Title: Heart rate variability and salivary cortisol in very preterm children during school age

Author: Natalie Urfer-Maurer [a], Sebastian Ludyga [b], Tobias Stalder [c], Serge Brand [b,d,e], Edith Holsboer-Trachsler [d], Markus Gerber [b], Alexander Grob [a], Peter Weber [f], Sakari Lemola [g]*

[a] University of Basel, Department of Psychology, Basel, Switzerland
[b] University of Basel, Department of Sport, Exercise and Health, Sport Sciences Section, Basel, Switzerland
[c] University of Siegen, Clinical Psychology, Siegen, Germany
[d] University of Basel Psychiatric Clinics, Center for Affective, Stress, and Sleep Disorders, Basel, Switzerland
[e] Kermanshah University of Medical Sciences (KUMS), Sleep Disorders Research Center; Kermanshah, Iran
[f] University Children's Hospital Basel, Division of Neuropediatrics and Developmental Medicine, Basel, Switzerland
[g] University of Warwick, Department of Psychology, Coventry, United Kingdom

Running title: HRV and salivary cortisol in very preterm children

* Corresponding author: Sakari Lemola, PhD
Department of Psychology
University of Warwick
University Road
Coventry, CV4 7AL, UK
Tel. +44 24 765 23177
Fax +44 24 765 24225
s.lemola@warwick.ac.uk

Summary

The autonomic nervous system (ANS) plays a major role in the human stress response and reflects physical and psychological adaptability to a changing environment. Long-term exposure to early life stressors may alter the function of the ANS. The present study examines differences in the ANS between children born very preterm and full-term as well as the association between the ANS and the hypothalamic-pituitary-adrenal (HPA) axis, the other main branch of the human stress system.

Fifty-four healthy children born very preterm (<32nd gestational week) and 67 full-term children aged 7–12 years provided data for the present study. Polysomnography (PSG) assessments were obtained during a night at the children's home in lying position at rest (wake) and during different sleep stages (stage 2 sleep, slow wave sleep, rapid-eye-movement sleep). Autonomic function was assessed by use of heart rate variability, specifically low frequency power (LF), high frequency power (HF), total spectral power (Tot Pow), and the LF/HF ratio. HPA axis activity was measured using salivary cortisol the next morning at awakening, 10, 20, and 30 min later.

Children born very preterm had lower LF/HF ratio during wake and stage 2 sleep compared to full-term children. Moreover, higher LF, Tot Pow, and LF/HF ratio during wake, stage 2 sleep, and REM sleep were related to more post-awakening cortisol secretion.

The present study provides evidence on long-term ANS alterations after very preterm birth. Moreover, findings suggest a relation between the ANS and the HPA axis and therefore support the notion of mutual feedback between the two human stress systems.

Keywords: preterm birth; heart rate variability; HPA axis activity, salivary cortisol; school-aged children; polysomnography

1. Introduction

The sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis are the two main branches involved in the human stress response. During acute stress, the autonomic nervous system (ANS), consisting of the SNS and the parasympathetic nervous system (PNS), induces immediate rapid bodily changes through modulation of noradrenergic and cholinergic neuronal communication and the quick release of adrenaline via the sympatho-adrenal medullary system (SAM; Charmandari et al., 2005; Stratakis and Chrousos, 1995). The SNS is involved in the so-called 'fight or flight' response – the immediate reaction to a stressor, while the complementary PNS regulates 'rest and digest' processes. In addition to stress response modulation, the ANS is involved in the regulation of various physiological functions, such as the heart rate. Autonomic function can be measured by electrocardiography (ECG) and the assessment of heart rate variability (HRV; Shaffer et al., 2014). HRV describes the change in beat-to-beat intervals over time and can be separated into different frequency domain bands, with low frequency power (LF: 0.04–0.15 Hz) reflecting a combination of sympathetic and parasympathetic nervous system activity, high frequency power (HF: 0.15–0.4 Hz) predominantly reflecting parasympathetic activity, total spectral power (Tot Pow: 0.0033-0.40 Hz) reflecting the global ANS activity, and the LF/HF ratio reflecting sympathovagal balance (Stein and Pu, 2012; Task Force, 1996). These frequency-domain HRV indices are assumed to be trait-like markers of autonomic function (Montano et al., 2009) and a change towards increased LF/HF ratio or decreased HF reflecting a dominance of sympathetic over parasympathetic activity has been shown to be associated with poor physical and mental health (Acharya et al., 2006; Friedmann, 2007). However, also overly increased HRV may be non-optimal since it may reflect non-efficient physiological functioning and energy utilization (Shaffer et al., 2014). The role of the balance between sympathetic behavioral activation and parasympathetic inhibition for behavioral and emotional regulation is object of theoretical models including the polyvagal and the neurovisceral model (Porges, 1995; 2007; Thayer et al., 2009). In their core, both models propose that fine-tuning of the parasympathetic system is important for psychosocial adjustment to a changing environment.

