38) = 3.230$, $p = 0.003$), and mother over-protection ($t(38) = -3.19$, $p = 0.003$). All other tests were not significant.

4.2 Psychological response

A LME regressing time^2 onto VAS scores found a significant effect of time^2 ($t(317) = -9.28$, $p = 0.000$) and time ($t(317) = 7.74$, $p = 0.000$) (see Figure 4). A Mann-Whitney-U test between SSES scores pre-MIST compared to post-MIST found a significant drop in scores across the treatment ($z = 2.28$, $p = 0.0112$) (see Figure 5).

4.3 Physiological response

Four separate measures of RSA were derived from physiological data (see Figure 7 for comparison). Table 2 shows the cross-correlation of these four signals. The coefficients indicate that the measures were highly similar and therefore all following analyses of RSA used only the RMSSD.

A LME with heart rate as the response variable found a significant effect of time^2 ($t(317) = -4.61$, $p = 0.000$) and time ($t(317) = 1.99$, $p = 0.048$) (see Figure 6). The LME of RSA found no significant effects of time^2 ($t(317) = 1.02$, $p = 0.307$) or time ($t(317) = 0.555$, $p = 0.579$). Similarly, the LME of cortisol found no significant effects of time^2 ($t(312) = -0.762$,

$p = 0.447$) or time ($t(312)$ = -1.10, $p = 0.271$). The LME of sAA found significant results of time^2 ($t(312)$ = -5.69, $p = 0.000$) and time ($t(312) = 5.36$, $p = 0.000$).

4.3.1 MC

To analyze possible differences in stress physiology and psychology between MC groups a MANOVA was performed using baseline measures of cortisol, sAA, and RSA as well as AUC_i scores for cortisol, sAA, RSA, and VAS, and the difference in pre- and post-SSES scores as dependent variables. Wilks' Λ test found no significant differences between MC groups ($F(8,31) = 0.823$, $p = 0.589$, $\Lambda = 0.825$) (see Table 3 for between subject comparisons).

Following the rationale of Ali and Pruessner[63] subsequent tests were conducted on the ratio of AUC_is between the stress systems as well as the ratio of their baseline values. Wilks' Λ test found significant multivariate differences ($F(4,35) = 3.06$, $p = 0.029$, $\Lambda = 0.741$). An examination of the univariate comparisons revealed only a significant result in the ratio of AUC_i sAA to AUC_i RSA ($F(1,38) = 11.3$, $p = 0.002$, partial-$\eta^2 = 0.229$) (see Table 4).

4.3.2 ACM clustering

A composite ELA score was derived using PCA. MC and the five subscales of the CTQ were used as the six components. All values were normalized before PCA was performed. Of the resultant components, the first two components were used as they accounted for a combined 94% of the variance in the original data.

To group participants according to the ACM, k-means clustering was performed using the k-means algorithm in Matlab using the squared Euclidean distance for clustering, clusters

computed over a max of 100 iterations with 10 replicates. To explore several possibilities clustering was performed for k's of 2, 3, and 4. After clustering, the groupings into groups of 3 and 4 were discarded as both resulted in groups with sizes of 2, and 2 and 1, respectively. Such small groups sizes would make meaningful statistical comparisons impossible therefore only the results of clustering into 2 groups was used in subsequent analyses. Using this clustering as the factor a MANOVA was performed with measures of MC, total CTQ score, a summed score of CTQ and MC, the two ELA scores derived from PCA, and finally the Mini-K as dependent variables. Wilks' Λ test indicated there were no differences in groups ($F(5,33) = 0.592$, $p = 0.706$, $\Lambda = 0.918$) (see Table 5 for between subject comparisons).

5 Discussion

5.1 Summary of the findings

The main purpose of this study was to investigate the effects of ELA on stress reactivity across all of the stress systems in response to the MIST. More specifically, the goal was to see if differences in the three main stress systems reflected predictions made by the ACM.

Results on psychological measures confirm that subjects found the MIST subjectively stressful. Increases in self-reported stress occurred following exposure to the MIST in a well defined, curvilinear manner. Furthermore, subjects reported drops in self-esteem associated with the MIST. This similarly validates that the psychological effects of the MIST were effective.

In comparing the MC groups across variables known to affect the stress response (chronic stress, depression, anxiety, age, BMI, self-esteem) no significant differences were found alleviating concern that these factors could be confounding potential effects of ELA. Significant differences were found between the groups in CTQ scores, levels of self-reported father care and mother over-protection, and on the results of the Mini-K. These results are unsurprising, as scores on the CTQ tend to be correlated with the PBI[119]. The most interesting result was the difference in Mini-K scores. Subjects in the high MC group had significantly higher Mini-K scores ($\mu = 1.08$) compared to those low in MC ($\mu = 0.24$). This is the expected direction of the relationship as those having experienced more stressful early environments are theorized to have faster (lower Mini-K scores) life history strategies. This evidence lends support to the ACM.

Initial analyses would suggest that the MIST failed to elicit a strong, physiological stress response. There was a significant, curvilinear change in sAA in response to the task. How-

ever neither RSA, or, more importantly, cortisol displayed reactivity profiles consistent with having evoked a stress response.

As it is possible that group differences would wash out the stress response for the overall sample we continued analyses to see if either baseline or reactivity measures of the stress systems differed between MC groups (see Figures 8, 9, and 10 for visual comparisons of stress responses separated by MC). No difference in any of the measures was found. Following the work of Ali & Pruessner[63] we created ratio measures of stress reactivity between the stress systems. This is interpreted as providing a measure of the activity of a system when accounting for the activity of another. Using this model there was a significant difference between the ratio of reactive sAA over reactive RSA between the two groups. As sAA is not a pure measure of the SNS, controlling for PSNS activity using RSA could be interpreted as filtering out any PSNS elements in the sAA signal, leaving only the pure SNS aspects. Using this framework, this finding would suggest that we have observed a difference in SNS reactivity tied to ELA. As differences in other measures of the SNS have been found[36, 61, 62], this seems a plausible interpretation of the data.

Finally, an attempt was made to derive groups based on physiological data using k-means clustering. This was an attempt to see if data driven methods could reveal groups that differed across measures of ELA. In doing this we followed the theoretical predictions made by the ACM which describes 4 different endophenotypes. We attempted to cluster subjects into either 2, 3, or 4 groups using their baseline measures and measures of reactivity. Results for grouping with k = 3 and 4 were discarded as they produced groups with sizes of 1 and 2 subjects, sizes that would render meaningful analyses impossible. Therefore we proceeded using only two groups.

Figure 11 shows the silhouette plot of the grouping. A silhouette plot is a plot of silhou-

ette scores by grouping. Silhouette scores gives a measure of how similar a data point is to the cluster it was assigned to compared to the next nearest cluster. The range of possible scores is -1 to 1, inclusive. A score of 1 suggests a very strong fit of a data point into the group it was placed in. Conversely, a score of -1 suggests that a data point was erroneously placed into its assigned group. A score of 0 means that the data point is equidistant from its assigned group and the next nearest group, suggesting no preference for group placement. Examining of silhouette scores provides a means to verify that the clustering algorithm successfully identified unique groups. The silhouette plot shown in Figure 11 shows that the k-means clustering into two groups fit well (groups sizes of 12 and 27), with only one point being marginally below zero.

Figure 12 shows the results of separating the participants by their assigned group. The graphs support the interpretation that two different groups of responders have been found by the clustering. There appeared to be a group with higher cortisol output that exhibited a strong sAA response. The second group had lower overall cortisol and did not exhibit a sAA response to the MIST. There did not appear to be strong differences in RSA response. However, the hypothesis that these physiological categories reflected differences in ELA ala the ACM was not found. Tests showed that the two groups did not differ in any measures of ELA, including a composite measure constructing using PCA. Thus it would seem that k-means clustering simply found a group of responders and non-responders with no evident underlying theoretical significance.

5.2 Interpretation & hypotheses

The above results found, unlike the majority of other studies, that there were no effects of ELA on HPA-reactivity. A handful of other studies have similarly found no effect of ELA[57, 58]. However, this lack of a difference could be attributable to a lack of significant cortisol

response as even subsequent analyses conducted when participants were split by MC group did not report an effect. We were able to find a significant response in sAA and subjective stress. This would suggest that the MIST was not stressful enough to elicit a strong response. Though subjects reported the MIST as being subjectively stressful, Figure 4 shows the response peaking around 30 - 35 points. The VASs all have a maximum possible value of 100 and a value of 30 is not particularly high on the scale. Similarly, though there was a significant effect of the MIST on heart rate, this change was, on average, an increase of only 4 beats per minute (see Figure 6). THis lends credence to the interpretation that, though stressful, the MIST paradigm employed in this study was not highly stressful (see section 5.3).

The response observed in sAA corroborates this view. As sAA is under ANS control, it responds to rather moderate stressors (*e.g.* sitting in a sauna for an extended period[66]). Cortisol, on the other hand, requires either acute physical activity[120] or strong aspects of social evaluative threat and uncontrollability[5]. It is entirely possible that the procedure was stressful enough to elicit a sAA response, but not produce a cortisol response. Subjects were given only two rounds of psychosocial feedback. This may have been insufficient as other MIST studies have opted for 3 or 4 rounds. Furthermore, the MIST may not have been unpredictable or uncontrollable enough. When standing, humans experience large changes in ANS activity[76]. In order to prevent these changes from possibly contaminating RSA measurements of stress reactivity participants were brought to the testing room 10 minutes before the MIST, and remained in the room 10 minutes after. This could provide a window for participants to acclimate to the setting, making it more normal and predictable, ergo less uncontrollable. Additionally, trials of the MIST lasted for 9 minutes. This may have been too long as participants are given enough time to interpret how the MIST works and become familiarized with its structure and patterns.

Evaluating the effect size for LMEs is a difficult task. Unlike their fixed effects cousins,

there is no straight forward manner in which to obtain an equivalent of an R^2 value to give an estimate of effect size. To address this problem Nakagawa & Schielzeth proposed modified versions of this statistic which they name the marginal-R^2 and the conditional-R^2[121]. By their account, the marginal-R^2 provides a measure of the amount of variance described by the fixed effects in the model, whereas the conditional-R^2 gives the variance described by both the fixed and random effects. Using the *piecewiseSEM* package in R 3.2.2 we derived these values for our model of sAA reactivity. It was found that the marginal-$R^2 = 0.018$ while the conditional-$R^2 = 0.881$. This means that less than 2% of the variance of the model was explained by the fixed effects alone and implies that there was more variability between subjects than between samples. This is part of the strength of LMEs, as they allow researchers to account for the random, inter-individual differences between participants to tease out effects. However, by any standards it is an incredibly small effect size. This further supports the interpretation that the MIST was not highly stressful.

In exploring differences in stress reactivity between MC groups the only significant result was found to be in the ratio of reactive sAA to reactive RSA. This methodology was first used by Ali & Pruessner[63] who reported significant differences between those high and low in MC in the ratio of reactive cortisol to reactive sAA, and its inverse. We did not find a similar result. As we did not elicit a strong cortisol response it is unsurprising that we did not see such a difference. However, extending this method we did find a significant difference in the ratio of reactive sAA to reactive RSA. Following the original authors' interpretation this is a measure of sAA reactivity when controlling for PSNS activity. As sAA is not a pure SNS measure controlling for PSNS activity would leave only the SNS component. From this interpretation it would follow that we found differences in SNS reactivity between ELA conditions.

Previous attempts have been made to produce a measure of SNS activity derived from

mixed ANS activity. In the HRV literature this is the low-frequency to high-frequency ratio ($\frac{LF}{HF}$). The HF component of HRV is purely mediated by PSNS activity. However, early work found that the LF component is a combination of SNS and PSNS input[113]. Extrapolating from this information, many researchers have used the $\frac{LF}{HF}$ as either a measure of SNS activity, or an indicator of ANS balance (*i.e.* informative of whether the SNS or PSNS is more active\dominant). Though widely used a recent comprehensive review has brought into question the empirical validity of the measure, arguing that LF-HRV is predominantly PSNS mediated[122]. For those reasons, the $\frac{LF}{HF}$ measure was not included in this study. However, the measure foudn in this study (the $\frac{sAA}{RSA}$) could be a fruitful replacement for this method. Future research will be necessary to further test and validate this measure.

