Effect of presentation rate on auditory processing in Rett Syndrome: ERP study

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Research Article

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Abstract

Rett Syndrome (RS) is a rare neurodevelopmental disorder characterized by mutations in the MECP2 gene. Patients with RS have severe motor abnormalities and are often unable to walk, use hands and speak. The preservation of perceptual and cognitive functions is hard to assess, while clinicians and caregivers point out that these patients need more time to process information than typically developing peers. Here we examine neurophysiological correlates of auditory processing in RS as a function of presentation rate. From previous literature we knew that auditory event-related potential (ERP) is increased with prolongation of interstimulus interval (ISI). We presented a repetitive stimulus (1000Hz) at three different ISI of 900 ms, 1800 ms, and 3600 ms in children with RS and their typical development peers (TD) aged 2.5–16 years while recording 28-channels electroencephalogram, EEG. The amplitude of N1 and P2 components of event-related potential (ERP) was smaller at ISI 900 than at longer ISIs in both groups, pointing out that the basic mechanism of adaptation in the auditory system is preserved in Rett Syndrome. At the same time the latency of these components was significantly delayed in the RS than in TD. Moreover, late components (P2 and N2) were drastically reduced in Rett Syndrome irrespective of the ISI, suggesting a severely affected mechanism of integration of upcoming sensory input with memory. Based on these ERP measures it was possible to differentiate RS from TD with great accuracy (0.922 ± 0.047), being maximal with shortest ISI, supporting its implication as potential output measures in clinical trials as well as pointing to the diminishing of the neurophysiological differences between RS and TD with slowing down the presentation rate.

Background

Rett syndrome (RS) is a neurodevelopmental disorder associated with mutations in the X-linked gene MECP2[1]. This disease is characterized by a variety of physiological, motor, and cognitive deficits[2]. As most children with RS is non-verbal[3–5] and have severe problems with goal-oriented motor actions[6], it is hardly possible to use standard tools to assess their cognitive abilities. Thus, limited data exist on the specifics of cognitive function including the ability to perceive and understand speech in RS.

Auditory event related potentials (ERP) is a convenient tool for assessing the processing of auditory information in the brain, as it does not require participants attention and can be used in challenging populations. It consists of positive and negative components (P1, N1, P2, and N2) whose prominence depends on many parameters including rate of presentation[7–9]. In particular, the N1 and P2 components become larger as the inter-stimulus interval increases at least up to 12 seconds[10,11]. Neuronal networks activated by the sounds do not come to their initial state immediately after sound termination but their activation fades slowly. If during this fading period the similar sound is presented it could not elicit the “full” response as the first stimuli but only its part (that might be also called adaptation). The more neurons return to their initial state the larger the response. That what we believe is captured by ERP modulation by the rate of presentation. These neurophysiological changes were linked to the decay of the memory representation of stimuli as it corresponds with behavioral results in psychophysical experiments[10–12]. In general, faster presentation rate is better for integration
information over many temporally segregated stimuli, while slower rate allows to process each stimuli thoroughly. However, the optimal limit for each type of analysis is varied across individuals. For example, adult dyslexics have faster memory trace decay than good readers that provide difficulties in integrating the information within several seconds while in autism the memory decay is longer putting less weight into the most recent event and increasing the ability to link together unrelated things[10].

In the current study we aim to examine modulation of auditory ERPs by the rate of stimulation in RS to dig into the mechanisms underlying Rett symptomatology. Previous studies showed that auditory ERPs are severely altered in RS: its late components P2 and N2 almost absent when the inter-stimulus interval is about 1 second - typical rate of presentation in neurophysiological experiments[13,14]. No study examined the auditory ERPs in RS in response to more slowly presented stimuli, with ISI longer than 2 seconds. However, as the speed of signal processing is reported to be low in RS as indicated with delayed ERP components[15–17] these patients might benefit from the slower presentation rate. Thus, we hypothesize that by increasing the inter stimulus interval from 0.9 to 3.6 seconds we might see recovery of auditory ERP components in RS that will get more typical. Simultaneously we assess if the ERP components are modulated by the rate of tone presentation, getting insight into the mechanism of basic memory function in this group. Additionally, we used machine learning techniques to examine if the auditory ERPs characteristics allow us to differentiate RS from typically developing children with sufficient accuracy.

**Methods**

**Participants:**

**Rett syndrome group**

24 children aged from 3 to 17 (Mean age = 9.0 ± 3.1) with Rett syndrome participated in this study. They were recruited during clinical visits to the Research Clinical Institute of Pediatrics in Moscow, Russian Federation. The diagnosis was based on current diagnostic criteria and was confirmed clinically by a medical doctor specializing in this population (V.V.) as well as via genetic testing. Severity of RS was measured using the Rett Syndrome Severity Scale (RSSS)[18].