The ANS matures during gestation and the first months after birth (David et al., 2007; Sahni et al., 2000) and stress during these sensitive phases may lead to long–term programming of the ANS (Fyfe et al., 2014). One major stress factor during this phase that occurs in around 10% of all children worldwide is preterm birth (Blencowe et al., 2013). In particular, children born very preterm are endowed with immature organs and are exposed to several illness-related adversities and invasive treatments (Lemola, 2015; Roberts and Dalziel, 2006). Later in their lives, very preterm children are more likely to suffer from physical disorders and psychological disturbances, such as depression, burnout, and anxiety disorders (Aarnoudse-Moens et al., 2009; Aylward, 2005; Lemola, 2015), which, in turn, may be associated with alterations in autonomic function (Kanthak et al., 2017; Licht et al., 2008; van Gestel and Steier, 2011). Compared to their peers born at term, infants born preterm show decreased HRV right after birth and at theoretical term (Landrot et al., 2007; Patural et al., 2008). These changes in HRV indicate a reduced regulatory capacity, so that infants born preterm may have more difficulty in adaptively responding to environmental stressors (Shaffer et al., 2014). Regarding the question how preterm birth affects ANS development at a later age up to 7 years, the evidence is more mixed. Specifically, studies reporting that preterm children either exhibited higher LF/HF ratio (Fyfe et al., 2015), lower LF (Yiallourou et al., 2013), or lower HF (Fyfe et al., 2015; Yiallourou et al., 2013), or failed to reveal differences in frequency domains at all (Fyfe et al., 2015; Landrot et al., 2007; Yiallourou et al., 2013). In addition, a recent study by Rakow et al. (2013) including nine-year old children showed rather global HRV reductions, characterized by lower very low frequency power (VLF), LF, HF, Tot Pow, and a trend towards a lower LF/HF ratio, in children born very preterm and small for gestational age (SGA) full-terms. The above–mentioned inconsistent findings regarding HRV differences between children born preterm and full-term might be due to differences in the age ranges studied as well as differences in the measurement setting, which might introduce variance to the findings. Specifically, studies with infants measured HRV during sleep (Fyfe et al., 2015; Yiallourou et al., 2013), while studies including older children conducted ECGs over a 24h period (Landrot et al., 2007; Rakow et al. 2013).

The second arm of the human stress system, the HPA axis, reacts more slowly by regulating the secretion of glucocorticoids, including cortisol (Clements, 2013) and is supposed to play a major role in chronic stress (Miller et al., 2007). The HPA axis can be measured through salivary cortisol assessments. Cortisol secretion increases across the first 30–45min after morning awakening, a

phenomenon termed the cortisol awakening response (CAR; Clow et al., 2004; Stalder et al., 2016). There is evidence that an increased CAR is associated with stress and a reduced CAR with fatigue, burnout, and exhaustion, despite considerable heterogeneity in findings (Chida and Steptoe, 2009). As both the HRV and the HPA axis are proposed to be trait-like characteristics that play a major role for emotional adaptability and regulation (Porges, 1995; 2007; Thayer et al., 2000; Chida and Steptoe, 2009), it is an important question how these two systems are interrelated. An earlier study by Stalder et al. (2011) on the relationship between HRV and the CAR found that lower global HRV was related to an elevated CAR in young adults. Interestingly, these CAR-HRV associations were consistently found for HRV assessments taken in a laboratory setting and over the pre- and post-awakening periods as well as for measures of overall HRV, LF, and HF (Stalder et al., 2011). There are only few studies, which have examined the relation between ANS and HPA axis activity in children. One study by Michels et al. (2013), including children aged 5–10 years, reported higher LF and LF/HF ratio during 10 min in supine position in a quiet room in the afternoon to be associated with a larger CAR. However, another study by Rotenberg and McGrath (2016) found no such associations in children and adolescents aged 8–18 years conducting 24h ECGs.

A first aim of the present study was thus to examine potential differences in HRV between school-age children born very preterm and those born full-term by measuring HRV during a wake episode in a lying position at rest before sleep onset and during sleep. There are two advantages of measuring HRV separately in the evening before sleep as well as during night sleep within different sleep stages. First, this involves a more standardized measurement situation across participants compared to 24h ambulatory assessments, since HRV is assessed during the same circadian phase and sleep stages, which may reduce the acute impact of daily experience on HRV measurement. Second, this strategy allows us to determine whether differences between very preterm and full-term children are consistent across these different psychophysiological states. Following Rakow et al. (2013), we examined HRV parameters in children born very preterm and full-term. In addition to prior studies (Landrot et al., 2007; Rakow et al. 2013), we measured HRV parameters in a lying position at rest before sleep onset and during separate sleep stages (stage 2 sleep, slow wave sleep [SWS], and rapid eye movement [REM] sleep). A second aim was to test the association between the ANS (measured through HRV) and HPA axis activity (measured as the post-awakening cortisol secretion in the morning after HRV assessment). Thereby, we address the apparent gap in research regarding the association between HRV and HPA axis activity in school-aged children.

2. Methods

2.1. Study population and procedure

The data for the present study comes from the second wave (May 2013 – September 2014) of the Basel Study of Preterm Children (BSPC). Recruitment procedures have been described elsewhere in detail (see Perkinson-Gloor et al. [2015], and Lemola et al. [2015], for reports on the first study wave and Urfer-Maurer et al. [in press] and Maurer et al. [2016] for reports on the second study wave, including results regarding HPA axis activity in children born very preterm). In total, 54 (44.6%) healthy very preterm children (<32 weeks of gestation; age: $M = 9.62$ years, $SD = 1.36$; range: 7.33 to 12.33 years) and 67 (55.4%) age and sex matched full-term children (age: $M = 9.66$ years, $SD = 1.51$; range: 7.5 to 12.92 years; see Table 1) are included in the present report as they had one night of in-home electroencephalography (EEG) and readable electrocardiogram (ECG) data. Children born very preterm were recruited from an initial cohort of 260 prematurely born children treated at the University Children's Hospital Basel (Switzerland). Full-term children were recruited from official birth notification. For each participant, parents gave written informed consent and assent was obtained from the child. Ethical approval was obtained from the Ethics Committee of Basel (Basel, Switzerland, 122/11) and the study was performed in accordance with the ethical standards laid down in the Declaration of Helsinki.