The final hypothesis of this thesis was that a data driven clustering approach using physiological data would yield useful information about ACM groupings when comparing them on measures of ELA. Using k-means clustering participants were grouped based on six measures: RSA, sAA, and cortisol baseline, and RSA, sAA, and cortisol reactivity (AUC_i). Only the clustering into 2 groups was used in further analyses, as the 3 and 4 group clusterings produced several group sizes of 1 to 2 participants. Figure 12 shows the response profiles across the three stress measures when grouping participants in this way. A cursory look would suggest that the algorithm found distinct groups with respect to their sAA and cortisol levels throughout the task, though these groups did not appear to differ RSA levels. However, when we probed these groups for differences in ELA measures and the Mini-K we found no differences.

The ACM predicts that different patterns of baseline and reactivity across the stress systems are adaptive changes to differences in early life environment and stress (see Figure 1). Our inability to find differences here could be explained by several possible factors. It could reflect the difficulties and limitations of the ACM. The ACM considers changes in stress en-

dophenotype to be due to an increase in early life stress in some unidimensional manner. It is never explicitly defined what this overall early life stress is, nor how researchers are supposed to measure it. As we chose to focus on maternal care, with additional supplementary data on abuse and neglect, our measure would not have captured all aspects of ELA. Alternatively, the ACM does not explicate whether these differences are qualitative or quantitative. In other words it does not specify if these changes occur gradually as an organism experiences more ELA, or if they behave in a categorical, switch like manner. By clustering into groups we inadvertently assume that they are categorical rather than continuous, an assumption that may be ill-founded (see section 5.3 for further discussion).

These results could also reflect a lack of variability within our sample. ELA scores obtained in this study were limited and did not cover a wide range of the possible values (see section 5.3 for further discussion). Consequently we may only have participants from one of the predicted groups and ergo would see no differences between them. Finally, it is also possible — as covered above — that we did not evoke a strong enough stress response. It is impractical to expect consistent grouping of participants by their physiological stress response in the absence of said response.

Overall, in evaluating the initial hypotheses we can conclude:

I We were unable to observe vagal withdrawal when exposing subjects to the MIST

II We observed no main effect of ELA on cortisol reactivity

III We found an effect of ELA on sAA when controlling for PSNS activity (using a ratio measure)

IV Grouping of subjects based on physiological data yielded groups

that were not observed to differ in measures of ELA.

5.3 Limitations

There exist a number of limitations in the design of this study. First and foremost is the small sample size. Though balanced in design, the small number of participants means that we are prone to making Type II errors. Estimating power in mixed models is difficult, but one study, using simulated data compared power between a study with a sample size of 20 and one of 100 reporting powers of 38.2% to 85.4%, respectively[123]. As the sample size of this study is closer to 20 (and is 20 per group) it is reasonable to assume that we have relatively low power. This means that we are unable to make strong assertions about null findings. A straight forward solution would be to increase the sample size by two or three fold to open the door to more powerful statistical methods.

Another clear limitation of this study is that we neglected to include variables that are either known or proposed to moderate the relationship between ELA and the stress response. These include, but are not limited to, genetic variation in GRs[124], the timing of the adversity in early life[125], the intentionality of the trauma[56], or the relationship between the qualitative aspects of the ELA and the experimental stressor being used in evoking a stress response[53]. The inclusion of all of these moderators would require great statistical power, but future studies would do well to include as many as methodologically possible.

Somewhat related to the issue of sample size is the issue of studying ELA itself. Part of the significance of studies of the effects of ELA on stress reactivity is that these changes can be seen in non-clinical, healthy subjects. It is obvious, therefore, that during recruitment we pre-screen for mental and physical illness. However, we additionally pre-screen for drug use, tobacco use, alcohol comsumption, and BMI, as all of these are known to effect the HPA-

axis and ANS[85, 91, 92, 94–96]. Critically it is also true that increased ELA itself makes people more prone to engage in these behaviours[126] or suffer from adverse health effects like obesity[127]. This leads to two primary issues in methodology. First is the difficulty in recruitment. By pre-screening for all of the factors associated with ELA, it becomes very hard to recruit people that are both high in ELA and meet all of the selection criteria. In fact in this study we had to relax selection criteria after the first 3 months of screening, as it became increasingly evident that we would be unable to recruit a sufficient number of high ELA participants in a manner consistent with completing a Master's project in a timely manner.

The second issue when dealing with ELA concerns resilience. Resilience is a concept researchers have deployed in order to understand how some people are vulnerable to the negative effects of ELA whereas others are not[128]. In other words, it is the answer to the question of why everyone whom experiences ELA does not go on to develop pathology. Much literature has been devoted to explaining how this happens. However, it is entirely possible that resilience to the effects of early life stress can be extended not just to pathology, but to the other factors we pre-screen for. In other words, in pre-screeing against behaviours and variables that are known to effect the stress response, but are collinearly related with ELA, we may be inadvertently adding a bias to our sample. In this case selecting only for those resilient to the effects of ELA. This is a highly intractable problem, with no clear solution.

Finally, there is the issue of variance in our measures of ELA. In recruiting participants to the two groups, only MC as used as a measure. Thus our other measure of ELA, the CTQ, was only gathered in the lab for possible post-hoc analyses. As seen in Figure **??** however, there is very little variability in the CTQ data. Our sample therefore may be biased to those with only moderate to mild ELA, rather than those with more severe histories.

5.4 Future directions

Further research can focus on many possible avenues. An obvious extension of this study would be to include female participants. A recent study did find effects of ELA on HPA-axis reactivity to the MIST in female participants[78]. However a balanced design including both male, and female participants — in different phases of the menstrual cycle — in one study would permit direct sex comparisons, as well as allowing for a direct test of sex differences predicted by the ACM (Figure 1).

With respect to the ACM, more research is needed into how the separate subgroups are delineated *i.e.* what constitutes mild as opposed to moderate early life stress. As discussed above, it is unclear as to how one explicitly defines and quantifies a single measure of early life stress, particularly given the multiplicity of measures that have been found to have effects. Thus research that focuses on gathering participants over an incredibly large range of early life experiences quantified over many different experimental measures would be indispensable in addressing this problem. Such a study could see if there exists an underlying, principle construct that would be of use in quantifying a total measure of ELA.

Finally, future research could explore the moment-to-moment changes in HRV (RSA) in response to stress. Due to sizeable missing segments of data in the EKG traces, continuous analysis of HRV was not possible in this study. However, methods do exist to examine second-to-second changes in HRV and hence PSNS activity[129–131]. As the initial PSNS response to stress occurs so rapidly[9] these methods could illuminate the immediate response to stress, one that may be washed out over longer time frames.

5.5 Conclusion

Extensive research has gone into investigating the effects that ELA has on the stress response. However, in looking at the stress response, researchers have historically neglected the branches of the ANS in favour of the HPA-axis. Along with an increasing number of studies, this project wanted to examine the effects of ELA on stress response across all of the stress systems. The results indicated, in contrast to the majority of studies, that there were no effects of ELA on HPA-axis reactivity. However, we lacked enough statistical power to make strong conclusions, particularly concerning null results. Importantly the effects ELA on sAA could only be seen when accounting for the PSNS, once more emphasizing the importance of studying the stress systems in tandem. However, the small effect sizes in this study mean further research is needed to verify the claims. Additionally, further research is needed to test if early life related changes in the stress systems fall in line with the ACM. Overall, this study suggests an important role in controlling for the response of the other stress systems when trying to parse the effects of ELA on stress reactivity.

Figures

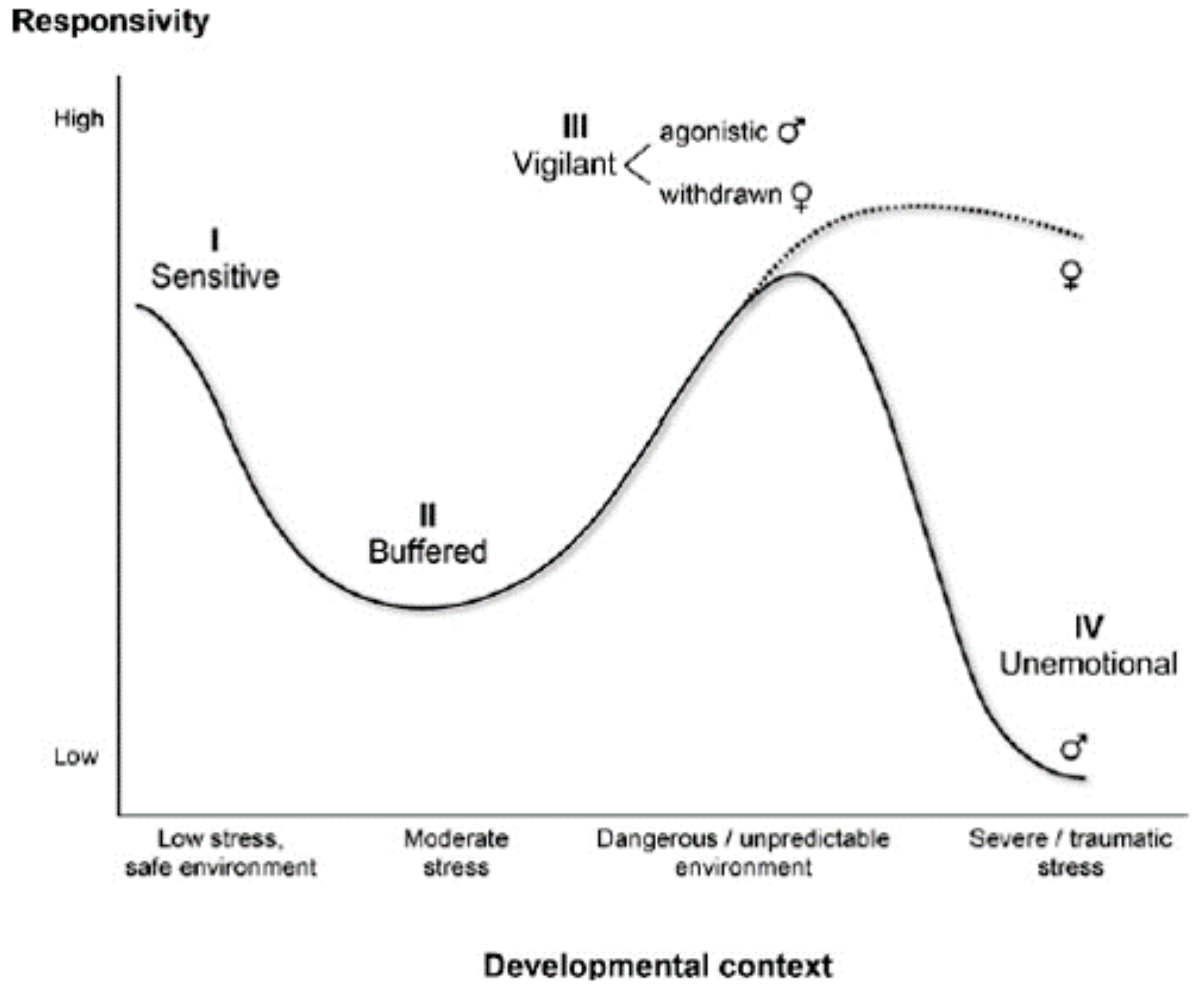

Figure 1: A figure from Del Giudice *et al.*(2011)[41] depicting how the general stress reactivity patterns of the systems change in accordance with the levels of stress in the early environment. This model predicts 4 distinct endophenotypes, as well as a difference between sexes at higher levels of early life stress.

