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·Method·

Evaluation of an automated spike-and-wave complex detection algorithm in the EEG from a rat model of absence epilepsy

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ABSTRACT

The aim of this prospective blinded study was to evaluate an automated algorithm for spike-and-wave discharge (SWD) detection applied to EEGs from genetic absence epilepsy rats from Strasbourg (GAERS). Five GAERS underwent four sessions of 20-min EEG recording. Each EEG was manually analyzed for SWDs longer than one second by two investigators and automatically using an algorithm developed in MATLAB®. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for the manual (reference) *versus* the automatic (test) methods. The results showed that the algorithm had specificity, sensitivity, PPV and NPV >94%, comparable to published methods that are based on analyzing EEG changes in the frequency domain. This provides a good alternative as a method designed to mimic human manual marking in the time domain.

Keywords: GAERS; seizure detection; epilepsy

INTRODUCTION

Epilepsy is a chronic neurological condition characterized by recurrent seizures. Its incidence in most developed countries is between 50 and 100 cases per 100 000 per year[1, 2]. As treatment with conventional anti-epileptic drugs provides adequate seizure control in only 2/3 of patients, more translational research in this field is urgently needed[1, 2]. The efficacy assessment of novel treatments in animal models typically relies on the evaluation of many hours of electroencephalogram (EEG) recordings. Manual evaluation of these recordings is time-consuming and very subjective, while the development of automatic seizure-detection methods can make these analyses quicker and more reproducible.

Genetic absence epilepsy rats from Strasbourg (GAERS) is a strain in which 100% of the animals exhibit recurrent generalized non-convulsive seizures[3]; and it has become the gold standard for studying the mechanisms of absence epilepsy. This model is characterized by the development of well-defined and consistent spike-and-wave discharges (SWDs) even though their duration and numbers vary between colonies[3, 4]. Spontaneous SWDs (7–11 Hz, 330–1 000 μV, 0.5–75 s) start and end abruptly in a normal background EEG[3].

The algorithm to automatically mark the onset and termination of SWDs was implemented using MATLAB® and Signal Processing Toolbox™ R2010b (The MathWorks, Inc., Natick, MA). Many groups have published methods that automatically detect seizures, but they are predominantly designed for studies in humans[5-9]. The morphology of SWDs in GAERS is noticeably more consistent than seizures in humans, so we hypothesized

that an algorithm based on the definition of SWD (referred to as the 'SWD detection algorithm') would mark the onset and termination of SWD accurately.

The aim of the present study was to determine whether the SWD detection algorithm can successfully replace the manual marking of EEG recording with high accuracy. A time-domain-based assessment of algorithm performance was used to compare the SWD detection algorithm with human markers[10].

MATERIALS AND METHODS

The study was designed as a prospective controlled masked experiment. All experiments were approved by St Vincent's Hospital (Melbourne) Animal Ethics Committee and conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).

Animals

Six-month-old female GAERS, obtained from the University of Melbourne (Parkville, Victoria, Australia), were used in experiments on novel techniques for the delivery of anti-epileptic drugs. The first five animals randomly allocated to the control group in that study were also used in the present study. These animals underwent surgery for the implantation of EEG recording electrodes and their epileptic activity was evaluated for 8 weeks. They were housed individually under a 12-h light/dark regime with *ad libitum* access to food and water.

Recording Electrode Implantation

Immediately before surgery, the rats were weighed and anaesthetized with an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (10 mg/kg). Following the induction of anesthesia, each rat was placed in a stereotaxic apparatus and given isoflurane (0.5% to 1% in oxygen, 1 L/min) *via* a nose-cone, along with subcutaneous carprofen (5 mg/kg) for pain relief and 0.9% sodium chloride (2 mL) for cardiovascular support. Then the EEG recording electrodes were implanted as follows: after preparation of the scalp, a single incision was made down the midline, the skull cleared of tissue, and the exposed bone dried with 3% hydrogen peroxide. Four extradural electrodes, consisting of small jeweler's screws, were implanted cranial to the interaural line (two on each side of the sagittal suture) and one caudal to the interaural line to the right of the sagittal suture (Fig. 1A). The electrodes were then connected to an adaptor secured with dental cement. The skin was sutured leaving only part of the dental cement exposed and each animal was placed on a heating pad for recovery. Post-operative treatment included subcutaneous buprenorphine (0.03 mg/kg, twice/day), saline (2 mL, once/day) and carprofen (5 mg/kg, once/day) for up to 3 days.