2.2. Variables

2.2.1. HRV assessment and analysis

Using Compumedics Somté PSG during a single night at the children's home, electrocardiogram was recorded in lying position at rest without movement (wake) and during sleep. The amplified signal was low-pass filtered (30 Hz) and digitized at 256 Hz. Collected data was exported to Kubios HRV Analysis Software 2.1 (Matlab, Kuopio, Finland) for offline processing and analysis. Procedures were

in line with the HRV analysis guidelines (Task Force, 1996) and the expert performing the analysis was blinded to the group allocation (prematurity status). Based on the adaptive QRS detection algorithm of Kubios (Tarvainen et al., 2014), R-wave time instants were automatically detected and verified by visual inspection. The resulting HRV time series were converted to equidistantly sampled series by using a 4 Hz cubic spline interpolation. To remove slow nonstationary trends from the signal, a linear detrend correction based on smoothness priors regularization (0.001 hz cut-off) was applied to the R-R series (Tarvainen et al., 2002). Frequency domain measures were estimated from short-term recordings collected during rest in bed before sleep onset (15 min), stage 2 sleep, and SWS as well as REM sleep. The classification of sleep stages was based on polysomnographic assessments (see 2.2.3). For each HRV recording period, five consecutive 5 min segments (25 min) containing no changes in sleep stages, artifacts or erratic heart beats were averaged. If more than one block of segments fulfilled these criteria, the first valid epoch after sleep onset was selected for further analysis. Averaged segments were subjected to Fast-Fourier transform by using Welch's periodogram (300 s with 50 % overlap) and divided into very low frequency (VLF: 0–0.04 Hz), low frequency (LF: 0.04–0.15 Hz), and high frequency (HF: 0.15–0.4 Hz) bands. In addition, LF/HF power ratio and the total spectral power were calculated. The LF band is modulated by a combination of sympathetic and parasympathetic nervous system inputs, whereas the HF is considered to reflect parasympathetic activity (Acharya et al., 2006; Stein and Pu, 2012).

2.2.2. Post-awakening cortisol secretion assessment

Parents collected four saliva samples from their children at 0, 10, 20, and 30 min after the child's awakening on a normal school-day. Children were not allowed to eat, drink, and brush their teeth until saliva sampling was completed. Saliva samples were collected using the "Salivette" device (Sarstedt, Nümbrecht/Germany). Analyses of free salivary cortisol concentrations were conducted with a time-resolved immunoassay with fluorometric detection "Coat-A-Count" Cortisol RIA from DPC (Diagnostics Products Corporation; obtained through H. Biermann GmbH, Bad Nauheim, Germany). Statistical analyses were conducted according to the published guidelines on post-awakening cortisol assessment (Stalder et al., 2016). The level of cortisol on awakening (S1), the area-under-the-curve with respect to increase (AUC_I) and with respect to ground (AUC_G; Pruessner et al., 2003) were estimated to quantify different aspects of post-awakening cortisol secretion. Salivary cortisol variables were log-transformed before building AUC_G and AUC_I. The endpoint of the pre-awakening cortisol increase was defined as S1 and the cortisol awakening response (CAR) was interpreted as AUC_I. In addition, the overall post-awakening cortisol secretion was defined as AUC_G (Stalder et al., 2016).

To evaluate the time point of saliva sampling, parents reported children's light-on-time (children woke up naturally or were woken up by alarm clock or by their parents; mode of awakening was not separately assessed) and awakening time was additionally measured by PSG. However, the time-point of saliva sampling was not additionally measured by electronic time stamp. Analyses including salivary cortisol were controlled for the difference between parent-reported light-on-time/cortisol sampling time and awakening time according to PSG ('parent reported light-on-time/cortisol sampling time' – 'in-home PSG awakening time' > 5 min; Stalder et al., 2016). Season and awakening time as measured by PSG were additionally controlled for. Please see supplemental Table S1 for further information regarding control variables according to the CAR consensus guidelines (Stalder et al., 2016).

2.2.3. Sleep assessment

Children's sleep was assessed using the Compumedics Somté PSG during a single night at the children's home. Polysomnogram signals C3/A2 and C4/A1 EEG, right and left electrooculogram and bipolar submental electromyogram were obtained. Two experienced raters analyzed the EEG recordings according to the standard procedures (Rechtschaffen and Kales, 1968).

