Sleep physiological correlates of autism spectrum disorders

Ph.D. theses

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Introduction

Asperger syndrome (AS) is a neurodevelopmental disorder characterized by impairment in social interactions, manifest repetitive and stereotyped behaviours and interests without significant delay in language or cognitive development. Genetic, neurophysiologic, cognitive and behavioural data support the hypothesis that AS is a variant of autism located at the milder end of the spectrum of autistic disorders.

Assessments of brain development revealed that autism is characterised by a period of unusually rapid rate of early brain growth followed by abnormally slow or arrested growth. It has been proposed that this early overgrowth interferes with the normal developmental trajectory of connectivity in the cortex. It has been hypothesized that these altered patterns of brain neural development result in a local overconnectivity in the frontal cortex and a reduction in long-distance cortical-cortical coupling. The frontal overconnectivity and the long-distance underconnectivity are thought to underlie the cognitive characteristics of autism spectrum disorders

Sleep holds promise as a sensitive indicator of changes in brain neuronal organization. EEG studies and analyses of the EEG during NREM in particular may provide further insight into functional brain connectivity in autism spectrum disorder (ASD).

Aims

General aim of the study

To investigate whether sleep macrostructure, NREM sleep dependent EEG spectral activity, sleep spindle activity, as well as phase coherence are different in Asperger Syndrome (AS) compared to typically developing children and adolescents.

Specific aims and hypotheses

Aims related to the analysis of sleep

1. **Aim:** To investigate whether second night sleep structure is different between the groups.

   Hypothesis: We hypothesize that the sleep of children and adolescent with AS will exhibit a qualitative difference compared to the Age and IQ matched healthy controls. This is in spite of the fact that these subjects are reported to be good sleepers.

Aims related to the analyses of absolute and relative power spectra of the entire second night NREM sleep

2. **Aims:** To investigate whether the second night NREM sleep dependent EEG power spectrum density in a broad frequency range **(**0.5-45) is different in AS compared to the healthy controls.

   a. Hypothesis: We assume that the pattern of EEG power spectra in AS will differ compared to the healthy controls in many frequency bands and in a region specific way.

   b. Hypothesis: We assume that the power of the spindle band EEG activity (sigma: 11-15 Hz) as a sensitive indicator of the thalamo-cortical resonance will differ between the studied groups.

Aims related to the analyses of NREM sleep dependent spindle activity

3. **Aim:** To investigate whether second night NREM sleep dependent sleep spindle activity is different between the groups.

   Hypothesis: We assume that the amplitude, duration and density of the sleep spindles being detected based on individually adjusted frequency and amplitude criteria are different in the AS group compared to the healthy controls.

Aims related to the analyses of inter- and intrahemispheric phase coherence

Aim: To investigate whether second night inter-, and intrahemispheric phase coherence in a broad frequency range is different between the studied groups.

a. **Hypothesis:** We hypothesize that the AS group will exhibit a region specific decrease in inter-, and intrahemispheric coherence compared to the healthy controls.

b. **Hypothesis:** We hypothesize, that the emerging decrease in the inter- and intrahemispheric coherence in AS will be more emphasized over the frontal region.

Methods

Subjects

Eighteen un-medicated subjects (all males) with AS frequenting the outpatient care of Vadaskert Child Psychiatric Hospital, Budapest and 14 control (CONT) subjects were recruited in a multicentre sleep study. Parents were interviewed extensively with respect to all behavioural and cognitive characteristics of their children.

