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Seizure prediction in genetic rat models of absence epilepsy: improved performance through multiple-site cortico-thalamic recordings combined with machine learning

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2 performance through multiple-site cortico-thalamic recordings combined with machine

3 learning

4

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6

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32

1

Abstract

Seizure prediction is the grand challenge of epileptology. Yet, effort was devoted to prediction of focal seizures, while generalized seizures were regarded as stochastic events. Long lasting LFP recordings containing several hundred generalized spike and wave discharges (SWDs), acquired at eight locations in the cortico-thalamic system of absence epileptic rats, were iteratively analyzed in all possible combinations of either two or three recording sites, by a wavelet-based algorithm, calculating the product of the wavelet-energy signaling increases in synchronicity. Sensitivity and false alarm rate of prediction were compared between various combinations and wavelet spectra of true- and false positive predictions were fed to a random forest machine learning algorithm to further differentiate between them. Wavelet analysis of intracortical and cortico-thalamic LFP traces showed a significantly smaller number of false alarms compared intrathalamic combinations, while predictions based on recordings in layer 4, 5 and 6 of the somatosensory-cortex significantly outreached all other combinations in terms of prediction sensitivity. In 24-hours out-of-sample recordings of 9 GAERS, containing diurnal fluctuations of SWD occurrence, classification of true and false positives by the trained random forest further reduced the false alarm rate by 71%, although at some tradeoff between false alarms and sensitivity of prediction, as reflected in relatively low F1-score values. Results provide support for the cortical-focus theory of absence epilepsy and allow the conclusion that SWDs are predictable to some degree. The latter paves the way for the development of closed-loop SWD prediction-prevention systems. Suggestions for a possible translation to human data are outlined.

2

3 **Significance statement**

4 Seizure prediction was declared the grand challenge of epileptology. While most effort was
5 devoted to the prediction of focal seizures, generalized seizures were regarded as stochastic
6 events. Results of this study demonstrate that above chance prediction of generalized spike
7 and wave discharges (SWDs) is possible in long lasting, pseudoprospective 24 hours
8 recordings of absence epileptic rats, by means of wavelet analysis of LFP traces acquired
9 near the proposed cortical initiation network in S1 and further classification of true and false
10 positive detections by a trained random forest machine learning algorithm. Moreover, as
11 lower SWD prediction performance was achieved by analysis of LFP traces distant to S1, the
12 study provides evidence supporting the cortical focus theory of absence epilepsy.

13

1. Introduction

Epilepsy is a neurological disorder characterized by infrequent, short lasting periods of either local or generalized, hypersynchronous brain activity which can be recorded in the electroencephalogram. Depending on the type and nature of these seizures they either go along with a loss of behavioral control in the form of tonic or clonic convulsions and/or with a loss of consciousness. As a majority of patients diagnosed with epilepsy report the uncertainty of when a seizure attack will happen to them as one of the most disabling aspects of the disease, seizure prediction was declared the grand challenge of epileptology (Seizure Gauge Challenge 2017; 2016 Community Survey of Epilepsy Innovation Institute (Ei2) 2016; Kiral-Kornek et al. 2018).

At present, most effort in the development of seizure prediction algorithms has been devoted to the prediction of focal seizures, in which, a local group of abnormally discharging neurons is assumed to gradually recruit a critical mass of neurons during a putative pre-seizure state. Results on seizure prediction performance are quite variable, with multi-variable methods taking measures of synchronization between brain structures into account usually outperforming uni-variable methods (Mormann et al. 2007). Part of this variability can be attributed to methodological shortcomings, and a list of criteria based on which prediction performance should be evaluated was established to guide good scientific practice (Mormann et al. 2007). Criteria include evaluation of prediction performance based on unselected continuous data, in-sample and out-of-sample testing with unseen (pseudo) prospective data, and evaluation with rigorous and solid statistical methods like Monte Carlo surrogate statistics to test prediction performance against chance level prediction (Mormann et al. 2007; Kuhlmann et al. 2018).

More recently developed algorithms evaluated against these criteria, employed machine learning or deep learning approaches, and were found to achieve above chance prediction (Khan et al. 2018; Eberlein et al. 2019; Kiral-Kornek et al. 2018). Both are feature extraction methods that have been proven successful in a number of pattern recognition tasks, like image and speech recognition in medical diagnosis, genomics, translation or robotics (Walter et al. 2019; Ratner 2015; Daily et al. 2017).

Comparatively little effort has been devoted to the prediction of generalized seizures, as they have long been regarded as stochastic events (Lopes Da Silva et al. 2003). In two validated genetic rat model of absence epilepsy (rats of the WAG/Rij strain and Genetic Absence Epilepsy Rats from Strasbourg (GAERS)), characterized by generalized spike and wave discharges (SWDs) and a concomitant decrease in the level of consciousness (Depaulis and

50 Charpier 2018; van Luijtelaar and Zobeiri 2014), several studies reported the presence of
51 pre-ictal changes in the corticothalamic system, that might be useful features for SWD
52 prediction (Polack et al. 2007; Sorokin et al. 2016; Pinault et al. 2001; van Luijtelaar et al.
53 2011; Lüttjohann and van Luijtelaar 2012). A first proof of principle for the predictability of
54 SWDs was provided by Maksimenko et al. (2017). To achieve a measure for synchronization
55 signaling SWD initiation, these authors calculated the product of the wavelet energy
56 assessed in local field potential (LFP) recordings taken at three locations in the cortico-
57 thalamic system of WAG/Rij rats. While this algorithm already reached a high sensitivity of
58 prediction, it still suffered from a large amount of false alarms, strongly reducing the
59 specificity of prediction.

60 The current study was designed to improve SWD prediction performance through (i) a
61 systematic variation of the multiple recording sites of SWDs in the cortico-thalamic system
62 and relation to SWD prediction sensitivity and false alarm rate, (ii) a thorough statistical
63 comparison of wavelet spectra corresponding to true positive- and false positive detections,
64 and (iii) training of a machine learning algorithm (random forest) to further differentiate
65 between both types of detections.

66 In line with the criteria of good scientific practice mentioned above, we assessed algorithm
67 performance in long lasting, non-selected, pseudo-prospective 24 hours recordings, taking
68 potential diurnal variations of seizure occurrence into account (Smyk and van Luijtelaar
69 2020), we incorporated in-sample and out-of-sample recordings (from two different genetic
70 rat models of absence epilepsy, rats of the WAG-Rij strain and GAERS), and we statistically
71 verified the results using surrogate statistics.

72

2. Material and Methods

73 **2. Material and Methods**
74 **2.1 Animals, surgery and acquisition of LFP recordings**
75 LFP recordings of a total of 22 male WAG/Rij rats and 15 male GAERS, two well validated
76 genetic rat models of absence epilepsy were analyzed. As both strains show several
77 hundred spontaneously occurring SWDs per day (Depaulis and van Luijtelaar 2006), the data
78 are potentially suited for training and evaluation of machine learning algorithms requiring a
79 large amount of training data.

80 Recordings of 16 WAG/Rij rats were taken from a previously published data set analyzing
81 pre-ictal network interactions in the cortico-thalamic system (Lüttjohann and van Luijtelaar
82 2012, 2015). In these rats, LFP signals were simultaneously measured in freely moving
83 animals in eight different brain structures within the cortico-thalamic system including the
84 posterior thalamic nucleus (Po), the ventral-postero-medial thalamic nucleus (VPM), caudal
85 and rostral part of reticular thalamic nucleus (cRTN and rRTN), anterior thalamic nucleus

86 (ATN) as well as layer IV, V, VI of the somatosensory cortex (S1) (coordinates are specified

87 in Lüttjohann & van Luijtelaar, 2012). LFP signals were gathered at a constant sample rate of

88 2048 Hz and filtered between 1 Hz high pass (HP) and 100 Hz (LP) low pass as well as by a

89 50 Hz notch filter, over a period of at least 4 hours. A WINDAQ-recording-system was used

90 to digitize EEG signals (DATAQ-Instruments Inc., Akron, OH, USA). Rat movement was

91 registered via a PIR detector (RK2000DPC LuNAR PR Ceiling Mount, Rokonet RISCO

92 Group S.A., Drogenbos, BE). In additional 6 WAG/Rij rats LFP recordings were acquired in

93 layer Va, Vb and layer VI of the secondary motor cortex (A/P +2.7 mm, M/L +1.2 mm, d -2.5,

94 2.6, 2.8 mm, respectively; coordinates relative to bregma). Coordinates were determined

95 relatively to bregma and according to the stereotactic atlas of Paxinos and Watson (1998)).

96

97 LFP recordings of GAERS were acquired in the Münster lab. Animals aged 3 to 9 months,

98 born and raised at the Institute of Physiology I, Westfälische Wilhelms-University Münster.