2.3. Statistical analysis

To test our first hypothesis, we performed analyses of covariance (ANCOVA) with prematurity status as independent and frequency-domain HRV measures (LF, HF, Tot Pow, LF/HF ratio) as dependent variables. Moreover, we performed repeated-measures ANCOVAs with HRV indices during the different sleep stages (wake, stage 2 sleep, SWS, REM sleep) as within subject factor and prematurity status as fixed factor. Analyses regarding differences in HRV were controlled for children's age, sex,

BMI, and first language, since these variables were correlated to HRV parameters. Effect sizes were calculated following Cohen (1969, 1988) with d = 0.20 and η^2_p = 0.01 indicating small, d = 0.50 and η^2_p = 0.06 indicating medium, and d = 0.80 and η^2_p = 0.14 indicating large effect sizes. To test our second hypothesis, multiple regression analyses with HRV parameters as independent variable and salivary cortisol as dependent variable were calculated, entering one HRV parameter and the covariates at a time. Bootstrap procedures were applied for robust estimation of standard errors (Chernick, 2008). Statistical analyses were performed with IBM® SPSS® Statistics 22 (IBM Corporation, Armonk NY, USA) for Apple Mac®.

3. Results

3.1. Preliminary analyses

Table 1 shows descriptive statistics and Pearson correlations of child age and sex with HRV indices and post-awakening cortisol secretion. Child age was negatively related to ECG-derived respiration (EDR) during sleep stage 2, SWS, and REM sleep. Girls showed higher LF, HF, and Tot Pow during wake and more EDR during REM sleep than boys. Regarding post-awakening cortisol secretion, girls had a smaller AUC_I than boys.

Regarding intra-individual changes in HRV during the transition from wake to stage 2 sleep, SWS, and REM sleep there was a significant quadratic effect regarding LF ($F(1,120) = 51.13$, $p < .001$, $\eta^2_p = .30$), Tot Pow ($F(1,120) = 40.86$, $p < .001$, $\eta^2_p = .25$), and LF/HF ratio ($F(1,120) = 255.73$, $p < .001$, $\eta^2_p = .68$). The shape of this quadratic relationship was such that LF, Tot, and LF/HF ratio decreased from wake to stage 2 sleep and SWS, which was followed by a somewhat less pronounced increase in the transition to REM sleep (see Figure 1). Regarding HF, there was a significant cubic effect ($F(1,120) = 30.64$, $p < .001$, $\eta^2_p = .20$), since HF increased with the transition from wake to stage 2 sleep, however, decreased during SWS and increased again during REM-sleep.

3.2. Differences in HRV between children born very preterm and full-term

Table 2 shows differences in HRV parameters between children born very preterm and full-term. Very preterm children had a lower LF/HF ratio during wake and during stage 2 sleep compared to full-term children. There were no differences in any of the HRV parameters during SWS and REM sleep between children born very preterm and full-term.

Regarding sleep stage × prematurity status interactions on HRV parameters, there was a significant interaction on LF/HF ratio by the transition from wake to stage 2 sleep and SWS. Moreover, there was a significant interaction on LF, HF, and Tot Pow by the transition from stage 2 sleep to SWS and REM sleep. Results are displayed in Figure 1.

3.3. Association between HRV and post-awakening cortisol secretion

Table 3 shows associations between HRV parameters and post-awakening cortisol secretion. Higher LF during wake, stage 2 sleep, and REM sleep and higher LF/HF ratio during wake and higher Tot Pow during REM sleep were associated with more AUC_G. Higher LF and Tot Pow during stage 2 sleep were related to more AUC_I. Moreover, higher LF/HF ratio during wake was associated with more S1. No other significant relation between HRV parameters and post-awakening cortisol secretion was found.

4. Discussion

This is the first study that examined HRV differences in school-aged very preterm and full-term born children during different sleep stages and considering the association between HRV parameters and HPA axis activity. The main findings were that children born very preterm had lower LF/HF ratio compared to full-term children during wake and stage 2 sleep. Moreover, across the whole sample, there was some indication that higher LF, Tot Pow, and LF/HF ratio at wake and during sleep were associated with increased overall morning cortisol secretion.

4.1. Differences in HRV between children born very preterm and full-term

Children born very preterm had a lower LF/HF ratio during wake and during stage 2 sleep compared to children born full-term, which is in line with Rakow et al. (2013) who found a tendency towards a lower LF/HF ratio in 9 year old children born very preterm and SGA full-term. However, and in

contrast to Rakow et al (2013), no differences were found in the other frequency domain parameters (LF, HF, and Tot Pow) between children born very preterm and full-term. As a difference between the two studies, Rakow et al. (2013) conducted 24h ECG, while we measured HRV parameters in lying position at rest without movement (wake) and during different sleep stages. Interestingly, Rakow et al. (2013) concluded that the HRV differences were due to processes associated with low birth weight rather than with gestational age, since they found no differences between children born very preterm and full-term SGA. Based on our sample, it is not possible to address this assumption, since no separate sample of SGA children was included in our study.

