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Diurnal changes in human brain glutamate + glutamine levels in the course of development and their relationship to sleep

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ABSTRACT

Sleep slow waves during non-rapid eye movement (NREM) sleep play a crucial role in maintaining cortical plasticity, a process that is especially important in the developing brain. Children show a considerably larger overnight decrease in slow wave activity (SWA; the power in the EEG frequency band between 1 and 4.5 Hz during NREM sleep), which constitutes the primary electrophysiological marker for the restorative function of sleep. We previously demonstrated in adults that this marker correlates with the overnight reduction in cortical glutamate + glutamine (GLX) levels assessed by magnetic resonance spectroscopy (MRS), proposing GLX as a promising biomarker for the interplay between cortical plasticity and SWA. Here, we used a multimodal imaging approach of combined MRS and high-density EEG in a cross-sectional cohort of 46 subjects from 8 to 24 years of age in order to examine age-related changes in GLX and its relation to SWA. Gray matter volume, GLX levels and SWA showed the expected age-dependent decrease. Unexpectedly, the overnight changes in GLX followed opposite directions when comparing children to adults. These age-related changes could neither be explained by the overnight decrease in SWA nor by circadian factors.

1. Introduction

Throughout life, the brain remains plastic and has the ability to shape neural circuits as a consequence of experience, which underpins learning and memory (Kolb and Gibb, 2014). Neural plasticity is not restricted to the waking brain but is also strongly dependent on sleep (Wang et al., 2011). Specifically, learning-related changes in synaptic strength modulate slow wave activity (SWA; EEG power during non-rapid eye movement (NREM) sleep in the frequency range of 1–4.5 Hz) during subsequent sleep (Hanlon et al., 2009; Huber et al. 2004, 2006), which was shown to be critical for maintaining learning capacity (Fattinger et al., 2017). These fundamental observations seem to be even more prominent during cortical maturation, as the developing brain matures over a sensitive period during which the capacity of plastic changes is extremely high (Knudsen, 2004). Correspondingly, the post-learning rise in SWA is even higher in children compared to adolescents and adults (Wilhelm et al., 2014). Additional evidence for a tight connection between SWA and cortical maturation comes from studies showing parallel temporal and spatial trajectories. Overall SWA increases until the age of about 8 years, plateaus thereafter and shows an exponential decline into adulthood (Feinberg and Campbell, 2012). This trajectory parallels markers of cortical maturation such as synaptic density and cortical gray matter volume (Giedd et al., 1999; Gogtay et al., 2004; Huttenlocher, 1979; Huttenlocher and Dabholkar, 1997). Moreover, the topographical cortical maturation from posterior to anterior regions of the brain (Sowell et al., 2004) is paralleled by a shift of maximal SWA in the same posterior-anterior direction (Kurth et al., 2010). SWA reflects not only experience and maturation dependent neural plasticity but also subserves the restorative function of sleep. The build-up of sleep pressure with time of prior wakefulness is mirrored in a corresponding increase of SWA at the beginning of the night. In the course of sleep, when sleep pressure decays, SWA decreases exponentially over consecutive NREM sleep episodes (Achermann and Borbély, 2003). Importantly, preventing this SWA decrease, e.g. by selectively suppressing slow wave sleep, increases sleep propensity the next day (Agnew et al., 1964). Interestingly, the decrease in SWA in the course of

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the night also shows age-dependent changes. From children to middle-aged adults, there is a steady attenuation of the overnight decline in SWA, suggesting a progressive reduction in homeostatic sleep pressure with age (Gaudreau et al., 2001; Jenni and Carskadon, 2004). However, we do not know how this age-dependent change in the recovery function of sleep is related to cortical plasticity.

A key neurotransmitter related to cortical plasticity is glutamate, which can be quantified non-invasively with proton magnetic resonance spectroscopy (^{1}H-MRS). Using this technique, we have recently demonstrated that glutamate + glutamine (GLX) shows diurnal changes in the left parietal cortex in healthy young adults (Volk et al., 2018). Most interestingly, the gradual decrease in sleep SWA was correlated with the decrease in GLX from evening to morning. Specifically, a higher decrease in SWA in the course of the night was associated with a larger reduction in GLX levels, indicating that MRS measured changes in GLX levels might represent a promising biomarker for the interplay between brain plasticity and sleep SWA. Given the massive structural and functional reorganization that occurs during brain maturation, in the present study we investigated whether these processes might be expressed as changes in MRS measured GLX levels. Since both SWA during initial sleep and its gradual decrease in the course of the night show a marked reduction with age, we hypothesized that GLX levels would show the same age-related dependency, resulting in a progressive reduction in overnight changes from childhood to young adulthood.

