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Association between antenatal glucocorticoid exposure and the activity of the stress system, cognition, and behavior in 8- to 9-year-old children: A prospective observational study

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Abstract

Introduction: Glucocorticoid (GC) -induced fetal programming of the activity of the hypothalamus–pituitary–adrenal axis (HPAA) and its associated cognitive and behavioral consequences in later life have been well characterized in several animal species. However, information on humans is scarce. In this study, we examined HPAA activity markers and associated outcomes at 8 to 9 years of age among children prenatally exposed to GC for suspected preterm birth. Our hypothesis was that antenatal exposure to the betamethasone (BM) is associated with exacerbation of HPAA activity in childhood.

Material and methods: Prospective observational study in 31 children whose mothers received single ($n = 19$) or multiple ($n = 12$) courses of BM for threatened preterm birth but born with normal weight appropriate for the gestational age (median $37+^6$ weeks of gestation) compared with 38 non-exposed, age-matched children. Primary end point was the activity of the HPAA in response to the Trier Social Stress Test. Secondary end points were changes in autonomic nervous system (ANS) activity, cognitive performance (IQ), attention-deficit/hyperactivity disorder (ADHD) symptoms, and electrocortical activity (EEG).

Results: There was no statistically significant difference in HPAA activity markers between antenatal BM exposed and unexposed groups. ANS activity in BM-exposed children shifted towards a higher parasympathetic tone reflected by a higher overall high-frequency band power of heart rate variability. IQ scores were within normal limits for both groups; however, BM-exposed children had lower IQ scores than the unexposed group. BM-exposed group had marginally more ADHD core symptoms

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Abbreviations: ADHD, attention-deficit/hyperactivity disorder; ANS, autonomic nervous system; BM, betamethasone; GC, glucocorticoid; HF, high frequency; HPAA, hypothalamus–pituitary–adrenal axis; IQ, intelligence quotient; LF, low frequency; SEF, spectral edge frequency; SES, socioeconomic status; TSST-C, Trier Social Stress Test version adapted for children.

Heike Hoyer and Carolin Ligges contributed equally to the study.

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and increased electrocortical activity in the occipital brain region compared with controls. A monotonic dose–response relation between BM exposure and activity of the ANS and IQ was estimated in post-hoc analyses.

Conclusions: Antenatal exposure to BM in the context of threatened preterm birth was not associated with changes in HPAA activity in childhood. However, BM exposure may be associated with changes in ANS activity. Antenatal GC prophylaxis is a valuable and often life-saving therapy, but its prescription may warrant a well-balanced risk–benefit assessment.

KEYWORDS

fetal physiology, fetal programming, glucocorticoids, neurodevelopment, preterm birth

1 | INTRODUCTION

Glucocorticoids (GCs) are involved in growth and maturation of many organ systems and are crucial for regular fetal development.[1] However, excessive fetal GC exposure, resulting either from maternal psychosocial stress or treatment with synthetic GCs, may be associated with life-long dysregulation of ~~fetal~~ hypothalamus–pituitary–adrenal axis (HPAA) activity.[1,2] Later in life, dysregulation of HPAA activity can have far-reaching consequences for the health of the affected individual. Because of their intimate neuroanatomical and functional relation, HPAA dysregulation can lead to alterations in autonomic nervous system (ANS) function,[3] which predicts the development of cardiovascular and psychiatric disease.[3,4] Moreover, dysregulation of HPAA activity is thought to underlie several cognitive and behavioral deficits, most notably cognitive impairment and attention-deficit/hyperactivity disorder (ADHD).[5,6]

The long-term consequences of excessive GCs on fetal HPAA development and associated outcomes have been well characterized in controlled animal experiments.[1,2,6] However, information on humans is less comprehensive and results often vary because individual severity of prenatal stress is difficult to objectify.[2] A small number of studies used antenatal GC prophylaxis in the context of threatened preterm birth as objective fetal GC exposure.[7–11] Yet, individuals in these cohorts were mostly born premature, which may interfere with the effects of GCs on HPAA development.[12]

To overcome these gaps, we aimed to examine HPAA activity in 8- to 9-year-old children exposed to antenatal GC prophylaxis but born at or near term with a normal birthweight appropriate for the gestational age. Our primary hypothesis was that antenatal exposure to the synthetic GC, betamethasone (BM), would be associated with exacerbation of HPAA activity in response to acute stress, as seen in animal experiments[1,5] and other human cohorts.[9,11,13] We evaluated as secondary outcomes whether exposure to BM was also associated with changes in ANS activity, cognitive performance, and the presence of ADHD core symptoms. We further determined electrocortical activity as a correlate of aberrations in cortical and subcortical brain function.

Key message

In 8- to 9-year-old children, antenatal exposure to betamethasone for suspected preterm birth was not associated with changes in hypothalamus–pituitary–adrenal axis activity but may be associated with alterations in autonomic nervous system activity and neurofunctional outcome.

2 | MATERIAL AND METHODS

2.1 | Research design

A prospective observational study to evaluate the effects of antenatal BM exposure on HPAA activity and associated outcomes in 8- to 9-year-old children (BM group) vs non-exposed, age-matched children (control group) was conducted. Children were born between July 1999 and August 2001 at Jena University Hospital or the affiliated teaching hospital Waldklinikum Gera in Germany. Outcome assessment took place on a single day at Jena University Hospital.