Differences in maturation of the two ANS branches could account for differences in LF/HF ratio between children born very preterm and full-term. During prenatal life sympathetic activity is predominant, while parasympathetic activity begins to increase rapidly between 25 and 32 weeks of gestation (David et al., 2007; Schneider et al., 2009). However, children born very preterm spend this crucial period outside of their mother's womb in the neonatal intensive care unit, where environmental conditions for the development of the ANS are markedly different. Children born very preterm are exposed to many distressing events after birth (Lemola, 2015; Roberts and Dalziel, 2006), which might provoke increased sympathetic activation. In this vein, previous studies focusing on preterm children at the beginning of their lives, reported less parasympathetic and more sympathetic activity (Landrot et al., 2007; Patural et al., 2008). During later development, there is evidence of a steady increase in parasympathetic activity compared to sympathetic activity until age 6-7 years, which is reflected in a declining LF/HF ratio. This decline was more pronounced in preterm than in full-term children (Landrot et al., 2007). A possible explanation for a reduced sympathetic activity in later life is a persistently down-regulation of the sympathetic arm of the ANS in a similar vein as it has been discussed for the other human stress system – the HPA axis (Feng et al., 2011; Kaseva et al., 2014; Maurer et al., 2016) – in response to the excessive activation in early life eventually leading to parasympathetic activity predominance. The assumption of long-term sympathetic down-regulation by early-life stress is further in line with evidence from twin studies showing that environmental factors play a major role for cardiovascular autonomic function (e.g. Osztovits et al., 2011). It is possible, that epigenetic mechanisms are involved in long-term programming of the cardiovascular autonomic function (Esler et al., 2008).

Regarding intra-individual changes in HRV during the transition from wake to SWS, sympathetic activity decreased progressively, while parasympathetic activity increased from wake to stage 2 sleep. From SWS to REM sleep sympathetic activity increased again. This is in line with previous research showing a decreased sympathetic activity with the transition from wake to non-REM sleep and an increased sympathetic activity with the transition from non-REM to REM sleep (Trinder et al., 2001; Versace et al., 2003). This pattern reflects the notion of increasing relaxation with the progression to deeper sleep stages.

With the transition from wake to stage 2 sleep and SWS children born very preterm showed a flatter decrease in LF/HF ratio than children born full-term. Relatedly, children born very preterm showed a flatter decrease in LF and Tot Pow from stage 2 sleep to SWS and a flatter increase from SWS to REM-sleep. Regarding HF, full-term children showed a stronger decline from stage 2 sleep to SWS and a stronger increase from SWS to REM-sleep than very preterm born children. Our results are in line with studies showing an overall lower wake-time sympathetic activity in children born preterm (e.g. Rakow et al., 2013). However, in SWS full-term children reached approximately the same level of LF, Tot Pow, and LF/HF ratio as very preterm children.

4.2. Association between HRV and post-awakening cortisol secretion

The present study is one of the few studies examining the association between the ANS and the HPA axis. We saw some indication that higher HRV characterized by a shift towards sympathetic dominance (higher LF, Tot Pow, and LF/HF ratio) during wake, stage 2 sleep, and REM sleep is associated with more overall post-awakening salivary cortisol secretion. No association was found between HF and post-awakening cortisol secretion. Our results are in line with Michels et al. (2013), who found higher LF in supine position in the afternoon to be associated with a larger AUC_I. Moreover and in line with Stalder et al. (2011), we found no association between LF/HF ratio and AUC_I. However, our findings are in contrast to Stalder et al. (2011) regarding the association between LF and HF and AUC_I. While they reported that lower LF and HF were related to an increased AUC_I, we found higher LF to be related to an increased AUC_I. Moreover, they found no significant

association between HRV parameters and S1. Differences in age between the studied samples could account for the discrepancies between our results and those from Stalder et al. (2011) who studied young adults (mean age: 23 years), while our study included children aged between 7 to 12 years.

Our results indicate an association between the ANS and the HPA axis – reflected by a co-occurrence of high LF, Tot Pow, and LF/HF ratio during wake, stage 2 sleep, and REM sleep and high post-awakening HPA axis activity. This coincides with evidence that both the ANS activity and the HPA axis are involved in emotion regulation (Porges, 1995; 2007; Thayer et al., 2000) and both have trait-like properties representing relatively stable biological markers for stress and related psychological outcomes. It is for instance known that general life stress and poor physical and mental health are associated with both increased HPA axis activity reflected in a higher AUC_I and AUC_G (Chida and Steptoe, 2009) as well as with a higher LF, Tot Pow, and LF/HF ratio (Acharya et al., 2006; Friedmann, 2007). Moreover, the relationship between ANS activity and the HPA axis may also reflect that they both receive input from the amygdala and hypothalamus (Tafet and Bernardini, 2003), which may align their activity.

4.3. Limitations

The current study is not without limitations. First, it is not possible to draw conclusions regarding causal relations due to the correlative study design. Second, only one night of HRV measurement and one morning of saliva sampling were conducted, which may reduce reliability. Third, while we sought to follow the guidelines of the CAR consensus report (e.g. consideration of objective awakening times and trait-related confounders), there were also some limitations in this regard. The study design did not include any objective verification of saliva sampling times and thus influences of participant non-compliance cannot be fully excluded. Furthermore, we did not have data on the full set of covariates specified in the consensus report available (Stalder et al., 2016). Moreover, for the wake-state measurement HRV parameters were assessed in lying position in the evening before sleep onset. These parameters may therefore not be directly comparable to other wake-state ECG recordings, such as 24h or stress ECGs. Finally, there was no simultaneous HRV and HPA axis activity assessment. Future studies may assess HRV and HPA axis concurrently at different stages of the diurnal cycle and during different situations (e.g. during stress vs. during relaxation) to unravel their diurnal co-variation. Finally, longitudinal assessment of the ANS and the HPA axis activity and their interplay from infancy to childhood in children born preterm and full-term would allow to examine differential trajectories of the two stress systems in children with different levels of early risk exposure.