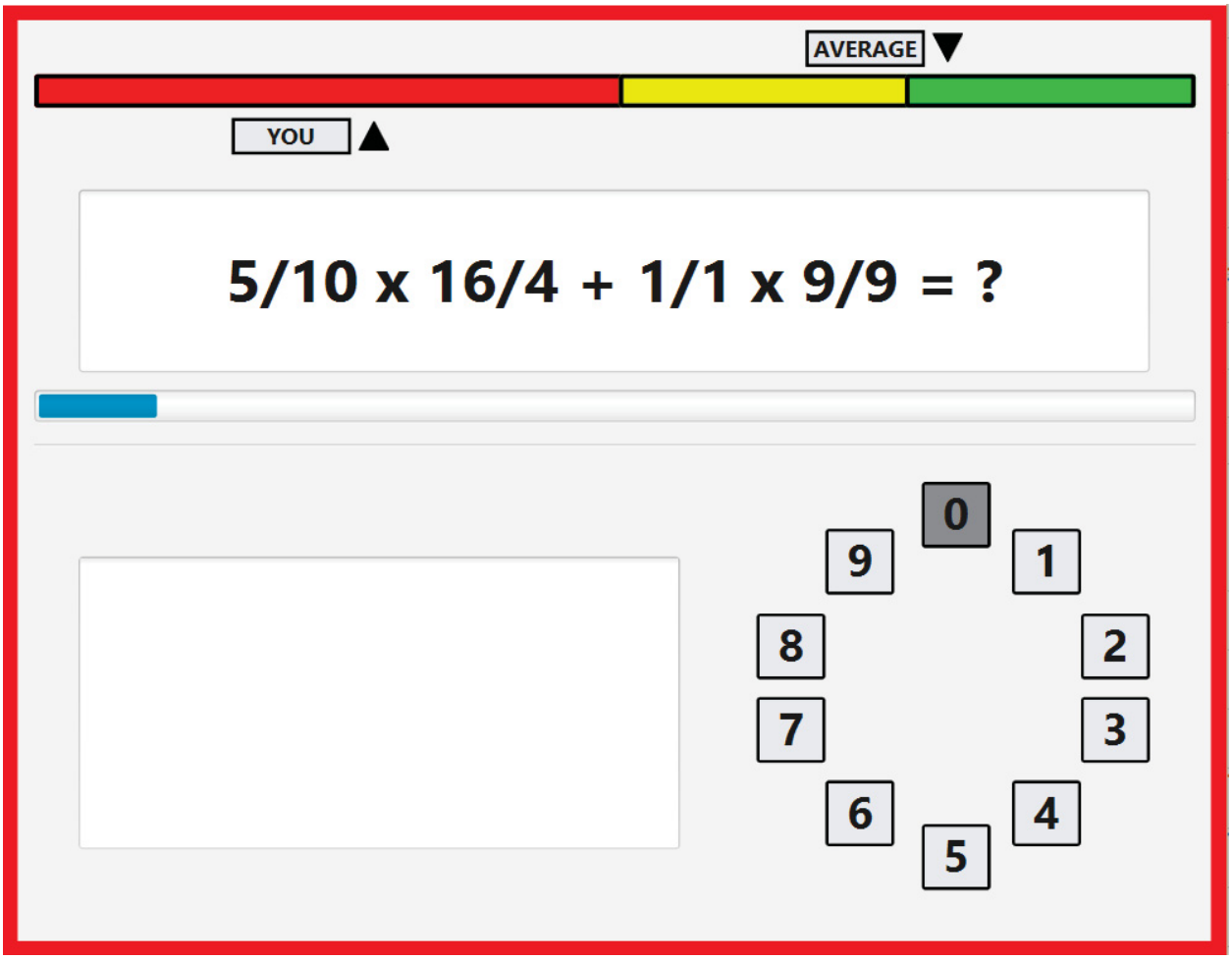

Figure 2: An example screen of the MIST during the experimental condition. Subjects answer using the keyboard to control the rotary dial located in the bottom right corner. Fabricated feedback of how the subject is doing relative to an "average" is presented in the top bar (the label of "YOU" being the subject's performance).

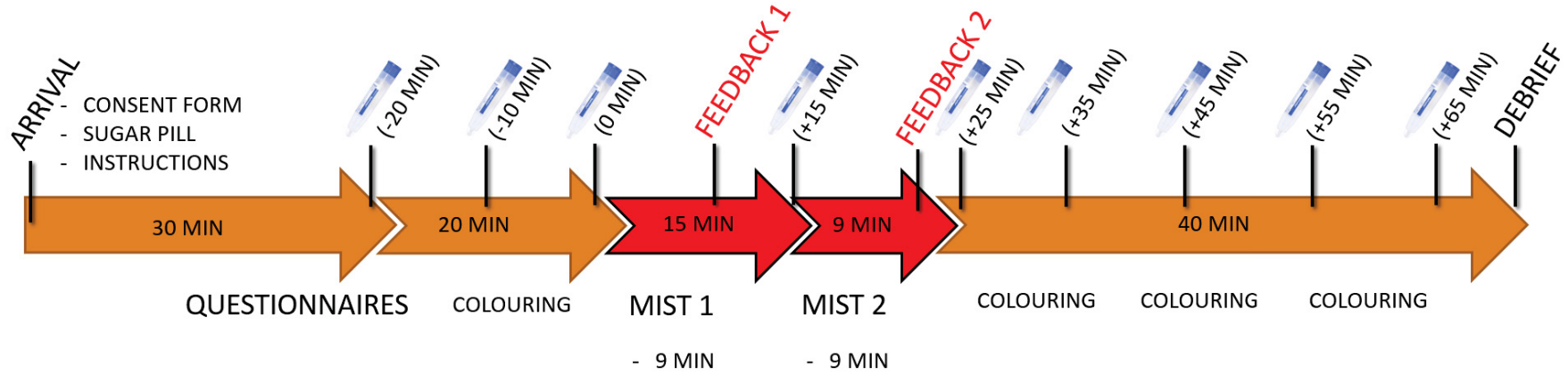

Figure 3: A time line of the events throughout the protocol. The small blue salivettes above the line indicate sampling times. The red portions of the time line represent when the MIST occurred (orange portions represent baseline and recovery periods).

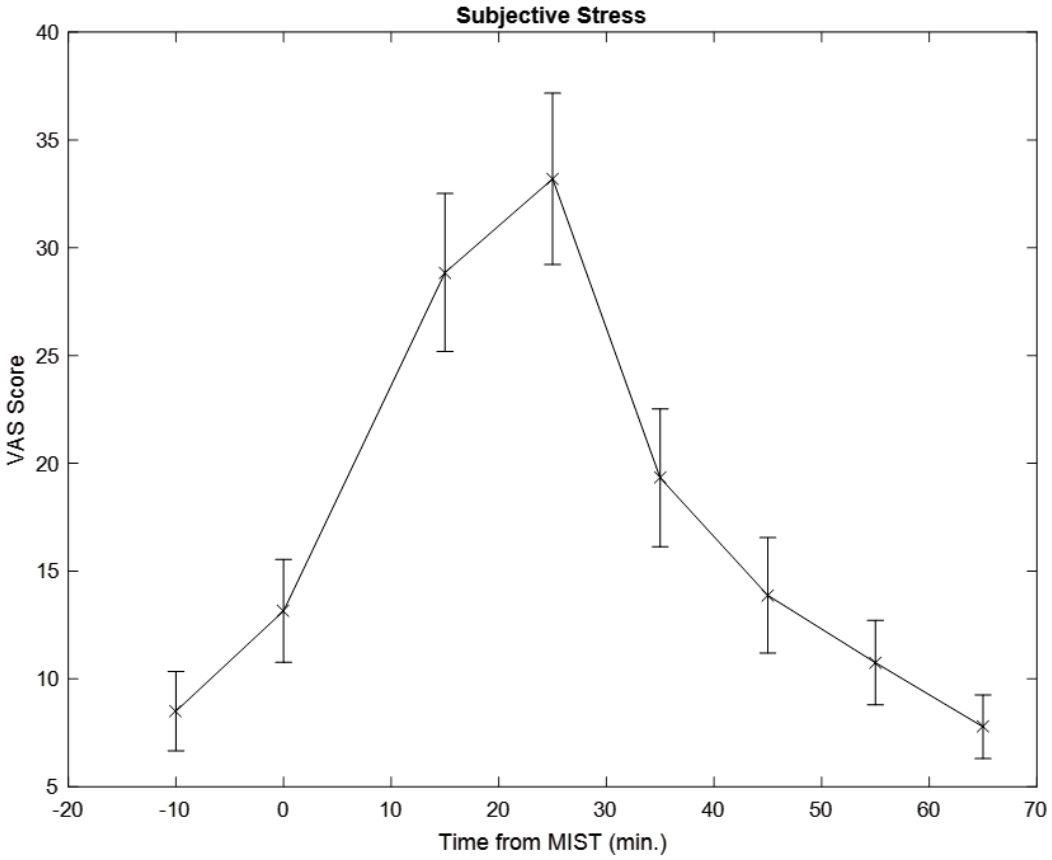

Figure 4: The self-reported VAS scores throughout the procedure in response to the question "In this moment how stressed do you feel?" Values peak following the second feedback session returning to baseline over the course of the recovery. All values are mean ± standard error.

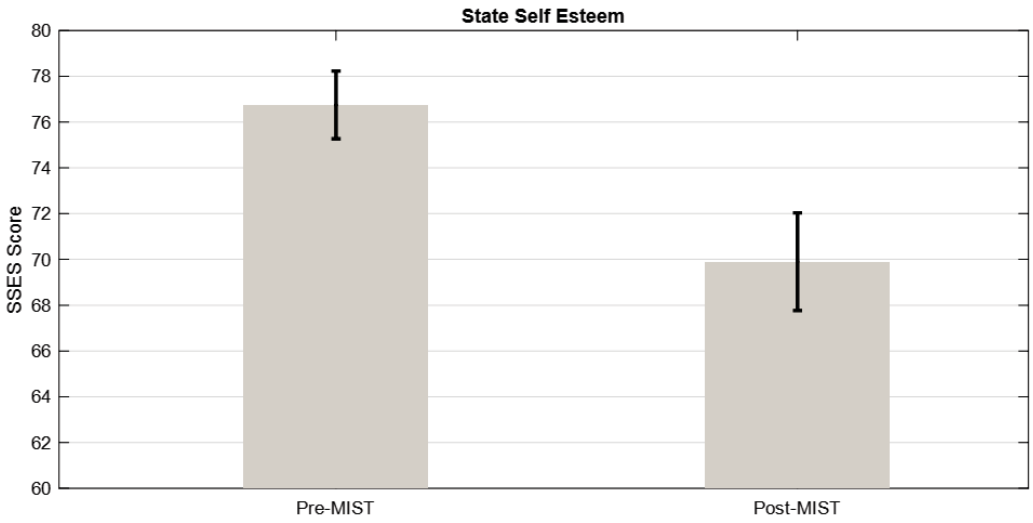

Figure 5: Measures of state self-esteem pre- (left bar) and post-MIST (right bar). The graph depicts that on average self-esteem dropped by 7 points after the MIST. All values are mean ± standard error.

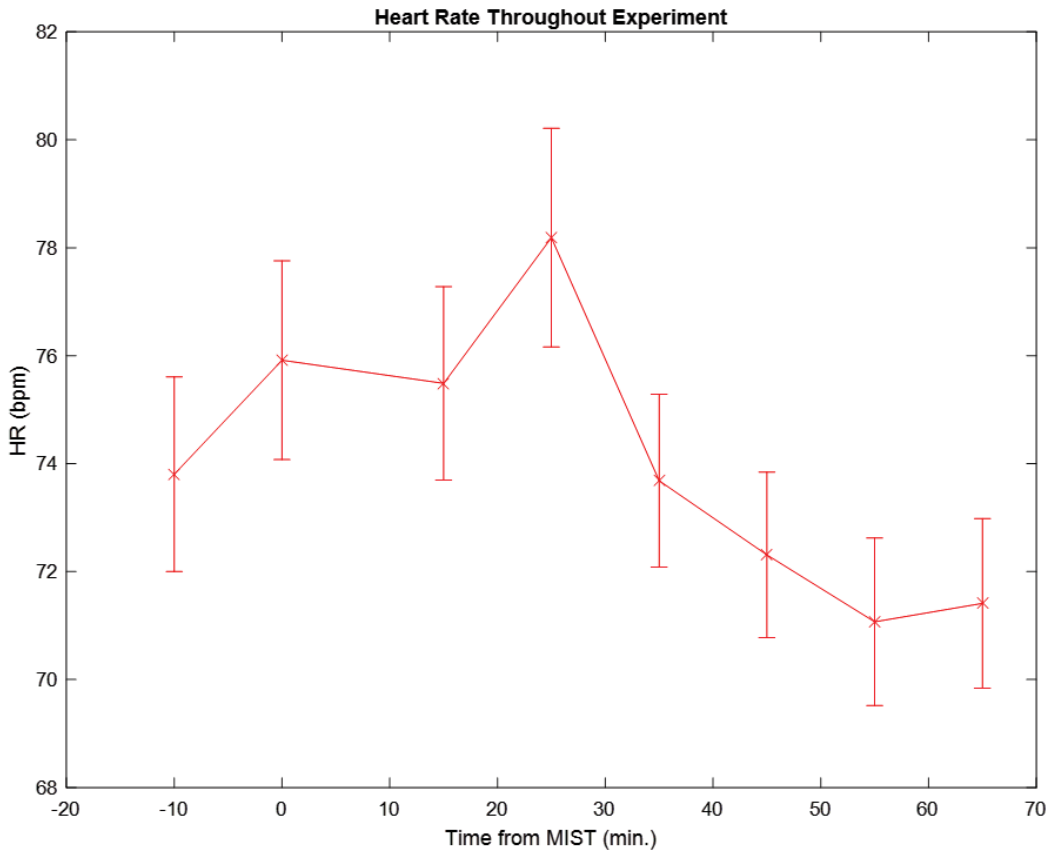

Figure 6: The heart rate of participants throughout the procedure. Participants experienced a significant, but small, increase in heart rate during the MIST. All values are mean ± standard error.