2.2 | Recruitment strategy

Children in the BM group were recruited from an existing cohort of mothers who had previously participated in a randomized controlled intervention trial after they had been admitted to one of the two study hospitals for high risk of preterm birth between 27^{+0} and 35^{+0} weeks of gestation.[14] The trial objective was to compare maternal and fetal adverse effects of two standard tocolytic treatments. Routinely, all study participants also received single or multiple courses of 2×8 mg BM 24 hours apart to induce fetal lung maturation. This dosage is slightly lower than the current recommended dosage of 2×12 mg but corresponds to the clinical routine in our clinic during the study period. Control group children did not participate in the interventional trial or were at risk for preterm birth but were born in the same study hospitals and received the same standard of care as BM group children.

2.3 | Recruitment process and study participants

The recruitment process is depicted in Figure 1.

2.3.1 | BM group

We only recruited BM-exposed children from the existing cohort (see above) who were initially thought to be at risk for preterm birth but subsequently born with an appropriate birthweight after at least 238 days/34^{+0} weeks of gestation. Exclusion criteria for the BM group included a birthweight below the 5th reference centile; severe perinatal complications requiring treatment in the intensive care unit; continuous GC treatment; intrauterine exposure to maternal smoking, alcohol, or drugs; and incomplete birth records. Of 105 mother–child pairs invited, 42 pairs agreed to participate. Three children met the exclusion criteria (see Figure 1 for details); the remaining 39 children were included in the BM group.

2.3.2 | Control group

Control group children were identified randomly using medical birth records from the same two hospitals where the BM group children were born. Initially, we matched control group children by current age, sex, and gestational age at a ratio of 1:1. Exclusion criteria were the same as for the BM group. Of 212 mother–child pairs invited, 53 agreed to participate. Eight children met the exclusion criteria of which five had

incomplete birth records. Six children had no matching partner in the BM group. Hence, 39 children were enrolled in the control group.

Eight children in the BM group and one child in the control group had to be excluded from the statistical analysis because additional information on exclusion criteria that were not accessible before enrollment was received post-matching (maternal smoking, perinatal complications) (Figure 1). The final analysis comprised data from 31 BM group and 38 control group children.

2.4 | Demographic and clinical baseline data

Socioeconomic status (SES; parental education level, unemployment status, and disposable household income)[15] and other demographic variables were collected using a parent questionnaire. Pregnancy and birth data were obtained from the hospital records or the German maternity passport (*Mutterpass*). The Zurich Life-Event List was used to quantify the frequency of 36 positive as well as negative life events during the past 12 months, which are known to correlate with indicators of behavioral and emotional abnormalities.[16]

2.5 | Outcomes of interest

Primary outcome was HPAA activity expressed as salivary free cortisol at rest and during acute stress. Secondary outcomes were (a) ANS activity during acute stress and included salivary α-amylase
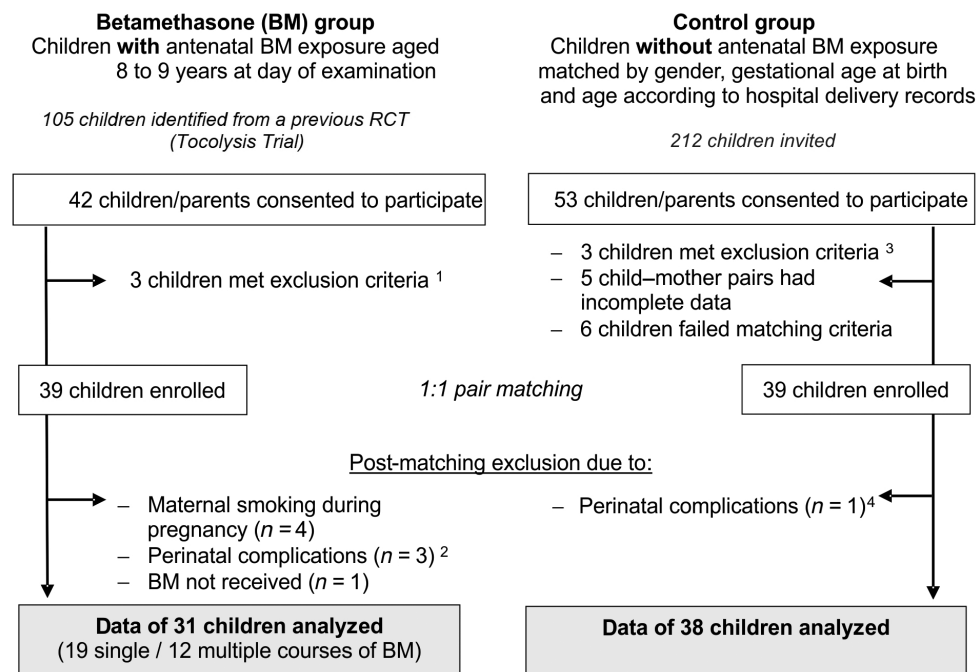
FIGURE 1 Flowchart of the study recruitment process

concentration, biomarkers of heart rate variability, and baroreflex sensitivity; (b) cognitive performance (intelligence quotient; IQ); (c) presence of ADHD core symptoms; and (d) electrocortical activity measured via electroencephalogram (EEG) signals.