Demographic characteristics of subjects

Groups	Age (months)	T-test	IQ (RAVEN*)	T-teszt
Asperger (N=18)	m = 158 SD = 48	*t* =1.67 p = 0.253	m = 49.9 Sd = 6.5	*t* = 0.34 p = 0.741
Control (N=14)	m = 177 SD = 41		m = 51 Sd = 8.8	

*. RAVEN raw scores

The diagnosis of AS was based on ICD-10 criteria, and also confirmed by the Autism Diagnostic Observation Schedule (ADOS) criteria and performed by experienced clinicians from the Autism Foundation and Research Group, Budapest. All subjects with AS participated in regular meetings for special education purposes in the framework of the Vadaskert Child Psychiatric Hospital. No subject had a history of verbal language delay, any neurological, or co-morbid psychiatric disorder. No subject exhibited spike wave EEG activity. No subjects with reported sleep problems were enrolled in the study. Subjects were matched in non-verbal IQ, gender and lateralization. The spectral and coherence analysis was based on a sample of 24 subjects assessed in the sleep laboratory of the Vadaskert Child Psychiatric Hospital. The age [CONT: 177 (SD = 41), vs. AS: 150 (SD = 52); t = 1.41, P = 0.172] and IQ [CONT: 49.9 (SD = 6.5) vs. AS: 52.9 (SD = 5.4), t = 0.97, P = 0.340] matching between control and patient groups in this sub-sample was similar to that of the combined sample.

Ethical aspects

Parents of the participating children and subjects above the age of 18 years signed the informed consent approved by the ethics committee of Semmelweis University, Budapest, Hungary. Principles of the Declaration of Helsinki were followed.

Polisomnography

EEG, EOG, EMG and ECG were recorded on two consecutive nights. A 10 channel (F3, F4, C3, C4, P3, P4, T3, T4, O1, O2) EEG montage was used. PSG assessment was performed by the same specialists and by the use of standard procedures in both laboratories. The timing of lights off was determined by subjects' sleep-wake habit, and the awakenings were spontaneous.

The first night served as an adaptation period to the sleep laboratory conditions. The EEGs obtained during the second night EEG recording were visually scored by an experienced scorer (ASL), with an epoch length of 20 seconds according to standard criteria. After visual artefact rejection, stages 2, 3 and 4 of NREM sleep were subjected to spectral, sleep spindle and coherence analyses. Absolute and relative power spectrum density (PSD) and coherence indices were computed for the delta (0.5–4 Hz), theta (4.25–7.75 Hz), alpha (8–10.75 Hz), sigma (11-15 Hz), beta (15.25–25 Hz), gamma1 (25.23-35 Hz) and gamma2 (35.25-45 Hz) frequency bands. The standard frequency bands were assigned in accordance with previous studies. Spindle analyses was based on the individually adjusted method of sleep spindle analyses (Bódizs et al., 2009)

Statistical analyses

Although the studied groups were comparable and there was only a slight and statistically non-significant difference in age, we nevertheless used age as a covariate in all statistical analyses concerning quantitative EEG measures in order to minimize the bias caused by this confounding factor in the assessment of inter-group differences with respect to EEG power and coherence.

In order to reduce the number of variables the gained absolute and relative PSD data from the ten derivations were grouped into six regions along a sagital region (sRegion: frontal, centro-temporal, and posterior) and hemisphere factor (left and right) and averaged over each region so as to reduce the number of statistical comparisons.

Statistical analyses for spectral measures were applied in two consecutive steps. First, we employed multivariate analysis of covariance in order to analyze group differences in absolute and relative power spectrum density for the entire frequency range (absolute total power) and then for each frequency band separately.

In order to analyze intrahemispheric coherence we employed the same two factors (sRegion and hemisphere) averaging coherence data from all intrahemispheric electrode pairs related to a specific area.

The statistical analyses of sleep spindling was based on a multivariate model including factor Group, factor sRegion (frontal and parietal) as well as factor Type of Spindle (slow and fast).