2. Methods

2.1. Participants

A total number of 46 subjects between the age of 8 and 24 years (mean ± s. e.m., 16.5 ± 0.78 years, 24 females) were included in this study. Data of 16 subjects was previously published (Volk et al., 2018). Morning spectroscopy data of 1 subject exceeded the threshold for outlier criteria and therefore morning spectroscopy data comprises 45 subjects only. All subjects were recruited via advertisement placed at the University and the social media page of the University Children's Hospital Zurich. Inclusion criteria comprised the following: No personal or family history of psychopathology, no sleep disorders, good sleepers (sleep efficiency > 80%), no chronic diseases, no current use of psychoactive agents or other medications, no travelling across time zones during the month before the study, non-smoker, no high caffeine (>160 mg caffeine/day) or alcohol consumption (>14 mg alcohol/day for adults, >3–4 standard alcoholic drinks for adolescents (16 years and older) and no alcohol consumption for subjects younger than 16 years of age). Adults and adolescents were not allowed to consume alcohol 48 h before the start of the measurements. Extensive exercise as well as visits to the sauna were not allowed on the day of the recording. The study was approved by the local ethics committee and subjects gave written informed consent before participating. In case the subjects were younger than 14 years of age, written informed consent was given by the legal guardian. Adult participants were compensated monetarily; children and adolescents were compensated according to choice either with coupons for the cinema or the bookstore.

2.2. Experimental procedure

One week before the scheduled measurement, participants were instructed to keep a regular sleep-wake rhythm adjusted to their habitual bedtime and were equipped with a wrist-worn motor actigraph (GENEActiv, activinsights Ltd., Kimbolton, Huntingdon, UK). A self-reported journal was used to log sleep times, exercise hours and intake of alcohol/caffeine containing beverages or food. The experimental part took place at the University Children's Hospital Zurich and started with the magnetic resonance imaging (MRI), performed between 2 and 3 h before lights off (mean ± s. e.m., 2.6 h ±3.6 min). Thereafter, subjects were equipped with a high-density (hd) EEG net and sleep was recorded all night. The following morning, after removal of the EEG net, the MRI was repeated (mean ± s. e.m 1.5 h ±2.5 min after lights on).

2.3. High-density electroencephalography

The high-density EEG nets (128 channels, Sensor Net for long-term monitoring; Electrical Geodesic Inc., EGI, Eugene, OR, USA) were adjusted to the vertex and mastoids and subsequently filled with electrolyte gel. Four additional electrodes were mounted for visual scoring (2 submental EMG electrodes and 2 electrodes at the earlobes; Grass Technologies, West Warwick, RI, USA). Impedances were kept below 50 kΩ. EEG was recorded across the whole night, sampled at 500 Hz (filtered between 0.01 and 200 Hz) and referenced to the vertex (Cz). Afterwards, data was band-pass filtered (0.5–40 Hz) and down sampled to 128 Hz. Sleep stages were scored for 20-sec epochs according to standard criteria (Iber et al., 2007) by a sleep expert and audited by a second expert. Artefact-containing epochs were excluded by visual inspection and if power exceeded a threshold based on a mean power value in the 0.75–4.5 or 20–30 Hz band. After removal of bad channels and channels below the ears (due to common contamination by muscle artefacts) data were re-referenced to the average value of all remaining channels. Sleep cycles were defined according to the criteria of Feinberg and Floyd, (1979) and adapted for children (as described by Jenni and Carskadon, 2004; Kurth et al., 2010) when skipped REM occurred). Spectral analysis was performed for consecutive 20 s epochs for each sleep cycle (fast Fourier transformation, Hanning window, average of five 4 s epochs, frequency resolution of 0.25 Hz). Missing data from excluded electrodes were interpolated using spherical linear interpolation (Delorme and Makeig, 2004) resulting in 109 channels per subject. SWA (1–4.5 Hz) and spindle power (12–15 Hz) was calculated as the mean power for every NREM sleep episode (stage N2 and stage N3).

2.4. Magnetic resonance imaging

MR imaging and spectroscopy scans were performed with a 3T GE MR 750 scanner, using an 8 channel receive-only head coil. Due to a scanner software upgrade in the course of the study, not all subjects were recorded with the same software version. However, data acquisition before and after scanner upgrade was nearly balanced (25 subjects before and 21 subjects after the upgrade). Subjects were instructed to stay awake during the scanning session and were asked before and after each scan if they were awake and if they had fallen asleep during the previous scan. Single voxel Point RESolved (PRESS) ^{1}H-MR spectra were acquired from a 20 × 20 × 20 mm^3 voxel of interest positioned in the left parietal lobe (see Fig. 1).