2.6 | Summary of study procedure, outcome assessment, and statistical analysis

Comprehensive methodological details can be found in Appendix S1.

To evaluate HPAA and ANS activity in response to acute stress, children were exposed to the Trier Social Stress Test version adapted for children (TSST-C).[17] The TSST-C includes a preparation period, speech, and mental arithmetic tasks in front of an audience and a recovery period (Figure 2). EEG, electrocardiogram, and blood pressure data were recorded during the TSST-C. Four saliva samples were collected to determine salivary free cortisol and α-amylase as surrogate biomarkers of HPAA and sympathetic ANS activity, respectively (Figure 2). To estimate HPAA activity at rest, parents collected two saliva samples from their child at home during a restful day. ANS activity was estimated using heart rate variability frequency indices (low frequency [LF] and high frequency [HF] band power, LF/HF ratio).[18] Cognitive performance was assessed using Raven's Colored Progressive Matrices test.[19] The raw test score was converted into a centile scale according to the norms for the respective age group and then to the IQ norm scale. ADHD core symptoms were determined using the FBB-ADHS questionnaire (*Fremdbeurteilungsbogen für Aufmerksamkeitsdefizit- / Hyperaktivitätsstörungen*, a German ADHD rating scale).[20] We performed a power spectral analysis of the EEG recordings to determine electrocortical activity as a physiological correlate of cortical and subcortical brain function.[21] The planned sample size of 40 children per group would have 80% power to detect a standardized effect size of 0.63 or greater using a two-group t test with a two-sided significance level of 0.05. Mixed linear models were used for analyses. To analyze the TSST-C stress response, two models were fitted. First, the overall-activity model comprised outcomes of all TSST-C periods including baseline and feedback. Second, the change-from-baseline model analyzed the baseline-adjusted differences between active periods of TSST-C and baseline. We accounted for differences in SES by including parental education as a proxy covariate in all analyses. Parental education is regarded as the main proxy measure for the family's SES.[15] The two-sided significance level was 0.05. We did not adjust the level of significance for multiple outcomes in our study of associations because we aimed at searching for adverse signals.[22]

2.7 | Ethical approval

Approval by the ethics committee of Jena University Hospital (Approval 2041–06/07/22.06.2007) and informed consent from all participants has been obtained.

3 | RESULTS

3.1 | Demographic and clinical baseline data and exposure characteristics

Study groups were similar for age, sex, and gestational age at birth (Table 1). All other non-matched variables were also well balanced, except for all measures of SES (parental education, employment, and income), which were lower in the BM group (Table 1). We statistically adjusted for this imbalance by adjusting for parental education. For BM exposure characteristics see Table 2.
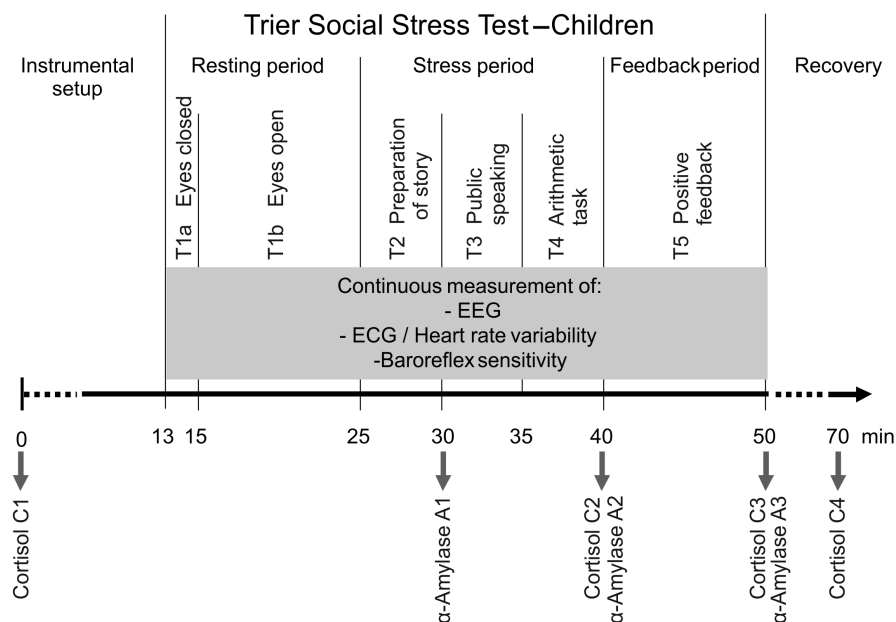

FIGURE 2 Course and timepoints of the Trier Social Stress Test version adapted for children and timepoints of saliva sample collection to determine cortisol and α-amylase concentrations.