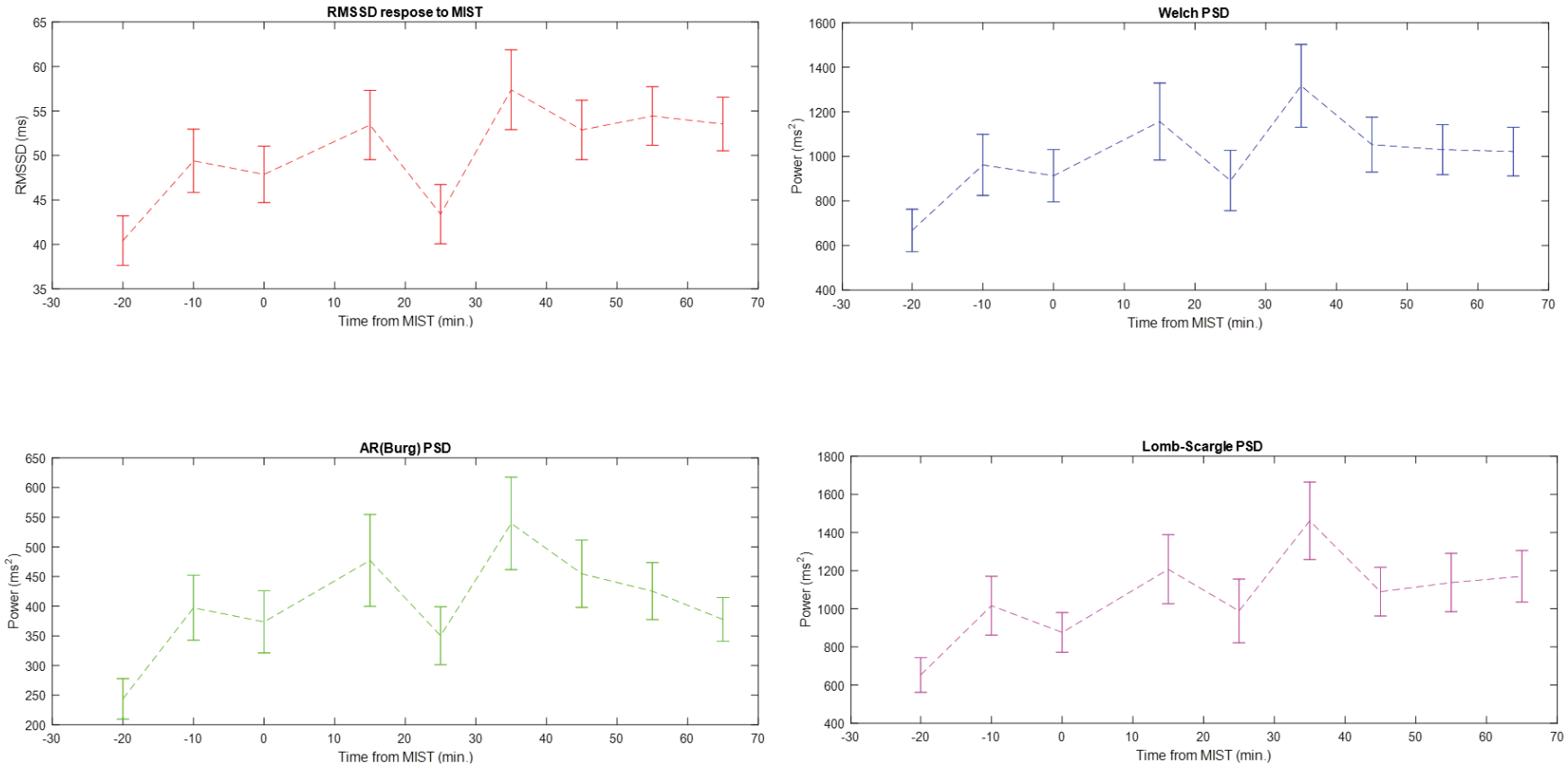

Figure 7: The RSA values across the task of four different RSA measures. There was high correlation between all 4 (see Table 2). **MEASURES:** upper left: RMSSD; upper right: Welch power spectral density (PSD); lower left auto-regressive Burgess PSD; lower right: Lomb-Scargle PSD. All values are mean ± standard error.

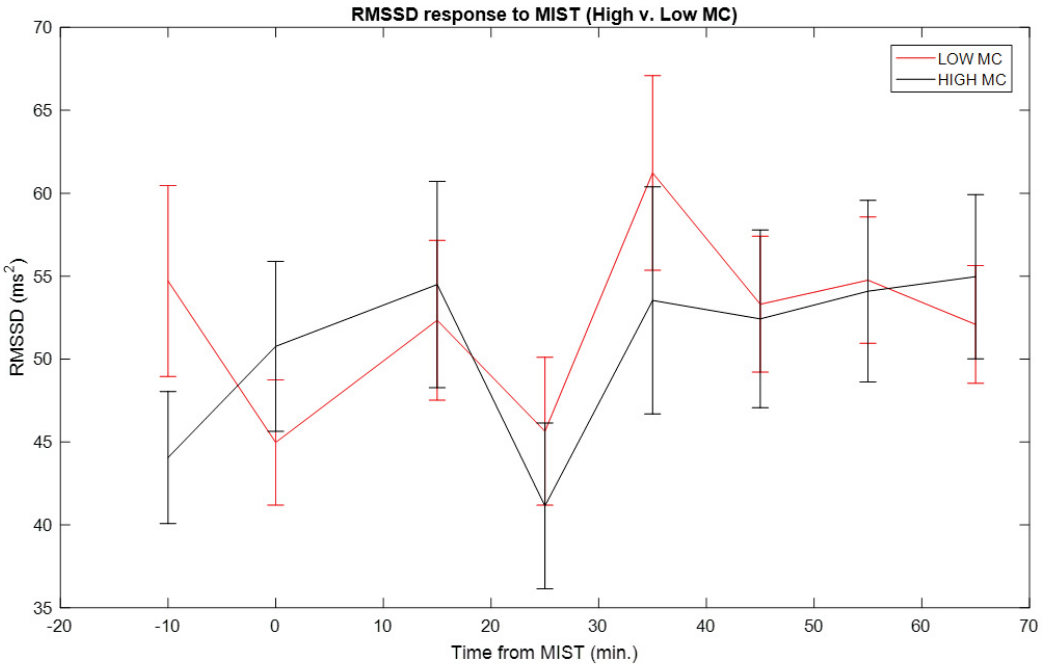

Figure 8: The RSA response profiles of the low MC and high MC groups. MIST trials occur at t = 0 and t = +15. All values are mean ± standard error.

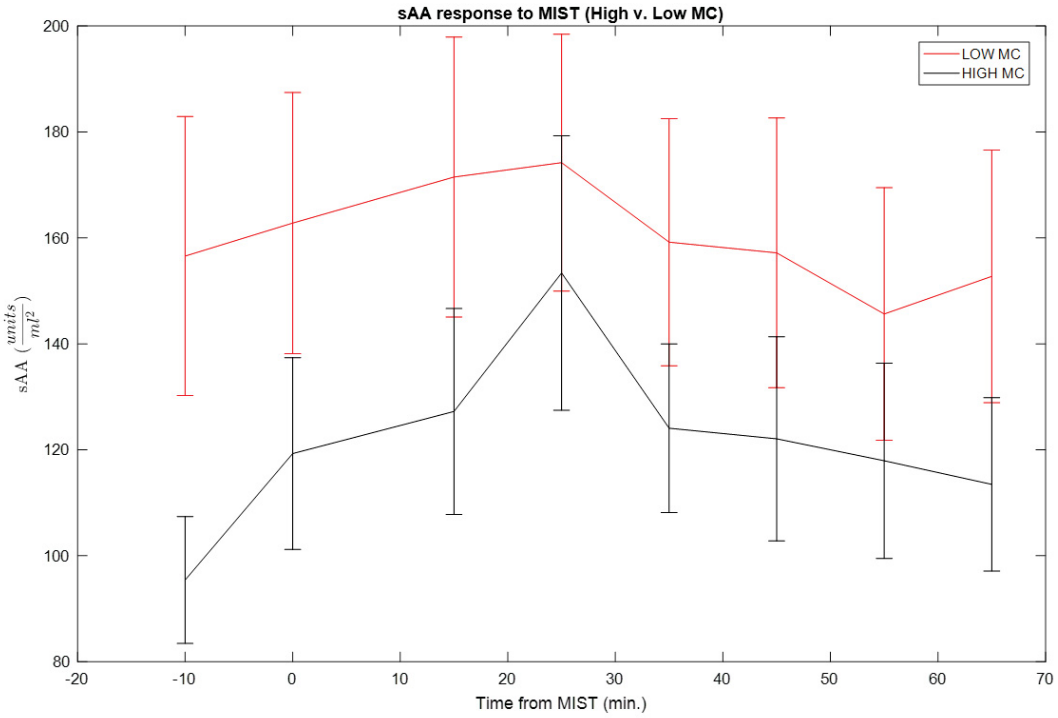

Figure 9: The sAA response profiles of the low MC and high MC groups. MIST trials occur at t = 0 and t = +15. All values are mean ± standard error.

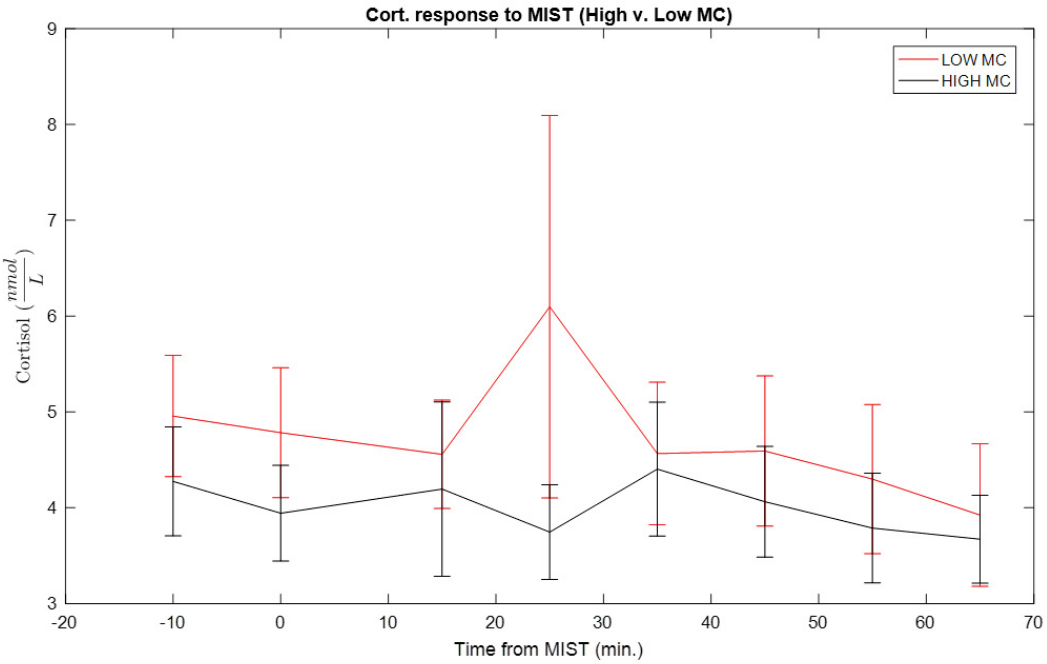

Figure 10: The cortisol response profiles of the low MC and high MC groups. MIST trials occur at t = 0 and t = +15. All values are mean ± standard error.