99 underwent stereotactic surgery under pentobarbital anesthesia (Narcoren, 50 mg/kg;

100 Boehringer Ingelheim Vetmedica GmbH, Ingeheim am Rhein, Germany) for the implantation

101 of recording electrodes (stainless steel, insolated with polyamide, impedance 0.1 MΩ;

102 diameter 0.005 inch; Plastics One, Roanoke, USA) in the deep layer (IV, V and VI) of S1

103 (A/P: -1.8, M/L: -3.6, d:-2.6, -2.9 -3.2). Reference and a ground electrode were placed on top

104 of the cerebellum. Carprofen (5mg/kg) was administered to the rats 30 minutes before as

105 well as 24 and 48 hours after surgery to ensure intra and postoperative analgesia.

106 Two weeks after surgery animals were placed in a 43x28x42 cm plexiglas recording box,

107 equipped with bedding material, cage enrichment (Enviro-Dri) and free excess to food and

108 water. Rats were connected to recording leads connected to a swivel commutator allowing

109 LFP recordings in freely moving animals. LFP signals were amplified by an amplifier (TD

110 90087, Radboud University Nijmegen, Electronic Research Group) filtered between 1 Hz

111 (HP) and 100 Hz (LP) as well as by a 50 Hz notch filter, and digitalized with a constant

112 sample rate of 500 Hz by WINDAQ-recording-system (DATAQ-Instruments Inc., Akron, OH,

113 USA). In addition, a PIR (Passive Infrared Registration, RK2000DPC LuNAR PR Ceiling

114 Mount, Rokonet RISCO Group S.A., Drogenbos, BE) registered rat movements. GAERS

115 were recorded for a total of 24 hours.

116 All experimental procedures were carried out according to the guidelines and regulations of

117 the council of the European Union (Directive 2010/63/EU) and were approved by local

118 authorities.

119

120 **2.2 Data processing and statistics**

121 **2.2.1. Wavelet-based SWD prediction by the Maksimenko et al (2017) algorithm –**

122 **comparison between combinations of recording sites in the cortico-thalamic system.**

4

123   In an attempt to determine the optimal recording sites for SWD prediction and to gain
124   additional insight into network interactions in the cortico-thalamic system in relation to the
125   generation of SWD, we assessed SWD prediction performance in all possible combinations
126   of two and three different recording sites in the cortico-thalamic system (Table I) using the
127   algorithm previously published by Maximenko et al (2017).

128

129   For SWD prediction, the Maksimenko et al (2017) algorithm determines in each LFP trace,
130   the mean wavelet energy within a time window of 500 ms shifting along the complete LFP
131   trace sample by sample. In each trace (i) and at each time step (t), the wavelet energy (W)
132   within the frequency range of 5-10 Hz corresponding to the precursor ($W_{(5\text{-}10\ Hz)}(t)$) is
133   calculated using wavelet transformation with a modified Morlet mother function (Maksimenko
134   et al. 2017; van Luijtelaar et al. 2016). This energy obtained in each trace is multiplied to
135   determine the occurrence of cortico-thalamic synchronization at each moment in time ($W_{(5\text{-}10}$
136     $_{Hz)}(t) = W1_{(5\text{-}10\ Hz)}(t)$ X $W2_{(5\text{-}10\ Hz)}(t)$ X $W3_{(5\text{-}10\ Hz)}(t)$). Moreover, wavelet energy is calculated and
137   multiplied in each channel for a frequency range of 3-5 Hz in accordance to the light slow
138   wave sleep ($W_{(3\text{-}5\ Hz)}(t) = W1_{(3\text{-}5\ Hz)}(t)$ X $W2_{(3\text{-}5\ Hz)}(t)$ X $W3_{(3\text{-}5\ Hz)}(t)$) and within a frequency
139   range of 7-20 Hz representing sleep spindles ($W_{(7\text{-}20\ Hz)}(t) = W1_{(7\text{-}20\ Hz)}(t)$ X $W2_{(7\text{-}20\ Hz)}(t)$ X
140     $W3_{(7\text{-}20\ Hz)}(t)$) (Figure 4A).
141   Decision on whether a SWD precursor is present is based on three criteria:

142   1. Energy of $W_{(5\text{-}10\ Hz)}(t)$ needs to exceed an individualized specific threshold.
143   2. Energy of $W_{(5\text{-}10\ Hz)}(t)$ must exceed energy of $W_{(3\text{-}5\ Hz)}(t)$
144   3. Energy of $W_{(5\text{-}10\ Hz)}(t)$ must exceed energy of $W_{(7\text{-}20\ Hz)}(t)$

145

146   For determination of optimal recording sites for SWD prediction, LFP recordings (duration 4
147   hours), simultaneously obtained within the cortico-thalamic system in GAERS and WAG/Rij
148   rats, were fed into the wavelet-based SWD prediction algorithm of Maksimenko et al. (2017),
149   testing data from the various recordings sites in all possible combinations (Table I). For
150   WAG/Rij rats, a total number of 57 combinations composed of LFP recordings from three
151   recording sites and 28 combinations, composed of LFP recordings from two recording sites
152   (see Table I), were presented to the algorithm. For GAERS, data from three recording sites
153   in layers IV, V and VI of S1 were used. Each combination of recording sites can be found in
154   Table I; 'C','T','M' globally refers to recording sites in somatosensory cortex (S1), thalamus
155   and secondary motor cortex, respectively.

156

157   Since SWD prediction quality depends on the above mentioned individualized threshold,
158   SWD prediction performance of each recording site combination was determined for a total

159   of 14 fixed threshold values ranging from 0.1 to 0.75 for all combinations of three recording
160   sites, and a total of 16 fixed threshold values ranging from 0.005 to 0.04 for all combinations
161   composed of two recording sites. Of note, the difference in magnitude in the threshold values
162   for two and three recording sites is attributed to the fact that detection relies on the product of
163   either two or three wavelet energy values (see above). For prediction based on two versus
164   three recording sites, the outer threshold levels (minimum and maximum) correspond to
165   saturated levels of either sensitivity or false alarm rates for all tested combinations.
166

167   Detections of the algorithm occurring within a 1s pre-ictal period before SWD onset were
168   regarded as true positives, while detections at interictal timepoints were regarded as false
169   positives. SWD onset was determined according to the criteria outlined by van Luijtelaar &
170   Coenen (1986), taking the peak of the first spike of twice the amplitude of the background
171   EEG as a reference to mark the onset of the SWD (Figure 1). In case of differences in spike
172   timing between recording sites, notably occurring in the range of milliseconds (Lüttjohann
173   and van Luijtelaar 2012), the peak of the first spike earliest in time was taken as SWD onset.

174

175   For each combination of recording sites, and for each of the threshold values, the sensitivity
176   (Sensitivity = number of correctly predicted SWDs / (number of correctly predicted SWDs +
177   number of unpredicted SWDs) × 100%) of SWD prediction as well as the false alarm rate
178   were determined.

179   Linear regression analysis (Pearson correlation) was used to determine the degree of
180   interdependence between the sensitivity of prediction and false alarm rate.

181   Statistical comparison of sensitivity and false alarm rate between different combinations of
182   recording sites were performed using ANOVA with sensitivity or false alarm rate as
183   dependent variable, combination of recording sites as between subject factor 1, number or
184   recording sites (2, 3) as between subject factor 2, threshold as covariate 1 and false alarm
185   rate or sensitivity as covariate 2.
186

187   To avoid multiple comparison problems all combinations of recording sites were grouped for
188   post-hoc analyses as follows. 1: two intracortical recording sites in S1 (CC), 2: one cortical
189   recording site in S1 and one thalamic recording site (CT), 3: two intrathalamic recording sites
190   (TT), 4: three intracortical recording sites in S1 (CCC), 5: two cortical recording sites in S1
191   and one thalamic recording site (CCT), 6: one cortical recording site in S1 and two thalamic
192   recording sites (CTT), 7: three intrathalamic recording sites (TTT) and 8: three intracortical

193   recording sites in the secondary motor cortex (MCCC). Post hoc analyses included: ANOVA

194   with sensitivity or false alarm rate as dependent variable, group of channel combinations

195   (CC, TC, TT, CCC, CCT, CTT, TTT, MCCC) as between subject factor 1, number of

196   recording sites (2, 3) as between subject factor 2, threshold as covariate 1 and false alarm

197   rate or sensitivity as covariate 2.

198

199   All statistical analyses were performed using IBM SPSS version 25. Data are expressed as

200   the arithmetic mean values ± standard error of the mean (S.E.M.). Differences were

201   considered statistically significant when $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***).

202

2.2.2 Comparison of wavelet spectra corresponding to true positive- and false positive predictions.

205   Irrespective of the combination of recording sites, the Maksimenko et al (2017) algorithm

206   results in relatively high false alarm rates. Therefore, we determined pre-existing differences

207   in spectra corresponding to either true positive- or false positive predictions. Wavelet spectra

208   of all true positive detections, and a total number of 50 randomly selected false positive

209   detections, as identified by the algorithm of Maksimenko et al. (2017), were calculated from

210   LFP traces acquired in the deep layer (IV, V and VI) of S1 in GAERS and WAG/Rij rats

211   (Figure 3). Timepoint zero indicates the timepoint of precursor detection at the end of a 500

212   ms analysis window (ranging from -0.5 to 0), in which either the true positive precursor or the

213   false positive was detected.