TABLE 1 Demographic and socioeconomic data documented at the day of examination and pregnancy-related data of the study groups

	BM (n = 31)	Controls (n = 38)	p value[a]
Demographic and socioeconomic data			
Child			
Age (y), mean (SD)[b]	8.4 (0.8)	8.4 (0.8)	[b]
Female sex, n (%)[b]	16 (51.6)	20 (52.6)	[b]
Weight (kg), mean (SD)	29.8 (7.4)	29.2 (5.6)	0.70
Height (cm), mean (SD)	133.5 (7.4)	136.3 (8.1)	0.15
Body mass index (kg/m^2), mean (SD)	16.5 (2.7)	15.6 (2.0)	0.14
Living together with parents, n (%)	22 (71.0)	27 (71.1)	1.00
Only child, n (%)	6 (19.4)	12 (31.6)	0.28
Number of unpleasant life events[c] (ZLEL), median (IQR)	3.5 (2–4)	3 (1–4)	0.67
Parents			
University degree (at least one parent), n (%)[c]	9 (29.0)	20 (52.6)	0.06
Unemployed (at least one), n (%)	10 (32.3)	2 (5.3)	<0.01
Net household income, n (%) <0.01			
<€2600	22 (71.0)	13 (34.2)	
≥ €2600	4 (12.9)	17 (44.7)	
Not reported	5 (16.1)	8 (21.1)	
Pregnancy and birth data			
Hyperemesis during pregnancy (%)	2 (6.5)	3 (7.9)	1.00
Bleeding during pregnancy, n (%)	8 (25.8)	3 (7.9)	0.05
Maternal age at birth (y), mean (SD)	26.8 (5.9)	29.0 (4.6)	0.08
Cesarean section, n (%)	9 (29.0)	10 (26.3)	1.00
Gestational age at birth (d), median (IQR)[b]	265 (254–272)	265 (257–270)	[b]
Birthweight (g), mean (SD)	2983 (520)	3096 (470)	0.34
Birth length (cm), mean (SD)	49.1 (3.3)	49.4 (2.5)	0.58
APGAR 10, median (IQR)	9 (9–10)	9 (9–10)	0.36
Neonatal monitoring, n (%)	4 (12.9)	6 (15.8)	1.00

Abbreviations: BM, betamethasone; IQR, interquartile range; SD, standard deviation; ZLEL, Zurich Life-Event List.

[a]p values resulting from Fisher's exact test, exact Wilcoxon two sample test or two-sample t test as appropriate.

[b]Primary matching criteria.

[c]Scored −2 on a five-item Likert-scale (−2 to +2), † including university of applied sciences.

3.2 | Outcomes

3.2.1 | HPAA activity (primary outcome)

Salivary cortisol concentration did not differ significantly between the BM group and controls at a restful day and in response to acute stress (Figure 3, Table 3). Non-significant standardized differences ranged from 0.1 to 0.49.

3.2.2 | ANS activity

Saliva α-amylase concentration did not differ significantly between the BM group and controls (Table 3). HF-band power during the TSST-C (all timepoints T1–T5) was higher in the BM group (Table 3) resulting in a lower LF/HF ratio (Table 3, Figure 4). Stress-induced changes from baseline (timepoints T1 vs T2–T4) in HF band power (Table 3) and LF/HF ratio (Table 3, Figure 4) were less marked in the BM group. Standardized differences for non-significant differences ranged from 0.08 to 0.49. (Table 3).

3.2.3 | Cognitive performance and ADHD core symptoms

BM-exposed children had significantly lower IQ scores (Table 3, Figure 5) and higher ADHD scores (Table 3, Figure 5). However, all 95% confidence intervals (CI) were still within the expected normal range.

TABLE 2 Exposure characteristics

	Single BM (n = 19)	Multiple BM (n = 12)	Total BM (n = 31)
Gestational age at treatment with 2×8mg BM 24h apart (d), median (IQR)	230 (224–234)	218 (215–224)	223 (216–233)
Number of BM courses[a], median (range)	1	2 (2–5)	1 (1–5)
Tocolytic treatment			
Transdermal glyceryl trinitrate, n (%)	9 (47)	7 (58)	16 (52)
Fenoterol per infusion, n (%)	10 (53)	5 (42)	15 (48)
Duration of tocolysis (d), median (IQR)	4 (3–10)	19 (7–28)	9 (4–16)
Prolongation of pregnancy (d), median (IQR)	38 (19–47)	52 (41–72)	46 (27–51)

Note: Maternal application of BM in relation to gestational age and tocolytic treatment.

Abbreviations: BM, betamethasone; IQR, interquartile range; SD, standard deviation.

[a]1 course = 2×8mg BM 24h apart.

FIGURE 3 Activity of the hypothalamus–pituitary–adrenal axis on the reference day and during the Trier Social Stress Test version adapted for children (timepoints C1–C4) expressed as salivary cortisol concentration in children exposed to antenatal betamethasone (BM; solid line) and in controls (dashed line). Data expressed as mean with 95% confidence interval.
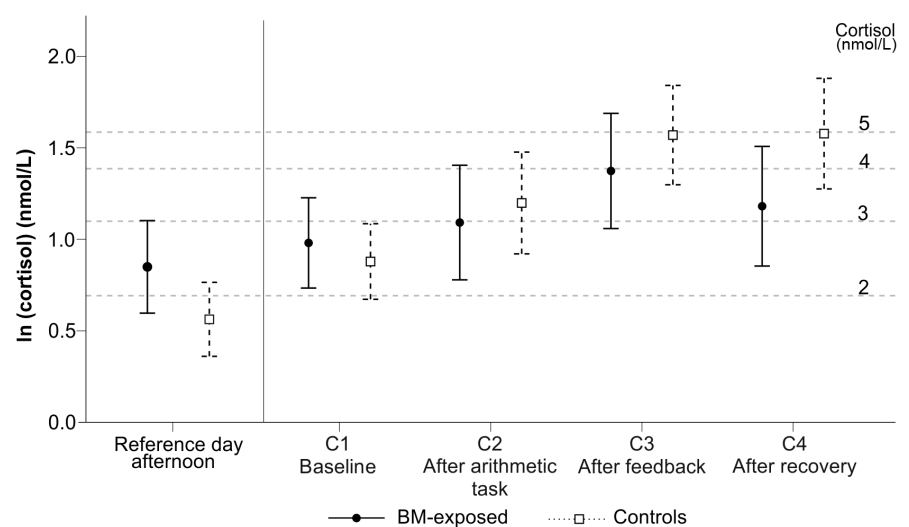
3.2.4 | Electrocortical activity

