COGNITIVE STIMULATION HAS POTENTIAL FOR BRAIN ACTIVATION IN INDIVIDUALS WITH RETT SYNDROME

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Conflict of interest

The authors have stated that they had no interests relevant to this article to disclose.

ABSTRACT

Background: Knowledge regarding neuropsychological training in Rett syndrome (RS) is scarce. The aim of this study was to assess the outcome and the duration of the effect of cognitive stimulation on topographic EEG data in RS.

Method: Twenty female children diagnosed with RS were included in the analysis. Girls with RS conducted a cognitive task using an eye-tracker designed to evaluate access and choice skills. EEG data was acquired during the experimental procedure including two 10-minutes baseline stages before and after the task. Topographical changes of several EEG spectral markers including absolute and relative powers, brain symmetry index and entropy were assessed.

Results: Topographic significance probability maps suggested statistical decreases on delta activity and increases on beta rhythm associated with the cognitive task. Entropy increased during and after the task, likely related to more complex brain activity. A significant positive interaction was obtained between brain symmetry index (BSI) and age showing that the improvement of interhemispheric symmetry was higher in younger girls (5-10 years).

Conclusions: According to our findings, significant alterations of brain rhythms were observed during and after cognitive stimulation, suggesting that cognitive stimulation may have effects on brain activity beyond the stimulation period. Finally, our promising results also showed an increased brain symmetry that was especially relevant for the younger group. This could suggest an interaction of the eye-tracking cognitive task, however, further studies in this field are needed to assess the relation between brain asymmetries and age.

Keywords: Rett syndrome; cognitive stimulation; electroencephalography (EEG); brain symmetry index; eye tracking.

Heading title: Assessing cognitive stimulation in Rett syndrome

Background

Rett syndrome (RS) is a neurodevelopmental disorder caused by mutations in the X-linked *MECP2* gene that mainly affects females (MECP2:OMIM*300005; Rett syndrome: OMIM#312750) (Amir et al., 1999; Balmer, Goldstine, Rao, & LaSalle, 2003). The prevalence of RS is estimated to vary around 1/15,000 of newborn females in all ethnic groups (Glaze, Kozinetz, Almes, Skender, & Percy, 1993). The most common clinical manifestation is recognized by apparently normal psychomotor development until 6 – 18 months of age, followed by severe regression in which hand skills and expressive language are lost (Neul et al., 2010). The most common accompanying symptoms are hand-washing stereotypies and gait apraxia. Seizures, scoliosis, and breathing disturbances such as periodic apnea during wakefulness may become evident (Trevathan & Naidu, 2017).

The progressive appearance of the signs and symptoms of RS has been classified in four stages, where the evolution of the clinical symptomatology and its severity is individual depending on the age of onset and the duration of each stage (Hagberg, 2002). The first stage arises between 6 to 18 months, where the first symptoms of neurological stagnation begin to manifest: low weight, cranial growth slowdown, decrease in communication and eye contact, difficulty in crawling and wavy movement of the hands. In stage II, starting at 18 months, patients show a regression in previously acquired skills: autistic behaviors, stereotyped hand movements, loss of motor skills and the normal sleep pattern, apraxia, ataxia, and intellectual disability appear. Stage III appears around three years of life and is characterized by a pseudo-stabilization of the disorder. Autistic manifestations regress and visual communication skills are restored. More pronounced epileptic seizures emerge. Finally, stage IV generally appears in late childhood where late motor impairment occurs. Patients present with severe scoliosis, cachexia, dystonia, loss of gait, and a reduction in seizures.

Assessment of the cognitive skills of RS patients is extremely challenging (Ahonniska-Assa et al., 2018). Cognitive processes and social aspects are difficult to explore in RS, mainly due to speech difficulties and loss of purposeful hand movements (Berger-Sweeney, 2011). Some studies report that many patients with RS could communicate using different ways of pre-intentional communicative abilities (Ahonniska-Assa et al., 2018; Berger-Sweeney, 2011; Sigafoos et al., 2009). Recently, the use of eye gaze has become increasingly appealing as a way to provide effective communication (Ahonniska-Assa et al., 2018; Schwartzman, Velloso, D'Antino, & Santos, 2017). Eye-tracking technology has allowed the possibility to evaluate cognitive and social aspect in RS in a measurable way. Several studies have demonstrated their utility to verify the level of understanding in RS during cognitive tasks (Ahonniska-Assa et al., 2018; Schwartzman et al., 2017) and to evaluate nonverbal cognitive social abilities and attention aspects (Djukic, Valicenti McDermott, Mavrommatis, & Martins, 2012; Rose, Djukic, Jankowski, Feldman, & Rimler, 2016), showing a preference for socially weighted stimuli and difficulty in endogenous attention.

Recently, the interest for assessing the effectiveness of the communication among the different brain areas in neurodevelopmental disorders using brain-mapping tools has increased. Electroencephalography (EEG) can be used as a low-cost neuroimaging tool that provides an excellent temporal resolution. During the last years, this tool was widely used in ADHD and autism disorders to identify prognostic factors that can help the clinical management, evaluate heterogeneity of cognitive spectrum, cognitive treatment responses, among other issues (Aldemir et al., 2018; Fabio et al., 2018; Machado et al., 2013; Nuwer, Buchhalter, & Shepard, 2016).

The aim of cognitive stimulation is to enhance the neuropsychological abilities of patients with RS. Few studies have evaluated the changes in neural-networks during neuropsychological stimulation or cognitive training in RS. Baptista et al. 2006 were the first to explore cognitive performances using eye tracking technology (Gorbachevskaya, Bashina, Gratchev, & Iznak, 2001). Vignoli et al. (2010) investigated the relationship among the attention, neuropsychological functions and neurophysiological impairment. Fabio et al. (2016) focused in the cognitive training during an eye-tracking discrimination task with simultaneous EEG recordings in short term and long term, indicating a positive effect on behavioural parameters and an improvement of neurophysiological measures after training (Roche et al., 2019). However, none of these studies has evaluated the topographical changes before and after the cognitive stimulation in patients with RS. Topographical maps allow to observe the distribution of the changes in spectral variables on the brain.

In this study, we have further explored the spectral changes due to the performance of an eye-tracking cognitive task in pediatric girls with RS. Fabio et al. (2016) assessed the changes on frequency power bands delta (δ, 1–4 Hz), theta (θ, 4–7 Hz), alpha (α, 8–13 Hz) and beta (β, 14–29 Hz) before and after cognitive stimulation. Previous studies have shown that RS patients presented an increased power in theta activity and decreased powers in alpha and beta bands (Gorbachevskaya et al., 2001). Furthermore, previous studies observed that alpha and beta powers increased while theta powers decreased after Cerebrolysin treatments (Gorbachevskaya et al., 2001) and after eye tracking visual tasks (Fabio et al., 2016). A comprehensive way to summarize the changes in bands activity is to use the median frequency (MF) and the spectral entropy (SE). These measures have been applied in different brain disorders: Bachiller et al. (2014) showed a deficit in MF and SE modulation as a response to a cognitive task for patients with schizophrenia. In Catarino et al. (2011), a SE decrease was observed in patients with Autistic disorder. However, there is no previous study evaluating the changes in complexity measures for Rett Syndrome patients.