214   Average wavelet energy within different frequency bands was statistically compared between

215   true and false detections using repeated measures ANOVA with average wavelet energy as

216   dependent variable, type of detection (true positive, false positive) as within subjects factor 1,

217   frequency band ($W_{(5\text{-}10 \text{ Hz})}$, $W_{(3\text{-}5 \text{ Hz})}$ and $W_{(7\text{-}20 \text{ Hz})}$) as within subjects factor 2 and rat strain

218   (GAERS, WAG/Rij rats) as between subject factor.

219

2.2.3 Random forest machine learning algorithm for differentiation between true positive - and false positive predictions

222   In an attempt to further differentiate between true and false positive predictions, we trained a

223   random forest machine learning algorithm. The wavelet energy extracted for true and false

224   detections was fed into a random forest (Birjandtalab et al. 2017) consisting of a total of 1000

225   decision trees (Figure 4A). Different numbers of trees were experimentally varied to

226   investigate the effect of forest size on classification performance (Figure 4E). For each true

227   and false positive prediction produced by the Maksimenko et al (2017) algorithm, 9 wavelet

228 energy values corresponding to the values assessed in the three frequency bands ($W_{(5\text{-}10 \text{ Hz})}$,

229 $W_{(3\text{-}5 \text{ Hz})}$ and $W_{(7\text{-}20 \text{ Hz})}$) at three different recording sites, were presented to the algorithm to

230 extract features for classification (Figure 4A). Majority voting of the different trees in the

231 random forest yielded final classification (Figure 4A).

232

233 Training of the random forest was performed with spectra obtained in 70% of all recorded

234 data in 6 WAG/Rij rats and 6 GAERS, and classification performance was evaluated on the

235 remaining 30% of unseen data of the same rats (i.e. in-sample testing). As epileptic seizures

236 or pre-ictal events are underrepresented compared to the vast number of inter-ictal

237 fragments or false positive predictions, a random undersampling approach was taken in a

238 first step in order to create a balanced training set and thereby ensure balanced learning

239 (Kubat et al. 1997). All true positive detections were fed into the algorithm, matched by an

240 equal number of randomly selected false positive detections. In this way a total of 100

241 random forest were trained. Of note, each random forest was fed with a different set of false

242 positive detections. Obtained results correspond to the performance of a single trained

243 random forest, which was found to reach an average performance of these 100 trained trees.

244

245 In order to allow an unbiased comparison of classification performance of the random forest

246 between different combinations of recording sites, we adjusted the detection threshold of the

247 Maksimenko et al algorithm (2017) for each combination to reach a 60% sensitivity of SWD

248 prediction for the extraction of the time points and wavelet features for training and

249 evaluation of classification.

250

251 To assess the classification performance of the random forest the balanced accuracy of

252 classification was calculated as (sensitivity of classification + specificity of classification) / 2),

253 with specificity = (number of false positives predicted as false positives / (number of false

254 positives predicted as false positives + number of false positives predicted as true positives))

255 * 100% and sensitivity = (number of true positives predicted as true positives / (number of

256 true positives predicted as true positives + number of true positives predicted as false

257 positives)) * 100%. Moreover an F1-score defined as F1 = 2 * ((precision * sensitivity) /

258 (precision + sensitivity)) * 100% was calculated, where precision equals (number of true

259 positives predicted as true positives / (number of true positives predicted as true positives +

260 number of false positives predicted as true positives)) * 100%.

261 Classification performance of the random forest was compared with ANOVA between the

262 different groups of recording sites in WAG/Rij rats: 1. Recordings in layers V and VI of S1,

263 referred to as "CC" (n=145); 2. Recordings in layers IV, V and VI of S1, referred to as "CCC"

264 (n=161); 3. Recordings in layers IV, VI of S1 and VPM,   referred to as "CCT" (n=161); 4.

265 Recordings in layer VI of S1, VPM and RTN, referred to as "CTT" (n=161); 5. Recordings in
266 VPM, cRTN and Po, referred to as "TTT" (n=145), 6. recordings in layer Va, Vb and VI of
267 secondary motor cortex, referred to as "MCCC" (n=161). In addition, classification
268 performance was assessed in recordings from layers IV, V and VI of S1 in GAERS, referred
269 to as "GCCC" (n=145, n=161, n=1844) and compared to results achieved in WAG/Rij rats
270 using ANOVA. Furthermore, classification performance of each group was evaluated against
271 chance level using surrogate statistics (see below).

272

273 **2.2.4 Probing the random forest machine learning algorithm for maximal SWD prediction**
274 **performance**

275 Next, the random forest machine learning combined with the Maximenko et al. (2017)
276 algorithm were probed for maximal prediction performance of SWD. Wavelet features for true
277 and false predictions were extracted in LFP recordings obtained in the deep layers (IV, V and
278 VI) of S1 of 6 GAERS at a threshold value reaching a 90% sensitivity for SWD prediction,
279 and were used for training and in-sample testing as described above. Moreover,
280 performance of random-forests trained in this approach were assessed in unseen 24 hours
281 recordings from a separate group of 9 GAERS rats (out-of-sample testing).

282

283 For in-sample testing and out-of sample testing, performance was statistically evaluated
284 against chance level prediction using surrogate statistics. To this end, training data of true
285 and false detections were randomly assigned to the two classes (total of 1000
286 randomizations), and for each randomization the average balanced accuracy achieved in the
287 unseen data was determined and displayed in a histogram. In case the achieved balanced
288 accuracy computed for the random forest trained with the real (i.e. non-randomized) training
289 data was positioned above the 95th quantile of the histogram, algorithm performance was
290 regarded as significant above chance level.

291

292 Lastly, as classification performance of the random forest was found to be reduced in the
293 out-of-sample testing, likely resulting from an insufficient amount of false positive predictions
294 presented to the algorithm during training, a separate set of random forests (n=100) was
295 trained in a (moderate) oversampling approach. A multiple (4) of all true positive predictions
296 and a matched number of randomly selected false positive predictions, derived in LFP
297 recordings of the deep layer (IV, V and VI) of S1 in 6 GAERS at a threshold value of 90%,
298 were used to train the random forests. Determination of an appropriate oversampling factor
299 was performed by comparison of classification performances achieved at different
300 oversampling factors, ranging between 2 to 7. Higher rates of oversampling were omitted to

301 avoid overtraining. As for the under-sampling approach, classification performance was
302 assessed in unseen 24 hours recordings from a separate group of 9 GAERS rats (out-of-
303 sample testing) and tested against chance level using surrogate statistics (see above).

304

305 Performance presented in the results corresponds to the performance of a single trained
306 random forest, reaching an average performance of these 100 trained trees.

307

308 **2.3 Histology**

309 At the end of the recordings, a direct current (9 V, 25 µA, 2 s duration) was pathed though
310 each electrode to create an electrolytic lesion at the location of the tip of the electrode.
311 Animals were killed with an intraperitoneal injection of pentobarbital (Narcoren, 150 mg/kg;
312 Merial GmbH, Münster, Germany). The brain was quickly removed and placed in a 4%
313 paraformaldehyde (PFA) solution for at least 24 h. Brains were fixated in a 30% sucrose
314 solution and cut into 60 µm slices with the aid of a microtome. Slices were mounted on
315 microscope slides, stained with cresyl violet, and inspected under a light-microscope (dnt,
316 DigiMicro Profi) for identification of the microlesions. Recording sites were extrapolated from
317 the center of the lesion relative to cortical depth and neighboring cortical layers. Only
318 recordings from verified recording positions were included in the analysis.

319

320 **2.4 Code Accessibility**

321 The random forest algorithm was programmed in Python and requires previous installation of
322 Python for execution. The code of the random forest algorithm is available as Extended Data.

323

324 **3. Results**

325 **3.1 Electrophysiological characteristics of SWDs in GAERS and WAG/Rij rats**

326 Exemplary LFP recordings of GAERS and WAG/Rij rats are displayed in Figure 1. LFP
327 signals of GAERS, recorded for 24 hours, displayed frequent (average of 17 per hour) SWDs
328 of 10 to 30 seconds duration at a main frequency of 5–7 Hz. Occurrence of SWDs showed
329 the well documented diurnal variation with highest rates of occurrence at the beginning of the
330 dark phase and lowest rates of occurrence at beginning of the light phase (Smyk and van
331 Luijtelaar 2020). LFP signals in WAG/Rij rats were acquired during four hours of the dark
332 phase. WAG/Rij rats showed on average 10 SWDs per hour, with a mean duration of 7 s and
333 a slightly higher internal frequency of 8–10 Hz. Spikes in thalamus typically possessed a
334 smaller amplitude (500 vs 700 µV) and broader form, with a reversed polarity as compared to
335 those in cortex. All differences of SWD morphology between strains (i.e. different internal

336   frequency) and recording sites (i.e. amplitude, polarity and sharpness of spike) are in
337   accordance with previously published data (Sitnikova and van Luijtelaar 2007; Lüttjohann
338   and van Luijtelaar 2012; Akman et al. 2010).