**Typical development group**

27 children aged from 2.5 to 16 (Mean age = 9.7 ± 3.4) years without neurological, psychiatric disorders, mental and speech delays, or hearing problems according to parental reports.

Parents or legal representatives have given written consent to the children's participation in the study, after the procedure was explained to them. Children have given verbal consent to participate. The research procedure was approved by the ethical committees of IHNA and Nph RAS (protokol №2 at April 30th, 2020) and Sirius University of Science and Technology amendment from April 15th, 2021.

**Experimental design:**
Stimuli

Pure tone 1000Hz (duration: 100ms, loudness: 65 dB) was presented in three experimental blocks with three interstimulus intervals (ISIs): 900, 1800, and 3600 ms. Stimuli with each type of ISI were presented in a separate block. Each tone was presented 150 times for 1800 and 3600 ISI conditions, and 300 times for 900 ms ISI conditions.

Procedure

Participants sat in a comfortable chair in a sound-attenuated room. Participants listened to sounds binaurally through earphones and watched a muted video of their or parent's choice. They were instructed to ignore sounds and avoid moving. In the short breaks between blocks, participants can change their positions.

EEG recordings

EEG was recorded from 28 electrodes placed according to the International 10–20 system guidelines ('Fp1', 'Fp2', 'F3', 'Fz', 'F4', 'F7', 'F8', 'Fc3', 'Fc5', 'Fcz', 'C3', 'Cz', 'C4', 'Cp3', 'Cpz', 'Cp4', 'P3', 'Pz', 'P4', 'T7', 'T8', 'T3', 'T4', 'T5', 'T6', 'O1', 'Oz', 'O2'). Data was recorded using left and right linked ears as reference and nasion as ground, and using 0.01–70Hz online filters. The data were sampled at 500 Hz. The electrode impedances were below 10 kΩ.

Data processing

EEG was filtered with 2–20 Hz offline filters. Bad channel interpolation was applied when necessary (0–2 channels per participant). Automatic raw data inspection with ±400µV thresholds was used for rejecting EEG segments with large artifacts, then for artifact rejection, the independent component analysis (ICA) was performed, in particular the ALICE platform was used[19]. The data were segmented into epochs starting 200 ms before a stimuli onset and lasting 500 ms after the onset. Automatic rejection of the bad segments with signals more than +100 µV was applied. ERPs were baseline corrected to -200 ms prestimulus intervals. Mean number of trials for each participant was approximately 120 for 1800ms and 3600ms ISI conditions and 230 for 900ms ISI conditions.

The FCz channel was chosen for the analysis, according to the literature as the auditory cortex response is observed in this area[7]. Also, for this channel ERP components were more pronounced and less affected by artifacts. Some participants from the RS group did not have a clear peaks' morphology, so it was hard to estimate absolute peak amplitude and latency. Thus, we calculated the mean amplitude of ERP components in the following time window based on TD group peaks: P1(63-88ms), N1(106-128ms), P2(154-192ms), N2(240-294ms). Peak to peak amplitudes for P1-N1, N1-P2 and P2-N2 were calculated as the difference in amplitude between two peaks.

All participants were further evaluated on the prominence of ERP’s components by two independent experts that were blind to the diagnosis (Fig. 1). Experts agreed that all participants from TD Group had a
clearly identified ERP component, participants from the RS group were divided into two groups: evident-ERP group (N = 12) and no-evident-ERP components (N = 10). Two RS group participants' data were excluded from the analysis because of a high number of bad channels. No significant differences that could be attributed to preprocessing features (e.g., number of trials (F(1,20) = 0.186, p = 0.671, eta2 = 0.009) or number of removed ICA components (F(1,20) = 3.274, p = 0.084, eta2 = 0.141)) were found between the two separated RS subgroups. Separate analysis of Rett syndrome girls with evident ERP components allowed the assessment of the latency of the peaks and provided the opportunity for deeper understanding of the pathophysiology of RS.

For TD and evident-ERP RS groups amplitude and latency measurements for the peaks were made using the MNE python tool and then verified by authors. Peak amplitudes were calculated relative to baseline and absolute peak latencies were calculated relative to stimulus onset.

The effects associated with the stimuli presentation rate and diagnosis were investigated by Two-way mixed analysis of variance (ANOVA) separately for the mean amplitude (for the whole group), and peak amplitude and latency for the evident-ERP subgroup for the P1, N1, P2, and N2 components and for peak-to-peak amplitude for P1-N1, N1-P2 and P2-N2 components. Statistical analysis was performed using Python 2 or 3 with the SciPy, pandas and Pingouin[20] packages. Two-way mixed ANOVA included Group as between-subjects factor (RS vs TD) and Presentation rate (ISI -inter-stimulus interval) as within-subjects factor (three levels: 900 ms, 1800 and 3600 ms) as well as their interaction. Estimation stats were performed using the Python package Dabestr[21].