5. Conclusions

In conclusion, the present study provides evidence of ANS alterations after very preterm birth during later childhood. Our study suggests that children born preterm show an altered balance between sympathetic and parasympathetic activity, with a relative increase in parasympathetic compared to sympathetic activity, during wake and stage 2 sleep, but not during SWS and REM sleep. Moreover, there is an association between HRV parameters during wake and sleep with salivary morning cortisol secretion implying mutual feedback between the SAM and the HPA axis – higher LF, Tot Pow, and LF/HF ratio are related to higher post-awakening cortisol secretion.

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Contributors

Sakari Lemola designed the study. Natalie Maurer and Sakari Lemola executed and supervised the data collection, provided the statistical analyses, and wrote the first draft of the manuscript. Natalie

Maurer, Sebastian Ludyga, Tobias Stalder, Serge Brand, Edith Holsboer-Trachsler, Markus Gerber, Alexander Grob, Peter Weber, and Sakari Lemola contributed to the interpretation of the data, planning the manuscript, internal revision and rewriting of the first draft of the manuscript, and approved the final manuscript.

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Table 1 Descriptive statistics for study variables and Pearson's correlation of child's age and sex with heart rate variability parameters and salivary cortisol secretion (N = 121).

	M/N	*(SD/%)*	*r*	
			Age	Sex
Age, years	9.64	(1.44)		
Sex, male[a]	72	(59.5)		
Prematurity status, born very preterm	54	(44.6)		
Gestational age, weeks	35.25	(5.11)		
HRV indices				
Wake				
LF Pow, ms^2	4238.12	(5125.02)	-.15	.24**
HF Pow, ms^2	2284.38	(2152.74)	-.11	.19*
Tot Pow, ms^2	25548.23	(39309.55)	-.17†	.26**
LF/HF ratio	1.78	(0.82)	-.06	.18†
EDR, Hz	0.23	(0.04)	-.05	.00
Stage 2 sleep				
LF Pow, ms^2	2585.51	(2773.13)	-.13	-.02
HF Pow, ms^2	3866.23	(3157.31)	-.17†	-.07
Tot Pow, ms^2	12498.09	(13247.04)	-.07	-.11
LF/HF ratio	0.84	(0.66)	-.00	-.02
EDR, Hz	0.25	(0.04)	-.26**	.03
SWS				
LF Pow, ms^2	1586.26	(1711.52)	-.13	.07
HF Pow, ms^2	3005.10	(2667.87)	-.07	.01
Tot Pow, ms^2	7656.72	(8416.52)	-.15†	.11
LF/HF ratio	0.67	(0.54)	.01	.01
EDR, Hz	0.26	(0.05)	-.29**	.07
REM sleep				
LF Pow, ms2	2743.49	(2618.58)	.02	-.03
HF Pow, ms2	3586.34	(2792.48)	-.00	.01
Tot Pow, ms2	13605.89	(15027.19)	.03	-.03
LF/HF ratio	1.03	(0.75)	-.02	-.06
EDR, Hz	0.25	(0.04)	-.22*	.21*
Salivary morning cortisol secretion[b]				
AUC_G	0.51	(0.07)	.01	.03
AUC_I	0.57	(0.07)	-.08	-.19*
First sample (S1)	0.87	(0.19)	.06	.16†
Second sample	0.98	(0.17)	-.01	.05
Third sample	1.09	(0.15)	-.05	-.06
Fourth sample	1.05	(0.20)	.07	.01

Note: SWS: slow wave sleep, REM: rapid eye movement, AUC_G: area-under-the-concentration-time-curve with respect to the ground, AUC_I: area-under-the-concentration-time-curve with respect to the increase.

[a] Coding of sex: 1 = male; 2 = female

[b] Log-transformed before building AUC_G/AUC_I

†p < .1, *p < .05, **p < .01 (two-tailed).

Table 2 Heart rate variability parameters in very preterm and full-term children.