339

340   - place figure 1 about here –
341   - place table I about here –

342

343

344   **3.2 Influence of cortico-thalamic recording sites on SWD prediction performance**
345   In a first set of experiments, we sought to identify the influence of LFP recording sites on
346   SWD prediction performance. LFP recordings were simultaneously obtained at multiple sites
347   in the cortico-thalamic system of WAG/Rij rats, specifically in the deep layers (IV, V and VI)
348   of the somatosensory cortex (S1), secondary motor cortex, and thalamic nuclei VPM, PO,
349   ATN, rostral and caudal RTN.
350   Recordings from either two or three sites in all possible combinations (yielding a total of 85
351   combinations) were fed into the wavelet-based algorithm (Maksimenko et al. 2017).
352   Sensitivity and false alarm rate of the algorithm were compared in these 85 combinations
353   (Table I). For post-hoc analysis combinations were grouped as either 'CC' (two intracortical
354   recording sites in S1), 'CT' (one cortical recording site in S1 and one thalamic recording site),
355   'TT' (two intrathalamic recording sites, 'CCC' (three intracortical recording sites in S1), 'CCT'
356   (two cortical recording sites in S1 and one thalamic recording site), 'CTT' (one cortical
357   recording site in S1 and two thalamic recording sites), 'TTT' (three intrathalamic recording
358   sites) or 'MCCC' (three intracortical recording sites in the secondary motor cortex),
359   respectively. Moreover, SWD prediction performance of each combination of recording sites
360   was determined at multiple threshold values employed for precursor detection. As ANOVA
361   revealed a significant influence of threshold on both sensitivity of prediction
362   $(F(1,10980)=3995, p<0.001, R^2=0.26)$ (the higher the threshold, the lower the sensitivity) and
363   false alarm rate $(F(1,10980)=10.7, p<0.05, R^2=0.1)$ (the higher the threshold, the lower the
364   false alarm rate), threshold was taken as a covariate factor into statistical analysis in order to
365   allow comparison of prediction performance between different combinations of recording
366   sites irrespective of any possible threshold effects.

367

368   ANOVA revealed significant differences in both the achieved sensitivity of prediction as well
369   as the produced false alarm rate between the different combinations of recording sites
370   $(F_{senitivity}(84, 10980) = 13.47, p<0.001, R^2=0.37; F_{nFP}(84, 10980) = 2.47, p<0.001, R^2=0.1)$
371   (Figure 2, Table I).

11

372   - place figure 2 about here -

373   On average, predictions based on three recording sites reached significantly higher

374   sensitivities (Figure 2A, Table I) and lower false alarm rates (Figure 2C, Table I) as

375   compared to predictions based on two recording sites ($F_{senitivity}$ (1, 10980) = 935.7, p<0.001,

376   R^2=0.07; F_{nFP} (1, 10980) = 116.3, p<0.001, R^2=0.02).

377   Regarding the false alarm rate (Figure 2C, Table I) predictions based on three intracortical

378   recordings in S1 (CCC) and predictions based on cortico-thalamic recording sites (CCT and

379   CTT) showed a significantly smaller number of false alarms compared to predictions based

380   on three intrathalamic recordings (TTT) (all p<0.001) (average false alarm rate of CCC =

381   85.2 ± 10.6, CTT= 94.7 ± 3.0, CCT= 70.6 ± 3.5 and TTT=110.2 ± 5.4). Predictions based on

382   three intracortical recordings acquired in the secondary motor cortex (MCCC), on the other

383   hand, resulted in significantly more false alarms (average false alarm rate MCCC = 129.8 ±

384   17.9) as compared to predictions based on CCC, CCT and CTT combinations (all p<0.05).

385   Highest false alarm rates with an average of 221.1 ± 6.2 were found for predictions based on

386   two intracortical recordings acquired in S1 (all p < 0.001).

387   Regarding the sensitivity of SWD prediction, predictions based on recordings in layer IV, V,

388   and VI of S1 significantly outreached all other combinations with an average sensitivity of

389   61.7 ± 1.5 % (all p<0.001) (Figure 2A, Table I).

390   Among the remaining combinations with three recording sites, MCCC, TTT and CTT showed

391   significantly lower sensitivities compared to predictions based on two recording sites in S1

392   combined with one thalamic site (CCT) (all p < 0.001) (Figure 2A, B, Table I). Lowest

393   sensitivity was reached for predictions on two thalamic recordings (average sensitivity TT =

394   13.7 ± 0.8%), while predictions based on two cortical recording sites in S1 reached a medium

395   sensitivity of 33.0 ± 0.9 % (Figure 2A, B, Table I).

396

397   To estimate the degree of interdependence between achieved sensitivity of SWD prediction

398   and resulting false alarm rate regression analysis was performed. Analysis revealed a

399   significant negative correlation between both indicators of SWD prediction performance (r = -

400   0.716; p<0.001) (Figure 2E), indicating that a higher SWD prediction sensitivity, achieved for

401   a given combination of recording sites, does not occur at the trade-off of a high false alarm

402   rate. The same clusters as described above could be identified in the regression pattern

403   including higher false alarm rates and lower sensitivities for predictions on two recording

404   sides within the cortico-thalamic system, highest false alarm rate and medium sensitivity for

405   predictions based of two intracortical recordings in S1, medium sensitivity and medium false

406   alarm rate for predictions based on three intracortical recordings in M2 and highest sensitivity

407   with a low false alarm rate for prediction based on three intracortical recordings in S1 (Figure

408   2). Of note, irrespective of recording site combination, algorithm performance remained at a
409   low level including only moderate sensitivities of SWD prediction and high false alarm rates.

410

411   **3.3 Out-of-sample testing: Comparison between rat strains**
412   Both, GAERS and WAG/Rij rats are well validated genetic rat models of absence epilepsy
413   sharing genetic, physiological and behavioral characteristics (Depaulis and van Luijtelaar
414   2006), although slight, but significant differences in electrophysiological parameters of SWDs
415   have been reported (Akman et al. 2010). Therefore, we evaluated the prediction performance
416   of the Maksimenko et al. (2017) algorithm also in GAERS. Prediction performance was
417   assessed in 4 hours lasting LFP recordings, obtained in layers IV, V and VI of S1 in GAERS
418   and WAG/Rij rats, and, was compared between the two strains. Significant differences
419   between rat strains were revealed for the produced false alarm rate, with significantly more
420   false alarms in WAG/Rij rats compared to GAERS (p<0.001) (Figure 3B). On the other hand,
421   no significant differences were seen between GAERS and WAG/Rij rats for the sensitivity of
422   prediction (p>0.05) (Figure 3A).

423

424   **3.4 Comparison of true and false positive detections**
425   Irrespective of the combination of recording sites, the Maksimenko et al (2017) algorithm
426   resulted in relatively high false alarm rates. Therefore, we determined pre-existing
427   differences in spectra corresponding to either true positive- or false positive predictions in a
428   next experimental step.
429   Figure 3 D and E depict exemplary spectrograms of true and false positive SWD predictions,
430   respectively. Time point -0.5 to 0 features the analysis window (window size 500 ms) in
431   which either the true positive precursor or the false positive was detected. The onset of the
432   SWD is depicted at time point 0.4 seconds on the x-axis (Figure 3C,D). At this point a strong
433   increase in the product of the wavelet energy can be noted in the main frequency band of the
434   SWD (i.e. 5-10 Hz). On average, precursor activity around 900 to 300 ms before SWD onset.

435

436   - place figure 3 about here -

437

438   Next, the product of wavelet energy, assessed in the frequency bands $W_{(5\text{-}10\ Hz)}$, $W_{(3\text{-}5\ Hz)}$ and
439   $W_{(7\text{-}20\ Hz)}$ (Maksimenko et al. 2017), was statistically compared between true and false
440   positives across the two rat strains. Data revealed significant differences between true and
441   false positives in the frequency bands $W_{(5\text{-}10\ Hz)}$ and $W_{(3\text{-}5\ Hz)}$. False positives possessed a
442   higher wavelet-energy product as compared to true-positives (all p<0.05). For both frequency

443 bands, this difference was significantly more pronounced in GAERS compared to WAG/Rij

444 rats ($F_{(2,28)}=7.3$, $p<0.05$, $R^2=0.3$) (Figure 3 F,G).