Machine learning models and feature analysis

We applied standard machine learning (ML) methods and pipelines[22] to assess the detectability of RS based on all our ERP measures obtained in the whole sample (thus, latency was not considered). We considered the most common ML models that are widely used for classification tasks. These include both linear models (logistic regression[23], support-vector machine[24] with linear kernel) and tree-based nonlinear models (random forest[25], gradient boosting[26]). The implementations from[27] and gradient boosting from[28] were used. For linear models, it is beneficial to scale the features into a similar range. We applied such a scaling during training so that each feature has zero mean and unit variance. The same scaling parameters were applied during evaluation. We used the ROC-AUC score to measure the performance of each model. It takes values in the range from 0.5 to 1 with 0.5 corresponding to the random classification and 1 corresponding to the ideal case. Each of the aforementioned models has a set of hyper-parameters that are set prior to training. These parameters can be tuned to improve the classification results. It is common to find the best parameters using cross-validation. In cross-validation, the data is split into several folds and for every such fold the classifier is fitted on the remaining data and tested on this fold. The final scores are obtained by aggregating the results of each fold. This technique allows us to use all of the data for training and to prevent overfitting which occurs when the same data is used for training and evaluation.
For each model, we considered a grid of parameters (See supplementary A). For each combination of hyperparameters, we performed 5-fold cross-validation and selected the combination with the highest aggregated ROC-AUC scores. We used the same procedure to obtain the final scores. We ran the outer 5-fold cross-validation to generate 5 splits. On each split, we supplied the training part of the data to the inner cross-validation to get the best model. We then fitted this model on the training data and measured the final scores on the test part of the data.

Results

The grand-averaged ERPs in response to tones presented with different ISI showed the expected pattern of identifiable P1, N1, P2 and N2 components in the TD participants (Fig. 2, FCz, for the topography and all channels response see Supplementary B). The adaptation of the component N1 was clearly observed (its amplitude was much smaller at the shortest interval). It is also notable that the increase in ISI from 1800 ms to 3600 ms had no impact on the evoked response component configuration. In the RS group, it was also possible to recognize the main components (especially the early ones). Comparing the evoked responses of the two groups, the early components of the ERP (P1, N1) in the RS group are relatively preserved. The later components (P2, N2), which were hardly expressed at the short interval (900ms), become more evident with an increase in the interstimulus interval but still evidently smaller and delayed than in TD. Below we prove these observations statistically.

The separate consideration of participants from the RS group with evident ERP components also support the delay and decrease in ERP components (Fig. 2, D, E, F). In no-evident ERP RS groups average ERP response showed large variability, making differentiation of the ERP components impossible. Evident and no-evident ERP components group were not significantly different in either age (F(1,20) = 0.002, p = 0.968, eta2 < 0.001) or RSSS (F(1,20) = 0.4545, p = 0.506, eta2 = 0.022).
Table 1
ANOVA effects on ERP components. Results for all participants mean amplitude and peaks amplitude of TD group and RS evident-ERP subgroup are shown divided by "//".