The statistical analyses of coherence was also performed in several consecutive steps progressing from 'global' to 'local' effects with respect to both frequency and topography. In the first step focusing at the entire frequency range (0.5 – 45 Hz) we used a multivariate model including the between factor Group and two within factors sRegion (frontal, centro-temporal, posterior) and Type of coherence (Inter- or intrahemispheric). In the next step still focusing upon the entire frequency range we analyzed group differences in intrahemispheric coherence including in the multivariate model the between factor Group and the within factors sRegion and Hemisphere. In a final step we aimed to identify the specific subregion (electrode pair) contributing to a specific regional group difference. For this purpose we applied univariate analysis of covariance. In order to avoid the increased risk of type I error due to multiple comparisons we resorted to Bonferroni-type corrections.

Results

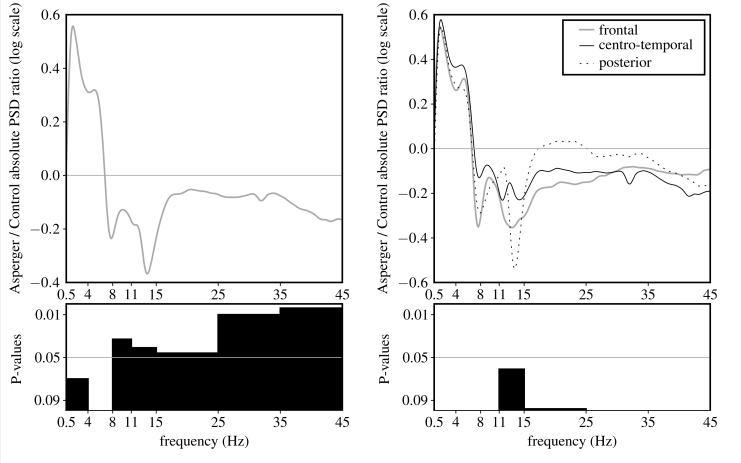
Sleep structure

The AS group spent significantly more time in bed, had a longer sleep onset latency, a somewhat lower sleep efficiency and longer wake after sleep onset.

Absolute power spectrum density

Analyses of the absolute total PSD revealed neither a main effect for the factor Group nor any interaction. Analyses of PSD per frequency

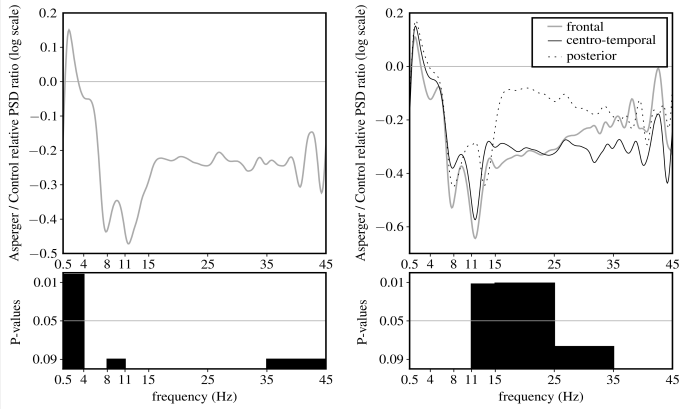
band revealed a significant overall decrease in alpha [F(1,21) = 5.232, P = 0.033], sigma [F(1,21) = 4.725, P = 0.041], beta [F(1,21) = 4.497, P = 0.046], gamma 1 [F(1,21) = 8.078, P = 0.01], and gamma 2 [F(1,21) = 10.772, P = 0.004] in the AS group compared to controls. We observed a considerable overall increase in delta power in AS [F(1,22) = 5.634, P = 0.027], which after controlling for age was no longer significant [F(2,42) = 3.632, P = 0.07].