The parietal lobe was chosen because it is one of a standard set of MRS regions (Bai et al., 2015; Hallahan et al., 2012; Robertson et al., 2001), where the data quality is usually high and the position can be calculated precisely by a standard set of measurements. On a midline sagittal localizer image, the voxel was located one third of the distance between the posterior commissure and the back of the brain (along a line defined by the angle of the corpus callosum), and halfway between the vertical distance from this line to the vertex. Subsequently, the voxel position was adjusted in the lateral (right-left) direction and visually inspected by overlying the voxel coordinates over the T1 image. Spectra were acquired with an echo time (TE) of 35 m s, a repetition time (TR) of 3 s, and 96 spectral averages. The scanning protocol also included a 3D high resolution T1-weighted IR-SPGR scan (TE = 3 m s, TR = 8 m s, inversion time = 600 m s, flip angle = 8°, voxel resolution = 1 mm^3), used for correction of partial volume cerebrospinal fluid (CSF) effects. Water-scaled concentrations were calculated with LCModel version 6.3-1k and corrected for partial volume CSF contamination after segmentation of the 3D T1-weighted images into gray matter (GM), white matter (WM), and CSF maps in SPM8 (Statistical Parametric Mapping; Wellcome Department of Imaging Neuroscience, Institute of Neurology, University College London) based on a unified segmentation model

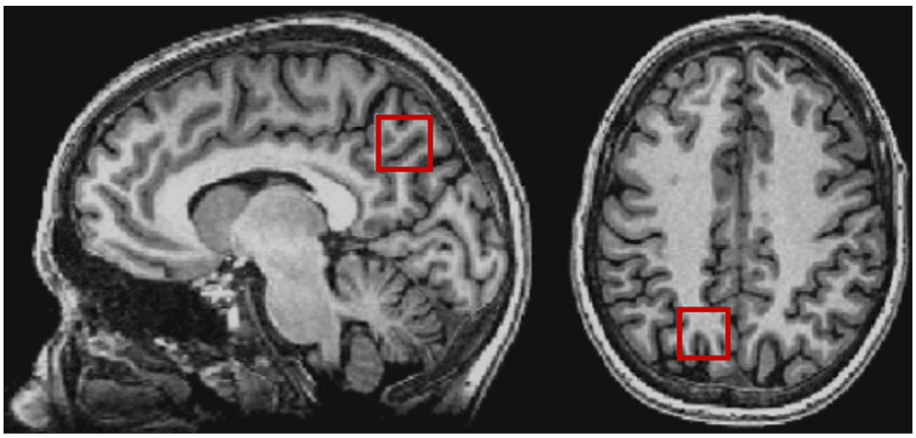

Fig. 1. Voxel position in the left parietal lobe (red rectangle on T1 image) in the sagittal plane (left) and the axial plane (right).

(Ashburner and Friston, 2005). The correction for partial volume CSF contamination was performed as described by Chowdhury (Chowdhury et al., 2015) using the formula Mcorr = M/(1 - CSF), where M is the uncorrected metabolite concentration, Mcorr is the corrected concentration and CSF is the CSF fraction within the spectroscopy voxel. Each spectrum was visually inspected for the presence of artefacts or fitting errors, and spectra with Cramer-Rao variance bounds of more than 10% for Creatine + Phosphocreatine or N-acetyl-aspartate, or more than 20% for glutamate were excluded from further analysis. Since glutamate and glutamine show overlapping resonance frequencies (chemical shifts) at 3T, we quantified the combined levels of glutamate and glutamine (GLX), as frequently done (e.g. Dlabac-de Lange et al., 2017).

2.5. Statistics

Correlation between age and metabolite concentrations were assessed using Spearman's rank order correlation coefficient. Differences in GLX concentrations between the age groups were tested using Kruskal-Wallis and post hoc pairwise comparisons using Mann-Whitney-U-Test. Values exceeding a threshold of three standard deviations from the mean were excluded, which led to the exclusion of GLX morning levels for 1 subject. Therefore, the calculation of the overnight change is based on 45 subjects. The decrease in SWA in the course of the night was calculated as the percentage reduction from the NREM sleep episode with maximal SWA (= 100%) to the last NREM sleep episode. Overnight changes in metabolite concentrations were calculated in the same way, with evening levels set to 100%. To assess the association between changes in GLX levels and SWA, Spearman's rank correlation coefficients between the decrease in SWA at every electrode and the overnight changes in GLX levels were calculated. All analyses were performed with the software package MATLAB (MathWorks) and the computing environment R (R Development Core Team, 2017). The significance level was set at $p < 0.05$ (two-tailed).