The overall spectral edge frequency (SEF) at the occipital electrodes during the TSST-C (all timepoints T1–T5) was higher in the BM group than in controls (Table 3, Figure 6). A slightly smaller difference was observed at the temporal electrodes (Table 3, Figure 6). The higher SEF can be explained by a higher relative band power of beta waves (exploratory post-hoc analysis, Table S1). There were no significant differences in the SEF-change from baseline between both groups. The standardized differences of the non-significant differences ranged from 0.08 to 0.39.

3.2.5 | Association between multiple BM courses and outcomes (post-hoc analysis)

In an exploratory post-hoc analysis, a monotonic dose–response relation was observed between number of BM courses and stress-induced ANS activity and cognitive performance (Table S2).

4 | DISCUSSION

Antenatal exposure to BM for threatened preterm birth was not associated with exacerbation of HPAA activity in childhood. In fact, HPAA activity during acute stress tended to be lower in BM-exposed children. In contrast to our study, Alexander et al. reported a more pronounced increase in salivary cortisol concentration in response to the TSST-C in BM-exposed vs non-exposed children aged 6–11 years[13] and later in adolescents.[9] The differences in HPAA activity may be attributed to the different dosing regimens of BM used. Although the studies of Alexander et al.[9,13] only included children antenatally exposed to a single course of BM, our study also included children exposed to repeated courses reflecting real-life prescription patterns. We know from studies in sheep that repeated, but not single, courses of antenatal BM cause HPAA hypo-activity in later life.[23] Similarly, in former preterm children at the age of 8–9 years, antenatal treatment with dexamethasone, but not hydrocortisone, was associated with blunted HPAA activation during the TSST-C.[10] In exploratory post-hoc analysis we identified a trend towards a negative dose–response relation between BM exposure

TABLE 3 Summary statistics of outcomes in betamethasone-exposed and control children

Outcomes	Estimated means		BM vs Controls			
	BM	Controls	Diffe-rence/Ratio	95% CI	SDIF	p value
HPAA activity (primary outcome)						
Cortisol (nmol/L)						
Reference day morning (nmiss = 11)	11.55[a]	12.01[a]	0.96[b]	0.77–1.20[b]	0.10 [b]	0.72
Reference day afternoon (nmiss = 10)	2.35[a]	1.75[a]	1.35[b]	0.97–1.86 [b]	0.49 [c]	0.07
Overall at TSST-C period C1–C4	3.33[a]	3.69[a]	0.90[b]	0.64–1.28 [b]	0.15[c]	0.56
Fold Change from baseline at TSST-C period C4–C6	1.33[a]	1.76[a]	0.76[b]	0.53–1.08 [b]	0.39[c]	0.13
ANS activity						
α-amylase overall at TSST-C period A1–A3 (U/mL)	314[a]	226[a]	1.39[b]	0.79–2.44 [b]	0.30 [c]	0.24
Overall activity at TSST-C period T1-T5						
Mean heart rate (beats/min)	89.1	90.0	−0.9	−6.9–5.0	0.08	0.76
LF band power (ms^2/Hz)	1247[a]	1028[a]	1.21[b]	0.87–1.69 [b]	0.28 [c]	0.24
HF band power (ms^2/Hz)	1313[a]	762[a]	1.72[b]	1.07–2.77 [b]	**0.57** [c]	**0.03**
LF/HF	1.30	1.86	−0.53	−0.88−−0.17	**0.75**	**<0.01**
Baroreflex sensitivity LF (ms/mm Hg)	13.3	12.7	0.53	−1.62–2.68	0.12	0.62
Baroreflex sensitivity HF (ms/mm Hg)	21.6	18.6	3.03	−2.60–8.65	0.27	0.29
Change from baseline at TSST-C period T2–T4						
Mean heart rate (beats/min)	5.06	8.85	−3.78	−7.69–0.13	0.49	0.06
LF band power (ms^2/Hz)	67	−228	295	−306–896	0.25	0.33
HF band power (ms^2/Hz)	−451	−1192	742	72–1412	**0.56**	**0.03**
LF/HF	0.33	1.05	−0.72	−1.06−−0.37	**1.05**	**<0.01**
Baroreflex sensitivity LF (ms/mm Hg)	−4.18	−3.90	−0.29	−1.92–1.35	0.08	0.72
Baroreflex sensitivity HF (ms/mm Hg)	−5.00	−6.73	1.73	−1.70–5.15	0.26	0.31
Cognition and behavior						
Cognitive performance (IQ)	96.9	108.0	−11.1	− 18.8−−3.4	**0.68**	**<0.01**
ADHD Core symptoms (DISYPS II, Stanine)	5.5	4.6	0.9	0.0–1.8	**0.51**	**0.04**
Electrocortical activity (EEG)						
Overall activity at TSST-C period T1–T5						
Spectral edge frequency 95 (Hz)						
Frontal	17.1	16.4	0.67	−0.85–2.18	0.20	0.38
Temporal	18.7	17.2	1.53	−0.15–3.21	0.44	0.07
Parietal	14.5	14.8	−0.33	−1.31–0.66	0.16	0.51
Occipital	15.5	14.1	1.42	0.28–2.55	**0.56**	**0.02**
Change from baseline at TSST-C period T2–T4						
Spectral edge frequency 95 (Hz)						
Frontal	0.42	1.05	−0.63	−1.44–0.18	0.39	0.12
Temporal	1.05	1.68	−0.64	−1.61–0.34	0.34	0.20
Parietal	0.39	0.57	−0.18	−0.61–0.25	0.21	0.40
Occipital	0.39	0.48	−0.09	−0.63–0.45	0.08	0.74