EEG absolute power spectral density in Asperger, expressed relative to Controls across each frequency band. The upper plots represent the ratio between the power spectral densities for the two groups calculated using the geometric averages over all subjects in the respective groups. The horizontal reference line at 0 reflects mean absolute spectral power density values for the Control group. Smoothing is applied with a twenty point Hanning window. Plots in the bottom panels reflect the levels of significance (p values) with respect to Group main effects (averaging over all channels) (left figure) and Group vs. sagital-Region (interactions averaging over each main sagital region: frontal, centro-temporal, posterior) (right figure);

Relative power spectrum density

Relative delta PSD exhibited a significant main effect for the factor Group [F(1,21) = 12.067, P = 0.002] for delta, pointing to an overall increase of the relative spectral power in AS group compared to the Controls. Furthermore, a significant interaction between the factors Group and sRegion in the sigma [F(2,42) = 4.875; P = 0.012] and beta [F(2,42)= 5.252, P = 0.011] frequency bands emerged. Post hoc analysis revealed a significant decrease of relative sigma PSD over the frontal (P = 0.02), and of relative beta PSD over the centro-temporal (P = 0.025) areas. Please note that in all of these analyses age was used as a co-variate.



EEG relative power spectral density in Asperger, expressed relative to Controls across each frequency band. The upper plots represent the ratio between the relative power spectral densities for the two groups calculated using the geometric averages over all subjects in the respective groups. The horizontal reference line at 0 reflects mean relative spectral power density values for the Control group. Smoothing is applied with a twenty point Hanning window. Plots in the bottom panels reflect the levels of significance (p values) with respect to Group main effects (averaging over all channels) (left figure) and Group vs. sagital-Region (interactions averaging over each main sagital region: frontal, centro-temporal, posterior) (right figure);

Sleep spindle analyses

In the first step analyzing the spectral activity in the individually adjusted sigma frequency bands, we found a Group main effect in the slow sigma band pointing to a global decrease in the power spectrum in the AS group compared to the healthy controls [$F(1, 21) = 14.105$, $p = 0.001$]. We also found a significant interaction between factor Group and sRegion [$F(2, 42) = 6.680$, $p = 0.003$] in this frequency band. The post hoc analyses revealed significantly lower absolute power over the frontal ($p = 0.0001$) and centro-temporal ($p = 0.025$) regions, as well as a non-significant tendency in the same direction over the posterior region ($p = 0.051$). Hemispheere factor apparently had no effect on group differences.

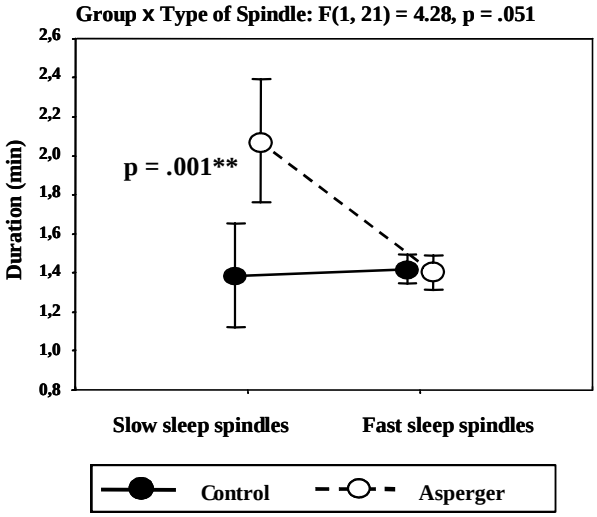
Sleep spindle density exhibited significant interaction between factor Group and factor Type of Spindle [$F(1, 21) = 6.144$, $p = 0.022$], marking a different behaviour of the two type of spindles (slow and fast) in the two groups.

However, post hoc analyses did not present significant group differences or any non-significant tendency in that direction.

We also found a group x Type of Spindle x sRegion interaction in spindle density ($F(1, 21) = 8.571$, $p = 0.008$). Post hoc analyses pointed to a non-significant tendency in group differences in the slow parietal spindles ($p = 0.065$), the AS group presenting slightly higher values in this regard.