3. Results

First, we assessed age-dependent changes in levels of GLX. GLX levels measured in the evening and in the morning showed a significant reduction with age ($p_{evening} = 0.01$, $r_{evening} = -0.4$; $p_{morning} < 0.001$, $r_{morning} = -0.7$; Fig. 2(A) and (B)). In order to identify if this is specific for GLX, morning and evening levels were examined also for NAA, NAA + NAAG, GPC + PCh, mI, Cr + PCr. In addition to GLX, there was a positive

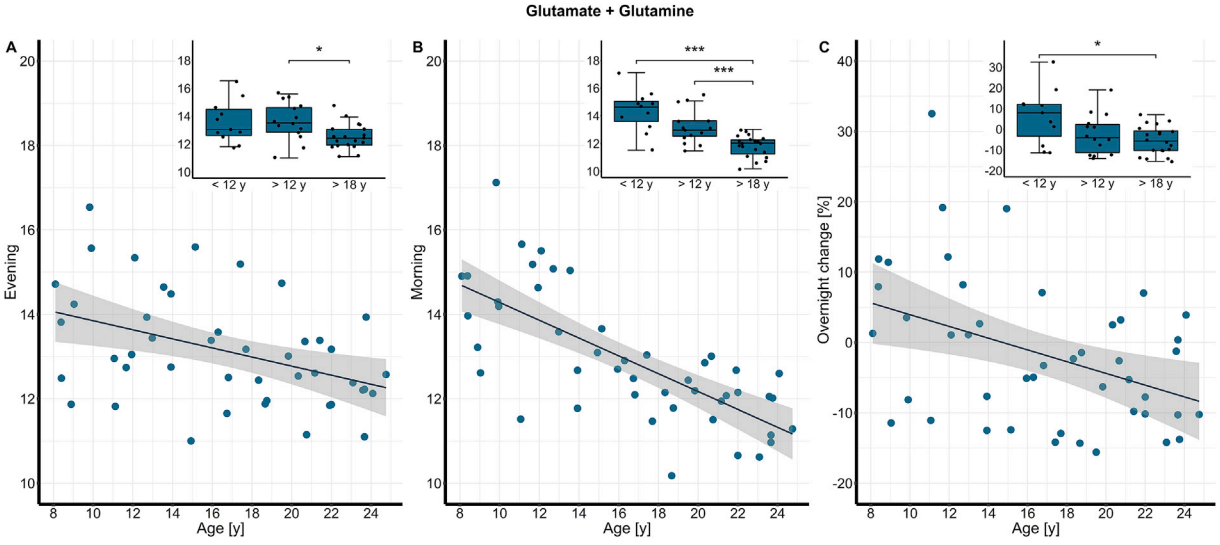

Fig. 2. Age-dependent changes in GLX levels. **(A)** Spearman's rank correlation of GLX levels in the evening ($p = 0.01$, $r = -0.4$) and between group analysis of children (<12 years), adolescents (>12 years) and adults (>18 years) as inset (Kruskal-Wallis χ^2 (2) = 7.4, p = 0.03). **(B)** Spearman's rank correlation of GLX levels in the morning ($p < 0.001$, $r = -0.7$) and between group analysis as inset (Kruskal-Wallis χ^2 (2) = 20.4, p < 0.001). **(C)** Spearman's rank correlation of GLX overnight changes (%, $p = 0.01$, $r = -0.4$) and between group analysis as inset (Kruskal-Wallis χ^2 (2) = 6.9, p = 0.03). Asterisks denote significant results of post-hoc pairwise comparisons using Mann-Whitney-U-Test. * < 0.05, ** < 0.01, *** < 0.001, **** < 0.0001.

correlation between age and NAA + NAAG, both in the evening and in the morning ($p_{evening} = 0.001$, $r_{evening} = 0.5$; $p_{morning} = 0.01$, $r_{morning} = 0.4$), and a trend towards a negative correlation between age and GPC + PCh evening levels ($p = 0.06$, $r = -0.3$). Next, we focused on one of our primary questions, namely how overnight changes in GLX change across development.