Note: Estimated by mixed linear models and adjusted for parental education as socioeconomic-proxy: Arithmetic means and their difference or geometric means ([a]) and their ratio ([b]) after transforming back logarithmic scaled variables to the original scale. [c]Standardized differences based on logarithmic scale. For better comparison, differences were standardized similar to Cohen's *d*.

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; BM, betamethasone; CI, confidence interval; DISYPS II, Diagnostic System for Psychiatric Disorders in Children and Adolescents II; HF, high frequency; IQ, intelligence quotient; LF, low frequency; nmiss, number of participants with missing data; SDIF, standardized difference; TSST-C, Trier Social Stress Test for children.

and HPAA activity. Although the mechanism remains speculative, it is possible that the dose-dependent fetal programming of HPAA activity is the result of changes in adrenal sensitivity. For example, in adult sheep, HPAA hypo-responsiveness following fetal exposure to multiple, but not single, maternal injections of BM is caused by reduced adrenal sensitivity to adrenocorticotropic hormone.[23]

FIGURE 4 Activity of the autonomic nervous system expressed as the low frequency/high frequency (LF/HF) ratio of the heart rate variability during the Trier Social Stress Test version adapted for children (timepoints T1–T5) in children exposed to antenatal betamethasone (BM; solid line) and in controls (dashed line). Data expressed as mean with 95% confidence interval.
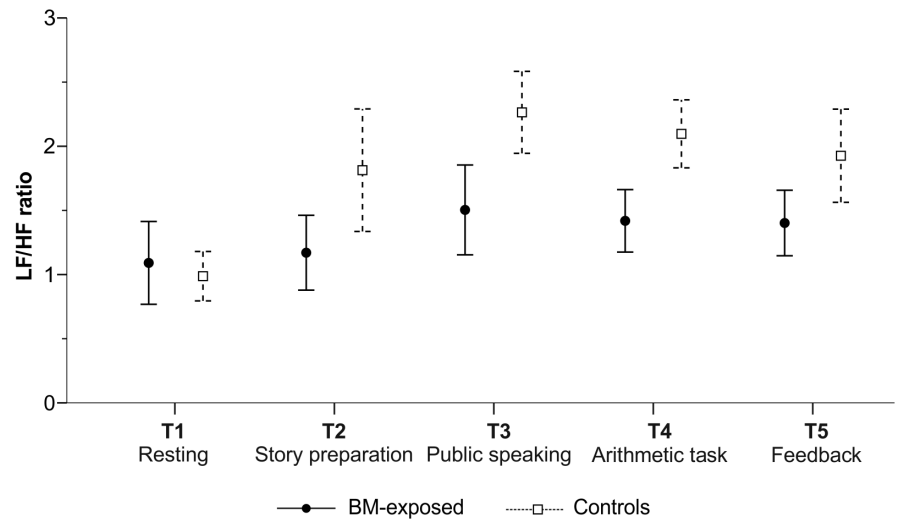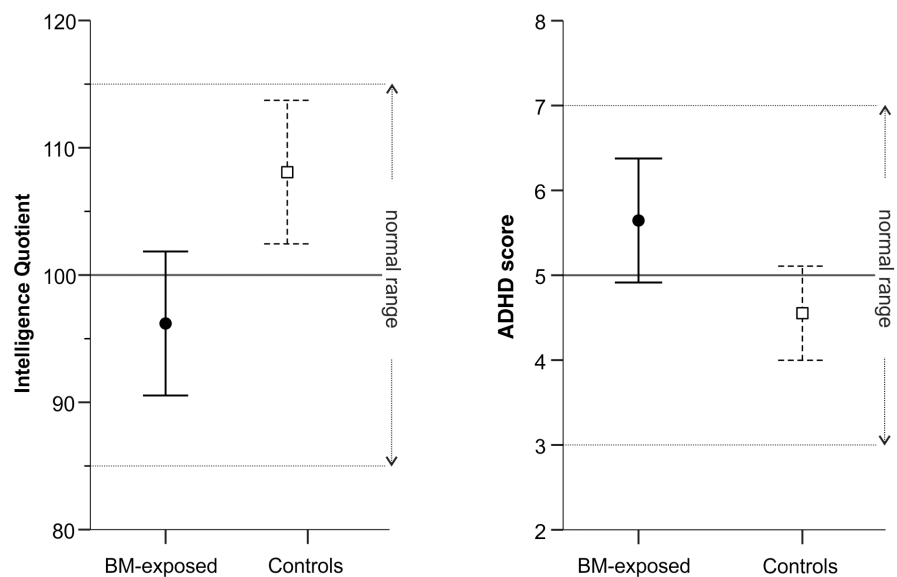
FIGURE 5 Cognitive performance (Intelligence Quotient, left) and ADHD core symptoms (German FBB ADHS rating questionaire on stanine scale, right) in children exposed to antenatal betamethasone (BM; solid line) and in controls (dashed line). Data expressed as mean with 95% confidence interval.