Based on these ideas, the aim of this study was to assess the effect of the cognitive stimulation on the EEG topographical patterns and the duration of the EEG alterations. Moreover, the correlation between the spectral findings with age, Rett stage and clinical measures were assessed.

Method

This is a cohort study, a prospective observational study without a control group. The study was conducted by the Department of Neurology at Sant Joan de Déu Children's Hospital in Barcelona. It was approved by the local ethics committee following the Declaration of Helsinki and written informed consent were obtained from all parents or legal caregivers.

Participants

Thirty young girls clinically diagnosed with RS were included in the study. Two subjects did not complete the whole recording session; hence, twenty-eight participants completed the whole assessment. Thereafter, eight subjects were rejected after an artifact rejection procedure.

All participants underwent a full medical and neurological evaluation in order to determine, according to the signs and symptoms, the stage of the disease (1 to 4). The molecular analysis confirmed the presence of a pathogenic mutation in *MECP2* in all patients. Fourteen participants had a history of epileptic seizures often associated with RS: 6 patients had monthly seizures, 4 weekly, 1 daily and the 3 patients declared sporadic seizures. Twenty-seven patients were pharmacologically treated with different anticonvulsant drugs. Three participants were medication free. This complementary information is included in Figure 1 and in the supplementary table (S1). The Vineland Adaptive Behavior Scales (VABS) was administered to parents or caregivers who knew the participant well in order to measure the patient's adaptive functioning level. VABS assesses the personal and social skills of individuals using four major domains: communication, daily living skills, socialization and gross motor abilities (Sparrow & Cicchetti, 1989).

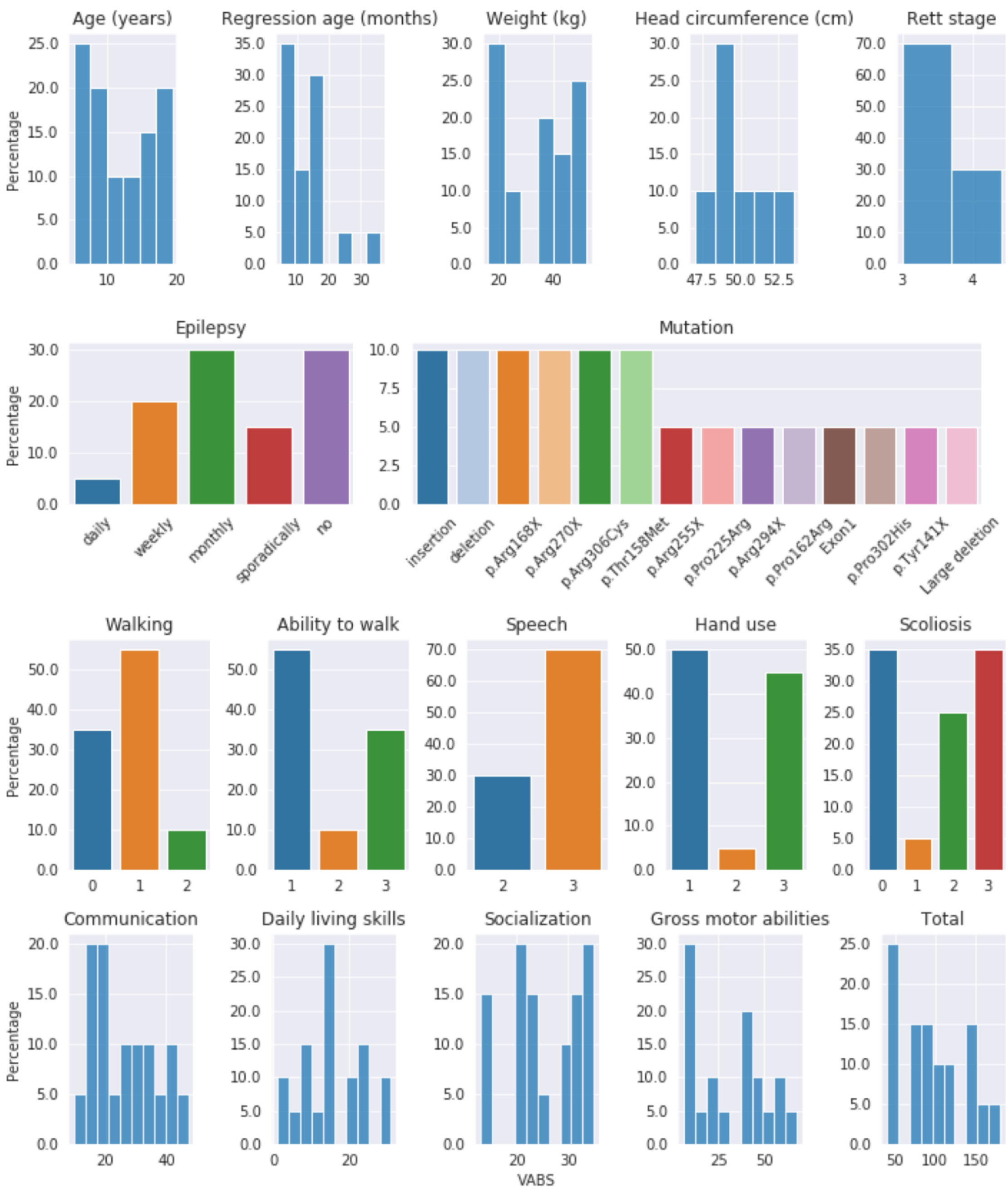

Figure 1. Demographic characteristics and distributions graphs of clinical scales for patients with RS finally included in the analysis (n=20). The first row includes demographic characteristics; the second row indicates the frequency of epilepsy seizures and the kind of gene mutation; the third row depicts functional impairment; and the last row shows the distribution of total VABS (Vineland Adaptive Behavior Scales) score.