We found that the percentage overnight changes in GLX correlated negatively with age ($p_{change} = 0.01$, $r_{change} = -0.4$; Fig. 2 C). The overnight changes in GLX were significantly different between children and adults ($p < 0.05$; pairwise comparisons using Mann-Whitney-U-Test, fdr adjusted) and changed its sign from positive to negative (mean children (<12 years) = 6.3%, mean adults (>18 years) = −5.4%)). No other metabolites showed a significant correlation for overnight changes with age (all $p > 0.2$). Because cortical gray matter (GM) fraction, as expected, showed a highly significant decrease with age in the evening ($p < 0.001$, $r = -0.5$) and in the morning ($p < 0.001$, $r = -0.6$; shown in Fig. 3(A) and (B)), correlation of metabolite levels with age were corrected for GM fraction of the respective scan (partial Spearman's rank correlation). Accordingly, correlations between overnight changes in metabolite concentration and age were corrected for the mean GM fraction of the evening and morning scans, respectively (GM between evening and morning showed no differences; Wilcoxon signed rank test, $p = 0.7$, $z = -0.3$). After correction, evening levels of GLX showed no correlation with age ($p = 0.23$, $r = -0.2$). Morning levels and percentage overnight changes in GLX were still significantly correlated with age ($p_{morning} < 0.001$, $r_{morning} = -0.6$; $p_{change} = 0.002$, $r_{change} = -0.5$). To further exclude white matter (WM) as a confounding factor for age related changes in GLX levels, we additionally performed the same analysis for the WM fraction within the voxel of interest. As for GM, when correcting for WM, GLX levels in the evening showed no correlation with age ($p = 0.82$, $r = -0.04$), morning levels and percentage overnight changes in GLX were still significantly correlated with age (p morning < 0.001, r morning = −0.6; p change = 0.004, r change = −0.4). In a next step, we correlated global SWA from the NREM sleep episode displaying maximal SWA (SWA max), the last NREM sleep episode (SWA last) and its percentage decrease (SWA percentage change) with age, confirming an age-dependent decrease in all three measures (SWA max: $p < 0.001$, $r = -0.9$; SWA last: $p < 0.001$, $r = -0.8$; SWA percentage change: $p = 0.001$, $r = 0.5$; Fig. 4).

Finally, we performed an electrode-wise correlation between percentage changes in GLX and the percentage reduction in SWA. This analysis did not reveal any significant relationship (when correcting for multiple testing, data not shown; correlation of mean SWA over all electrodes and percentage changes in GLX: $p = 0.4$, $r = -0.1$). In an exploratory approach, we performed a correlation analysis with power in the spindle frequency range, the second characteristic of NREM sleep, which was shown to exhibit age-dependent changes (e.g., Gaudreau et al., 2001). As expected we found a strong negative correlation between global spindle power and age ($p < 0.001$, $r = -0.73$). No significant relationship between spindle power and overnight changes in GLX were observed when correcting for age ($p > 0.5$). Further analyses were performed to exclude obvious potential masking effects. First, we performed correlations between sleep architecture parameters and changes in GLX. When controlling for age, because of the well-known age-related changes in sleep architecture, no significant correlations were found (all $p > 0.1$). In a second analysis, we focused on REM sleep in more detail, since it was previously shown that glutamate levels increase during REM sleep and wake in the rat cortex (Dash et al., 2009). Because both REM sleep and wake are more abundant towards the end of the night, we examined if there is a relation between changes in GLX and wake or REM sleep during the last hour of time in bed. Neither wake time nor the amount of REM sleep correlated with GLX morning levels (wake: $p = 0.2$, $r = 0.2$; REM sleep: $p = 0.8$, $r = -0.04$) or with GLX percentage change (wake: $p = 0.4$, $r = -0.1$; REM sleep: $p = 0.6$, $r = -0.1$). Additionally, we tested if changes in GLX might be explained by the sleep midpoint, an established marker of circadian rhythmicity. Again, as expected, the sleep midpoint significantly correlated with age ($p < 0.001$, $r = 0.6$). However, when controlling for age, no correlation between the sleep midpoint and GLX levels or the changes were found (all $p > 0.5$). In a last step, we explored if timing of the MR spectroscopy or the hours subjects have been awake before the spectroscopy was performed had an influence on GLX levels (see Table 1). None of these variables showed a significant association with measured GLX levels.