<table>
<thead>
<tr>
<th>Factors</th>
<th>ISI</th>
<th>F</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>ISI</td>
<td>0.344//2.090</td>
<td>0.560//0.131</td>
<td>0.007//0.053</td>
</tr>
<tr>
<td></td>
<td>ISI * Group</td>
<td>2.464//4.674</td>
<td>0.090//0.012</td>
<td>0.050//0.112</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>1.744//0.734</td>
<td>0.180//0.397</td>
<td>0.036//0.019</td>
</tr>
<tr>
<td>N1</td>
<td>ISI</td>
<td>28.615//30.211</td>
<td>&gt;0.001//&gt;0.001</td>
<td>0.378//0.449</td>
</tr>
<tr>
<td></td>
<td>ISI * Group</td>
<td>5.427//1.042</td>
<td>0.006//0.358</td>
<td>0.104//0.027</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>0.333//0.522</td>
<td>0.567//0.474</td>
<td>0.007//0.014</td>
</tr>
<tr>
<td>P2</td>
<td>ISI</td>
<td>0.532//6.168</td>
<td>0.589//0.003</td>
<td>0.011//0.143</td>
</tr>
<tr>
<td></td>
<td>ISI * Group</td>
<td>2.201//1.731</td>
<td>0.116//0.184</td>
<td>0.045//0.045</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>19.128//8.317</td>
<td>&gt;0.001//0.006</td>
<td>0.289//0.184</td>
</tr>
<tr>
<td>N2</td>
<td>ISI</td>
<td>10.036//0.823</td>
<td>&gt;0.001//0.443</td>
<td>0.176//0.022</td>
</tr>
<tr>
<td></td>
<td>ISI * Group</td>
<td>1.545//1.723</td>
<td>0.219//0.186</td>
<td>0.032//0.044</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>23.059//7.964</td>
<td>&gt;0.001//0.008</td>
<td>0.329//0.178</td>
</tr>
<tr>
<td>P1N1</td>
<td>ISI</td>
<td>17.746//22.230</td>
<td>&gt;0.001//&gt;0.001</td>
<td>0.274//0.375</td>
</tr>
<tr>
<td></td>
<td>ISI * Group</td>
<td>3.138//0.664</td>
<td>0.048//0.518</td>
<td>0.063//0.018</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>0.754//1.165</td>
<td>0.390//0.287</td>
<td>0.016//0.031</td>
</tr>
<tr>
<td>N1P2</td>
<td>ISI</td>
<td>20.721//40.887</td>
<td>&gt;0.001//&gt;0.001</td>
<td>0.306//0.525</td>
</tr>
<tr>
<td></td>
<td>ISI * Group</td>
<td>10.143//3.045</td>
<td>&gt;0.001//0.053</td>
<td>0.178//0.076</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>10.973//3.48</td>
<td>0.002//0.070</td>
<td>0.189//0.086</td>
</tr>
<tr>
<td>P2N2</td>
<td>ISI</td>
<td>2.146//2.126</td>
<td>0.123//0.127</td>
<td>0.044//0.054</td>
</tr>
<tr>
<td></td>
<td>ISI * Group</td>
<td>0.270//0.677</td>
<td>0.764//0.511</td>
<td>0.006//0.018</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>43.858//13.901</td>
<td>&gt;0.001//&gt;0.001</td>
<td>0.483//0.273</td>
</tr>
</tbody>
</table>
### Table 2
ANOVA effects of comparing peaks latency of TD group and RS evident-ERP subgroup.

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>ISI</td>
<td>4.986</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>ISI * Group</td>
<td>1.238</td>
<td>0.296</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>2.985</td>
<td>0.092</td>
</tr>
<tr>
<td>N1</td>
<td>ISI</td>
<td>0.483</td>
<td>0.619</td>
</tr>
<tr>
<td></td>
<td>ISI * Group</td>
<td>1.612</td>
<td>0.206</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>6.455</td>
<td>0.015</td>
</tr>
<tr>
<td>P2</td>
<td>ISI</td>
<td>4.620</td>
<td>0.0129</td>
</tr>
<tr>
<td></td>
<td>ISI * Group</td>
<td>0.262</td>
<td>0.771</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>17.277&lt; 0.001</td>
<td>0.318</td>
</tr>
<tr>
<td>N2</td>
<td>ISI</td>
<td>11.432&lt; 0.001</td>
<td>0.236</td>
</tr>
<tr>
<td></td>
<td>ISI * Group</td>
<td>3.537</td>
<td>0.068</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>2.172</td>
<td>0.121</td>
</tr>
</tbody>
</table>

### P1

Mean amplitude value, whole group

No significant differences between groups or ISI were found for the P1 component.

Peak amplitude value, evident-ERP subgroup: Significant ISI*Group interaction was found for peak amplitude of P1 component comparing TD and evident-ERP RS groups: (F (2;74) = 4.674, p = 0.012, eta2 = 0.112). Post hoc analysis by ISIs and Groups shows significant ISI effect only in TD group (P1 component decreases in longer ISI conditions in TD (F (2;52) = 3.735, p = 0.030, eta2 = 0.126), but in RS group this effect was less significant (F (2;22) = 3.399, p = 0.051, eta2 = 0.236). (See supplementary C)

Latency value, evident-ERP subgroup

For the P1 latency ISI effect was observed (F (2;74) = 4.986, p = 0.002, eta2 = 0.119). This component was later in short ISI condition in both groups (See supplementary C).

### N1


Mean amplitude value, whole group: Significant ISI effect (F(2;94) = 28.614 p < 0.001, eta2 = 0.378) and also for ISI*Group interaction (F(2;94) = 5.427, p = 0.005, eta2 = 0.103) was found for component N1. Post hoc analysis indicates that ISI effect is larger and significant in TD group (F(2;78) = 8.701, p < 0.001, eta2 = 0.182) - smaller N1 amplitude in shorter ISI (900ms), than in RS group that is even not reaching significance in the current sample (F(2;63) = 2.752, p = 0.071, eta2 = 0.080). Post-hoc analysis for each ISI separately showed no significant differences between the two groups of participants (for 900 ms ISI condition: (F(2;47) = 1.125, p = 0.294, eta2 = 0.033); for 1800 ms ISI condition: (F(2;47) = 1.752, p = 0.191, eta2 = 0.036); for 3600 ms ISI condition: (F(2;47) = 1.125, p = 0.294, eta2 = 0.023)) (Fig. 3(A-B)).