445

3.5 A random forest machine learning algorithm for improvement of SWD prediction

447 Since significant differences in the wavelet spectra of true and false positives were revealed,

448 a random forest machine learning algorithm was trained to differentiate between true positive

449 and false positive detections. In a first step, a random undersampling approach was used to

450 create a training data set. Here, true positives detected in 70% of recordings from 6 WAG/Rij

451 or 6 GAERS rats and an equal amount of randomly selected false positives derived from

452 70% of recordings in the same rats were used as training data. For in-sample performance

453 evaluation, the algorithm was confronted with the remaining 30% of unseen data (see

454 methods section for more details). As in the paragraphs above, classification performance of

455 the random forest was compared between different combinations of recording sites in

456 WAG/Rij rats and between rat strains (Figure 4).

457   - place figure 4 about here -

458 In WAG/Rij rats, classification performance of the random forest was significantly above

459 chance level for all combinations of recording sites (average balanced accuracy CCC =

460 71.5%, CCT = 66,7%, CTT = 63,2%, CC = 62,5%) (all $p<0.05$) except for spectra derived

461 from three intrathalamic recording sites (average balanced accuracy TTT = 56,2%) ($p>0.05$)

462 and spectra derived from recordings in layer Va, Vb and VI of the secondary motor cortex

463 (average balanced accuracy MCCC = 49,9%) ($p>0.05$) (Figure 4B). Highest classification

464 accuracies were derived from three intracortical recordings acquired in S1, as was seen

465 using the Maksimenko algorithm above (all $p<0.05$). Classification accuracies for spectra

466 derived from three intracortical recordings in S1 from GAERS were significantly higher

467 (balanced accuracy GCCC1841 = 78,8%) compared to those in WAG/Rij rats (balanced

468 accuracy GCCC1841 = 78,8% vs. balanced accuracy CCC = 71,5%, $p<0.05$). Of note, this

469 strain difference could not be attributed to the difference in the amount of training samples

470 (i.e. 1841 derived from 70% of the six 24 hours recordings of GAERS vs 161 derived from

471 70% of the six 4 hours recordings of WAG/Rij rats), as a reduction of the training data in

472 GAERS still resulted in higher classification accuracies than in WAG/Rij rats (balanced

473 GCCC161 = 73.6% vs balanced accuracy CCC = 71,5%, $p<0.05$) (Figure 4B).

474 In order to evaluate if classification accuracy of the random forest depends on the level of

475 sensitivity achieved by the Maksimenko algorithm, classification performance in GAERS and

476 WAG/Rij rats achieved at sensitivities of 60% and 90% were compared for spectra derived in

477 recordings of layer IV, V and VI in S1. In both strains, a small but significant reduction in

478   classification accuracy was noted for spectra derived at a 90% sensitivity as compared to
479   spectra derived at a 60% sensitivity (balanced accuracy CCC = 71.5% vs CCC90% = 63.3%
480   p<0.001; GCCC1841 = 78.8% vs GCCC90% = 73.1% p<0,001). Of note, classification
481   accuracies for spectra derived at a sensitivity of 90% significantly exceeded chance level
482   classification as indicated by surrogate statistics (both p<0.01) (Figure 4B). Moreover,
483   accuracies gradually increased towards a maximum at around 16 trees (Figure 4E).

484   For out-of-sample evaluation, the random forest trained on spectra derived from three
485   intracortical recordings in S1 of GAERS at a sensitivity of 90% was confronted to spectra
486   derived from 24 hours recordings in a separate group of GAERS (n=9).

487   Table II depicts the achieved balanced accuracies of each rat as well as the average
488   confusion matrix, specifying the relative percentage of true positives that had been classified
489   as such (lower right corner), true positives that had been incorrectly classified as false
490   positives (lower left corner), false positives correctly classified as such (upper left corner),
491   and false positives incorrectly classified as true positives (upper right corner). Classification
492   performance drastically dropped and above chance classification tested by permutation
493   statistics was only achieved in a single rat (i.e. rat 5, balanced accuracy 59,62%, p<0.05).

494

495   - place Table II about here -

496   As the low performance of the random forest in the out-of-sample evaluation might be
497   attributed to random undersampling (i.e. the algorithm was trained with a training set which
498   does not adequately represent the full spectrum/variance of the false positive spectra), we
499   next evaluated the performance of an random forest, which was trained in a (moderate)
500   oversampling approach. In this approach the random forest was trained with four times all
501   true positive detections and a matched number of randomly selected false positive
502   detections, derived in three intracortical recordings in S1 of GAERS at a sensitivity of 90%
503   (see methods for details). Again, for out-of-sample evaluation, the trained random forest was
504   confronted to spectra derived from 24 hours recordings in a separate group of nine GAERS.

505   Table III depicts the achieved balanced accuracies of each individual rat as well as the
506   average confusion matrix.

507   - place Table III about here -

508   Taking this (moderate) oversampling approach, the achieved balanced accuracies of the
509   random forest significantly increased (F(1,8)=26.8, p<0.001, R^2=0.7), and above chance
510   classification could be achieved in all subjects except one (permutation statistics, all but one
511   p<0.05) (Table III).

512   Classification of the random forest trained with the (moderate) oversampling approach
513   resulted in a strong reduction in the false alarm rate. While the Maximenko et al (2017)

514   algorithm alone produced an average number of 9388 false alarms within the 24 hours,

515   sorting of the random forest reduced the false alarm rate by 71,4 ± 2.6%. Reduction of the

516   false alarm rate, however, occurred at some tradeoff between false alarm rate and

517   sensitivity. Here, Maksimenko et al (2017) on average correctly predicted 368 out of 409

518   SWD, while 40 SWD were not detected (corresponding to a sensitivity of 90%). Following

519   sorting by the random forest, an average of 200 out of 409 SWD were correctly predicted

520   (corresponding to a sensitivity of 49%). It has to be mentioned, however, that rather large

521   inter-individual differences occurred in prediction performance using the combined

522   "Maksimenko et al + random forest" algorithm. Highest performance was seen in a rat in

523   which 349 out of 520 SWD were correctly predicted (corresponding to a sensitivity of 67%).

524

525   **4. Discussion**

526   The current study was designed to improve the prediction of SWDs, a type of generalized

527   seizures seen in several forms of absence epilepsy (Panayiotopoulos et al. 1992). While

528   these types of seizures have long been regarded as stochastic events (Lopes Da Silva et al.

529   2003), a recent study by Maksimenko et al. (2017) aimed at prediction of SWDs through the

530   use of a dedicated algorithm, which calculates the product of the wavelet energy in LFP

531   recordings taken at three locations in the cortico-thalamic system of absence epileptic rats. A

532   drawback was that this algorithm suffered from a large amount of false positive detections.

533   Therefore, the current study was designed to improve prediction performance, as quantified

534   by sensitivity, specificity and balanced accuracy of prediction. The rational was to

535   systematically vary the sites of simultaneous recordings in the cortico-thalamic system,

536   including somatosensory and motor cortices, rostral and caudal RTN, specific (VPM) and

537   higher order thalamic nuclei (PO, ATN), in view of their distinct role in initiation, spread and

538   synchronization of SWDs (Depaulis et al. 2016; Lüttjohann and van Luijtelaar 2015; Crunelli

539   et al. 2020). Results were iteratively analyzed, in that all possible combinations of the 2-3

540   simultaneous recording sites were compared by using the algorithm of Maksimenko et al.,

541   (2017). Moreover, a thorough comparison of wavelet spectra corresponding to true and false

542   positive detections was performed and a random forest machine learning algorithm was

543   trained to further differentiate between true and false positives. Algorithm performance was

544   evaluated according to the guidelines of good scientific practice (Mormann et al. 2007;

545   Kuhlmann et al. 2018) (long lasting, non-selected, pseudo-prospective 24 hours recordings

546   with both in-sample and out-of-sample periods, evaluation against chance level prediction

547   using surrogate statistics), and it was found to reduce the false alarm rate by on average

548   71.4%

549

550

4.1 Highest SWD prediction performance is achieved with analysis of LFP signals in the close proximity of the seizure initiation network in S1