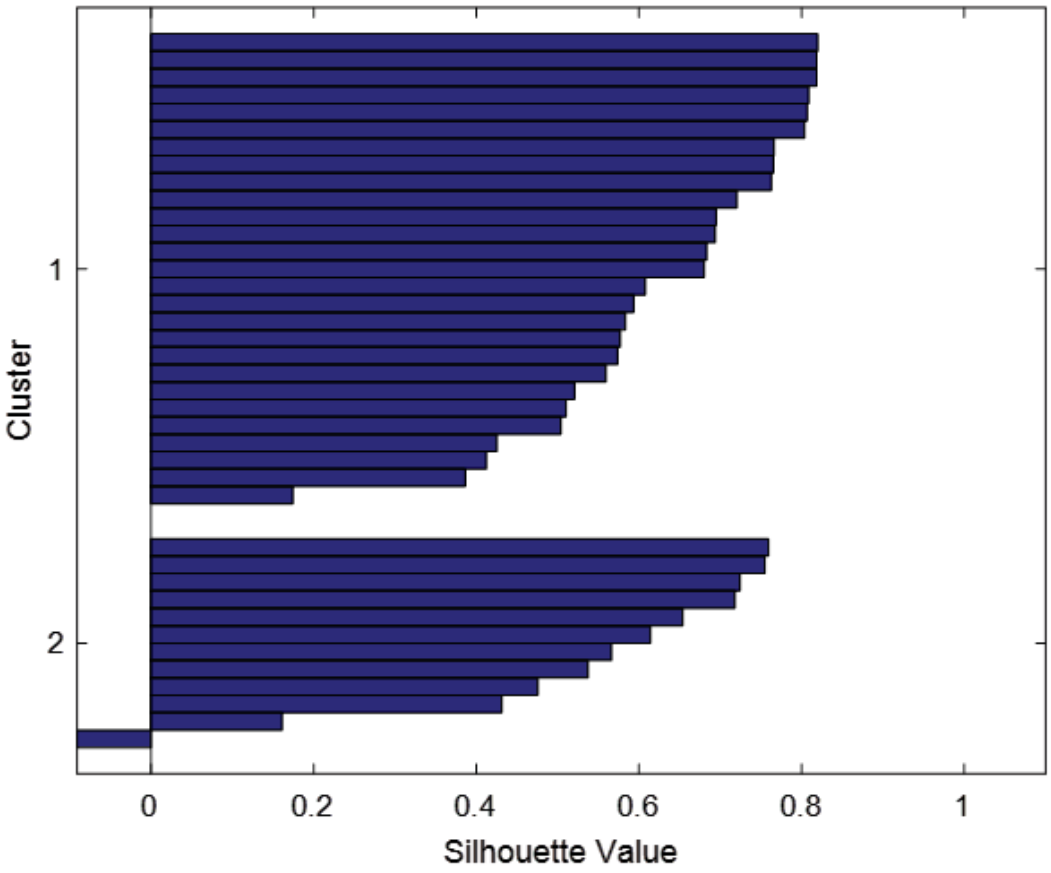

Figure 11: A silhouette plot of the two groups produced by k-means clustering. The majority of data points fit very well in their assigned cluster, with only one point being bearing a slightly negative score.

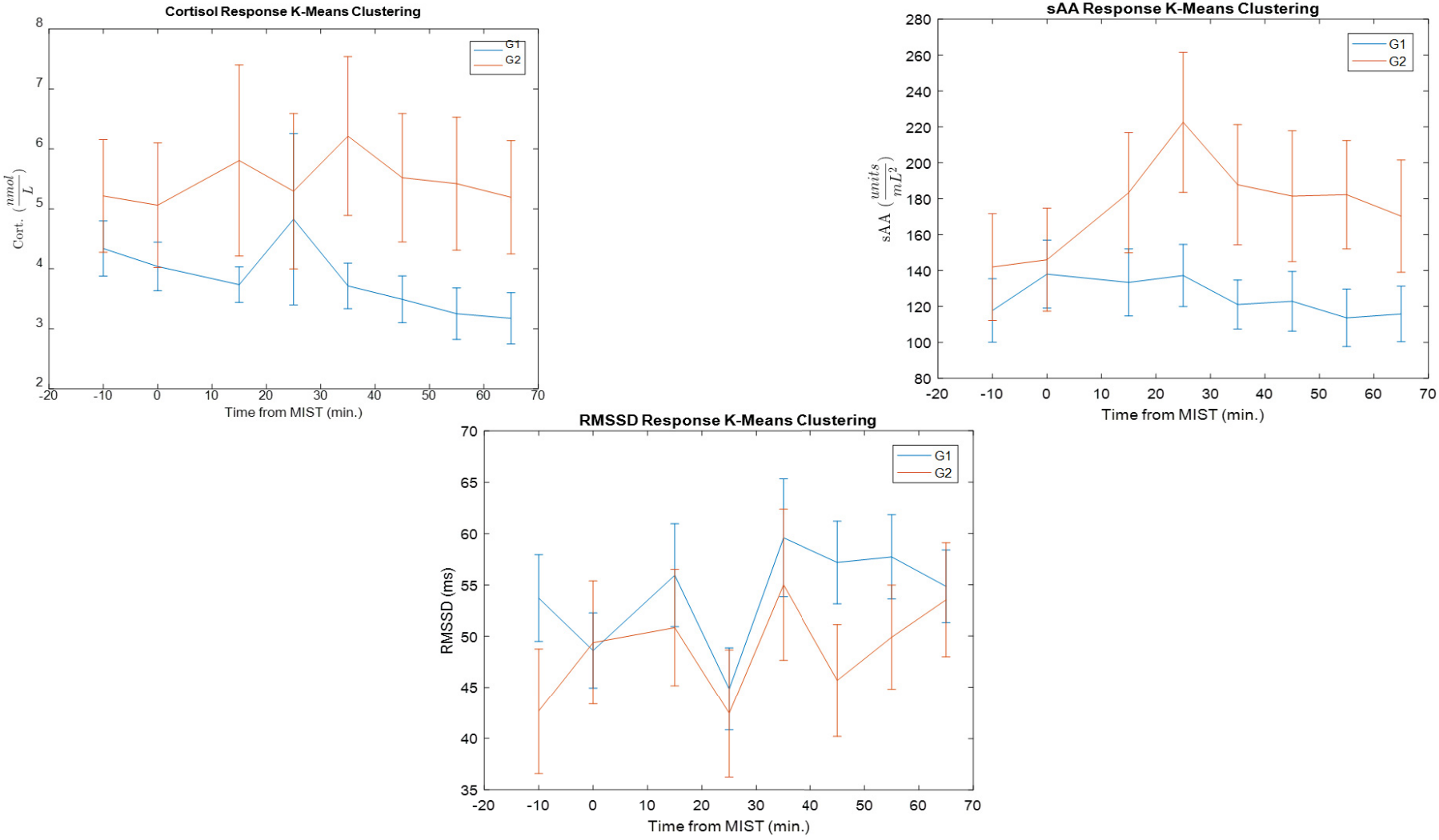

Figure 12: Graphs showing the physiological measures throughout the task based on clustering participants by their baseline and reactivity measures into two groups. Left most graph: cortisol; middle graph: RSA; right most graph: sAA. All values are mean ± standard error.

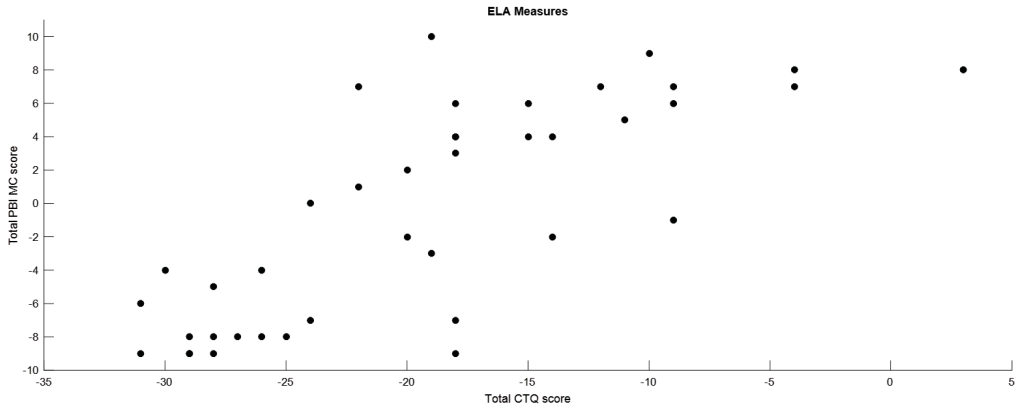

Figure 13: A scatter plot of ELA measure with MC (Maternal Care) on the y-axis and the total CTQ score on the x-axis. All values were centered around their cut-off scores and, in the case of MC, mirrored so that higher values correspond to higher levels of ELA. The possible range of MC scores is -9 to 27, inclusive. The possible range of CTQ scores is -31 to 69, inclusive.

Tables

Variable Name	MC Group		*t-stat*	*p-value*
	Low MC	High MC		
Age	23.2(3.3)	22.5(3.3)	-0.670	0.507
BMI	22.4(1.8)	22.4(1.9)	-0.052	0.959
CTQ	-14.0(7.0)	-24.5(6.1)	-5.067	0.000**
Father Care	18.5(5.1)	25.4(8.0)	3.230	0.003**
Mother Over-Protection	18.1(7.0)	11.6(6.1)	-3.194	0.003**
Father Over-Protection	9.4(6.3)	9.6(5.3)	0.082	0.935
Mini-K	0.24(0.40)	1.1(0.63)	5.031	0.000**
RSES	29.1(5.3)	29.3(8.5)	0.134	0.894
TICS	47.3(11.1)	42.2(20.0)	-0.995	0.326
State Anxiety	33.1(8.6)	28.7(5.9)	-1.885	0.067
Trait Anxiety	40.2(8.7)	36.2(10.3)	-1.310	0.198
Neuroticism	18.5(6.7)	16.5(8.7)	-0.794	0.432
Extraversion	25.5(7.0)	28.0(5.5)	1.233	0.225
Openness	31.4(5.8)	29.4(7.3)	-0.961	0.343
Agreeableness	27.9(4.7)	28.1(5.2)	0.128	0.899
Conscientiousness	29.2(6.1)	32.8(7.3)	1.678	0.102
BDI	8.3(5.2)	8.5(8.2)	0.115	0.909

Table 1: A comparison of outcome measures between the MC groups. All values (in MC Group column) are mean(stand deviation). Significant differences are marked by '**'. All t-tests are at 38 degrees of freedom. Values are presented as mean(standard deviation).

	RMSSD	AR(Burg)	Welch	L-S
RMSSD	1	-	-	-
AR(Burg)	0.9938	1	-	-
Welch	0.9965	0.9990	1	-
L-S	0.9937	0.9970	0.9987	1

Table 2: Table showing the normalized, cross-correlation coefficients between all of the measures of RSA. RMSSD = Root Mean Square of Successive Differences; AR(Burg) = Auto-Regressive Burgess model; Welch = Welch power spectral density; L-S = Lomb-Scargle power spectral density.