Peak amplitude value, evident-ERP subgroup

In the subgroup with evident ERP peaks only general effect of ISI was observed - significant decreasing of N1 peak in shortest ISI condition (900ms) - (F(2;74) = 30.211 p < 0.001, eta2 = 0.449), but there was not any interaction or difference between groups.

Latency value, evident-ERP subgroup

Significant differences between groups were found for the N1 component latency (F(2;37) = 6.456, p = 0.015, eta2 = 0.148). Latencies were longer in the RS group (mean = 144.4, std = 30.3) than in TD (mean = 124.8, std = 17.7) (Fig. 3 (C)).

Peak-to-peak amplitude between P1 and N1 fully mirrors those for N1 absolute amplitude (See supplementary D).

P2

Mean amplitude value, whole group

P2 component amplitude significantly decreases in the RS group (main effect of Group F (1;47) = 19.128, p < 0.001, eta2 = 0.289). Such reduction of this component is observed in all ISI conditions (Fig. 4(A)).

Peak amplitude value, evident-ERP subgroup: Peak P2 amplitude also significantly decreased in the RS group (main effect of Group: F(1;37) = 8.317, p = 0.006, eta2 = 0.184) in all ISI conditions (Fig. 4 (B)).

Additionally, main effects of ISI that did not differentiate between groups were observed for the peak P2 amplitude. Amplitude was smallest in shortest ISI conditions (F(1;37) = 6.168, p = 0.003, eta2 = 0.143) (Fig. 4 (C)).

Latency value, evident-ERP subgroup: The P2 latency was longer in the RS (mean = 206.4, std = 31.1) as compared in TD group (mean = 178.3, std = 11.3) (main effect of Group: F(2;37) = 17.276, p < 0.001, eta2 = 0.318) (Fig. 4 (D)).

Also the main effect of the ISI was detected: P2 component was observed significantly later in the longest ISI condition irrespective of group (F(2;74) = 4.620, p = 0.012, eta2 = 0.111) (Fig. 4 (E)).
Peak-to-peak amplitude analysis for N1-P2 peaks (Mean amplitude value, whole group) confirmed both ISI \( (F(2;94) = 20.721, p < 0.001, \eta^2 = 0.306) \) and group effects \( (F(2;47) = 10.972, p = 0.002, \eta^2 = 0.182) \) found when the absolute amplitude were considered. However, P2 amplitude relative to N1 also shows interaction between these effects \( (F(2;94) = 10.143, p < 0.001, \eta^2 = 0.178) \). In TD group amplitude significantly decreased in short ISI condition \( (F(2;78) = 4.593, p = 0.013, \eta^2 = 0.105) \), in RS group this effect was not observed \( (F(2;69) = 0.936, p = 0.397, \eta^2 = 0.028) \), (See supplementary D, Fig. 7). For the peak amplitude value, evident-ERP subgroup even Group effects lose significance.

**N2**

Mean amplitude value, whole group: For the N2 component both ISI \( (F(2;94) = 10.035, p < 0.001, \eta^2 = 0.175) \) and Group \( (F(1;47) = 23.059, p < 0.001, \eta^2 = 0.329) \) effects were found. N2 amplitude was smaller in the RS group. Also this component increases in longest ISI condition (3600ms) irrespective of the group. ISI*Group interaction was not significant: \( (F(2;94) = 1.545, p = 0.219, \eta^2 = 0.032) \) (Fig. 5 (A)).

Peak amplitude value, evident-ERP subgroup: N2 component amplitude in the RS evident-ERP decreases too (main effect of group: \( F(1;37) = 7.964, p = 0.008, \eta^2 = 0.178 \) ) (Fig. 5 (B)).

**Latency value, evident ERP subgroup**

For N2 latency observed significant ISI effect \( (F(2;74) = 11.431, p < 0.001, \eta^2 = 0.236) \). Latency increases with the increase of ISI irrespective of the group.

Group differences of the peak-to-peak amplitude between P2 and N2 mirrors that with absolute N2 (Fig. 5 (C)). ISI effects disappeared pointing to its origin in the P2 amplitude differences (See supplementary E)

Thus, the P1-N1-P2 complex is modulated by the interstimulus interval, both in Rett syndrome and during typical development. N1 and P2 latency is delayed and the P2-N2 complex is significantly reduced in Rett syndrome and is unaffected by the ISI.

In our study we did not observe any significant correlation between ERP components characteristics and Rett syndrome severity (See supplementary F).