551
552

553   Comparison of a total of 85 combinations of recording sites within the cortico-thalamic
554 system (Table I), revealed that prediction performance was best when based on analysis of
555 the wavelet energy of recordings obtained by three recording electrodes within the deep
556 layers of the somatosensory cortex. SWDs are well known to be generated in the cortico-
557 thalamic system. While the exact interactions between cortex and thalamus are still a matter
558 of debate, accumulating evidence indicates that SWDs originate from a local intracortical
559 initiation network in the peri-oral region of the somatosensory cortex (Crunelli et al. 2020;
560 Jarre et al. 2017; Meeren et al. 2002). In GAERS, the crucial role of layer V and VI of S1 has
561 been highlighted, as theses layers were found to contain abnormally (i.e. hyperactively)
562 discharging neurons, which drove neuronal activity in other cortical layers as well as thalamic
563 activity (Lüttjohann and Pape 2019; Polack et al. 2007). These epileptogenic neurons
564 display activity patterns strikingly similar to the precursor oscillations detected by the
565 algorithm in the present study, including an increase in activity within up to two seconds
566 before SWD onset and a firing frequency of around 10 Hz (Polack et al. 2007). Highest
567 sensitivity of prediction was achieved by the Maksimenko et al (2017) algorithm based on
568 analysis of wavelet energy in the deep layers of S1 (IV, V, VI), which significantly outreached
569 all other cortico-thalamic- and intrathalamic combinations of recording sites (Figure 2A).
570 Moreover, further classification of true and false positive detections by a trained random
571 forest also reached highest, above chance balanced accuracies for spectra derived in the
572 deep layers of S1, while classification based on intrathalamic-spectra failed to achieve above
573 chance balanced accuracies (Figure 4B). These data are in line with the concept of a local
574 intracortical initiation network in S1 (Meeren et al. 2002; Polack et al. 2007).
575 Interestingly, prediction performance of the Maksimenko et al. (2017) algorithm significantly
576 dropped upon reducing the number of simultaneous recordings sites in the deep
577 somatosensory layers from three to two (Figure 2), further demonstrating the importance of
578 local intracortical synchronization in S1 for SWD generation. The concurrent increase in the
579 false alarm rate might indicate a lack of information concerning the generation of other
580 synchronized oscillations, which might be transmitted to the deep cortical layers by other
581 subcortical structures (Sitnikova et al. 2009; Depauls et al. 1990).
582 The sensitivity of SWD prediction based on three simultaneous recordings in S1 also
583 outreached the one achieved in deep layers of M2. In view of long-range intracortical
584 connections between S1 and M2, specifically from layer V/VI of S1 to layer V of M2 (Condé
585 et al. 1995; Zhang and Deschênes 1997; Zakiewicz et al. 2014; Reep and Corwin 1999), the
586 high SWD prediction performance in S1 compared to M2 suggests that SWD precursor

587 activity is a locally restricted cortical phenomenon, at least with regard to the initiation zone in
588 S1.

589 Prediction performance of the Maksimenko et al. (2017)-algorithm was found to differ
590 between the two genetic model strains, in that prediction performance was generally more
591 accurate and spectra corresponding to true and false positive detections were more
592 differentiated in GAERS compared to WAG/Rij rats. Differences between the two models and
593 even between different colonies of the same strain have been described for distinct
594 electrographic features of the SWDs (Akman et al. 2010; Powell et al. 2014). It is likely that
595 that the frequency band $W_{(5\text{-}10\ Hz)}$, employed by the algorithm for precursor detection, better
596 suits detection of 5-9 Hz oscillations, which have been described to preceded SWDs in
597 GAERS (Pinault et al. 2001). In WAG/Rij rats, on the other hand, precursor activity has been
598 described in both theta and delta frequency bands (van Luijtelaar et al. 2016; van Luijtelaar
599 et al. 2011), implying that improved SWD prediction performance in WAG/Rij rats might
600 require additional fine-tuning of the frequency band width applied by the Maksimenko et al.
601 2017 algorithm.

602

603 **4.2 Random forest machine learning algorithm for the reduction of false alarms.**
604 Irrespective of the combination of recording sites, false alarm rates remained at a relatively
605 high level. However, statistical comparison between wavelet spectra of true positive and
606 false positive predictions were revealed to significantly differ in their wavelet energies in both
607 strains and a random forest machine learning algorithm could be trained to detect such pre-
608 existing spectral differences to further differentiate between true and false positive
609 predictions. In long lasting, out-of sample, 24 hours recordings in the deep layers of S1 in
610 nine GAERS, which cover the full diurnal variation reported for SWD occurrence (Smyk and
611 van Luijtelaar 2020), this additional classification of a trained random forest reduced the false
612 alarm rate for SWD prediction by an average of 71,4%

613

614 Of note, the balanced accuracy of classification depended on the approach of training (i.e.
615 oversampling vs. undersampling) introduced to the random forest. Machine learning
616 algorithms require a balanced training set in order for unbiased assessments of error rates to
617 be achieved (Khan et al. 2018). With respect to SWDs, precursor and true positive
618 predictions are an underrepresented class compared to the much larger group of interictal
619 and false positive predictions. For balance training, random undersampling and (moderate)
620 random oversampling (Chawla et al. 2002; Kubat et al. 1997) were used, and classification
621 performance of two differentially trained random forests were compared. Significantly higher
622 balanced accuracies were found for the random forest trained in the moderate oversampling
623 approach as compared to the under-sampling approach, suggesting that undersampling

624   does not include the full spectrum of variance among different types of false positive
625   detections.

626

627   Another common source of error in machine learning algorithms is the choice of the
628   dataset on which algorithm performance is evaluated. As mentioned above, in line with
629   guidelines of good scientific practice (Kuhlmann et al. 2018; Mormann et al. 2007), algorithm
630   performance was evaluated both in unseen in-sample recordings of the same rats (30% of
631   unseen data) as well as in lasting, non-selected, pseudo-prospective 24 hours recordings
632   acquired in a separate group of GAERS (out-of sample evaluation). The importance of such
633   an additional validation step can readily be inferred from the drop in algorithm performance
634   between in-sample and out-of sample testing. Furthermore, our attempt to confront the
635   algorithm with the full range of diurnal variations necessitated these 24 hours recordings.

636

637   Unfortunately, classification by the random forest also went along, to some degree, with a
638   decrease in prediction sensitivity, in that 200 out of 409 SWD were correctly predicted
639   (corresponding to a decrease in sensitivity by 41%). The prediction of SWDs thus lacks
640   behind the performance of prediction systems aimed at focal convulsive seizures, reaching
641   sensitivities of prediction up to around 90% (Kiral-Kornek et al. 2018; Khan et al. 2018;
642   Kuhlmann et al. 2018). Of note, SWDs in absence epilepsy constitute a type of seizure that
643   is fundamental different from focal convulsive seizures, in terms of pharmacological profile,
644   frequency of occurrence, pathomechansms, and interictal spike patterns (Depaulis and van
645   Luijtelaar 2006). Moreover, the moderate performance of SWD prediction may relate to inter-
646   individual differences, which are visible in both in-sample and out-of sample validation.
647   Spatial variance between the position of the recording electrodes relative to the initiation
648   zone in S1, or neurobiological differences in the cortical initiation network between
649   individuals (Meeren et al. 2002) may explain these findings. As a corollary, individualized
650   training of the random forest on long-term data obtained from a single individual may fine-
651   tune and improve random forest approaches to SWD prediction.

652

653   **4.3 Possible translation to prediction of SWDs in human absence epilepsy**
654   SWD prediction performance of the Maksimenko et al (2017) and combined classification
655   performance of the random forest was best for intracortical recordings obtained in close
656   proximity to the seizure initiation network in S1. These findings provide an interesting
657   perspective for SWD prediction in humans using surface EEG recordings. As in the genetic
658   rat models, a local cortical initiation site of SWDs has been identified using EEG and MEG
659   recordings combined with non-linear association analysis in children with absence epilepsy

660 (Westmijse et al. 2009; Ossenblok et al. 2019). Moreover, Gupta and colleagues (2011)

661 identified pre-ictal sources of activity, occurring approximately 1 second prior to SWDs. Of

662 note, the exact location of the cortical SWD onset zone is variable between individual

663 children and pre-ictal activity was reported to be most pronounced in the delta frequency

664 range. Fine tuning of the frequency bands analyzed by the Maksimenko et al algorithm, and

665 training of the random forest on long-lasting EEG recordings in an individual child, are thus

666 promising possibilities paving the way for SWD prediction in children.

667 Wavelet analysis is a fast and reliable method for assessing non-stationary signals like LFP-

668 or EEG recordings (Hramov et al. 2015). Together with the fast temporal precision of EEG

669 and LFP recordings, this approach allows a detection of fast and short lasting events like

670 SWD precursors and opens the door for an implementation in an on-line setting aimed at real

671 time prediction and prevention (Maksimenko et al. 2017) with as little interference to the

672 overall brain activity as possible (van Luijtelaar et al. 2017; Osterhagen et al. 2010). Such a

673 treatment approach might go along with a strong relief of side-effects often reported for the

674 commonly used chronic pharmaceutical interventions (Crunelli et al. 2020).

675

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Figure legends and tables

Figure 1: Exemplary local field potential recordings in the deep somatosensory cortex of a GAERS (right) as well as simultaneously recorded LFPs the deep somatosensory cortex and vertral-postero-medial thalamic nucleus (VPM) of a WAG/Rij rat (upper left panel and lower left panel, respectively). Arrows indicates the onset of the SWD, determined according to the criteria outlined by van Luijtelaar & Coenen (1986), taking the peak of the first spike of twice the background as reference for SWD onset.