4. Discussion

Our cross-sectional study using multimodal neuroimaging confirmed the age-related decrease in 1) cortical gray matter volume; 2) sleep SWA in the maximal and the last NREM sleep episode and 3) GLX levels in the evening and in the morning. As expected, we also found a larger overnight decrease of SWA in children compared to adults. Unexpectedly, the overnight reduction of GLX was larger in adults compared to children,

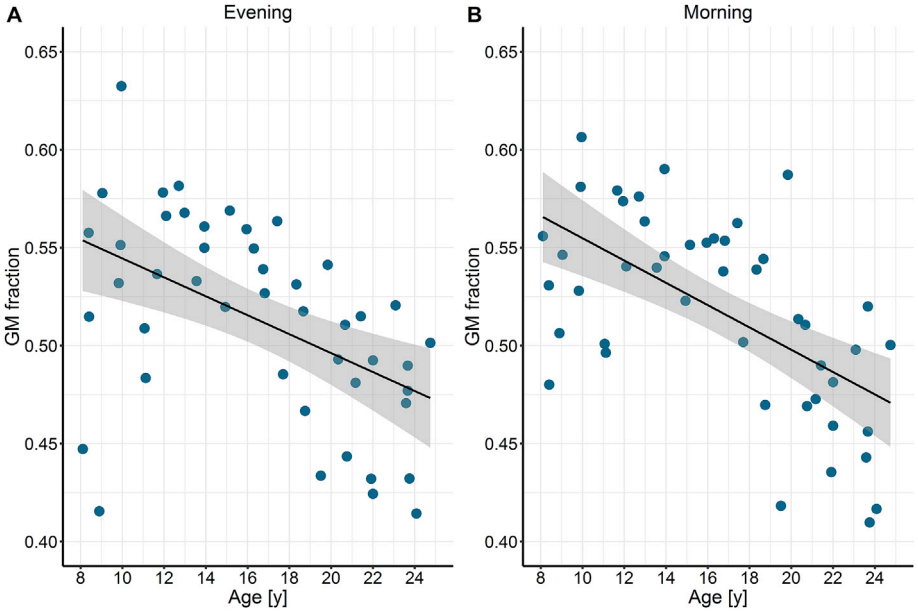

Fig. 3. Age-dependent changes of the GM fraction within the voxel of interest (A) in the evening ($p < 0.001$, $r = -0.5$) and (B) in the morning ($p < 0.001$, $r = -0.6$).