Procedure

The experiment was conducted in a quiet room with reduced ambient light to diminish distractions. Participants were seated approximately 45 cm from a 29-inch computer monitor. A portable eye tracker (Tobii Dynavox PCEye Explore) was used in a similar way than a computer mouse, allowing the navigation and control of the computer by tracking the gaze. Each participant required her own calibration for interacting adequately with the display. The cognitive processes targeted by the task are basically attentional processes, since the attention span is severely impaired in RS patients. The experimental session was divided into several stages. Figure 2 shows an outline of the experimental procedure. Firstly, a 3-min EEG recording with eyes open was recorded as a baseline. Because it is difficult for girls with RS to be still, the next stage (7-min) consisted in the presentation of an audiovisual distractor, with the aim of achieving a relaxed atmosphere where the patient felt quiet and comfortable. The active intervention consisted in a 10-min cognitive task activity using the software Look to Learn from Smartbox Assistive Technology to encourage the user to engage the whole screen and to teach “cause and effect”. In particular, the user had to focus the eyes on an object on the screen for 1-2 seconds to be successful and reveal feedback. During the final stage, the audiovisual distractor was again presented for 10 minutes. In the analysis, this final stage was split into two equal parts in order to assess the duration of the cognitive task effects. The patients were guided by a neuropsychologist who regularly evaluated whether the patient was following the task. EEG signals were continuously recorded using a 13-channel EEG system (Starstim 20 wireless device, Neuroelectrics; Barcelona, Spain). Dry electrodes were placed in accordance with the International 10-20 system (F3, F7, Fz, F8, F4, C3, Cz, C4, T7, P3, Pz, P4, T8). Additionally, Starstim system allows to acquire the information of the head movement through an accelerometer placed on the EEG cap. EEG signals were acquired during 30 minutes referenced to the right ear lobule with a sample frequency of 500 Hz.

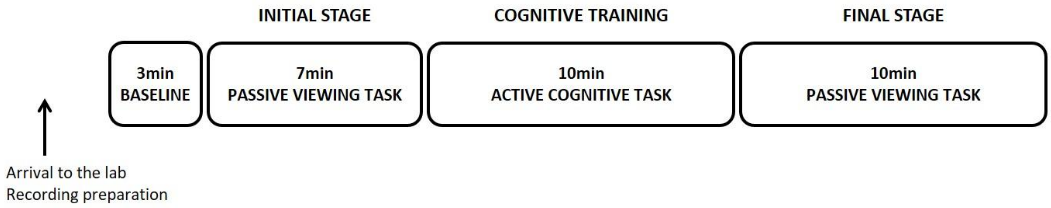

Figure 2. Schematic diagram of the time course of the experiment procedure. EEG acquisition had a 30 minutes-length: i) a 3-min EEG recording with eyes open was recorded as a baseline; ii) the initial stage (7 min) consisted in the presentation of an audiovisual distractor; iii) the active intervention consisted in a 10-min cognitive task activity using the software Look to Learn from Smartbox Assistive Technology; and iv) an audiovisual distractor was again presented for 10 minutes in the final stage.

EEG analysis

Firstly, EEG data was segmented into 5 seconds-length epochs. Next, an off-line pass-band Chebyshev FIR filter (0.5-45 Hz) was applied to EEG signals. Thereafter, data epochs were clustered into three categories: initial, task and final stages. In this way, each period comprised 10 min of duration (120 epochs).

As RS is characterized by a large series of abnormal movements, it was necessary to design a user-dependent artifact rejection procedure. An automatic adaptive algorithm based on information provided by the EEG and the accelerometer was performed to reject epochs containing amplitude artifacts (movements, eye activity, saturation, etc.) and muscular contamination. The artifact reduction method was divided in three phases: the first one detected and eliminated the outliers caused by movement, the second one removed the peaks above an amplitude threshold and finally, the last one removed muscle artifacts. To detect movement, the derivative of the accelerometer signal was calculated to accentuate the slope changes and determine the points that corresponded to sudden movements. The mean and standard deviation (SD) of this signal was then computed. The threshold was calculated as the mean+3*SD. The second step consisted in the computation of the mean and SD of the EEG signal that was already free of motion artifacts, obtaining a second threshold (mean+5*SD) that detected outliers according to their amplitude. The last step was to remove muscle artifacts, which are mostly found in the frequency band between 35 and 45 Hz (Anderer, Semlitsch, Saletu, & Barbanoj, 1992). To accentuate the highest energy peaks, the sum of the 13 channels was calculated. From this signal, the mean and *SD* of the signal between 25 and 75 quartiles was calculated and the energy threshold was obtained as mean+5**SD,* being able to detect those outliers that still remained in the signal and were caused by muscle contractions.

The short-time Fourier transform (STFT) was used to estimate the power spectral density (PSD) of the EEG data. Several spectral parameters were assessed from PSD data: total power (0.5 – 45 Hz) and absolute and relative powers in the conventional EEG frequency bands: delta (δ, 1–4 Hz), theta (θ, 4–7 Hz), alpha (α, 8–13 Hz) and beta (β, 14–29 Hz). Relative variables were estimated with respect to the total power.

As it was introduced, the median frequency and the spectral entropy were commonly used to summarize the changes in spectral brain activity. Median frequency was computed as the frequency at half of the accumulative PSD. The increases in the median frequency would be associated with the signal power increasing, suggesting that the slow states (theta) decrease and the fast states (alpha and beta) increase.

Shannon entropy (SE) was previously used as a measure of signal complexity under physiological and pathological conditions (Catarino et al., 2011; Costa, Goldberger, & Peng, 2005; Ouyang, Dang, Richards, & Li, 2010). SE was also calculated in order to evaluate alterations in the irregularity of the signals due to the cognitive task. SE has shown its usefulness in EEG studies for several pathologies, such as epilepsy, schizophrenia or autism (Acharya, Fujita, Sudarshan, Bhat, & Koh, 2015; Bachiller et al., 2014b; Djemal, AlSharabi, Ibrahim, & Alsuwailem, 2017). Decreases in entropy indicate decreases in signal complexity, which in turn are associated with pathological states of the brain.

Finally, Brain Symmetry Index (BSI) was calculated as a measure of asymmetry in spectral power between the two hemispheres. Asymmetry between both hemispheres is commonly associated with pathological states of the brain and could be related to the diminished ability from one part of the brain to generate EEG rhythms (Stroganova et al., 2007). BSI is computed as the difference on the EEG squared absolute power between homologous contralateral electrodes (Van Putten et al., 2004). BSI ranges between 0 (perfect symmetry) and 1 (maximal asymmetry).

Results

After the artifact rejection procedure, we established a minimum of 24 free-artifact epochs (2 min) in each one of the 3 stages (initial, task and final) in order to obtain enough signal information representative of each stage condition. Eight out of twenty-eight patients were excluded from the analysis because they did not meet this condition. The average number of 5-s free-artifact epochs were 62.14 ± 19.78 for the initial phase, 63.81 ± 20.88 for the task, and 64.76 ± 19.88 for the final stage.

The last row of Figure 1 shows the distribution of the VABS scores by the RS stage for all patients. VABS has shown its usefulness to support diagnoses, decide suitability for special services and report progress or deterioration. VABS domains as daily ability and motor skills differences were statistically significant between RS stages. However, communication and social skills values were uncorrelated with RS stages. Communication and socialization domains could be linked to the cognitive ability of the patients, but these cognitive abilities were not directly linked to the RS stages present the database of our patients (RS stages 3 and 4), since the differences between RS stages 3 and 4 are more related with a motor decay than a cognitive deterioration. No correlation between any VABS domain and age was significant. However, significant correlations were found between VABS communication domain and relative beta ($r = 0.45$; $p<0.05$) and entropy ($r = 0.44$; $p<0.05$) calculated from the baseline.