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Figure 2: Wavelet analysis for SWD prediction. Relative sensitivity (A) and average false alarm rate (C) of SWD prediction for different combinations of recording sites in the cortico-thalamic system, obtained by the Maksimenko et al. (2017) algorithm. LFPs, simultaneously recorded in the cortico-thalamic system of WAG-Rij rats (see methods), were analyzed in combinations of either two or three recording sites. Results from all 85 combinations are presented in Table I. To avoid type II errors, all combinations of recording sites were grouped as either 'CC' (two intracortical recording sites in S1), 'CT' (one cortical recording site in S1 and one thalamic recording site), 'TT' (two intrathalamic recording sites, 'CCC' (three intracortical recording sites in S1), 'CCT' (two cortical recording sites in S1 and one thalamic recording site), 'CTT' (one cortical recording site in S1 and two thalamic recording sites), 'TTT' (three intrathalamic recording sites) or 'MCCC' (three intracortical recording sites in the secondary motor cortex), respectively. B, C: Results of post-hoc comparison verified by ANOVA, with *** indicating significance at a $p<0.001$ level for sensitivity of prediction (B) and false alarm rate (D), respectively. E: Relationship of false alarm rates and average sensitivity of SWD prediction for different combinations of recording sites in the cortico-thalamic system of WAG/Rij rats, analyzed by the Maksimenko et al. (2017) algorithm. Note highest sensitivity with a low false alarm rate for prediction based on three intracortical recordings in S1 (blue triangle) that outperforms all other combinations of recording sites. Further note the negative correlation between both indicators of SWD prediction performance ($r = -0.716$; $p<0.001$), indicating that higher SWD prediction sensitivity at any given combination of recording sites does not occur at the trade-off of a high false alarm rate.

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885 **Figure 3:** SWD prediction in two genetic rat models of absence epilepsy. A, B: Average
886 sensitivity of SWD prediction (A) and false alarm rate expressed in number false positives
887 per hour (nFP/h) (B) achieved by the Maksimenko et al. (2017) algorithm assessed in 4
888 hours lasting LFP recordings, obtained in layers IV, V and VI of S1 in GAERS and WAG/Rij
889 rats.

890 C - E: Comparison of wavelet spectra of true and false positive predictions. An exemplary
891 LFP trace depicting a pre-SWD -> SWD transition is presented in (C). Onset of SWD is
892 marked by red vertical line termed 2. The corresponding spectrogram of a true positive
893 detection identified in intracortical LFP recordings in S1 of a GAERS is shown in (D). Time
894 point -0.5 to 0 (red rectangle termed 1) features the analysis window (window size 500 ms) in
895 which the true positive precursor is detected. An exemplary spectrogram of a false positive
896 detection is shown in (E). Again, time point -0.5 to 0 features the analysis window (window
897 size 500 ms) in which the false positive precursor is detected. F: Statistical comparison of the
898 product of wavelet energy, assessed in the frequency bands $W_{(5-10\ Hz)}$, $W_{(3-5\ Hz)}$ and $W_{(7-20\ Hz)}$
899 (Maksimenko et al. 2017), between true and false positives in WAG/Rij rats. E: Statistical
900 comparison of the product of wavelet energy, assessed in the frequency bands $W_{(5-10\ Hz)}$, $W_{(3-5\ Hz)}$
901 $_{5\ Hz)}$ and $W_{(7-20\ Hz)}$ (Maksimenko et al. 2017), between true and false positives in GAERS. ***
902 indicates a significant difference verified by ANOVA at level of p<0.001; ** at a level of
903 p<0.01; and * at level of p<0.05.

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905 **Figure 4:** Differentiation between true- and false positives by a random forest machine
906 learning algorithm. A: Schematic representation of the random forest machine learning
907 algorithm for differentiation between true positive and false positive predictions. After wavelet
908 analysis of either two or three simultaneously recorded LFP traces, the wavelet energies
909 ($W_{(5-10\ Hz)}$, $W_{(3-5\ Hz)}$ and $W_{(7-20\ Hz)}$) extracted in each trace are fed to a random forest composed
910 of 1000 decision trees. Final classification of the random forest is yielded from a majority
911 voting of the different trees (see methods for detail). B: Out-of-sample performance
912 (expressed as balanced accuracy) of random forests. Training in an undersampling
913 approach on wavelet spectra derived from recordings in layers V and VI of S1 (CC),
914 recordings in layers IV, V and VI of S1 (CCC), recordings in layers IV, VI of S1 and VPM
915 (CCT), recordings in layer VI of S1, VPM and RTN (CTT), recordings in VPM, cRTN and Po
916 (TTT) of WAG/Rij rats at a sensitivity of 60%, and recordings in layers IV, V and VI of S1 of
917 GAERS at a sensitivity of 60% (GCCC) or 90% (GCCC90%). Numbers in GAERS groups
918 (1844, 161, 145) refer to the different amount of true/false positive fragments, with which the
919 random forest was trained. Stars in B indicate a significant classification above chance as
920 validated by surrogate statistics with * indicating significance at a p<0.05, ** p<0.01 and ***

921 p<0.001 level. C: Table of achieved average balanced accuracies achieved by analysis of
922 the different combinations of recording sites. D: Statistics between group comparison of
923 balanced accuracies performed with ANOVA with * indicating significance at a p<0.05, **
924 p<0.01 and *** p<0.001 level. E: Relation between classification accuracy and the number of
925 incorporated trees in the random forest.

926 **Table I: Combinations of recording sites analyzed by the Maksimenko et al. algorithm and**
927 **achieved average sensitivities of prediction and false alarm rates.** Abbreviations: ctx4: layer 4 of
928 somatosensory cortex, ctx5: layer 5 of somatosensory cortex, ctx6: layer 6 of somatosensory cortex,
929 ATN: anterior thalamic nucleus, VPM: vertral-postero-medial thalamic nucleus, PO: posterior thalamic
930 nucleus, rRTN: rostral reticular thalamic nucleus, cRTN: caudal reticular thalamic nucleus, Mctx5a:
931 layer 5a of secondary motor cortex, Mctx5b: layer 5b of secondary motor cortex, Mctx6: layer 6 of
932 secondary motor cortex

number of simultaneous recording sites	combination number	area 1	area 1	area 3	abbreviation in text and figures	average sensitivity	average nFP/h
3	1	ctx 4	ctx 5	ctx 6	CCC	61,755	85,962
	2	ctx 4	ctx 5	Po	CCT	48,392	65,199
	3	ctx 4	ctx 5	ATN	CCT	45,974	85,363
	4	ctx 4	ctx 5	rRTN	CCT	44,230	69,947
	5	ctx 4	ctx 5	cRTN	CCT	50,600	58,431
	6	ctx 4	ctx 5	VPM	CCT	46,007	61,826
	7	ctx 4	ctx 6	Po	CCT	50,718	68,470
	8	ctx 4	ctx 6	ATN	CCT	48,932	82,776
	9	ctx 4	ctx 6	rRTN	CCT	45,690	71,436
	10	ctx 4	ctx 6	cRTN	CCT	51,823	60,125
	11	ctx 4	ctx 6	VPM	CCT	50,269	58,889
	12	ctx 5	ctx 6	Po	CCT	48,880	79,424
	13	ctx 5	ctx 6	ATN	CCT	48,345	95,587
	14	ctx 5	ctx 6	rRTN	CCT	51,354	65,995
	15	ctx 5	ctx 6	cRTN	CCT	48,963	72,081
	16	ctx 5	ctx 6	VPM	CCT	48,708	62,217
	17	ctx 4	Po	ATN	CTT	36,121	98,180
	18	ctx 4	Po	rRTN	CTT	35,171	97,276
	19	ctx 4	Po	cRTN	CTT	35,430	95,410
	20	ctx 4	Po	VPM	CTT	34,470	99,526

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21	ctx 4	ATN	rRTN	CTT	38,536	82,294
22	ctx 4	ATN	cRTN	CTT	34,133	101,225
23	ctx 4	ATN	VPM	CTT	32,981	99,376
24	ctx 4	rRTN	cRTN	CTT	35,892	93,150
25	ctx 4	rRTN	VPM	CTT	33,018	101,039
26	ctx 4	cRTN	VPM	CTT	37,588	83,424
27	ctx 5	Po	ATN	CTT	38,046	96,665
28	ctx 5	Po	rRTN	CTT	36,549	93,522
29	ctx 5	Po	cRTN	CTT	36,114	97,002
30	ctx 5	Po	VPM	CTT	34,702	99,814
31	ctx 5	ATN	rRTN	CTT	40,655	77,191
32	ctx 5	ATN	cRTN	CTT	36,485	98,925
33	ctx 5	ATN	VPM	CTT	33,716	98,891
34	ctx 5	rRTN	cRTN	CTT	37,172	90,429
35	ctx 5	rRTN	VPM	CTT	33,526	100,798
36	ctx 5	cRTN	VPM	CTT	38,023	82,687
37	ctx 6	Po	ATN	CTT	40,751	95,255
38	ctx 6	Po	rRTN	CTT	38,563	93,038
39	ctx 6	Po	cRTN	CTT	38,292	95,827
40	ctx 6	Po	VPM	CTT	36,516	101,606
41	ctx 6	ATN	rRTN	CTT	43,434	72,403
42	ctx 6	ATN	cRTN	CTT	37,946	100,546
43	ctx 6	ATN	VPM	CTT	35,950	98,257
44	ctx 6	rRTN	cRTN	CTT	40,527	84,918
45	ctx 6	rRTN	VPM	CTT	35,363	100,356
46	ctx 6	cRTN	VPM	CTT	38,784	87,363
47	Po	ATN	rRTN	TTT	35,880	103,088
48	Po	ATN	cRTN	TTT	31,342	115,496
49	Po	ATN	VPM	TTT	30,849	115,094
50	Po	rRTN	cRTN	TTT	33,263	109,348
51	Po	rRTN	VPM	TTT	31,252	116,632
52	Po	cRTN	VPM	TTT	30,485	116,111