**Group classification**

The results of classification based on all feature conditions are shown on Fig. 6. In general, every set of features produced high scores. The best scores were obtained on the features corresponding to ISI 900, the mean-fold ROC-AUC for them was 0.92 ± 0.05 with accuracy of 0.933 ± 0.059. The features of ISI 1800 and 3600 resulted in lower scores (mean-fold ROC-AUC 0.84 ± 0.1 and 0.77 ± 0.14 with accuracy of 0.856 ± 0.113 and 0.778 ± 0.137, respectively). In fact, when combined, these features appeared to offset the performance of the ISI 900 condition, which resulted in the combined scores being lower at 0.88 ± 0.07 with accuracy of 0.922 ± 0.047. All of the results however, are statistically significant.
Discussion

Our main goal was to examine the neurophysiological characteristics of auditory processing stages in girls with Rett Syndrome as well as their modulation by the rate of stimulus presentation. We confirmed that early stages of auditory processing are generally preserved in RS, while the later stages, reflected in the P2 and N2 components of ERP, are severely affected being both delayed and attenuated. At the same time, main auditory components of ERP are typically modulated by the rate of stimulation in Rett Syndrome showing increase with the prolongation of inter-stimulus interval at least in the evident-ERP subgroup of RS patients. Below we consider these findings in detail.

Delayed and decreased ERP components in RS

In our study significant group effect was shown for P2 and N2 components amplitude. The attenuation of these components in the RS group is evident when considering both peak and mean amplitudes, and is also observed when the related peak-to-peak amplitudes are taken in analysis. There was also a group effect for N1 and P2 latency. Latency delay in Rett syndrome may be the reason for increased differences between groups when considering mean amplitude, because some participants may have peaks beyond the selected time windows. Thus, when the peak amplitude is considered, the group effect for the amplitude of the late components (P2 and N2), as well as their peak-to-peak amplitude, decreases. At the same time, the effect for the average peak-to-peak N1P2 amplitude is absent when the peak amplitude is taken in the evident-ERP subgroup of RS patients. It indicates that P2 decrease might be predominantly triggered not by changes in amplitude, but by a latency shift of both N1 and P2 peaks in the RS group.

Previous studies describing the specifics of auditory evoked potentials in patients with Rett syndrome often highlight their attenuation and delay in comparison to typically developing children[14,16,29]. In particular, Sysoeva’s study demonstrated a decrease in the amplitude of the P2 and N2 components of auditory ERP in response to simple (tones) and more complex (phonemes) types of stimuli[13], with the former being confirmed in our current study. An atypical decrease of P2 measured as N1P2 amplitude was also reported in a multisite study of Saby and colleagues, however, unlike our results their RS patients showed also a decrease in N1 amplitude (P1N1 peak-to-peak)[14]. This discrepancy may be due to the slightly different experimental conditions (e.g. varied ISI from 0.6-2.0 s) or the wider age range of their patients (2–37 years old), since ISI variability as well as age was shown to increase N1 amplitude[30]. While peak delay was not reported in the study of Saby and colleagues, the Mismatch negativity component of ERP (MMN) in Rett syndrome was delayed by about 40 ms as compared to the control group[16], corresponding to the N1 and P2 delays observed in our study.

At the cognitive level these effects can be linked to a disturbance of information processing in the late stages. The P2 component is associated with the consolidation in the auditory memory[31]. Meanwhile, N2 is associated with the inhibition of irrelevant information[32]. Thus, a decrease in both of these components in Rett syndrome may be related to the violation of these abilities. From the other hand it could be an effect of the need for a large amount of time in Rett syndrome. The increase in these
components with an increase in the inter-stimulus interval may indicate that a longer period of time is required for the successful implementation of the described processes. This may be due to the increased refractory period of neurons in Rett syndrome. This assumption is also supported by the fact that latency of some ERP components (N1 and P2) is larger in Rett syndrome. Information processing in the auditory system is extremely fast, so small delays in time can be critical, especially for complex stimuli such as speech.

Noteworthy, the ERP abnormalities similar to what we found in patients with RS is also observed in RS translational models: Mecp2-deficient animals demonstrated delayed and reduced auditory cortex response[33–35]. Moreover, ERP component delay is evident in RS not only in auditory modality, but also observed in response to visual stimuli[36–38]. So this pattern is quite consistent for Rett syndrome and related Mecp2 damages.

**Preserved modulation of ERP components by the rate of presentation in RS**

As shown previously our study confirms that ERP components (N1 and P2) became more pronounced with increasing interstimulus intervals. The novel result is that ERPs increase was mostly between the ISI 900 ms and ISI 1800 ms conditions, while further increase in ISI up to 3600 ms did not influence ERPs. This is a rather surprising result as previous studies reported the ERP continuous increase for the ISI at least up to 12 s, however, they were all conducted in adults[10,11]. For the children population the studies of the effects of ISI on ERP are quite limited and none of the studies included in their design the ISI longer than 2400 ms[39,40]. The absence of further increase in the ERP components after 1800 ms ISI may be related to the fact that in children the response recovery period is faster, and, as a result, sensory memory is shorter. In line with this result, it has been shown that in children, previous experience has less influence on perception[41]. However, this result needs further investigation.