The time course of spectral variables showing statistically significant differences between post- and pre-task stages were assessed. Figure 3 shows the electrode-averaged values across the different temporal stages. No statistical differences for any spectral variable (absolute and relative powers, median frequency, entropy and BSI) were found between the baseline condition (open eyes) and the initial stage (passive viewing task) assessed by paired sample t-test. The final (post-task) stage was divided into two 5-min periods in order to assess the evolution of the time effects related to the cognitive task. The cognitive task induced a spectral shift to higher frequencies: a significant decrease in relative delta power ($t = -3.327$, $p < 0.004$) and increase in relative beta power ($t = 2.328$, $p < 0.031$). This effect was also exhibited by the significant increases in median frequency ($t = 2.687$, $p < 0.015$) and entropy ($t = 3.325$, $p < 0.004$; the spectral content was spread resulting in an increase of the complexity). The outcome of cognitive stimulation was maintained at the first 5 minutes after the task: decrease of relative delta ($t = -2.789$, $p < 0.012$); and increases of relative beta ($t = 2.100$, $p < 0.049$), median frequency ($t = 2.331$, $p < 0.031$) and entropy ($t = 3.656$, $p < 0.002$). These effects were also observed at the second 5 minutes after the task: decrease of relative delta ($t = -1.988$, $p < 0.062$); and increases of relative beta ($t = 3.048$, beta $p < 0.007$), median frequency ($t = 1.999$, $p < 0.061$) and entropy ($t = 2.567$, $p < 0.019$).

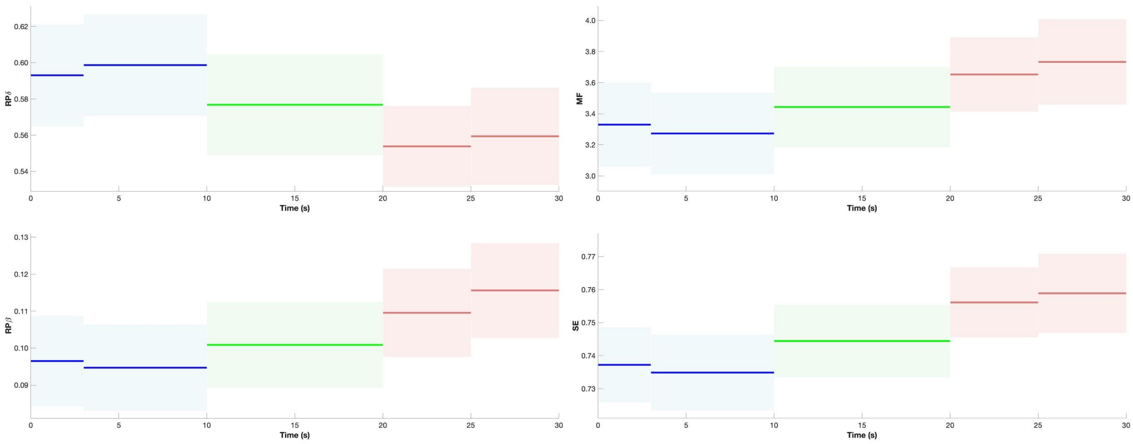

Figure 3. Subject-averaged values ± standard error of the mean (SEM) for each pre-defined temporal period (basal ([0-3] min), initial ([3-10] min), activity ([10-20] min), final1 ([20-25] min), and final2 ([25-30] min). Spectral variables were selected based on its electrode-averaged statistically significant performance: i) delta relative power; ii) beta relative power; iii) MF; iv) SE. Blue color indicates the first stage (baseline), green color indicates the second stage (cognitive task), and orange color indicates the third stage (baseline).

Figure 4 shows topographic maps corresponding to relative power variables, median frequency and entropy for each stage condition. Percentage differences between separated final stage time periods (first and second half of final stage) vs initial stages were calculated in order to better assess the changes. Statistical changes using paired-sample t-tests were shown using topographic significance probability maps (SPM). Significant decreases of delta activity and increases of beta activity were found for both 5-min periods. Absolute powers showed the same change trends (increases/decreases) as relative powers but with higher variability and hence less statistical significance. The ratio between delta and beta activities was also depicted. Hence, median frequency values indicated a statistically significant shift to higher frequencies. A statistically significant increase of the Shannon entropy was also observed in both 5-min periods after the cognitive task, indicating EEG signals were more irregular and therefore more unpredictable.

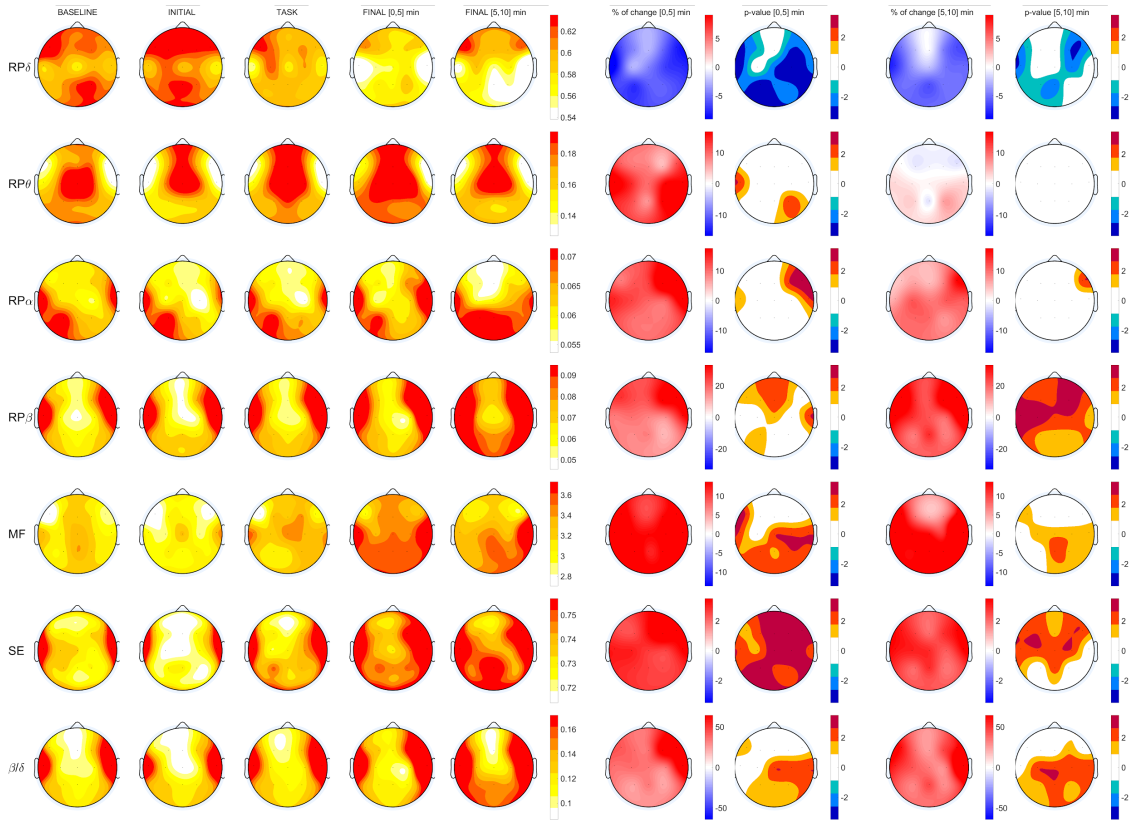

Figure 4. Topographic maps showing the averaged distribution of several spectral parameters (relative powers in delta, theta, alpha and beta, median frequency and Shannon entropy) over the scalp for the different stages of the experiment: baseline, initial, task, first half and second half of the final stage. Statistical probability maps for the differences after applying the cognitive stimulation (both final periods versus initial condition) were included. Black dots indicate electrode positions. Increases and decreases are depicted by hot and cold colors, respectively.