	53	ATN	rRTN	cRTN	TTT	36,646	89,893
	54	ATN	rRTN	VPM	TTT	34,497	98,061
	55	ATN	cRTN	VPM	TTT	30,137	110,576
	56	rRTN	cRTN	VPM	TTT	30,907	115,390
	57	Mctx 5a	Mctx 5b	Mctx 6	MCCC	33,330	129,803
2	1	ctx 4	ctx 5		CC	31,173	211,365
	2	ctx 4	ctx 6		CC	34,619	209,386
	3	ctx 5	ctx 6		CC	33,612	242,989
	4	ctx 4	VPM		CT	21,408	123,705
	5	ctx 4	ATN		CT	20,799	148,887
	6	ctx 4	Po		CT	21,987	151,854
	7	ctx 4	cRTN		CT	23,729	122,750
	8	ctx 4	rRTN		CT	25,276	130,967
	9	ctx 5	VPM		CT	23,357	120,332
	10	ctx 5	ATN		CT	22,728	158,520
	11	ctx 5	Po		CT	24,474	151,471
	12	ctx 5	cRTN		CT	24,874	130,418
	13	ctx 5	rRTN		CT	29,267	121,645
	14	ctx 6	VPM		CT	23,514	146,704
	15	ctx 6	ATN		CT	24,906	174,084
	16	ctx 6	Po		CT	25,886	171,314
	17	ctx 6	cRTN		CT	25,948	145,599
	18	ctx 6	rRTN		CT	31,349	137,519
	19	VPM	ATN		TT	10,411	157,414
	20	VPM	Po		TT	10,741	186,945
	21	VPM	cRTN		TT	14,999	151,043
	22	VPM	rRTN		TT	15,703	155,311
	23	ATN	Po		TT	12,648	179,928
	24	ATN	cRTN		TT	10,670	165,252
	25	ATN	rRTN		TT	20,339	142,317
	26	Po	cRTN		TT	11,267	171,176

27	Po	rRTN	TT	17,575	166,227
28	cRTN	rRTN	TT	21,339	142,157

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937   Table II: Out-of-sample performance of the random forest (trained in an undersampling approach on
938   spectra derived from three intracortical recordings in S1 of GAERS at a sensitivity of 90%) confronted
939   to spectra derived from 24 hours recordings in a separate group of GAERS (n=9).
940   Depicted in the upper panel is the average confusion matrix (+/- SEM), specifying the percentage of
941   true positives correctly classified as true positives (lower right corner), true positives incorrectly
942   classified as false positives (lower left corner), false positives correctly classified as false positives
943   (upper left corner) and false positives incorrectly classified as true positives (upper right corner).
944   Lower panel depicts the balanced accuracies and F1-scores for each individual rat. Note that the F1
945   score reflects the tradeoff between false alarm rate/sensitivity. Low F1 scores are reflecting the drop of
946   sensitivity associated to the drop of false alarm rate. As our goal in this work is the latter, the low
947   scores are justified by the high balanced accuracies. * denotes an above chance balanced accuracy of
948   classification as verified by surrogate statistics.
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average confusion matrix		
	predicted as false positive	predicted as true positive
false positive	52.46 % +/- 9.38	47.54 % +/- 9.38
true positive	50.66 % +/- 8.95	49.34 % +/- 8.95

	balanced accuracy	F1-score
rat 1	47,37%	14,53%
rat 2	53,68%	6,89%
rat 3	47,44%	11,74%
rat 4	49,07%	4,82%
rat 5	59,62% *	9,14%
rat 6	51,06%	5,44%
rat 7	51,93%	7,18%
rat 8	50,13%	4,25%
rat 9	47,82%	9,68%

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Table III: Out-of-sample performance of the random forest (trained in an oversampling approach on spectra derived from three intracortical recordings in S1 of GAERS at a sensitivity of 90%) confronted to spectra derived from 24 hours recordings in a separate group of GAERS (n=9).

Depicted in the upper panel is the average confusion matrix (+/- SEM), specifying the percentage of true positives correctly classified as true positives (lower right corner), true positives incorrectly classified as false positives (lower left corner), false positives correctly classified as false positives (upper left corner) and false positives incorrectly classified as true positives (upper right corner).

Lower panel depicts the balanced accuracies and F1-scores for each individual rat. Note that the F1 score reflects the tradeoff between false alarm rate/sensitivity. Low F1 scores are reflecting the drop of sensitivity associated to the drop of false alarm rate. As our goal in this work is the latter, the low scores are justified by the high balanced accuracies. * denotes an above chance balanced accuracy of classification as verified by surrogate statistics.

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	average confusion matrix	
	predicted as false positive	predicted as true positive
false positive	71.38 % +/- 2.56	28.62 % +/- 2.56
true positive	46.00 % +/- 4.00	54.00 % +/- 4.00
	balanced accuracy	**F1-score**
rat 1	70,28% *	46,88%
rat 2	55,14%	7,59%
rat 3	60,13% *	16,60%
rat 4	63,98% *	12,21%
rat 5	63,15% *	12,02%
rat 6	59,70% *	8,64%
rat 7	68,47% *	13,14%
rat 8	59,00%*	6,51%
rat 9	64,38% *	19,71%

977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992

WAG/Rij

GAERS

SWD onset

SWD onset

cortex (S1)

cortex (S1)

700 μV

thalamus
(VPM)

1sec

A

sensitivity [%]

80

60

40

20

0

CC CCC CCT CT CTT MCCC TT TTT

B

	CC	CCC	CCT	CT	CTT	MCCC	TT	TTT
CC		***	***	***	***	.918	***	.532
CCC			***	***	***	***	***	***
CCT				***	***	***	***	***
CT					***	***	***	***
CTT						.179	***	***
MCCC							***	.680
TT								***
TTT								

C

nFP/h

300

200

100

0

CC CCC CCT CT CTT MCCC TT TTT

D

	CC	CCC	CCT	CT	CTT	MCCC	TT	TTT
CC		***	***	***	***	***	***	***
CCC			.181	***	.377	*	***	*
CCT				***	***	***	***	***
CT					***	.396	***	***
CTT						*	***	**
MCCC							*	.270
TT								***
TTT								

E

nFP/h

300

250

200

150

100

50

0

10 15 20 25 30 35 40 45 50 55 60 65

Sensitivity [%]

● CC   ▲ CCC
● CT   ▲ CCT
● TT   ▲ CTT
        ▲ TTT
        ▲ MCCC

A

B

C

1 2

D

frequency (1/Hz)

0.20

0.10

-2.0 -1.5 -1.0 -0.5 0.0 0.5 1.0 1.5 2.0

0.015

0.010

0.005

(mV²/s)

E

frequency (1/Hz)

0.20

0.10

-2.0 -1.5 -1.0 -0.5 0.0 0.5 1.0 1.5 2.0

time

0.010

0.0075

0.005

0.0025

(mV²/s)

F WAG/Rij

G GAERS

average wavelet energy

0.2

0.1

* *

W
(5-10Hz)

W
(3-5Hz)

W
(7-20Hz)

average wavelet energy

1.0

0.5

* *

W
(5-10Hz)

W
(3-5Hz)

W
(7-20Hz)

true
false

A

B

C

combination	balanced accuracy
CC	62.50%
CCC	71.47%
CCC 90%	63.30%
CCT	66.70%
CTT	63.20%
TTT	56.20%
MCCC	49.94%
GCCC 1841	78.80%
GCCC 161	73.58%
GCCC 145	73.00%
GCCC 90%	73.10%

D

	CC	CCC	CCC 90%	CCT	CTT	TTT	MCCC	GCCC 1844	GCCC 161	GCCC 145
CC		***	0.398	0.844	***	***	***	***	***	***
CCC			***	***	***	***	***	**	**	***
CCC 90%				0.516	***	***	***	***	***	***
CCT					***	***	***	***	***	***
CTT						***	***	***	***	***
TTT							***	***	***	***
MCCC								***	***	***
GCCC1844									***	***
GCCC161										0.349
GCCC145										

E