Electroencephalographic Recording

Beginning on day 7 or 8 after surgery, rats were monitored for at least 60 min (30 min for recovery from anesthesia/acclimation and 30 min for EEG recording), on at least 2 days per week for the following 7 weeks. For the purpose of developing the semi-automatic detection algorithm, only the first two and the last two recordings were analyzed (Fig. 1C) and only the first 20 min of each recording were studied. At each monitoring session, rats were briefly anesthetized with isoflurane (4% in oxygen, 2 L/min) in an induction cage, and shielded cables were used to connect the recording electrodes to the EEG acquisition system (TDT processors; Tucker-Davis Technologies, Alachua, FL) and high-impedance head-stages driven by custom-designed software. The EEGs were sampled at 3051.76 Hz.

The rats were allowed to fully recover from anesthesia before recording. During the recording sessions, their behavior was observed and the EEGs were visualized using a custom-designed MATLAB® program. If the rats were perceived as being asleep and after confirmation of no seizure activity on the EEG, noise stimuli of 94 and 98 dB were delivered (Fig. 2). At the end of each recording session, the rats were briefly anaesthetized with isoflurane (4% in oxygen, 2 L/min) for disconnection from the shielded cable.

Evaluation of EEG Recordings

Preparation of Raw Data

The EEG data were transcribed using a graphical interface developed in MATLAB®. They were first band-pass filtered between 3 and 30 Hz using a second-order Butterworth filter, and then filtered in both the forward and the reverse directions to avoid introducing a delay in the signal, which effectively doubled the filter order to fourth order. During the