Figure 5 shows the distribution of the differences between pre- and post-task on BSI as a function of age. The decrease on BSI after the cognitive task (decreases mean more symmetry) was higher in younger girls (range 5-10 years; n=9) showing less effect and a greater variability as age increased (range 11-15 years; n=5 and range 16-20 years; n=6). A statistical difference was obtained for the comparison between 5-10y and 16-20y groups ($t = 2.607$, $p = 0.013$). We also examined whether the improvement of BSI after the cognitive stimulation correlated with age, obtaining a significant correlation ($r = 0.39$, $p < 0.05$).

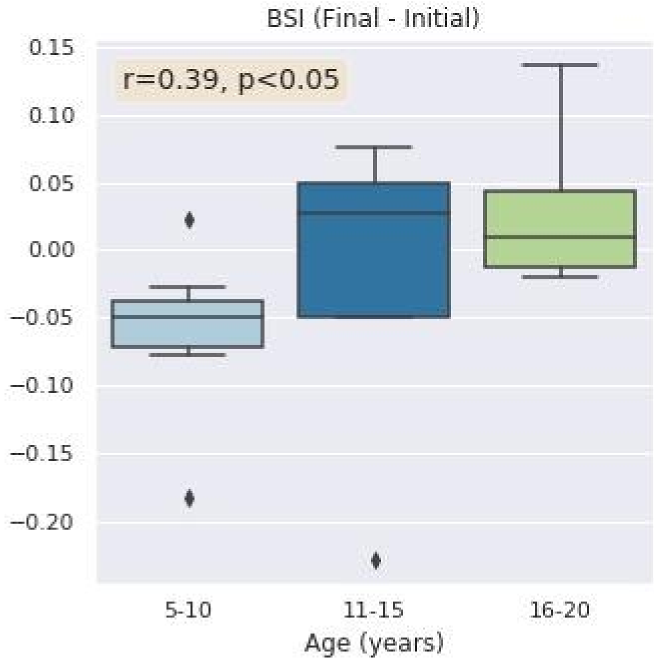

Figure 5. Boxplot for the improvement of BSI after the cognitive task (difference between final and initial baseline conditions) as a function of age. As BSI is a measure of interhemispheric spectral power asymmetry, decreases of BSI indicate a sustained improvement of brain symmetry after the cognitive task.

Discussion

Rett syndrome is a genetic disorder considered the second most common cause of mental disability in women. However, the effects at the neuro-cognitive level have not been studied in depth. Our study confirms the results found in recent studies that focused their research on the changes in brain networks during neuropsychological stimulation (Fabio et al., 2016; Vignoli et al., 2010). In this study, we evaluated the topographical changes of different EEG markers before and after a cognitive task, carried out using an eye-tracking system including a relatively large sample for a rare disorder. This study shows the potential of using EEG measures as biomarkers for the classification of RS severity and for the objective evaluation of not only cognitive tasks, but also other potential therapies such as electrical stimulation or pharmacological treatments.

Changes between final and initial baseline stages in several spectral parameters were performed to evaluate the effect of cognitive stimulation. Likewise, the final stage was divided into two 5 minute-periods to provide more information about the effect's duration. We observed statistically significant reductions and improvements of delta and beta powers respectively, concluding that the effect of the cognitive stimulation remained after the end of the task. These changes could be linked to a general increase in cognitive competences (Ward, 2003). In general, the predominance of low frequency oscillations is associated with pathological states. Low frequency oscillations encompass larger neuronal populations and general brain functions, whereas higher frequency oscillations are originated from a smaller neuronal population and they have been associated with cognitive functions such as attention, arousal, object recognition and top-down modulation of sensory processes (Schnitzler & Gross, 2005; Ward, 2003). Previous studies highlighted the prominent role for beta oscillations in long-range cortico-cortical synchronization (Schnitzler & Gross, 2005). Moreover, the deficiency of beta activity could be an indicator of compromised cortical functions, since beta rhythms are involved in conscious thought, cognitive processes and increased alertness (Kučikienė & Praninskienė, 2018). Beta/delta ratio, MF and SE measures provided a summary of these main effects: decrease of delta oscillations and increase of beta rhythms after the cognitive task. These findings suggested that the eye-tracking activity could be promoting the cognitive activation, leading girls to more active brain states. The increase in SE supported this hypothesis; SE measures the complexity of the signal and the more complex signals were also associated with less pathological states (Acharya et al., 2015; Djemal et al., 2017). "Brain regions with significant changes after cognitive stimulation do not have a specific anatomic pattern but mostly the involvement of extensive and multiple areas. These findings may lead to the hypothesis that cognitive stimulation triggers a generalized functional interconnectivity in the brain of RS patients. Severe intellectual disability and previous poor cognitive training in these patients may have produced a lack of task related specialization of diverse brain regions."

The BSI quantifies the asymmetry between interhemispheric EEG activities. Previous studies have found a strong correlation between BSI and several neurological disorders as stroke, autism or Alzheimer (Liu et al., 2018; Xin, Chang, Gao, & Shi, 2017). While none of the EEG computed measures correlated with RS stages, we found a significant interaction between BSI and age. After the task, the improvement of interhemispheric symmetry was higher in younger girls suggesting that younger girls (range 5-10 years; n=9) responded better to the cognitive tasks in terms of BSI than the pre-adolescent (range 10-15 years; n=5) and adolescent (range 15-20 years; n=6) group. A recent study assessed the relationship between age and several factors related to RS (Cianfaglione et al., 2016). Although it concluded that the analysis by age could not give a definitive picture, they found that the strongest indication of age-related deterioration was found on mood: the child groups had more positive mood and therefore higher interest and pleasure in conducting tasks. The correlation found in our work between BSI and age could be related to this fact. Another explanation for these differences in the BSI could be given by the changes between neural networks associated with the age of the patients. Several studies have evaluated the effect of age on brain networks, observing that younger brains have a greater number of generalized connections while adolescent and adult brains have fewer connections but with stronger interaction between them (Cao et al., 2014; Fransson, Åden, Blennow, & Lagercrantz, 2011; Gozdas, Holland, & Altaye, 2019; Meunier, Lambiotte, & Bullmore, 2010; Wu et al., 2013). Consequently, the BSI outcome could be linked to the cerebral plasticity of the younger girls, reinforcing the idea that it is more appropriate to start an early training with eye-tracking systems. These findings suggest the already known idea that early training using different rehabilitation strategies can be more convenient in children with neurodevelopmental disorders regardless of the etiology.