Variable Name	MC Group		*F-stat*	*p-value*
	Low MC	High MC		
ΔSSES	-4.45(9.38)	-9.25(11.5)	2.09	0.156
AUC_i VAS	893(839)	628(919)	0.850	0.362
Baseline RSA	54.7(25.7)	44.1(17.8)	0.907	0.347
Baseline sAA	157(118)	95.4(53.6)	0.008	0.931
Baseline cortisol	4.78(3.03)	3.94(2.23)	0.258	0.615
AUC_i RSA	-24.0(297)	-138(340)	2.02	0.163
AUC_i sAA	$192(1.89 \times 10^3)$	$530(1.03 \times 10^3)$	0.220	0.642
AUC_i cortisol	20.5(125)	18.5(83.1)	0.146	0.704

Table 3: Tests of the effect of MC group on baseline and stress reactivity measures, and psychological measures of stress. All tests at 1 and 38 degrees of freedom. ΔSSES = difference in pre-MIST and post-MIST SSES scores. Values are presented as mean(standard deviation).

Variable Name	MC Group		*F-stat*	*p-value*
	Low MC	High MC		
$\frac{Cort_{AUC_i}}{RSA_{AUC_i}}$	0.089(1.10)	0.446(2.60)	0.069	0.794
$\frac{Cort_{AUC_i}}{sAA_{AUC_i}}$	0.202(0.951)	0.220(0.987)	0.006	0.939
$\frac{sAA_{AUC_i}}{RSA_{AUC_i}}$	3.90(12.9)	-11.8(17.4)	11.3	0.002**
$\frac{sAA_{AUC_i}}{Cort_{AUC_i}}$	0.784(121)	14.4(35.8)	0.017	0.897

Table 4: Tests of the effect of MC group on the ratio of stress measures. All tests at 1 and 38 degrees of freedom. '**' indicates statistical significance. Values are presented as mean(standard deviation).

Variable Name	K-Means Group		*F-stat*	*p-value*
	Group 1	Group 2		
MC	0.370(6.53)	-2.42(6.52)	1.52	0.226
CTQ	-18.8(7.92)	-19.8(9.93)	0.117	0.734
Composite ELA	-18.4(13.5)	-22.3(15.9)	0.593	0.446
ELA#1 (PCA)	0.0232(0.204)	-0.0547(0.217)	1.16	0.288
ELA#2 (PCA)	-0.0089(0.0730)	0.0259(0.128)	1.17	0.286
Mini-K	0.580(0.657)	0.871(0.704)	1.56	0.219

Table 5: Tests of ELA measures between groups clustered by physiological measures. All tests at 1 and 37 degrees of freedom. '**' indicates statistical significance. Values are presented as mean(standard deviation).

Bibliography

1. Jackson, M. in *Stress, Shock, and Adaptation in the Twentieth Century* (eds Cantor, D. & Ramsden, E.) 21–48 (Boydell & Brewer, Apr. 2014).

2. Johnson, E. O., Kamilaris, T. C., Chrousos, G. P. & Gold, P. W. Mechanisms of stress: A dynamic overview of hormonal and behavioral homeostasis. *Neuroscience & Biobehavioral Reviews* **16,** 115–130. ISSN: 0149-7634 (1992).

3. Chrousos, G. P. Stress and disorders of the stress system. *Nature Reviews Endocrinology* **5,** 374–381. ISSN: 1759-5029 (July 2009).

4. Sterling, P. & Eyer, J. in *Handbook of Life Stress, Cognition, and Health* (eds Fisher, J. & Reason, J.) 629–649 (John Wiley & Sons, Inc., New York, NY, 1988).

5. Dickerson, S. S. & Kemeny, M. E. Acute Stressors and Cortisol Responses: A Theoretical Integration and Synthesis of Laboratory Research. *Psychological Bulletin* **130,** 355–391. ISSN: 1939-1455 0033-2909 (2004).

6. Dedovic, K., D'Aguiar, C. & Pruessner, J. C. What Stress Does to Your Brain: A Review of Neuroimaging Studies. *The Canadian Journal of Psychiatry* **54,** 6–15. ISSN: 0706-7437 (Jan. 1, 2009).

7. Dedovic, K., Duchesne, A., Andrews, J., Engert, V. & Pruessner, J. C. The brain and the stress axis: The neural correlates of cortisol regulation in response to stress. *NeuroImage. Brain Body Medicine* **47,** 864–871. ISSN: 1053-8119 (Sept. 2009).

8. Grossman, P. & Taylor, E. W. Toward understanding respiratory sinus arrhythmia: Relations to cardiac vagal tone, evolution and biobehavioral functions. *Biological Psychology. Special Issue of Biological Psychology on Cardiac Vagal Control, Emotion, Psychopathology, and Health.* **74,** 263–285. ISSN: 0301-0511 (Feb. 2007).

9. Berger, R. D., Saul, J. P. & Cohen, R. J. Transfer function analysis of autonomic regulation. I. Canine atrial rate response. *American Journal of Physiology - Heart and Circulatory Physiology* **256,** H142–H152. ISSN: 0363-6135, 1522-1539 (Jan. 1, 1989).

10. Jänig, W. *The Integrative Action of the Autonomic Nervous System: Neurobiology of Homeostasis* (Cambridge University Press, Cambridge, UK, 2006).

11. Cannon, W. B. *Bodily changes in pain, hunger, fear and rage; an account of recent researches into the function of emotional excitement* 2nd ed. (D. Appleton and Co., New York ; London, 1929).

12. Morilak, D. A. *et al.* Role of brain norepinephrine in the behavioral response to stress. *Progress in Neuro-Psychopharmacology and Biological Psychiatry. Experimental Stress: from Basic to Clinical AspectsExperimental Stress: from Basic to Clinical Aspects* **29,** 1214–1224. ISSN: 0278-5846 (Dec. 2005).

13. Cecchi, M., Khoshbouei, H. & Morilak, D. A. Modulatory effects of norepinephrine, acting on alpha1 receptors in the central nucleus of the amygdala, on behavioral and neuroendocrine responses to acute immobilization stress. *Neuropharmacology* **43,** 1139–1147. ISSN: 0028-3908 (Dec. 2002).

14. Loewy, A. D. & Spyer, K. M. *Central Regulation of Autonomic Functions* ISBN: 978-0-19-976311-5. `http://site.ebrary.com/lib/alltitles/docDetail.action?docID=10142089` (2017) (Oxford University Press (US), Cary, US, 2005).

15. Damasio, A. R., Tranel, D. & Damasio, H. Individuals with sociopathic behavior caused by frontal damage fail to respond autonomically to social stimuli. *Behavioural Brain Research* **41,** 81–94. ISSN: 0166-4328 (Dec. 14, 1990).

16. Joëls, M. & Baram, T. Z. The neuro-symphony of stress. *Nature Reviews Neuroscience* **10,** 459–466. ISSN: 1471-003X (June 2009).

17. Knight, B. The history of child abuse. *Forensic Science International* **30,** 135–141. ISSN: 0379-0738 (Feb. 1, 1986).

18. Freud, S., Breuer, J. & Luckhurst, N. *Studies in hysteria* OCLC: 53820634. ISBN: 978-0-14-243749-0 978-0-14-118482-1 (Penguin Books, London; New York, 2004).

19. Gunnar, M. & Quevedo, K. The Neurobiology of Stress and Development. *Annual Review of Psychology* **58,** 145–173. ISSN: 0066-4308 (Dec. 6, 2006).

20. Lupien, S. J., McEwen, B. S., Gunnar, M. R. & Heim, C. Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience* **10,** 434–445. ISSN: 1471-003X (June 2009).

21. Heim, C. & Nemeroff, C. B. Neurobiology of early life stress: clinical studies. *Seminars in Clinical Neuropsychiatry* **7,** 147–159. ISSN: 1084-3612 (Apr. 2002).

22. Nugent, N. R., Tyrka, A. R., Carpenter, L. L. & Price, L. H. Gene–environment interactions: early life stress and risk for depressive and anxiety disorders. *Psychopharmacology* **214,** 175–196. ISSN: 0033-3158, 1432-2072 (Jan. 12, 2011).

23. Ehlert, U. Enduring psychobiological effects of childhood adversity. *Psychoneuroendocrinology* **38,** 1850–1857. ISSN: 0306-4530 (Sept. 2013).

24. Eriksson, M., Räikkönen, K. & Eriksson, J. G. Early life stress and later health outcomes—findings from the Helsinki Birth Cohort Study. *American Journal of Human Biology* **26,** 111–116. ISSN: 1520-6300 (Mar. 4, 2014).

25. Suzuki, A., Poon, L., Papadopoulos, A. S., Kumari, V. & Cleare, A. J. Long term effects of childhood trauma on cortisol stress reactivity in adulthood and relationship to the occurrence of depression. *Psychoneuroendocrinology* **50,** 289–299. ISSN: 0306-4530 (Dec. 2014).

26. Dong, M. *et al.* Insights Into Causal Pathways for Ischemic Heart Disease. *Circulation* **110,** 1761–1766. ISSN: 0009-7322, 1524-4539 (Sept. 28, 2004).

27. Chartier, M. J., Walker, J. R. & Naimark, B. Separate and cumulative effects of adverse childhood experiences in predicting adult health and health care utilization. *Child Abuse & Neglect* **34,** 454–464. ISSN: 0145-2134 (June 2010).

28. Westfall, N. C. & Nemeroff, C. B. The Preeminence of Early Life Trauma as a Risk Factor for Worsened Long-Term Health Outcomes in Women. *Current Psychiatry Reports* **17,** 90. ISSN: 1523-3812, 1535-1645 (Sept. 18, 2015).

29. Chiang, J. J., Taylor, S. E. & Bower, J. E. Early adversity, neural development, and inflammation. *Developmental Psychobiology* **57,** 887–907. ISSN: 1098-2302 (Dec. 1, 2015).

30. Pechtel, P. & Pizzagalli, D. A. Effects of early life stress on cognitive and affective function: an integrated review of human literature. *Psychopharmacology* **214,** 55–70. ISSN: 0033-3158, 1432-2072 (Sept. 24, 2010).

31. Paivio, S. C. & Cramer, K. M. Factor structure and reliability of the Childhood Trauma Questionnaire in a Canadian undergraduate student sample. *Child Abuse & Neglect* **28,** 889–904. ISSN: 0145-2134 (Aug. 2004).

32. Wright, K. D. *et al.* Factorial validity of the Childhood Trauma Questionnaire in men and women. *Depression and Anxiety* **13,** 179–183. ISSN: 1520-6394 (Jan. 1, 2001).

33. Carpenter, L. L. *et al.* Decreased Adrenocorticotropic Hormone and Cortisol Responses to Stress in Healthy Adults Reporting Significant Childhood Maltreatment. *Biological Psychiatry. Stress and Anxiety: Developmental and Therapeutic Perspectives* **62,** 1080–1087. ISSN: 0006-3223 (Nov. 15, 2007).

34. Carpenter, L. L., Shattuck, T. T., Tyrka, A. R., Geracioti, T. D. & Price, L. H. Effect of childhood physical abuse on cortisol stress response. *Psychopharmacology* **214,** 367–375. ISSN: 0033-3158, 1432-2072 (Sept. 14, 2010).

35. Dietz, L. J. *et al.* Cortisol Response to Social Stress in Parentally Bereaved Youth. *Biological Psychiatry. Dimensions and Subtypes of Anxiety Disorders* **73,** 379–387. ISSN: 0006-3223 (Feb. 15, 2013).

36. McLaughlin, K. A. *et al.* Causal effects of the early caregiving environment on development of stress response systems in children. *Proceedings of the National Academy of Sciences* **112,** 5637–5642. ISSN: 0027-8424, 1091-6490 (May 5, 2015).

37. Engert, V. *et al.* Perceived early-life maternal care and the cortisol response to repeated psychosocial stress. *Journal of Psychiatry & Neuroscience : JPN* **35,** 370–377. ISSN: 1180-4882 (Nov. 2010).

38. Bernstein, D. P. & Fink, L. *Childhood trauma questionnaire: a retrospective self-report: Manual* (The Psychological Corporation, San Antonio, TX, 1998).

39. Parker, G., Tupling, H. & Brown, L. B. A Parental Bonding Instrument. *British Journal of Medical Psychology* **52,** 1–10. ISSN: 2044-8341 (Mar. 1, 1979).

40. Bremner, J. D., Bolus, R. & Mayer, E. A. Psychometric Properties of the Early Trauma Inventory - Self Report: *The Journal of Nervous and Mental Disease* **195,** 211–218. ISSN: 0022-3018 (Mar. 2007).