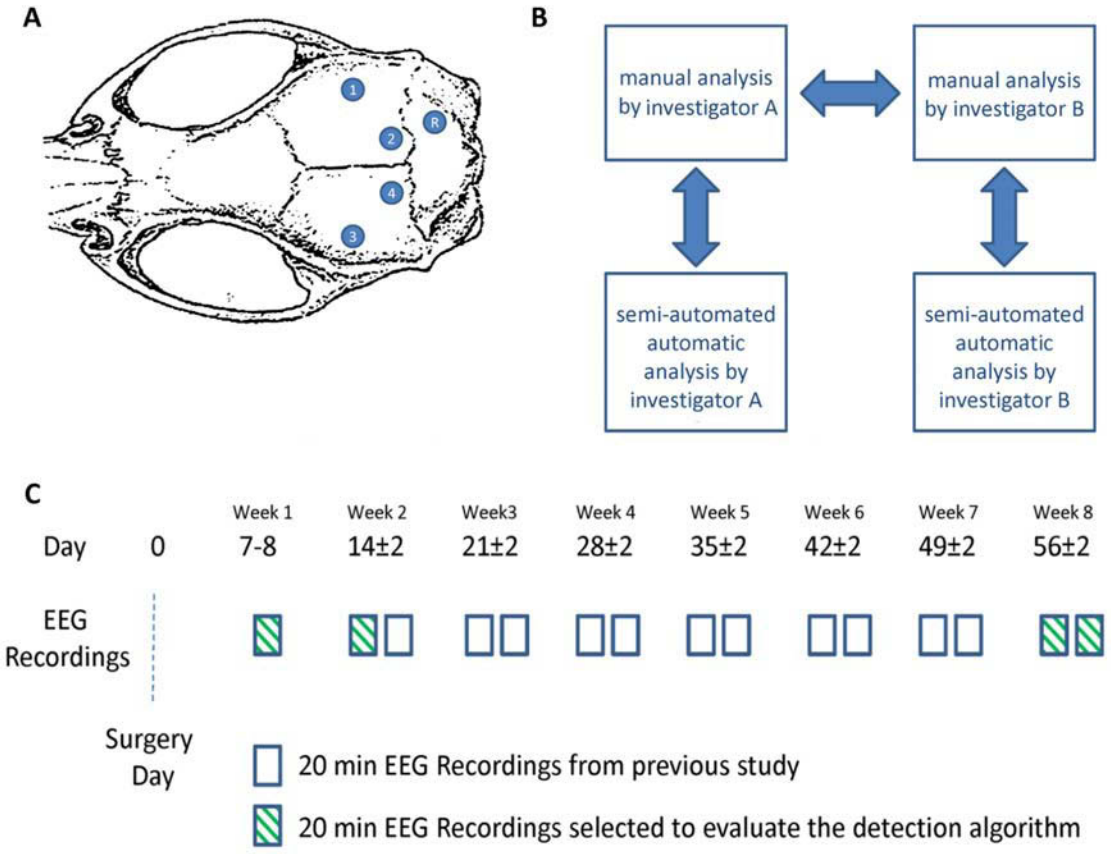

Fig. 1. (A) Schematic diagram illustrating the positioning of epidural recording screw electrodes (1-4) and the reference electrode (R). (B) Comparisons were made between the manual EEG recording evaluations of the two researchers, and between the manual evaluations and the semi-automated SWD detection algorithm. (C) Experimental design: 20-min EEG recording began at day 7 or 8 after surgery and 2 days per week for the following 7 weeks; for the purpose of developing the semi-automatic detection algorithm only the first two and the last two recordings were analyzed.

second step, all recorded EEG channels were displayed for the observer to select the one with the best signal for SWD detection (i.e., largest SWD amplitude relative to background EEG amplitude) and without signal dropout (i.e., poor physical connection resulting in occasional flat lines) (Fig. 3A). The selected channel was used for manual and semi-automated marking.

Manual Analysis of EEG Data

EEG recordings were independently analyzed manually by two experienced researchers. While reviewing the recorded EEG, the researcher marked the beginning and the end of all identified SWDs that were more than one second in duration (Fig. 3 B) and an Excel data sheet reporting the start and end times of all the SWDs was generated. After completing the manual marking, each researcher applied the semi-automated SWD detection algorithm to the selected channel of EEG recording.

Semi-Automated SWD Detection Algorithm

The semi-automated SWD detection algorithm was developed within the same graphical interface as that noted above. This algorithm required human intervention to select the best EEG channel and to set an amplitude threshold for SWD detection. In the latter step, non-parametric thresholds were generated by evaluating the empirical cumulative distribution function of the voltage magnitude (from 90% to 100% at 0.5% intervals) and were plotted against the filtered EEG data, so that the observer could select, by visual inspection, a percentile threshold