Nevertheless, it is important to consider that although the adolescent group did not improve their symmetry after the task, all patients significantly increased the high frequency activity and decreased the powers in low frequencies, resulting in more complex EEG signals. This suggests that although the improvement of the interhemispheric symmetry seemed to be linked to age, the development of cognitive tasks with eye-tracking systems could improve the cognitive skills of patients of all ages. Further studies are needed to assess the relation between brain modulation and the age. Therefore, neurorehabilitation must continue during all stages of life. This is relevant in order to implement educational programs in children with special needs, not only in early childhood but also throughout life.

Some limitations of this research merit further consideration. Firstly, the maintained activation was observed only in a short term, thus, future works should evaluate how these improvements are sustained through time. Secondly, our conclusions are based on a cognitive task, as a first step of a neuropsychological training, which is more complex and articulated. Thus, it would be interesting to evaluate the spectral changes of EEG activity in a longitudinal study that includes a neuropsychological training and different test sessions. In a recent study, (Rosa Angela Fabio, Giannatiempo, Semino, & Caprì, 2021) evaluated the cognitive training effects in girls with RS. The topographical measures presented in this study, could work as external biomarkers to evaluate the improvement of the periodic sessions, defining how continuous cognitive training could change clinical practice of patients with RS. Also, it would be interesting to evaluate the most suitable cognitive stimulation for each patient, as RS girls have shown the ability to make consistent choices among alternatives (Rosa A. Fabio, Magaudda, Caprì, Towey, & Martino, 2018).

Finally, the inclusion of additional information about the performance of cognitive tasks for each subject could improve the relevance of our findings. Patient's parents appreciated a subjective improvement in concentration and motivation but no objective measures were performed. The severity of cognitive impairment in RS patients made these evaluations very difficult. The patients are currently being followed clinically on specific cognitive and behavioral functions, in order to validate the long-term efficacy of the training program. Finally, future studies will complement the evaluation of cognitive stimulation using additional EEG electrodes, including relevant areas such as prefrontal and temporal ones, and combining cognitive stimulation with other types, such as electrical or transcranial magnetic stimulations (Rosa Angela Fabio et al., 2020). The analysis of higher-density EEG data will allow us to assess brain connectivity measures and to estimate brain sources.

Conclusion

This study showed the potential benefits of cognitive stimulation in RS patients, highlighting that the younger the therapy is started, the better the outcome. According to our findings, significant alterations of brain rhythms were observed due to cognitive stimulation: EEG analysis depicted a decrease of low frequency oscillations and an increase of higher frequency rhythms, resulting in more complex EEG signals. Currently, the evaluation of the efficacy of RS therapies is only done by clinical scales, EEG could add an extra objective measure and be used as a potential biomarker. In our study, the changes in EEG variables were observed not only during the task but also maintained after finishing the task, suggesting that cognitive stimulation may have lasting effects on brain activity. Finally, our results also showed an improvement of brain asymmetries that were especially relevant for the younger group.

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REFERENCES

Acharya, U. R., Fujita, H., Sudarshan, V. K., Bhat, S., & Koh, J. E. W. (2015). Application of entropies for automated diagnosis of epilepsy using EEG signals: A review. *Knowledge-Based Systems*. https://doi.org/10.1016/j.knosys.2015.08.004

Ahonniska-Assa, J., Polack, O., Saraf, E., Wine, J., Silberg, T., Nissenkorn, A., & Ben-Zeev, B. (2018). Assessing cognitive functioning in females with Rett syndrome by eye-tracking methodology. *European Journal of Paediatric Neurology*, *22*(1), 39–45. https://doi.org/10.1016/j.ejpn.2017.09.010

Aldemir, R., Demirci, E., Bayram, A. K., Canpolat, M., Ozmen, S., Per, H., & Tokmakci, M. (2018). Evaluation of two types of drug treatment with QEEG in children with ADHD. *Translational Neuroscience*, *9*(1), 106–116. https://doi.org/10.1515/tnsci-2018-0017

Amir, R. E., Van den Veyver, I. B., Wan, M., Tran, C. Q., Francke, U., & Zoghbi, H. Y. (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nature Genetics*, *23*(2), 185–188. https://doi.org/10.1038/13810

Anderer, P., Semlitsch, H. V., Saletu, B., & Barbanoj, M. J. (1992). Artifact processing in topographic mapping of electroencephalographie activity in neuropsychopharmacology. *Psychiatry Research: Neuroimaging*. https://doi.org/10.1016/0925-4927(92)90002-L

Bachiller, A., Díez, A., Suazo, V., Domínguez, C., Ayuso, M., Hornero, R., … Molina, V. (2014a). Decreased spectral entropy modulation in patients with schizophrenia during a P300 task. *European Archives of Psychiatry and Clinical Neuroscience*. https://doi.org/10.1007/s00406-014-0488-6

Bachiller, A., Díez, A., Suazo, V., Domínguez, C., Ayuso, M., Hornero, R., … Molina, V. (2014b). Decreased spectral entropy modulation in patients with schizophrenia during a P300 task. *European Archives of Psychiatry and Clinical Neuroscience*, *264*(6), 533–543. https://doi.org/10.1007/s00406-014-0488-6

Balmer, D., Goldstine, J., Rao, Y. M., & LaSalle, J. M. (2003). Elevated methyl-CpG-binding protein 2 expression is acquired during postnatal human brain development and is correlated with alternative polyadenylation. *Journal of Molecular Medicine*, *81*(1), 61–68. https://doi.org/10.1007/s00109-002-0396-5

Berger-Sweeney, J. (2011). Cognitive deficits in Rett syndrome: What we know and what we need to know to treat them. *Neurobiology of Learning and Memory*. https://doi.org/10.1016/j.nlm.2011.05.006

Cao, M., Wang, J.-H., Dai, Z.-J., Cao, X.-Y., Jiang, L.-L., Fan, F.-M., … He, Y. (2014). Topological organization of the human brain functional connectome across the lifespan. *Developmental Cognitive Neuroscience*, *7*, 76–93. https://doi.org/10.1016/j.dcn.2013.11.004

Catarino, A., Churches, O., Baron-Cohen, S., Andrade, A., & Ring, H. (2011). Atypical EEG complexity in autism spectrum conditions: A multiscale entropy analysis. *Clinical Neurophysiology*. https://doi.org/10.1016/j.clinph.2011.05.004