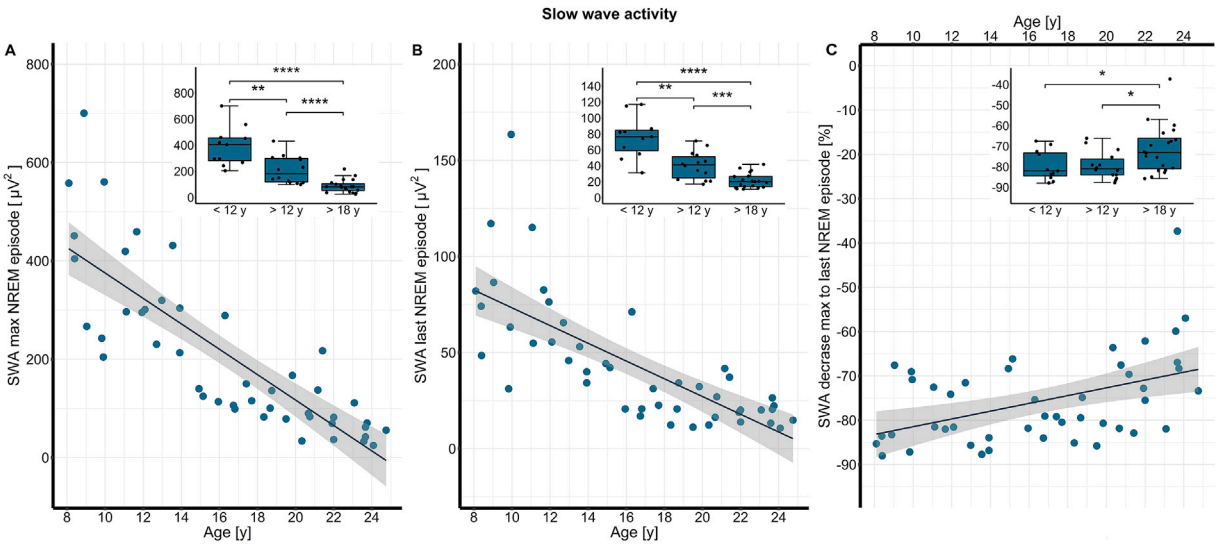

Fig. 4. Age-dependent changes in Slow wave activity. **(A)** Spearman's rank correlation of the sleep cycle with maximal SWA ($p < 0.001$, $r = -0.9$) and between group analysis of children (<12 years), adolescents (>12 years) and adults (>18 years) as inset (Kruskal-Wallis χ^2 (2) = 29.1, p = < 0.0001). **(B)** Spearman's rank correlation of SWA in the last sleep cycle ($p < 0.001$, $r = -0.8$) and between group analysis as inset (Kruskal-Wallis χ^2 (2) = 27.2, p < 0.0001). **(C)** Spearman's rank correlation of the percentage change in SWA from maximal to last cycle ($p = 0.001$, $r = 0.5$) and between group analysis as inset (Kruskal-Wallis χ^2 (2) = 6.5, p = 0.04). Asterisks denote significant results of post-hoc pairwise comparisons using Mann-Whitney-U-Test. * < 0.05, ** < 0.01, *** < 0.001, **** < 0.0001
SWA =
slow wave activity
NREM sleep =
Non-rapid eye movement sleep.

Table 1
Spearman's rank correlation coefficients between scanner times and glutamate + glutamine levels. In case age correlated with scanner times, partial Spearman's rank correlation.

	Time MRS eve				Time MRS mor		Minutes being awake at eve MRS				Minutes being awake at mor MRS	
	p	*r*	*p* partial	*r* partial	*p*	*r*	*p*	*r*	*p* partial	*r* partial	*p*	*r*
Age	<0.001				1.00		<0.001				0.24	
GLX eve	0.26	−0.17	0.22	0.19	0.33	−0.15	0.07	−0.28	1.00	<0.001	0.71	−0.06
GLX mor	<0.001	−0.51	0.62	0.08	0.51	−0.10	<0.001	−0.56	0.91	−0.02	0.67	−0.06
GLX % change	0.03	0.33	0.65	0.07	0.41	−0.13	0.05	0.30	0.97	0.01	0.66	0.07

who showed a mean change towards a positive direction. As a consequence, we found no significant relationship between the overnight reduction of GLX and SWA. These latter findings contradict our hypothesis derived from our previous work in adults which showed that GLX decreases with the gradual decline of SWA in the course of the night. While this relationship might be true in adults, we could not confirm the same relationship of GLX and SWA when extending our sample to children and adolescents.

Our results of an age-related change in GM fractions within the parietal voxel fit to the well-described developmental decrease observed in GM volume (Giedd et al., 1999; Giedd, 2004; Giedd and Rapoport, 2010; Lenroot et al., 2007). These changes in GM might reflect a remodeling of synaptic strength/density and might also include changes in white matter (Giedd and Rapoport, 2010). In parallel with these changes in cortical GM, sleep SWA also changes in the course of development. Several studies have shown that SWA is highest during childhood and decreases during adolescence (Campbell and Feinberg, 2009; Feinberg and Campbell, 2012; Kurth et al., 2010). We were able to confirm an age related decrease for both SWA during the NREM sleep episode displaying maximal SWA and SWA in the last NREM sleep episode. When it comes to age-dependent changes in GLX, a more functional anatomical marker of brain maturation, evidence is scarce and depends on the brain region and the age of included subjects. Two studies that have included a similar population to that of the present study demonstrated an age-related decrease of glutamate in the basal ganglia (Ghisleni et al., 2015) and a decrease of GLX in the occipital and frontal cortex (Shimizu et al., 2017). Other studies investigating older cohorts have confirmed that the age-related decrease in glutamate continues throughout adulthood in a healthy aging population, in various brain regions including frontal and parietal cortices (Gao et al., 2013; Kaiser et al., 2005; Marsman et al., 2013). The similar developmental trajectory of the presented parameters, that show a decline with age, might be explained by similar underlying mechanisms, i.e. synaptic pruning/refinement (Huttenlocher, 1979; Giedd, 2004; Hoel et al., 2016). Consequently, we would expect direct correlations between GM volume, SWA and GLX. Indeed, we found a positive correlation between GM fractions derived from evening and morning scans and SWA in the maximal ($p_{eve} = 0.001$, $r_{eve} = 0.5$; $p_{mor} < 0.001$, $r_{mor} = 0.6$) and the last NREM episode ($p_{eve} = 0.002$, $r_{eve} = 0.5$; $p_{mor} < 0.001$, $r_{mor} = 0.5$). This fits well to the results of a previous study demonstrating a similar positive correlation between GM and SWA (Buchmann et al., 2011). We also found a relation of evening and morning GLX levels with max SWA ($p_{eve} = 0.01$, $r_{eve} = 0.4$; $p_{mor} < 0.001$, $r_{mor} = 0.6$) and last SWA ($p_{eve} = 0.03$, $r_{eve} = 0.3$; $p_{mor} < 0.001$, $r_{mor} = 0.6$). Additionally, GM fraction was highly correlated with evening and morning GLX levels ($p_{eve} < 0.001$, $r_{eve} = 0.5$; $p_{mor} = 0.001$, $r_{mor} = 0.5$). Thus, GLX levels might present a functional marker of synaptic plasticity during cortical maturation. A suggested mechanism behind the link of neural plasticity and sleep SWA is that increased synaptic strength leads to an increased number of neurons oscillating synchronously, which in turn generates a rise in SWA (Esser

et al., 2007; Vyazovskiy et al., 2009).