	Very preterm (n = 54)			Full-term (n = 67)			d	p^a
	M	*(SD)*	*BC 95%-CI*[a]	*M*	*(SD)*	*BC 95%-CI*[a]		
Wake								
LF Pow, ms^2	3514.57	3755.39	[2638.21, 4522.33]	4821.28	5971.38	[3524.30, 6293.15]	0.27	.172
HF Pow, ms^2	2401.25	2258.27	[1859.69, 3008.39]	2190.19	2076.17	[1718.73, 2693.39]	0.14	.463
Tot Pow, ms^2	18968.22	25435.34	[12884.98, 25856.73]	30851.53	47180.80	[20579.03, 42452.38]	0.32	.093
LF/HF ratio	1.62	0.95	[1.39, 1.86]	1.91	0.68	[1.76, 2.07]	0.41	.047
EDR, Hz	0.24	0.04	[0.23, 0.25]	0.23	0.04	[0.22, 0.24]	0.37	.072
Stage 2 sleep								
LF Pow, ms^2	2140.55	1788.46	[1691.20, 2621.86]	2944.14	3334.84	[2227.57, 3785.92]	0.37	.124
HF Pow, ms^2	3869.30	3504.67	[3047.55, 4853.99]	3863.76	2874.26	[3229.76, 4530.58]	0.03	.731
Tot Pow, ms^2	10785.25	9935.15	[8429.67, 13503.81]	13878.59	15344.77	[10669.86, 17598.68]	0.28	.245
LF/HF ratio	0.72	0.42	[0.61, 0.83]	0.94	0.79	[0.76, 1.14]	0.43	.035
EDR, Hz	0.26	0.04	[0.25, 0.27]	0.25	0.03	[0.24, 0.25]	0.33	.108
SWS								
LF Pow, ms^2	1828.74	2027.15	[1391.65, 2352.29]	1390.84	1392.55	[1094.75, 1723.08]	0.27	.150
HF Pow, ms^2	3430.19	2906.15	[2702.94, 4237.49]	2662.49	2427.30	[2135.10, 3235.01]	0.33	.066
Tot Pow, ms^2	9144.79	10573.40	[6888.57, 11889.72]	6457.37	5978.97	[5209.92, 7904.29]	0.33	.096
LF/HF ratio	0.67	0.59	[0.53, 0.82]	0.67	0.50	[0.56, 0.78]	0.02	.991
EDR, Hz	0.27	0.06	[0.26, 0.29]	0.25	0.04	[0.25, 0.26]	0.34	.129
REM sleep								
LF Pow, ms2	2485.14	1985.09	[2014.31, 3038.83]	2951.72	3033.84	[2322.68, 3719.68]	0.25	.307
HF Pow, ms2	3559.81	2840.99	[2869.88, 4345.49]	3607.72	2774.10	[2946.60, 4308.72]	0.07	.798
Tot Pow, ms2	12437.62	11803.27	[9750.92, 15777.18]	14547.48	17224.48	[11064.39, 18778.80]	0.21	.402
LF/HF ratio	1.00	0.55	[0.86, 1.16]	1.05	0.88	[0.88, 1.26]	0.08	.728
EDR, Hz	0.25	0.04	[0.24, 0.26]	0.24	0.03	[0.24, 0.25]	0.25	.242

Note: SWS: slow wave sleep, REM: rapid eye movement, BC 95%-CI: bias-corrected 95% bootstrap confidence intervals.

[a] Bootstrapped values based on 5000 samples.

all variables adjusted for first language, children's age, sex, and BMI.

Table 3 Multiple regression results with heart rate variability parameters predicting post-awakening cortisol measures.

	Post-awakening cortisol secretion														
	First sample (S1)					AUC_I					AUC_G				
	β	*B*	*SE*[a]	*BC 95%-CI*[a]	*p*[a]	β	*B*	*SE*[a]	*BC 95%-CI*[a]	*p*[a]	β	*B*	*SE*[a]	*BC 95%-CI*[a]	*p*[a]
Wake															
LF Pow, ms^2	.10	0.00	0.00	[0.00, 0.00]	.336	.09	0.00	0.00	[0.00, 0.00]	.341	.22	0.00	0.00	[0.00, 0.00]	.036
HF Pow, ms^2	-.06	0.00	0.00	[0.00, 0.00]	.572	.16	0.00	0.00	[0.00, 0.00]	.081	.07	0.00	0.00	[0.00, 0.00]	.500
Tot Pow, ms^2	.10	0.00	0.00	[0.00, 0.00]	.397	.10	0.00	0.00	[0.00, 0.00]	.261	.23	0.00	0.00	[0.00, 0.00]	.050
LF/HF ratio	.23	0.05	0.02	[0.01, 0.12]	.028	-.10	-0.01	0.01	[-0.03, 0.01]	.344	.23	0.02	0.01	[0.00, 0.04]	.027
Stage 2 sleep															
LF Pow, ms^2	.02	0.00	0.00	[0.00, 0.00]	.805	.16	0.00	0.00	[0.00, 0.00]	.030	.18	0.00	0.00	[0.00, 0.00]	.047
HF Pow, ms^2	-.14	0.00	0.00	[0.00, 0.00]	.193	.11	0.00	0.00	[0.00, 0.00]	.277	-.10	0.00	0.00	[0.00, 0.00]	.345
Tot Pow, ms^2	-.07	0.00	0.00	[0.00, 0.00]	.336	.19	0.00	0.00	[0.00, 0.00]	.027	.08	0.00	0.00	[0.00, 0.00]	.254
LF/HF ratio	.12	0.03	0.03	[-0.01, 0.10]	.198	.00	0.00	0.01	[-0.03, 0.02]	.997	.17	0.02	0.01	[0.00, 0.03]	.055
SWS															
LF Pow, ms^2	.12	0.00	0.00	[0.00, 0.00]	.344	.04	0.00	0.00	[0.00, 0.00]	.617	.19	0.00	0.00	[0.00, 0.00]	.116
HF Pow, ms^2	-.07	0.00	0.00	[0.00, 0.00]	.511	.04	0.00	0.00	[0.00, 0.00]	.613	-.07	0.00	0.00	[0.00, 0.00]	.511
Tot Pow, ms^2	.03	0.00	0.00	[0.00, 0.00]	.770	.04	0.00	0.00	[0.00, 0.00]	.692	.07	0.00	0.00	[0.00, 0.00]	.575
LF/HF ratio	.01	0.00	0.04	[-0.07, 0.09]	.898	-.02	0.00	0.01	[-0.03, 0.03]	.874	.01	0.00	0.02	[-0.03, 0.04]	.955
REM sleep															
LF Pow, ms2	.14	0.00	0.00	[0.00, 0.00]	.072	.04	0.00	0.00	[0.00, 0.00]	.675	.22	0.00	0.00	[0.00, 0.00]	.008
HF Pow, ms2	-.08	0.00	0.00	[0.00, 0.00]	.428	.08	0.00	0.00	[0.00, 0.00]	.394	-.05	0.00	0.00	[0.00, 0.00]	.630
Tot Pow, ms2	.10	0.00	0.00	[0.00, 0.00]	.152	.02	0.00	0.00	[0.00, 0.00]	.832	.14	0.00	0.00	[0.00, 0.00]	.047
LF/HF ratio	.09	0.02	0.02	[-0.02, 0.09]	.238	-.01	0.00	0.01	[-0.02, 0.02]	.894	.11	0.01	0.01	[-0.01, 0.04]	.230