Cianfaglione, R., Clarke, A., Kerr, M., Hastings, R. P., Oliver, C., & Felce, D. (2016). Ageing in Rett syndrome. *Journal of Intellectual Disability Research*. https://doi.org/10.1111/jir.12228

Costa, M., Goldberger, A. L., & Peng, C. K. (2005). Multiscale entropy analysis of biological signals. *Physical Review E - Statistical, Nonlinear, and Soft Matter Physics*, *71*(2).

https://doi.org/10.1103/PhysRevE.71.021906

Djemal, R., AlSharabi, K., Ibrahim, S., & Alsuwailem, A. (2017). EEG-Based Computer Aided Diagnosis of Autism Spectrum Disorder Using Wavelet, Entropy, and ANN. *BioMed Research International*, *2017*, 1–9. https://doi.org/10.1155/2017/9816591

Djukic, A., Valicenti McDermott, M., Mavrommatis, K., & Martins, C. L. (2012). Rett syndrome: Basic features of visual processing - A pilot study of eye-tracking. *Pediatric Neurology*, *47*(1), 25–29. https://doi.org/10.1016/j.pediatrneurol.2012.04.009

Fabio, R. A., Billeci, L., Crifaci, G., Troise, E., Tortorella, G., & Pioggia, G. (2016a). Cognitive training modifies frequency EEG bands and neuropsychological measures in Rett syndrome. *Research in Developmental Disabilities*, *53–54*, 73–85. https://doi.org/10.1016/j.ridd.2016.01.009

Fabio, R. A., Caprì, T., Mohammadhasani, N., Gangemi, A., Gagliano, A., & Martino, G. (2018). Frequency bands in seeing and remembering: Comparing ADHD and typically developing children. *Neuropsychological Trends*, (24). https://doi.org/10.7358/neur-2018-024-fabi

Fabio, R. A., Gangemi, A., Semino, M., Vignoli, A., Canevini, M. P., Priori, A., … Caprì, T. (2020). Effects of combined Transcranial direct current stimulation with cognitive training in girls with Rett syndrome. *Brain Sciences*, *10*(5). https://doi.org/10.3390/brainsci10050276

Fabio, R. A., Giannatiempo, S., Semino, M., & Caprì, T. (2021). Longitudinal cognitive rehabilitation applied with eye-tracker for patients with Rett Syndrome. *Research in Developmental Disabilities*, *111*. https://doi.org/10.1016/j.ridd.2021.103891

Fabio, R. A., Magaudda, C., Caprì, T., Towey, G. E., & Martino, G. (2018). Choice behavior in Rett Syndrome: The consistency parameter. *Life Span and Disability*, *21*(1).

Fransson, P., Åden, U., Blennow, M., & Lagercrantz, H. (2011). The functional architecture of the infant brain as revealed by resting-state fMRI. *Cerebral Cortex*, *21*(1), 145–154. https://doi.org/10.1093/cercor/bhq071

Glaze, C., Kozinetz, A., Almes, J., Skender, L., & Percy, K. (1993). Epidemiology of Rett Syndrome : A Population-Based methodology. *Paediatrics*, *91*(2).

Gorbachevskaya, N., Bashina, V., Gratchev, V., & Iznak, A. (2001). Cerebrolysin therapy in Rett syndrome: Clinical and EEG mapping study. In *Brain and Development*. https://doi.org/10.1016/S0387-7604(01)00349-7

Gozdas, E., Holland, S. K., & Altaye, M. (2019). Developmental changes in functional brain networks from birth through adolescence. *Human Brain Mapping*, *40*(5), 1434–1444. https://doi.org/10.1002/hbm.24457

Hagberg, B. (2002). Clinical manifestations and stages of Rett syndrome. *Mental Retardation and Developmental Disabilities Research Reviews*, *8*(2), 61–65. https://doi.org/10.1002/mrdd.10020

Kučikienė, D., & Praninskienė, R. (2018). The impact of music on the bioelectrical oscillations of the brain. *Acta Medica Lituanica*. https://doi.org/10.6001/actamedica.v25i2.3763

Liu, H., Zhang, L., Xi, Q., Zhao, X., Wang, F., Wang, X., … Lin, Q. (2018). Changes in brain lateralization in patients with mild cognitive impairment and Alzheimer's disease: A resting-state functional magnetic resonance study from Alzheimer's disease neuroimaging initiative. *Frontiers in Neurology*. https://doi.org/10.3389/fneur.2018.00003

Machado, C., Estévez, M., Leisman, G., Melillo, R., Rodríguez, R., DeFina, P., … Beltrán, C.

(2013). QEEG Spectral and Coherence Assessment of Autistic Children in Three Different Experimental Conditions. *Journal of Autism and Developmental Disorders*, *45*(2), 406–424. https://doi.org/10.1007/s10803-013-1909-5

Meunier, D., Lambiotte, R., & Bullmore, E. T. (2010). Modular and Hierarchically Modular Organization of Brain Networks. *Frontiers in Neuroscience*, *4*. https://doi.org/10.3389/fnins.2010.00200

Neul, J. L., Kaufmann, W. E., Glaze, D. G., Christodoulou, J., Clarke, A. J., Bahi-Buisson, N., … Percy, A. K. (2010). Rett syndrome: Revised diagnostic criteria and nomenclature. *Annals of Neurology*. https://doi.org/10.1002/ana.22124

Nuwer, M. R., Buchhalter, J., & Shepard, K. M. (2016). Quantitative EEG in attention-deficit/hyperactivity disorder. *Neurology: Clinical Practice*, *6*(6), 543–548. https://doi.org/10.1212/CPJ.0000000000000308

Ouyang, G., Dang, C., Richards, D. A., & Li, X. (2010). Ordinal pattern based similarity analysis for EEG recordings. *Clinical Neurophysiology*, *121*(5). https://doi.org/10.1016/j.clinph.2009.12.030

Roche, K. J., Leblanc, J. J., Levin, A. R., O'Leary, H. M., Baczewski, L. M., & Nelson, C. A. (2019). Electroencephalographic spectral power as a marker of cortical function and disease severity in girls with Rett syndrome. *Journal of Neurodevelopmental Disorders*. https://doi.org/10.1186/s11689-019-9275-z

Rose, S. A., Djukic, A., Jankowski, J. J., Feldman, J. F., & Rimler, M. (2016). Aspects of attention in rett syndrome. *Pediatric Neurology*, *57*, 22–28. https://doi.org/10.1016/j.pediatrneurol.2016.01.015

Schnitzler, A., & Gross, J. (2005). Normal and pathological oscillatory communication in the brain. *Nature Reviews Neuroscience*. https://doi.org/10.1038/nrn1650

Schwartzman, J. S., Velloso, R. de L., D'Antino, M. E. F., & Santos, S. (2017). The eye-tracking of social stimuli in patients with Rett syndrome and autism spectrum disorders: a pilot study. *Arquivos de Neuro-Psiquiatria*, *73*(5), 402–407. https://doi.org/10.1590/0004-282x20150033

Sigafoos, J., Green, V. A., Schlosser, R., O'eilly, M. F., Lancioni, G. E., Rispoli, M., & Lang, R. (2009). Communication intervention in Rett syndrome: A systematic review. *Research in Autism Spectrum Disorders*, *3*(2), 304–318. https://doi.org/10.1016/j.rasd.2008.09.006

Sparrow, S. S., & Cicchetti, D. V. (1989). The Vineland Adaptive Behavior Scales. In *Major psychological assessment instruments* (Vol. 2, pp. 199–231).