Important finding is that N1 and P2 component modulation by ISI is generally preserved in patients with RS, even in spite of the severely delayed and attenuated P2 component. This result points to the similar functional meaning of these components and preservation of basic learning ability, reflected in sensitivity to the presentation rate in RS. As ERP components typically increase with the increase of ISI, we can differentiate major ERP components in RS much better with longer ISI suggesting that auditory information processing is less affected when tones are presented at a slow rate (e.g. with ISI 1800 and 3600 ms). However, clear between group differences is evident even with these longer ISIs.

The effect of presentation rate on auditory processing in Rett syndrome has been demonstrated previously in the oddball paradigm. MMN in response to frequency deviant occurs in girls with RS only when the interval between stimuli is short (450ms), while with a longer interval between stimuli (900ms and 1800 ms) MMN was not observed anymore[29]. This could indicate that the stimulus representation in Rett syndrome persists for a shorter period of time and vanishes with increasing ISI, which may be due to a variation in the neuronal refractory period. At the same time, in our current study - the increase in ISI slightly normalizes the basic component of auditory ERP. Thus, there is a trade-off for the presentation
rate in RS. With a quick presentation rate (e.g. ISI 450 ms) the main ERP components are hardly distinguishable but MMN response reflecting deviation from the auditory memory representation is typical. When we slow down the presentation (e.g. ISI 1800 ms), the main ERP components become clearer, but MMN vanishes. Practitioners might try to account for such neurophysiological abnormalities: present information that needs to be linked together within a short time frame and at the same time to provide enough time for the information processing that is clearly delayed. Probably such neurophysiological discrepancy might be among crucial deficits in RS that prevent effective processing of information.

Contamination of the ERPs components can explain other ISI effects as increase in one component can lead to latency shift and decrease in the neighboring components of opposite polarity. In particular, an increase in the N1 amplitude with ISI prolongation could lead to 1) delay in the next component (P2), which appeared as an increase in its latency with increasing ISI as well as 2) shortening of the previous component latency, which appears as P1 latency decrease with ISI prolongation. Amplitude of the N2 component demonstrates the opposite effect of ISI (large in short ISI condition) which may be due to the increase of P2 amplitude by ISI.

**Auditory ERP as biomarker of Rett Syndrome**

Machine learning algorithms applied to auditory ERP features allows segregating patients with RS from typically developing peers with high accuracy (0.922 ± 0.047), pointing to its clinical relevance. The best classification results were achieved for our shortest interstimulus presentation rate of 900 ms, confirming that slowing down the speed of presentation has a beneficial effect on auditory processing in Rett Syndrome, as neurophysiological differences between the RS and TD diminishes. However, the significant difference between RS and TD groups is evident even with the longest ISI of 3600 ms used in our study.

**Approach to minimize patients exclusions**

It is a continuous struggle for researchers, especially those who work with a challenging population, to get as much data as possible preferentially without compromising on signal to noise ratio (SNR). However, quite often a lot of data needed to be excluded from the final sample due to various, sometimes subjective, reasons. For example, if you exclude participants without evident ERP components - that seems reasonable as it might represent some problems with EEG recording - you might exclude the patients that indeed have very small and not very pronounced ERP and this absence of evident ERP might be important characteristics of the population considered. Thus, as our sample contained about half of the RS group without evident ERPs, we decided to implement two different strategies for participants inclusion: one to include all patients with RS who have sufficient number of trials for averaging and the other - to select only those who have evident ERPs with measurable ERP components. From one side, the abnormalities that are revealed in the evident-ERP group cannot be attributed to the poor SNR or other technical problems, and considered in our study as the genuine neurophysiological atypicalities that characterizes RS. Moreover, in this group we can examine not only the amplitude but also latency of the ERP components providing a more in depth view on the origin of the observed changes. From the other
side, the inclusion of all RS patients seems to be more representative of the RS population as we did not reveal any difference in clinical measures, as well as in the subjective and technical aspects of raw EEG between RS with evident and not-evident ERPs. It also should be noted that generally girls with RS were very tolerable to the ERP procedure and did not have much movements, thus substantial artifacts were not typical. This all-inclusion mean amplitude approach is also more easily implemented in clinical settings where there is a need to automatize as many steps of the analysis as possible. Detection of the individual peaks can be a challenging task, so estimation of the peak in the predefined time window is much easier and also more powerful in separation of the TD and RS group as it unites two factors - decrease in the amplitude as well as delay in latency of the component that are both observed in RS.