Note: SWS: slow wave sleep, REM: rapid eye movement, AUC_G = area-under-the-concentration-time-curve with respect to the ground, AUC_I = area-under-the-concentration-time-curve with respect to the increase.

[a] Bootstrapped values based on 5000 samples.

all variables adjusted for children's age, sex, prematurity status, season, awakening time, BMI, and awakening time difference.

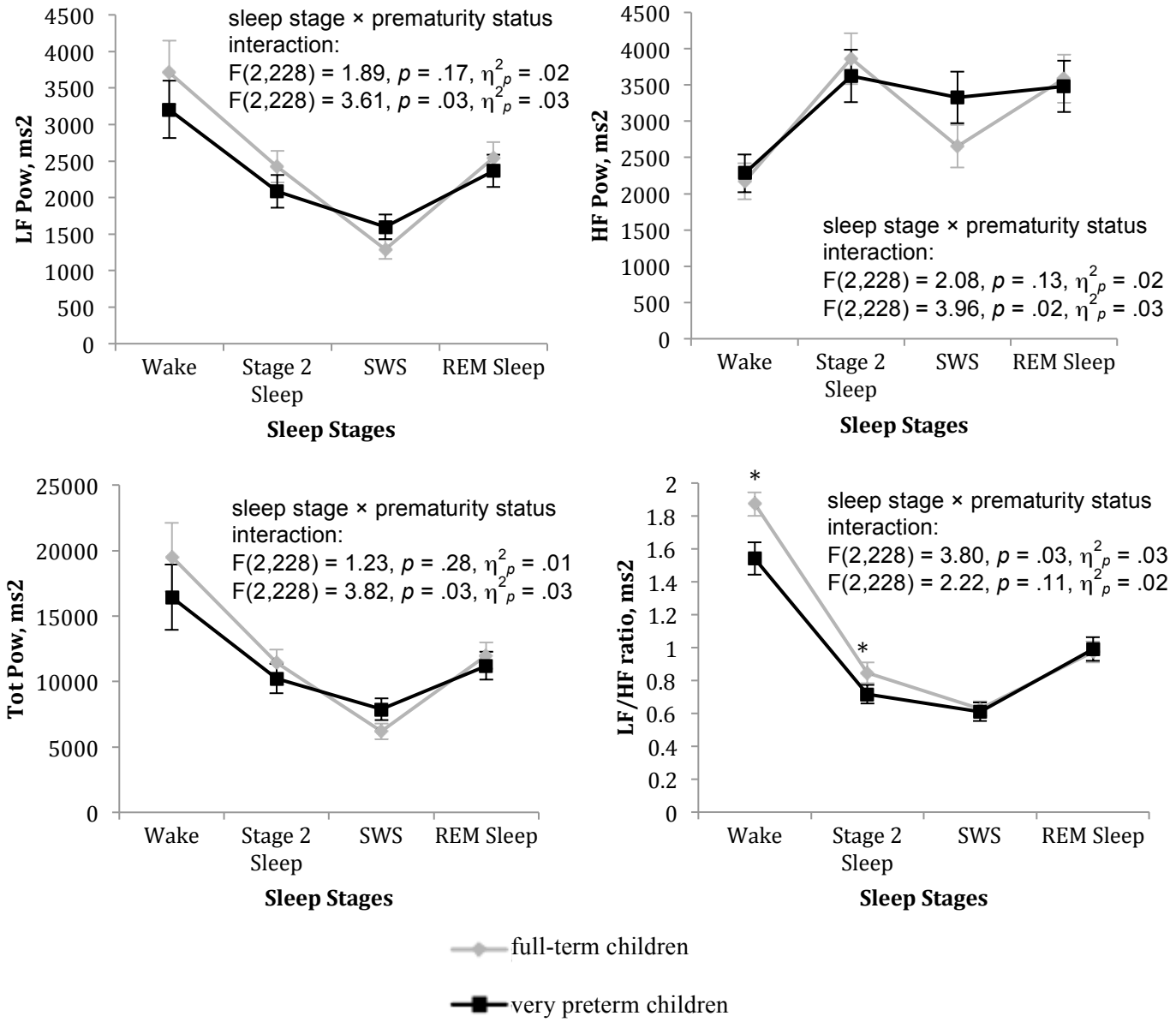

Fig. 1. Sleep stage × prematurity status interaction on HRV parameters.

upper line: sleep stage × prematurity status regarding the transition from wake to stage 2 sleep and SWS, lower line: sleep stage × prematurity status regarding the transition from stage 2 sleep to SWS and REM sleep.
* significant differences in HRV parameters between children born very preterm and full-term.
all variables adjusted for first language, children's age, sex, and BMI.