Consistent with the results of other human cohort studies,[9,13] basal HPAA activity did not differ between the BM group and controls.

Although we did not detect any significant changes in HPAA activity, antenatal BM exposure was independently associated with alterations in some secondary outcomes.

Regarding ANS activity, HF band power during the TSST-C was higher in the BM group and its stress-induced decrease was less pronounced than in controls. As the efferent cardiac vagal nerve activity is the major contributor to the HF band power,[24] this finding suggests the possibility of an overall increased vagal tone in BM-exposed children, and may suggest an accelerated maturation of the parasympathetic ANS.[3] Furthermore, LF/HF ratio was lower in the BM group than in controls. The LF/HF ratio is thought to represent the sympatho-vagal balance because it is assumed that sympathetic nerve activity is the main factor modulating the LF component of heart rate variability.[25] In this context, our results suggest a potential parasympathetic shift of the sympatho-vagal balance in the BM group and developmental or functional alterations of the parasympathetic ANS independently of changes in HPAA activity. In agreement with this observation, saliva α-amylase as a humoral marker of the sympathetic activity did not differ between groups. However, it has been suggested that the sympatho-vagal balance is not fully reflected in the LF/HF ratio,[24] hence interpretation of our data merits caution.

IQ as a marker of general cognitive function was on average 11.1 points lower in the BM group compared with controls. However, IQ scores were still within the expected normal range in both groups suggesting that statistical differences may not be clinically relevant. In contrast to our results, previous follow-up studies of well-controlled randomized controlled trials did not reveal any association between antenatal GC prophylaxis and cognitive performance in preterm-born individuals aged 20–31 years of age.[8,26] Furthermore, a recent meta-analysis of three studies reporting on
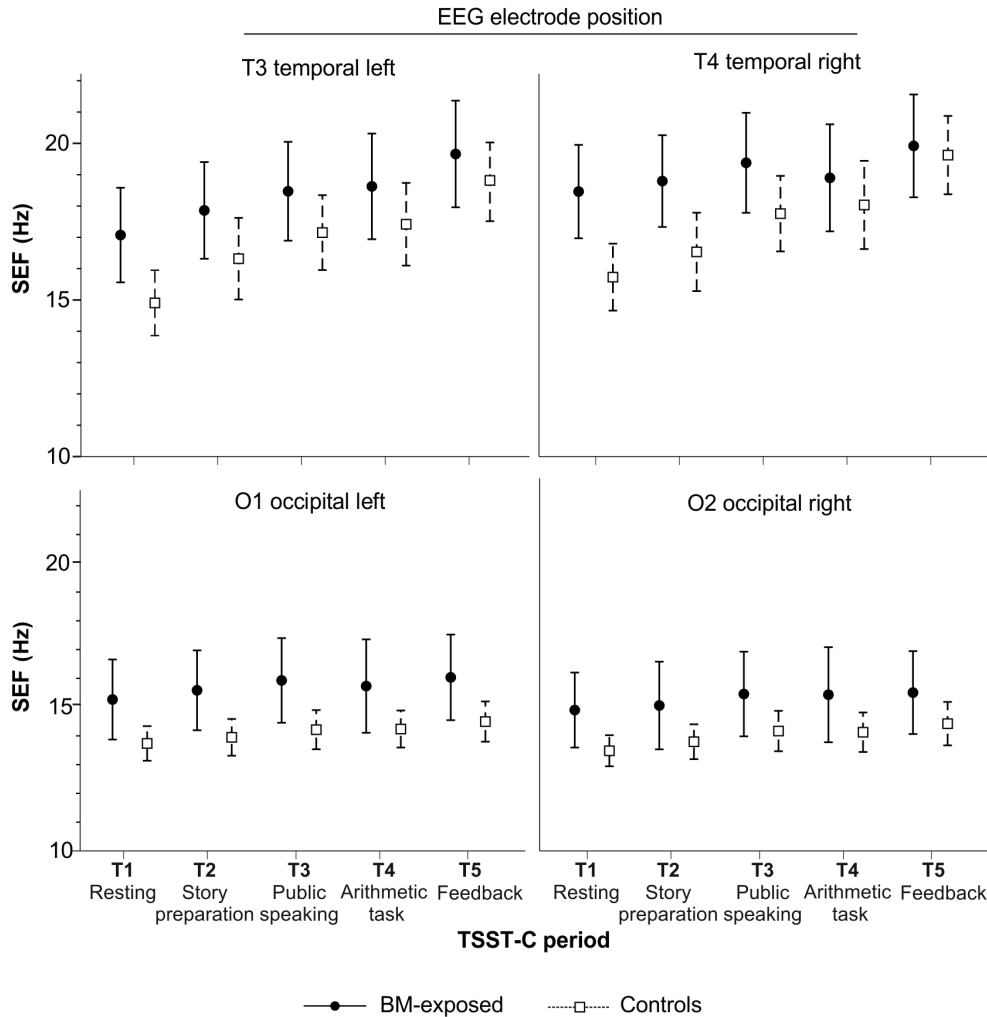
FIGURE 6 Electrocortical activity expressed as the spectral edge frequency (SEF) of the EEG at the temporal and occipital electrodes during the Trier Social Stress Test version adapted for children (timepoints T1–T5) in children exposed to antenatal betamethasone (BM, solid line) and in controls (dashed line). Data expressed as mean with 95% confidence interval.