Stroganova, T. A., Nygren, G., Tsetlin, M. M., Posikera, I. N., Gillberg, C., Elam, M., & Orekhova, E. V. (2007). Abnormal EEG lateralization in boys with autism. *Clinical Neurophysiology*. https://doi.org/10.1016/j.clinph.2007.05.005

Trevathan, E., & Naidu, S. (2017). The Clinical Recognition and Differential Diagnosis of Rett Syndrome. *Journal of Child Neurology*, *3*, S6–S16. https://doi.org/10.1177/0883073888003001s03

Van Putten, M. J. A. M., Peters, J. M., Mulder, S. M., De Haas, J. A. M., Bruijninckx, C. M. A., & Tavy, D. L. J. (2004). A brain symmetry index (BSI) for online EEG monitoring in carotid endarterectomy. *Clinical Neurophysiology*, *115*(5), 1189–1194. https://doi.org/10.1016/j.clinph.2003.12.002

Vignoli, A., Fabio, R. A., La Briola, F., Giannatiempo, S., Antonietti, A., Maggiolini, S., & Canevini,

M. P. (2010). Correlations between neurophysiological, behavioral, and cognitive function in Rett syndrome. *Epilepsy and Behavior*, *17*(4), 489–496. https://doi.org/10.1016/j.yebeh.2010.01.024

Ward, L. M. (2003). Synchronous neural oscillations and cognitive processes. *Trends in Cognitive Sciences*. https://doi.org/10.1016/j.tics.2003.10.012

Wu, K., Taki, Y., Sato, K., Hashizume, H., Sassa, Y., Takeuchi, H., … Fukuda, H. (2013). Topological Organization of Functional Brain Networks in Healthy Children: Differences in Relation to Age, Sex, and Intelligence. *PLoS ONE*, *8*(2), e55347. https://doi.org/10.1371/journal.pone.0055347

Xin, X., Chang, J., Gao, Y., & Shi, Y. (2017). Correlation between the Revised Brain Symmetry Index, an EEG Feature Index, and Short-term Prognosis in Acute Ischemic Stroke. *Journal of Clinical Neurophysiology*. https://doi.org/10.1097/WNP.0000000000000341

Table S1. Specific detailed patient information

ID	AGE	WEIGHT	RETT STAGE	MUTATION	WALKING	ABILITY TO WALK	SPEECH	HAND USE	EPILEPSY	SCOLIOSIS	VABS				
											COM	DA	SOC	MA	TOT
1	6.7	20.3	4	p.Arg168X	0	3	3	3	weekly	0	10	7	21	10	48
2	15.1	34.3	3	deletion	0	3	3	3	monthly	3	30	20	32	23	105
3	14.2	52.1	3	p.Arg270X	1	1	3	1	monthly	1	26	19	35	38	118
4	8.9	20.6	4	p.Arg270X	0	3	3	3	monthly	3	25	14	25	6	70
5	19.4	46.2	3	p.Thr158Met	2	2	3	3	sporadically	3	23	8	20	24	75
6	11.6	43.2	3	p.Arg306Cys	1	1	2	1	weekly	2	47	31	34	68	180
7	12.0	25.0	3	p.Arg255X	1	1	3	1	no	3	20	15	29	30	94
8	19.5	38.7	3	p.Pro225Arg	1	1	3	1	no	2	18	15	29	45	107
9	19.5	52.2	3	p.Arg294X	2	2	2	2	daily	2	14	10	20	39	83
10	9.4	35.5	4	insertion	0	3	3	3	weekly	3	18	4	22	8	52
11	8.6	26.1	3	p.Arg168X	1	1	3	3	no	0	18	13	13	8	52
12	5.2	18.8	3	p.Pro162Arg	1	1	3	1	no	0	29	13	23	46	111
13	5.1	17.0	4	p.Thr158Met	0	3	3	3	weekly	0	15	2	15	8	40
14	8.5	41.1	3	Exon1	1	1	2	1	monthly	2	37	24	32	53	146
15	16.0	42.1	4	p.Pro302His	0	3	3	3	monthly	3	34	8	23	13	78
16	19.1	50.3	3	Insertion	1	1	2	1	no	0	42	29	35	42	149
17	14.0	36.0	4	p.Tyr141X	0	3	3	3	monthly	3	17	1	21	8	47
18	16.2	52.2	3	Large deletion	1	1	3	1	no	2	43	22	33	61	159
19	6.3	16.1	3	p.Arg306Cys	1	1	2	1	sporadically	0	33	22	32	58	145
20	5.4	20.0	3	Deletion	1	1	2	1	sporadically	0	16	14	14	39	83
21	8.7	20.2	4	p.Thr158Met	0	3	3	3	daily	3	22	16	27	9	74
22	11.1	33.5	4	p.Lys352fs	0	3	3	2	monthly	3	22	3	31	17	73
23	10.6	23.0	3	p.X487Gly	1	1	2	1	no	0	25	13	18	52	108
24	17.6	40.4	3	p.Arg255X	2	2	3	1	daily	0	28	15	37	69	149
25	3.8	12.5	2	p.Thr158Met	0	3	3	3	weekly	0	28	11	33	14	86
26	3.8	14.0	2	p.Thr158Met	0	3	3	3	monthly	0	22	6	23	8	59
27	17.0	39.3	4	p.Ser229fs	0	3	3	2	monthly	3	33	22	39	14	108
28	16.4	35.0	4	p.Gly237fs	0	3	3	3	monthly	3	14	9	22	9	54
29	2.3	12.6	2	p.Arg255X	0	3	3	3	daily	0	22	2	28	10	62
30	12.5	32.7	3	p.Arg294X	1	1	3	1	no	3	24	17	30	27	98

Walking: 0 no walking; 1 unsupported walking; 2 walking with support. Ability to walk: 0 normal gait; 1 mildly apraxic; 2 severely apraxic; 3 requiring support to stand and/or wheelchair bound. Speech: 0 normal; 1 sentences/phrases; 2 single words; 3 non-verbal. Hand use: 0 normal; 1 purposeful grasping; 2 tapping for needs; 3 no hand use. Scoliosis: 0 not present; 1 less than 20 degrees; 2 less than 30 degrees; 3 greater than 30 degrees or surgical correction had taken place. VABS standard scores for each subdomain have a mean of 100 and standard deviation of 3. Shading rows include the subject rejected after artifact rejection procedure.