41. Del Giudice, M., Ellis, B. J. & Shirtcliff, E. A. The Adaptive Calibration Model of stress responsivity. *Neuroscience & Biobehavioral Reviews. Resilience and Adaptive Aspects of Stress in Neurobehavioural Development* **35,** 1562–1592. ISSN: 0149-7634 (June 2011).

42. Hill, K. Life history theory and evolutionary anthropology. *Evolutionary Anthropology: Issues, News, and Reviews* **2,** 78–88. ISSN: 1520-6505 (Jan. 1, 1993).

43. McEwen, B. S. & Stellar, E. Stress and the Individual: Mechanisms Leading to Disease. *Archives of Internal Medicine* **153,** 2093–2101. ISSN: 0003-9926 (Sept. 27, 1993).

44. Meaney, M. J., Aitken, D. H., Berkel, C. v., Bhatnagar, S. & Sapolsky, R. M. Effect of neonatal handling on age-related impairments associated with the hippocampus. *Science* **239,** 766–768. ISSN: 0036-8075, 1095-9203 (Feb. 12, 1988).

45. Bhatnagar, S. & Meaney, M. J. Hypothalamic-Pituitary-Adrenal Function in Chronic Intermittently Cold-Stressed Neonatally Handled and Non Handled Rats. *Journal of Neuroendocrinology* **7,** 97–108. ISSN: 1365-2826 (Feb. 1, 1995).

46. Liu, D. *et al.* Maternal Care, Hippocampal Glucocorticoid Receptors, and Hypothalamic-Pituitary-Adrenal Responses to Stress. *Science* **277,** 1659–1662. ISSN: 0036-8075, 1095-9203 (Sept. 12, 1997).

47. Aisa, B., Tordera, R., Lasheras, B., Del Río, J. & Ramírez, M. J. Cognitive impairment associated to HPA axis hyperactivity after maternal separation in rats. *Psychoneuroendocrinology* **32,** 256–266. ISSN: 0306-4530 (Apr. 2007).

48. Pruessner, J. C., Champagne, F., Meaney, M. J. & Dagher, A. Dopamine Release in Response to a Psychological Stress in Humans and Its Relationship to Early Life Maternal Care: A Positron Emission Tomography Study Using [11C]Raclopride. *The Journal of Neuroscience* **24,** 2825–2831. ISSN: 0270-6474, 1529-2401 (Mar. 17, 2004).

49. Schmid, B. *et al.* Maternal stimulation in infancy predicts hypothalamic–pituitary–adrenal axis reactivity in young men. *Journal of Neural Transmission* **120,** 1247–1257. ISSN: 0300-9564, 1435-1463 (Jan. 20, 2013).

50. Blair, C., Granger, D., Willoughby, M., Kivlighan, K. & The Family Life Project Investigators. Maternal Sensitivity Is Related to Hypothalamic-Pituitary-Adrenal Axis Stress Reactivity and Regulation in Response to Emotion Challenge in 6-Month-Old Infants. *Annals of the New York Academy of Sciences* **1094,** 263–267. ISSN: 1749-6632 (Dec. 1, 2006).

51. Blair, C. *et al.* Maternal and child contributions to cortisol response to emotional arousal in young children from low-income, rural communities. *Developmental Psychology* **44,** 1095–1109. ISSN: 1939-0599 0012-1649 (2008).

52. Fernald, L. C. H., Burke, H. M. & Gunnar, M. R. Salivary cortisol levels in children of low-income women with high depressive symptomatology. *Development and Psychopathology* **20,** 423–36. ISSN: 09545794 (2008).

53. Sturge-Apple, M. L., Davies, P. T., Cicchetti, D. & Manning, L. G. Interparental violence, maternal emotional unavailability and children's cortisol functioning in family contexts. *Developmental Psychology* **48,** 237–249. ISSN: 1939-0599 0012-1649 (2012).

54. Hackman, D. A. *et al.* Selective Impact of Early Parental Responsivity on Adolescent Stress Reactivity. *PLoS ONE* **8.** ISSN: 1932-6203. doi:10.1371/journal.pone.0058250. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3596401/ (2016) (Mar. 13, 2013).

55. Heim C, Newport D, Heit S & et al. Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. *JAMA* **284,** 592–597. ISSN: 0098-7484 (Aug. 2, 2000).

56. Kuhlman, K. R., Geiss, E. G., Vargas, I. & Lopez-Duran, N. L. Differential associations between childhood trauma subtypes and adolescent HPA-axis functioning. *Psychoneuroendocrinology* **54,** 103–114. ISSN: 0306-4530 (Apr. 2015).

57. Murali, R. & Chen, E. Exposure to violence and cardiovascular and neuroendocrine measures in adolescents. *Annals of Behavioral Medicine* **30,** 155–163. ISSN: 0883-6612, 1532-4796 (2005).

58. Maria, M. M. M.-S. *et al.* Influence of cocaine dependence and early life stress on pituitary–adrenal axis responses to CRH and the Trier social stressor. *Psychoneuroendocrinology* **35,** 1492–1500. ISSN: 0306-4530 (Nov. 2010).

59. Bauer, A. M., Quas, J. A. & Boyce, W. T. Associations Between Physiological Reactivity and Children's Behavior: Advantages of a Multisystem Approach. *Journal of Developmental & Behavioral Pediatrics* **23,** 102–113 (2002).

60. Newlin, D. B. & Levenson, R. W. Pre-ejection Period: Measuring Beta-adrenergic Influences Upon the Heart. *Psychophysiology* **16,** 546–552. ISSN: 1469-8986 (Nov. 1, 1979).

61. Ellis, B. J., Essex, M. J. & Boyce, W. T. Biological sensitivity to context: II. Empirical explorations of an evolutionary–developmental theory. *Development and Psychopathology* **17,** 303–328. ISSN: 1469-2198, 0954-5794 (June 2005).

62. Del Giudice, M., Benjamin, J., Ellis, B. J. & El-Sheikh, M. Adaptive patterns of stress responsivity: A preliminary investigation. *Developmental Psychology* **48,** 775–790. ISSN: 1939-0599 0012-1649 (2012).

63. Ali, N. & Pruessner, J. C. The salivary alpha amylase over cortisol ratio as a marker to assess dysregulations of the stress systems. *Physiology & Behavior. Allostasis and Allostatic Load* **106,** 65–72. ISSN: 0031-9384 (Apr. 12, 2012).

64. Taylor, Z. E. *et al.* Sociodemographic risk, parenting, and effortful control: Relations to salivary alpha-amylase and cortisol in early childhood. *Developmental Psychobiology* **55,** 869–880. ISSN: 1098-2302 (Dec. 1, 2013).

65. Nater, U. M. & Rohleder, N. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: Current state of research. *Psychoneuroendocrinology* **34,** 486–496. ISSN: 0306-4530 (May 2009).

66. Chatterton, R. T., Vogelsong, K. M., Lu, Y.-c., Ellman, A. B. & Hudgens, G. A. Salivary α-amylase as a measure of endogenous adrenergic activity. *Clinical Physiology* **16,** 433–448. ISSN: 1365-2281 (July 1, 1996).

67. Van Ockenburg, S. L. *et al.* Effects of adverse life events on heart rate variability, cortisol, and C-reactive protein. *Acta Psychiatrica Scandinavica* **131,** 40–50. ISSN: 1600-0447 (Jan. 1, 2015).

68. Meyer, P.-W. *et al.* Heart rate variability in patients with post-traumatic stress disorder or borderline personality disorder: relationship to early life maltreatment. *Journal of Neural Transmission* **123,** 1107–1118. ISSN: 0300-9564, 1435-1463 (June 16, 2016).

69. McLaughlin, K. A., Alves, S. & Sheridan, M. A. Vagal regulation and internalizing psychopathology among adolescents exposed to childhood adversity. *Developmental Psychobiology* **56,** 1036–1051. ISSN: 1098-2302 (July 1, 2014).

70. Rottenberg, J., Clift, A., Bolden, S. & Salomon, K. RSA fluctuation in major depressive disorder. *Psychophysiology* **44,** 450–458. ISSN: 1469-8986 (May 1, 2007).

71. Bylsma, L. M., Salomon, K., Taylor-Clift, A., Morris, B. H. & Rottenberg, J. Respiratory Sinus Arrhythmia Reactivity in Current and Remitted Major Depressive Disorder: *Psychosomatic Medicine* **76,** 66–73. ISSN: 0033-3174 (Jan. 2014).

72. Yaroslavsky, I., Rottenberg, J. & Kovacs, M. Atypical patterns of respiratory sinus arrhythmia index an endophenotype for depression. *Development and Psychopathology* **26,** 1337–1352. ISSN: 0954-5794, 1469-2198 (Nov. 2014).

73. Patriquin, M. A., Lorenzi, J., Scarpa, A., Calkins, S. D. & Bell, M. A. Broad implications for respiratory sinus arrhythmia development: Associations with childhood symptoms of psychopathology in a community sample. *Developmental Psychobiology* **57,** 120–130. ISSN: 1098-2302 (Jan. 1, 2015).

74. Smeets, T. Autonomic and hypothalamic–pituitary–adrenal stress resilience: Impact of cardiac vagal tone. *Biological Psychology* **84,** 290–295. ISSN: 0301-0511 (May 2010).

75. Kirschbaum, C., Pirke, K.-M. & Hellhammer, D. H. The 'Trier Social Stress Test' – A Tool for Investigating Psychobiological Stress Responses in a Laboratory Setting. *Neuropsychobiology* **28,** 76–81. ISSN: 0302-282X, 1423-0224 (1993).

76. Young, F. L. & Leicht, A. S. Short-term stability of resting heart rate variability: influence of position and gender. *Applied Physiology, Nutrition, and Metabolism* **36,** 210–218. ISSN: 1715-5312 (Apr. 1, 2011).

77. Dedovic, K. *et al.* The Montreal Imaging Stress Task: using functional imaging to investigate the effects of perceiving and processing psychosocial stress in the human brain. *Journal of Psychiatry and Neuroscience* **30,** 319–325. ISSN: 1180-4882 (Sept. 2005).

78. Voellmin, A. *et al.* Blunted endocrine and cardiovascular reactivity in young healthy women reporting a history of childhood adversity. *Psychoneuroendocrinology. This issue includes a Special Section on Biomarkers in the Military - New Findings from Prospective Studies* **51,** 58–67. ISSN: 0306-4530 (Jan. 2015).

79. Sullivan, R. M. & Gratton, A. Prefrontal cortical regulation of hypothalamic–pituitary–adrenal function in the rat and implications for psychopathology: side matters. *Psychoneuroendocrinology* **27,** 99–114. ISSN: 0306-4530 (Jan. 2002).

80. McKlveen, J. M., Myers, B. & Herman, J. P. The Medial Prefrontal Cortex: Coordinator of Autonomic, Neuroendocrine and Behavioural Responses to Stress. *Journal of Neuroendocrinology* **27,** 446–456. ISSN: 1365-2826 (June 1, 2015).

81. Wallis, J. D. Cross-species studies of orbitofrontal cortex and value-based decision-making. *Nature Neuroscience* **15,** 13–19. ISSN: 1097-6256 (Jan. 2012).

82. Cintra, A. *et al.* Mapping and computer assisted morphometry and microdensitometry of glucocorticoid receptor immunoreactive neurons and glial cells in the rat central nervous system. *Neuroscience* **62,** 843–897. ISSN: 0306-4522 (Oct. 1994).

83. Ratka, A., Sutanto, W., Bloemers, M. & Kloet, R. d. On the Role of Brain Mineralocorticoid (Type I) and Glucocorticoid (Type II) Receptors in Neuroendocrine Regulation. *Neuroendocrinology* **50,** 117–123. ISSN: 0028-3835, 1423-0194 (1989).

84. Francis, D. *et al.* The Role of Early Environmental Events in Regulating Neuroendocrine Development: Moms, Pups, Stress, and Glucocorticoid Receptors. *Annals of the New York Academy of Sciences* **794,** 136–152. ISSN: 1749-6632 (Sept. 1, 1996).