A number of the effects we observed (ISI effect for N1 amplitude, group effect for P2 and N2 amplitude) were both at whole group analysis and at evident-ERP subgroup analysis. Some effects were observed only at whole group analysis. For example ISI*group interaction for N1 amplitude at the whole group analysis might be at least partially related to the suboptimal time-window for peak detection in the whole RS group that may prevent detection of its modulation by the inter-stimulus interval when measured as the mean amplitude in the typical N1 latency range. Also some effects (ISI effect for P2 amplitude) were observed only at evident-ERP subgroup analysis. The absence of the effect in the whole group could be caused by data noisiness due to participants with no-evident ERP. Or there might be a subgroup of RS patients without clear ERP response, however, in the current study we could not find clinical measures to segregate these groups. Thus, the combination of both approaches to the analysis opens up some additional details of the results as well as allow to highlight the most consistent effects.

The causes of the fact that only half of the participants with Rett syndrome had evident components are currently unclear. This specific pattern was not due to age and the severity of the symptoms of the disease. One of the possible causes of this could be abnormal background EEG, which has always been considered one of the features of this syndrome[42,43]. The epileptiform activity in Rett syndrome is expressed by the appearance of spikes and sharp waves in the central temporal regions and a slowing of the theta-band background EEG in the frontal-central regions[15,44–46]. These features are unrelated to seizures and occur even in patients without a history of epilepsy[44,45,47,48]. Thus, against the background of an epileptiform activity or just increased low-frequency oscillations, the detection of evoked potentials could be problematic. Whether this “ERP absence” is a true characteristic of some RS patients or a result of technical problems in detection of ERPs in the presence of atypical background EEG activity is an important direction for future research.

**Limitations**

As a limitation, it is important to point out the absence of a control clinical group. This contrast would allow us to distinguish whether the findings are actually neuromarkers specifically for Rett syndrome or shared with other neurological or psychiatric conditions.

**Conclusions**
To sum up, the interstimulus interval modulates the amplitude and latency of the main auditory ERP components similarly in typical development and in Rett syndrome. At the same time, characteristics of auditory ERP are clearly disturbed in Rett syndrome, with the most pronounced abnormalities observed with the most rapid presentation rate.

**Abbreviations**

RS – Rett syndrome  
TD – typical development  
ISI – interstimulus interval  
ERP – Event related potentials  
RSSS – Rett Syndrome Severity Scale  
EEG – Electroencephalography  
ICA - independent component analysis  
ANOVA - Analysis of variance  
ML – Machine learning  
ROC - Receiver operating characteristic  
AUC - Area under the curve

**Declarations**

**Ethical Approval**

The research procedure was approved by the ethical committees of IHNA and Nph RAS (protocol №2 at April 30th, 2020) and Sirius University of Science and Technology amendment from April 15th, 2021. Parents or legal representatives have given written consent to the children's participation in the study, after the procedure was explained to them. Children have given verbal consent to participate.

**Competing interests**

The authors declare that they have no conflict of interest.

**Authors' contributions**
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Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

References


Figures

**Figure 1**

Grouping of the participants enrolled in the study
Figure 2

ERP of RS (all participants) (red line) and TD groups (blue line) (FCz electrode) in different ISI conditions: (A) 900ms, (B) 1800ms, (C) 3600ms. ERP of TD (blue line), “evident ERP” (green line) and “no-evident ERP” (orange line) RS groups (FCz electrode) in different ISI conditions: (D) 900ms, (E) 1800ms, (F) 3600ms.

Figure 3

N1 characteristics: Similar N1 amplitude modulation by ISI (A) in TD and (B) RS whole group. While the ISI effect is insignificant in the whole RS group you can clearly see a typical trend that gets significant in the evident-ERP group. (C) Evidently delayed N1 in RS as compared to TD. Dots represent individual values, lower panel shows effect size (Hedges' g).
Figure 4

P2 characteristics: Patients with RS showed lower P2 amplitude (A) in whole group (mean value) and (B) in evident ERP group (peak value) At the same time at the evident ERP group, (C) P2 amplitude modulation by ISI were similar across groups. P2 latency (D) larger in patients with RS and (E) modulate by ISI; Dots represent individual values, lower panel shows effect size (Hedges' g).

Figure 5

N2 characteristics: general decrease of N2 amplitude relative to baseline (A) in whole RS group (B) in evident ERP group and (C) relative to P2 in the whole group. Dots represent individual values, lower panel shows effect size (Hedges' g).
Figure 6

The aggregated ROC curves (solid blue) for each of the ISI values 900 (a), 1800 (b), and 3600 (c) and the combined set of features. In each case, we also provide the per-fold ROC curves (thin lines) along with the confidence intervals (gray area). Each ROC curve is accompanied by an AUC score which indicates the overall performance of a given classifier (higher is better).

Supplementary Files

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