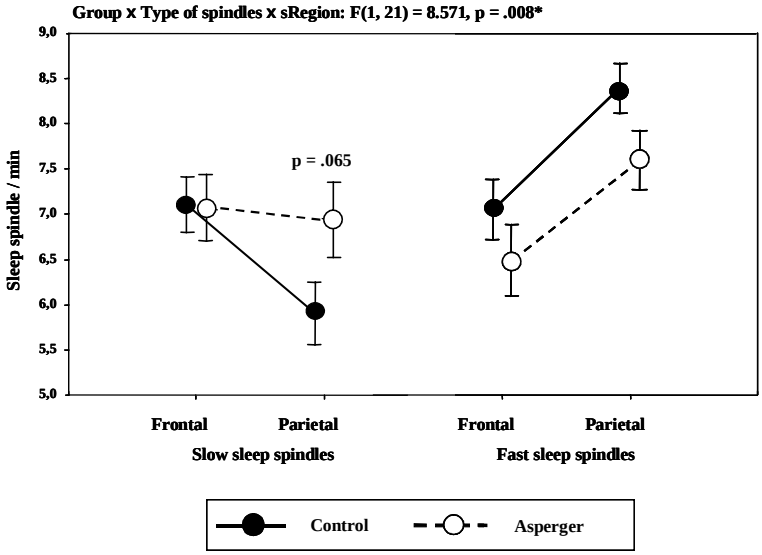
Regarding spindle duration we found a group main effect [$F(1, 21) = 6.494$, $p =0.019$], pointing to a globally increased spindle duration in the AS group compared to the controls. There was also

present a marginally significant interaction between factor Group and factor Type of Spindle [F(1, 21) = 4.280, p = 0.051]. Post hoc analyses revealed significantly longer slow sleep spindles in the clinical group (p=0 .001).



Group differences in sleep spindle duration

Spindle amplitude exhibited a Group main effect [F(1, 21) = 7.079, p = 0.015], pointing to a significantly higher amplitude of the individually detected sleep spindles. There was also present a tendency towards a non-significant Group x Type of Spindle x sRegion interaction [F(1, 21) = 3.383, p = 0.08]. Post hoc analyses revealed significantly lower amplitude of the slow sleep spindles (p = 0.007).



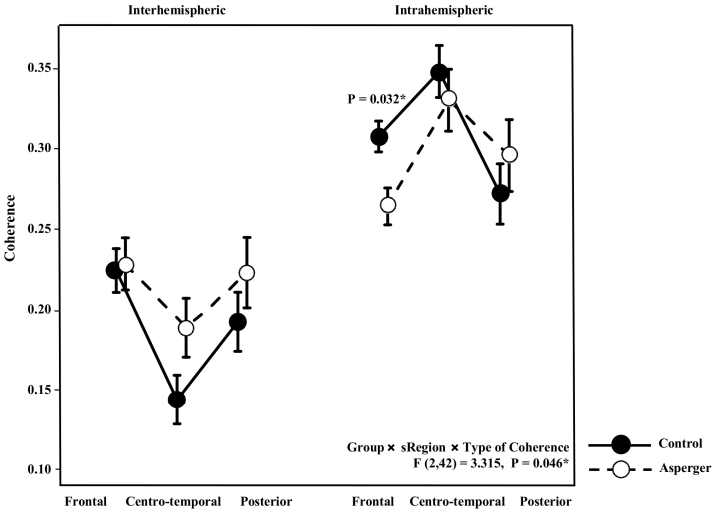
Group differences in sleep spindle density

Coherence analyses

In the first step, analyzing group differences in coherence with respect to the entire frequency range we found a significant Group × sRegion × Type of coherence interaction [$F(2,42) = 3.315$, $P = 0.046$]. Post hoc analysis revealed significantly lower values of intrahemispheric coherence over the frontal region ($P = 0.032$) in the AS group compared to Controls, while interhemispheric coherence was not significantly different between the groups.