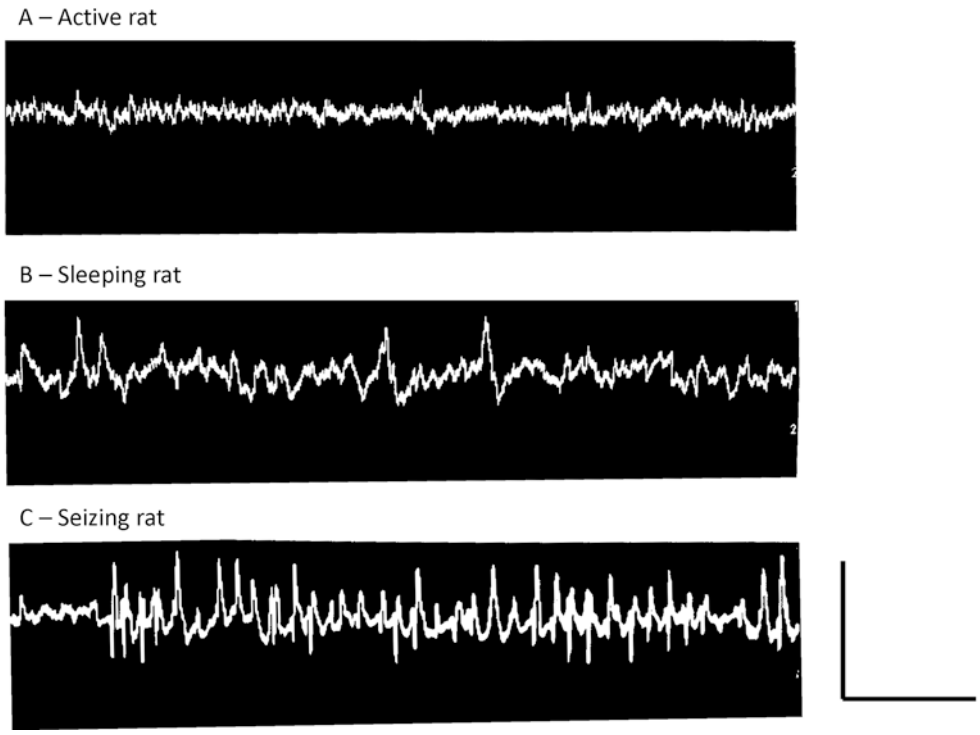

Fig. 2. During a recording session, the EEGs were visualized using a custom-designed MATLAB® program. The visualization of the EEGs allowed confirmation of the status of the rat: active (A), sleeping (B), or seizing (C). If the rats were perceived as being asleep and seizure activity on the EEG was confirmed, a noise stimulus of 94 to 98 dB was applied by knocking on the Plexiglas cage. The three examples were recorded at the same scale. Calibration 1 s, 1 mV.

that would best differentiate the SWD activity from the background EEG (Fig. 3).

Once the threshold was selected, the commands of the program were to: (1) construct a binary array corresponding to whether the filtered EEG amplitude was above the user-set threshold (i.e., 0 for below and 1 for above); (2) evaluate the derivative of the binary array to identify spike rises and spike falls; (3) iterate through the derivative array, data-point by data-point, and alternatively search for SWD onsets and terminations; and (4) when the end of the data set was reached, to evaluate the number of SWDs (number of SWD onsets) and their durations (time between onset and termination) (Fig. 4).

The first sample of the 1-s observation window was classified as a SWD onset if it met the following criteria (Fig. 5): (1) the first data point of the window corresponded to a spike rise; (2) the number of spike rises within the window was between 5 and 13; and (3) the interspike intervals were between 40 and 300 ms. The first data point of the 1-s observation window was classified as a SWD termination if it met the following criteria (Fig. 5): (1) the first sample of the window corresponded to a spike fall; and (2) apart from the first sample, all other samples in the window were below the threshold amplitude. So, a SWD event had to be at least 1 s in duration and two SWD events were considered separate if they were >1 s apart.

Data Analysis and Performance Metrics

Comparisons were made between the manual evaluations of EEG recordings by the researchers and evaluation by the semi-automated SWD detection algorithm. A previously-published, rigorous method of assessing performance was used (Fig. 6), where only the time intersection of an automatically detected event with one that was manually marked as a positive SWD was considered a true positive time window[10]. Likewise, only when the automatic time intersection of the absence of an event coincided with a manually marked negative SWD was considered a true negative time window. When a time window was evaluated to be SWD-positive by the detection algorithm, but not