85. Thayer, J. F., Hall, M., Sollers III, J. J. & Fischer, J. E. Alcohol use, urinary cortisol, and heart rate variability in apparently healthy men: Evidence for impaired inhibitory control of the HPA axis in heavy drinkers. *International Journal of Psychophysiology. Cortisol and the Addictions* **59,** 244–250. ISSN: 0167-8760 (Mar. 2006).

86. Thayer, J. F. & Sternberg, E. Beyond Heart Rate Variability. *Annals of the New York Academy of Sciences* **1088,** 361–372. ISSN: 1749-6632 (Nov. 1, 2006).

87. Kirschbaum, C., Kudielka, B. M. M., Gaab, J. M., Schommer, N. C. M. & Hellhammer, D. H. Impact of Gender, Menstrual Cycle Phase, and Oral Contraceptives on the Activity of the Hypothalamus-Pituitary-Adrenal Axis. *Psychosomatic Medicine* **61,** 154–162. ISSN: 0033-3174 (Apr. 1999).

88. Sato, N., Miyake, S., Akatsu, J. & Kumashiro, M. Power spectral analysis of heart rate variability in healthy young women during the normal menstrual cycle. *Psychosomatic Medicine* **57,** 331–335 (1995).

89. Stein, P. K., Kleiger, R. E. & Rottman, J. N. Differing Effects of Age on Heart Rate Variability in Men and Women. *The American Journal of Cardiology* **80,** 302–305. ISSN: 0002-9149 (Aug. 1, 1997).

90. Yildirir, A., Kabakci, G., Akgul, E., Tokgozoglu, L. & Oto, A. The effects of menstrual cycle on cardiac autonomic innervation as assessed by heart rate variability. *Journal of the American College of Cardiology* **39,** 208. ISSN: 0735-1097 (Mar. 6, 2002).

91. Rohleder, N. & Kirschbaum, C. The hypothalamic–pituitary–adrenal (HPA) axis in habitual smokers. *International Journal of Psychophysiology. Cortisol and the Addictions* **59,** 236–243. ISSN: 0167-8760 (Mar. 2006).

92. Manetti, L., Cavagnini, F., Martino, E. & Ambrogio, A. Effects of cocaine on the hypothalamic–pituitary–adrenal axis. *Journal of Endocrinological Investigation* **37,** 701–708. ISSN: 1720-8386 (May 23, 2014).

93. Zhang, J. Effect of Age and Sex on Heart Rate Variability in Healthy Subjects. *Journal of Manipulative and Physiological Therapeutics* **30,** 374–379. ISSN: 0161-4754 (June 2007).

94. Karason, K., Mølgaard, H., Wikstrand, J. & Sjöström, L. Heart rate variability in obesity and the effect of weight loss. *The American Journal of Cardiology* **83,** 1242–1247. ISSN: 0002-9149 (Apr. 15, 1999).

95. Casu, M. *et al.* Spectral analysis of R-R interval variability by short-term recording in anorexia nervosa. *Eating and Weight Disorders - Studies on Anorexia, Bulimia and Obesity* **7,** 239–243. ISSN: 1124-4909, 1590-1262 (Sept. 1, 2002).

96. Poliakova, N. *et al.* Influence of obesity indices, metabolic parameters and age on cardiac autonomic function in abdominally obese men. *Metabolism* **61,** 1270–1279. ISSN: 0026-0495 (Sept. 2012).

97. Kroenke, K., Spitzer, R. L. & Williams, J. B. W. The PHQ-9. *Journal of General Internal Medicine* **16,** 606–613. ISSN: 1525-1497 (Sept. 1, 2001).

98. Swinson, R. P. The GAD-7 scale was accurate for diagnosing generalised anxiety disorder. *Evidence Based Medicine* **11,** 184–184. ISSN: , 1473-6810 (Dec. 1, 2006).

99. Figueredo, A. J. *et al.* Consilience and Life History Theory: From genes to brain to reproductive strategy. *Developmental Review. Evolutionary Developmental Psychology* **26,** 243–275. ISSN: 0273-2297 (June 2006).

100. Schulz, P. & Schlotz, W. Trierer Inventar zur Erfassung von chronischem Streß (TICS): Skalenkonstruktion, teststatistische Überprüfung und Validierung der Skala Arbeitsüberlastung. *Diagnostica* **45,** 8–19. ISSN: 0012-1924 (Jan. 1, 1999).

101. Kumar, Y., Agarwal, V. & Gautam, S. Heart Rate Variability During Examination Stress in Medical Students. *International Journal of Physiology* **1,** 83–86. ISSN: 23206039 (June 2013).

102. Beck, A. T., Ward, C. H., Mendelson, M. M., Mock, J. J. & Erbaugh, J. J. An inventory for measuring depression. *Archives of General Psychiatry* **4,** 561–571. ISSN: 0003-990X (June 1, 1961).

103. Beck, A. T., Steer, R. A. & Brown, G. K. *Manual for the Beck Depression Inventory–II* (The Psychological Corporation, San Antonio, TX, 1996).

104. Speilberger, C. D., Gorsuch, R. L. & Lushene, R. E. *Manual for the State-Trait Anxiety Inventory* (Consulting Psychologists Press, Pal Alto, CA, 1970).

105. Chu, D. A., Williams, L. M., Harris, A. W. F., Bryant, R. A. & Gatt, J. M. Early life trauma predicts self-reported levels of depressive and anxiety symptoms in nonclinical community adults: Relative contributions of early life stressor types and adult trauma exposure. *Journal of Psychiatric Research* **47,** 23–32. ISSN: 0022-3956 (Jan. 2013).

106. Rosenberg, M. *Society and the adolescent self-image* (Princeton University Press, Princton, NJ, 1965).

107. Pruessner, J. C., Lord, C., Meaney, M. & Lupien, S. Effects of Self-Esteem on Age-Related Changes in Cognition and the Regulation of the Hypothalamic-Pituitary-Adrenal Axis. *Annals of the New York Academy of Sciences* **1032,** 186–194. ISSN: 1749-6632 (Dec. 1, 2004).

108. Lupis, S. B., Sabik, N. J. & Wolf, J. M. Role of shame and body esteem in cortisol stress responses. *Journal of Behavioral Medicine* **39,** 262–275. ISSN: 0160-7715, 1573-3521 (Nov. 17, 2015).

109. Heatherton, T. & Polivy, J. Development and Validation of a Scale for Measuring State Self-Esteem. *Journal of Personality and Social Psychology* **60.** WOS:A1991FQ40200007, 895–910. ISSN: 0022-3514 (June 1991).

110. Dressendörfer, R. A., Kirschbaum, C., Rohde, W., Stahl, F. & Strasburger, C. J. Synthesis of a cortisol-biotin conjugate and evaluation as a tracer in an immunoassay

for salivary cortisol measurement. *The Journal of Steroid Biochemistry and Molecular Biology* **43,** 683–692. ISSN: 0960-0760 (Dec. 1992).

111. Lorentz, K., Gütschow, B. & Renner, F. Evaluation of a Direct α-Amylase Assay Using 2-Chloro-4-nitrophenyl-α-D-maltotrioside. *Clinical Chemistry and Laboratory Medicine* **37,** 1053–1062 (2005).

112. Berntson, G. G. *et al.* Heart rate variability: Origins, methods, and interpretive caveats. *Psychophysiology* **34,** 623–648. ISSN: 1469-8986 (Nov. 1, 1997).

113. Electrophysiology, T. F. o. t. E. S. o. C. t. N. A. S. o. P. Heart Rate Variability. *Circulation* **93,** 1043–1065. ISSN: 0009-7322, 1524-4539 (Mar. 1, 1996).

114. Clifford, G. D. & Tarassenko, L. Quantifying errors in spectral estimates of HRV due to beat replacement and resampling. *IEEE Transactions on Biomedical Engineering* **52,** 630–638. ISSN: 0018-9294 (Apr. 2005).

115. Penttilä, J. *et al.* Time domain, geometrical and frequency domain analysis of cardiac vagal outflow: effects of various respiratory patterns. *Clinical Physiology* **21,** 365–376. ISSN: 1365-2281 (May 14, 2001).

116. Berntson, G. G., Lozano, D. L. & Chen, Y.-J. Filter properties of root mean square successive difference (RMSSD) for heart rate. *Psychophysiology* **42,** 246–252. ISSN: 1469-8986 (Mar. 1, 2005).

117. Singh, D., Vinod, K. & Saxena, S. C. Sampling frequency of the RR interval time series for spectral analysis of heart rate variability. *Journal of Medical Engineering & Technology* **28,** 263–272. ISSN: 0309-1902 (Dec. 2004).

118. Pruessner, J. C., Kirschbaum, C., Meinlschmid, G. & Hellhammer, D. H. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology* **28,** 916–931. ISSN: 0306-4530 (Oct. 2003).

119. Rikhye, K. *et al.* Interplay between childhood maltreatment, parental bonding, and gender effects: Impact on quality of life. *Child abuse & neglect* **32,** 19–34. ISSN: 0145-2134 (Jan. 2008).

120. Luger, A. *et al.* Acute Hypothalamic–Pituitary–Adrenal Responses to the Stress of Treadmill Exercise. *New England Journal of Medicine* **316,** 1309–1315. ISSN: 0028-4793 (May 21, 1987).

121. Nakagawa, S. & Schielzeth, H. A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution* **4,** 133–142. ISSN: 2041-210X (Feb. 1, 2013).

122. Reyes del Paso, G. A., Langewitz, W., Mulder, L. J. M., van Roon, A. & Duschek, S. The utility of low frequency heart rate variability as an index of sympathetic cardiac tone: A review with emphasis on a reanalysis of previous studies. *Psychophysiology* **50,** 477–487. ISSN: 1469-8986 (May 1, 2013).

123. Samson, A., Lavielle, M. & Mentré, F. The SAEM algorithm for group comparison tests in longitudinal data analysis based on non-linear mixed-effects model. *Statistics in Medicine* **26,** 4860–4875. ISSN: 1097-0258 (Nov. 30, 2007).

124. Sumner, J. A., McLaughlin, K. A., Walsh, K., Sheridan, M. A. & Koenen, K. C. CRHR1 genotype and history of maltreatment predict cortisol reactivity to stress in adolescents. *Psychoneuroendocrinology* **43,** 71–80. ISSN: 0306-4530 (May 2014).

125. Bosch, N. M. *et al.* Timing matters: Long term effects of adversities from prenatal period up to adolescence on adolescents' cortisol stress response. The TRAILS study. *Psychoneuroendocrinology* **37,** 1439–1447. ISSN: 0306-4530 (Sept. 2012).

126. Iakunchykova, O. P. *et al.* The impact of early life stress on risk of tobacco smoking initiation by adolescents. *Addictive Behaviors* **50,** 222–228. ISSN: 0306-4603 (Nov. 2015).

127. Kaufman, D. *et al.* Early-Life Stress and the Development of Obesity and Insulin Resistance in Juvenile Bonnet Macaques. *Diabetes* **56,** 1382–1386. ISSN: 0012-1797, 1939-327X (May 1, 2007).

128. Daskalakis, N. P., Bagot, R. C., Parker, K. J., Vinkers, C. H. & de Kloet, E. R. The three-hit concept of vulnerability and resilience: Toward understanding adaptation to early-life adversity outcome. *Psychoneuroendocrinology* **38,** 1858–1873. ISSN: 0306-4530 (Sept. 2013).

129. Bozhokin, S. V. & Suslova, I. B. Analysis of non-stationary HRV as a frequency modulated signal by double continuous wavelet transformation method. *Biomedical Signal Processing and Control* **10,** 34–40. ISSN: 1746-8094 (Mar. 2014).

130. Toledo, E., Gurevitz, O., Hod, H., Eldar, M. & Akselrod, S. Wavelet analysis of instantaneous heart rate: a study of autonomic control during thrombolysis. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* **284,** R1079–R1091. ISSN: 0363-6119, 1522-1490 (Apr. 1, 2003).

131. Barbieri, R., Matten, E. C., Alabi, A. A. & Brown, E. N. A point-process model of human heartbeat intervals: new definitions of heart rate and heart rate variability. *American Journal of Physiology - Heart and Circulatory Physiology* **288,** H424–H435. ISSN: 0363-6135, 1522-1539 (Jan. 1, 2005).