In the second step, analyzing group differences in intra-hemispheric coherence over the entire frequency range, we found a

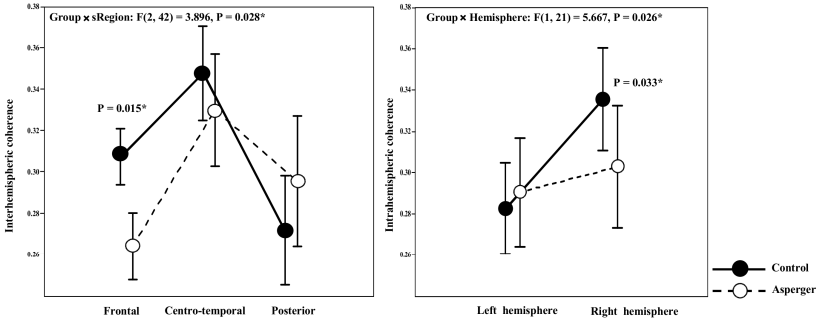
significant interaction between the factors Group and sRegion [F(2,42) = 3.896, P = 0.028] and the factors Group and Hemisphere [F(1,21) = 5.667, P = 0.027]. Fisher LSD Post hoc analyses revealed significantly lower values of intrahemispheric coherence in AS compared to the Controls over the frontal (P = 0.015) region and the right hemisphere (P = 0.033).



Group differences in inter- and intrahemispheric coherence

Using univariate analysis of covariance we computed group differences along the electrode pairs belonging to the affected regions as revealed by the multivariate analysis of covariance. Because all significant differences along the factor sRegion emerged

exclusively over the frontal region we included all intrahemispheric electrode pairs related to this region (F3-C3, F3-T3, F3-P3, F3-O1, F4-C4, F4-T4, F4-P4, F4-O2) in the analysis. We found significantly lower ($P < 0.002$) coherence values in the left (F3-C3) and right (F4-C4) fronto-central areas with respect to the entire frequency band (0.5-45 Hz).



Group differences in intrahemispheric coherence

After we applied Bonferroni correction for the 56 comparisons (8 channels x 7 frequency bands) we found significantly decreased intrahemispheric coherence values over the F4-C4 area in delta ($P = 0.004$), theta ($P = 0.017$), and sigma ($P = 0.001$) as well as in the F3-C3 region in alpha ($P = 0.049$) and sigma ($P = 0.030$) frequency bands.

Conclusions

This is a first study in which whole night NREM sleep dependent EEG spectra and its topography as well as phase coherence in children and adolescents with AS is compared with age and IQ matched controls. Marked changes in both absolute and relative power density values as well as changes in intrahemispheric coherence were observed in AS.

In conclusion, our sleep EEG findings indicate an altered brain connectivity pattern involving primarily the connection between the frontal region with other posterior cortical areas in children and adolescents with AS. This interpretation of these sleep EEG findings is in accordance with the underconnectivity hypothesis of autistic spectrum disorders affecting primarily the long distance fronto-cortical connections. Our results are also in accordance with theories claiming a globally delayed brain maturation in AS, as being reflected by the increased delta activity, as well as with those assumptions which predict a more specific developmental deficit affecting the thalamo-cortical system, as this being reflected by the modified sleep spindle parameters.

However, this is the first study to provide evidence in this regard on the basis of an exhaustive linear quantitative analysis of the whole night NREM sleep dependent oscillatory activity involving a large range of frequencies in children and adolescent with AS.

List of publications

Publications related to the dissertation

Lázár AS, Lázár ZI, Bíró A, Győri M, Tárnok Z, Keszei A, Stefanik K, Prekop C, Gádoros J, Halász P, Bódizs R. Reduced fronto-cortical brain connectivity during NREM sleep in Asperger syndrome: An EEG spectral and phase coherence study. Clin Neurophysiol. *In press.*

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Other publications

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Bódizs R, Sverteczki M, **Lázár AS**, Halász P. (2005) Human parahippocampal activity: non-REM and REM elements in wake-sleep transition. Brain Res Bull. 65: 169-76.

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