With dissipating sleep pressure, SWA decreases exponentially over consecutive NREM sleep episodes (Achermann and Borbély, 2003). Therefore, the decline in SWA in the course of the night is a reliable EEG marker for the restorative function of sleep (Tononi and Cirelli, 2006, 2014). In young adults, we have previously demonstrated a link between this decline in SWA and the overnight reductions in GLX, suggesting GLX as a promising biomarker for the interplay between brain plasticity and the recovery function of sleep. So far, less is known about how cortical plasticity during development is linked to the restorative function of sleep. Given that children and adolescents show a higher decline in SWA in the course of the night, we expected that GLX reductions would be more pronounced during development. Here, we find the contrary, namely a more pronounced overnight decrease in adults compared to children Thus, it is not surprising that changes in GLX and the decline in SWA were not correlated. Based on the present findings and the literature investigating the link between synaptic plasticity and SWA in humans, we can only speculate why children do not show the expected decrease. One potential cause might be found in the massive structural changes that take place during development. Childhood is associated with an overall increase in synaptic strength, which might be reflected in the overall increased levels of GLX as observed in the present study. Thus, the diurnal changes in GLX levels during development might be masked by the increase in GM or synaptic strength. Additionally, cortical reorganization includes different mechanisms from changes on the pre- and postsynaptic level to shifts in the balance of excitatory and inhibitory inputs (Turrigiano and Nelson, 2004). Therefore, changes in SWA might reflect different processes during development and an open question is if concurrent changes in SWA and GLX or GM are a mere epiphenomenon or if they indicate a functional relationship with importance for cortical maturation.

Our study suffers from some limitations that should be considered in future studies. First, we measured developmental changes using a cross-sectional approach. However, the vast structural changes during development might be disentangled more precisely in a longitudinal approach. Further, we cannot draw any conclusions regarding GLX changes outside the parietal lobe. Both, structural and functional reorganizations show a posterior - anterior trajectory. Thus, future studies should investigate how markers of cortical plasticity change with the degree of maturation across the brain. Another limitation of our study is that we only assessed whether subjects were awake in between the scanning sequences and therefore we cannot exclude that subjects might have fallen asleep during the MRS measurements. All subjects were awake before and after the MRS sequence, however 5 out of the 46 subjects reported afterwards that they might have fallen asleep shortly at some point during the scanning session. Yet, after exclusion of these subjects from the analysis, main results do not change (data not shown). Nonetheless, this assessment is based on subjective reporting and we therefore cannot ensure that other subjects might not have slept in the scanner. Additionally, some technical limitations should be considered. First, we did not distinguish between glutamate and glutamine. Glutamine represents the main precursor of neuronal glutamate and GABA (Rae, 2014). Shifts from glutamate towards its inactive metabolic form glutamine, or vice versa, are thus not detectable in our study. However, even with separation of glutamate, MRS measured glutamate represents the whole glutamate content in the region of interest and thus it cannot be distinguished between glutamate used for metabolic purposes and glutamate used as a neurotransmitter. Second, evening and morning measures differed in respect to the circadian phase. However, we did not find a correlation between sleep midpoint, a well-described marker for circadian rhythmicity, and GLX levels or GLX overnight changes.

In conclusion, we demonstrated that there are age-related differences in the overnight changes in GLX that cannot be explained by the main sleep electrophysiological markers, previous sleep-wake history or circadian factors. Although these results contradict our hypothesis, namely that children would show a more pronounced overnight reduction in GLX levels, it is important to note, that GLX was the only metabolite demonstrating age-dependent diurnal changes. These age-dependent changes might be of particular interest for the "neuroimaging community", since the influence of the time of day on GLX measures should be considered differently across age. These changes might also become interesting with respect to clinical populations. Cortical development is marked by extensive changes in the brain, which makes it extremely vulnerable for deviations that might result in pathological changes (Paus et al., 2008). Consequently, there is an increased need for multi-modal imaging to map and connect normal and abnormal maturational processes in order to enhance early treatment options.

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuroimage.2019.04.040.

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