neurodevelopment concluded with moderate-certainty of evidence that exposure to antenatal steroids is associated with reduction in developmental delay in childhood (relative risk 0.51, 95% CI 0.27–0.97) and not associated with intellectual impairment (relative risk 0.86, 95% CI 0.44–1.69).[27] Comparably, another recent meta-analysis of up to four randomized controlled trials came to the conclusion that repeated vs single courses of GCs probably result in no difference in neurocognitive impairment in early and mid-childhood.[28] However, as the randomized controlled trials and studies included in the meta-analyses are largely performed in formerly preterm children, results are not readily comparable. Further data are needed to understand the effects of antenatal GC on neurodevelopment.

Parent-reported ADHD scores were within the expected normal range in both groups. Within this normal range, BM-exposed children displayed a higher ADHD score than controls. A higher risk of mental and behavioral disorders including ADHD was also reported in a large population-based cohort study from Finland following antenatal exposure to maternal GC prophylaxis.[29] It should be noted, however, that not all available studies have found a

significant association between prenatal BM exposure and ADHD symptoms[8,26] and statistically significant difference may not have any clinically important difference.

Compared with controls, children in the BM group displayed a higher overall electrocortical activity in the occipital brain regions. SEF represents an effective estimate of the frequency content of the EEG power spectrum.[21] During physiological development, the proportion of higher frequencies in the EEG power spectrum increases from childhood to adolescence, reflecting maturational changes of the thalamo-cortical and cortico-cortical networks.[30] The higher frequencies start to develop in the occipital brain regions first.[30] Hence, the higher SEF at the occipital electrodes observed in BM-exposed children may suggest maturational acceleration of subcortical and cortical structures.[30] In agreement with our study, in preterm infants, antenatal GC prophylaxis was negatively associated with EEG slow delta and theta activity at rest.[31] Animal studies in fetal sheep revealed lasting complex changes in neuronal activity after GC administration with some reports of increased high frequencies.[32]

The strength of our study is that we included only healthy children born term or near term (median 37^{+6} weeks of gestation) who had normal birthweight and showed no signs of intrauterine growth restriction. In contrast to previous studies examining the individual stress responsiveness in terms of HPAA[10,11] or ANS activity[33] and cognition and behavior[8,26] in former preterm children following antenatal GC exposure, we can exclude the effect of preterm birth on outcome measures.

In addition, we considered parental education as a proxy measure for SES[15] as a potential confounder that has not been considered in most studies to date. Parental SES is independently associated with HPAA activity, cognition, and behavior in later life. As we noticed a significant difference in SES between BM group and controls potentially reflecting participation bias, we statistically adjusted for this imbalance.

However, there are several limitations of our study. First, prenatal maternal stress associated with the risk of preterm birth may have modified our outcomes.[2] Future studies may add a separate control group with comparable maternal stress elicited for example by adverse life events to overcome this issue. Second, we cannot rule out an independent effect of the tocolytic treatment on our results. However, because two pharmacologically different tocolytics were used in a balanced manner, a significant bias is unlikely. Third, as a result of post-matching exclusions, sample size and precision of the estimated differences were lower than planned. Fourth, we did not adjust the level of significance for multiple comparisons, which potentially raises the risk of false-positive results. Fifth, our post-hoc analysis on the dose–response relation between BM and outcomes is exploratory. Observed dose–response relation may also be attributed, in part, to maternal stress associated with a prolonged hospital stay necessary to receive multiple BM courses. Sixth, the observational study design does not allow us to conclude a causal relation between BM exposure and outcomes. Seventh, it was not possible to assess the extent to which the postnatal environment influenced outcome variables apart from education, employment, and income levels of BM-exposed children's parents, which were significantly lower in the control group suggesting the possibility of "healthy volunteer bias". Eighth, given the complex and long-lasting nature of brain development, we acknowledge that some residual confounding is highly likely.

5 | CONCLUSION

Antenatal exposure to BM for threatened premature birth, was not associated with changes in HPAA activity in 8- to 9-year-old children born near or at term. However, antenatal BM exposure may be associated with changes in ANS activity. Since HPAA activity was not altered, our results suggest that neurofunctional differences in outcomes may be the result of changes in structural or functional brain development as seen in animal experiments and human studies after antenatal GC exposure[34,35] rather than due to HPAA dysregulation.

Despite these findings, we want to point out that our study should not discourage obstetricians from prescribing GC prophylaxis, which is often life-saving for the unborn.[27] However, because not all children antenatally treated with GCs go on to be born preterm, prescription of these therapies should always be preceded by a well-balanced risk–benefit assessment also considering the potential consequences for the fetus.

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