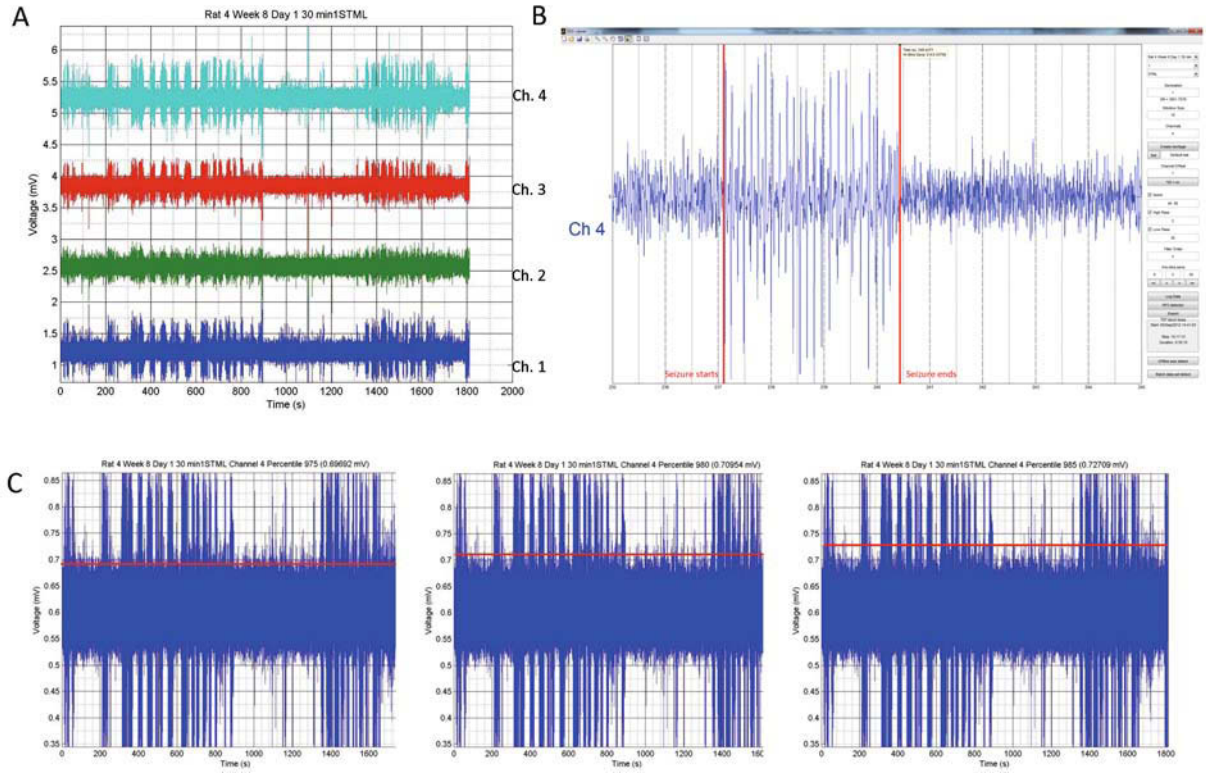

Fig. 3. EEG analysis using a semi-automated SWD detection requiring human intervention to select the best EEG channel/electrode and set an amplitude threshold for SWD detection. (A) Example of EEG recording: in this example, channel four was selected by the investigator as having the best signal-to-noise ratio, so subsequent analysis was done using this channel. (B) Example of manual marking: the EEG was analyzed using a 10-s window and the start and end of the SWD were noted. (C) Example of threshold selection: the traces show the EEG of the selected channel (channel 4 in this example) with superimposed automatically-calculated thresholds. To be selected, the threshold red line had to be just above the baseline EEG signal. In this example a threshold of 0.71 mV was selected (middle panel).

by the reviewer, it was considered to be a false-positive. When the detection algorithm evaluated a time window as SWD-negative, but the reviewer evaluated it as SWD-positive, it was considered to be a false-negative. Then, the performance metrics of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using equations 1 to 4. The performance metrics were assessed to a precision of 0.1 s.

$$Sensitivity = \frac{TruePositive}{TruePositive + FalseNegative} \quad (1)$$

$$Specificity = \frac{TrueNegative}{TrueNegative + FalsePositive} \quad (2)$$

$$PositivePredictiveValue = \frac{TruePositive}{TruePositive + FalsePositive} \quad (3)$$

$$NegativePredictiveValue = \frac{TrueNegative}{TrueNegative + FalseNegative} \quad (4)$$

Comparisons were also made between the two researchers using the same approach (with one being considered the detection algorithm and the other as the reviewer). In total, three comparisons were made for each EEG recording (Fig. 1B).

Data were analyzed with Microsoft Excel 2010 and graphic representations generated with GraphPad Prism 6. The computer used to run the detection algorithm was a desktop PC (twelve Intel® Xenon® CPUs X560 at 3.47 GHz running Windows 7, 64-bit).

RESULTS

The first recording from one of the rats was missing, so only 19 EEG recordings were evaluated, representing a total of 6 h and 20 min of recording. The median number

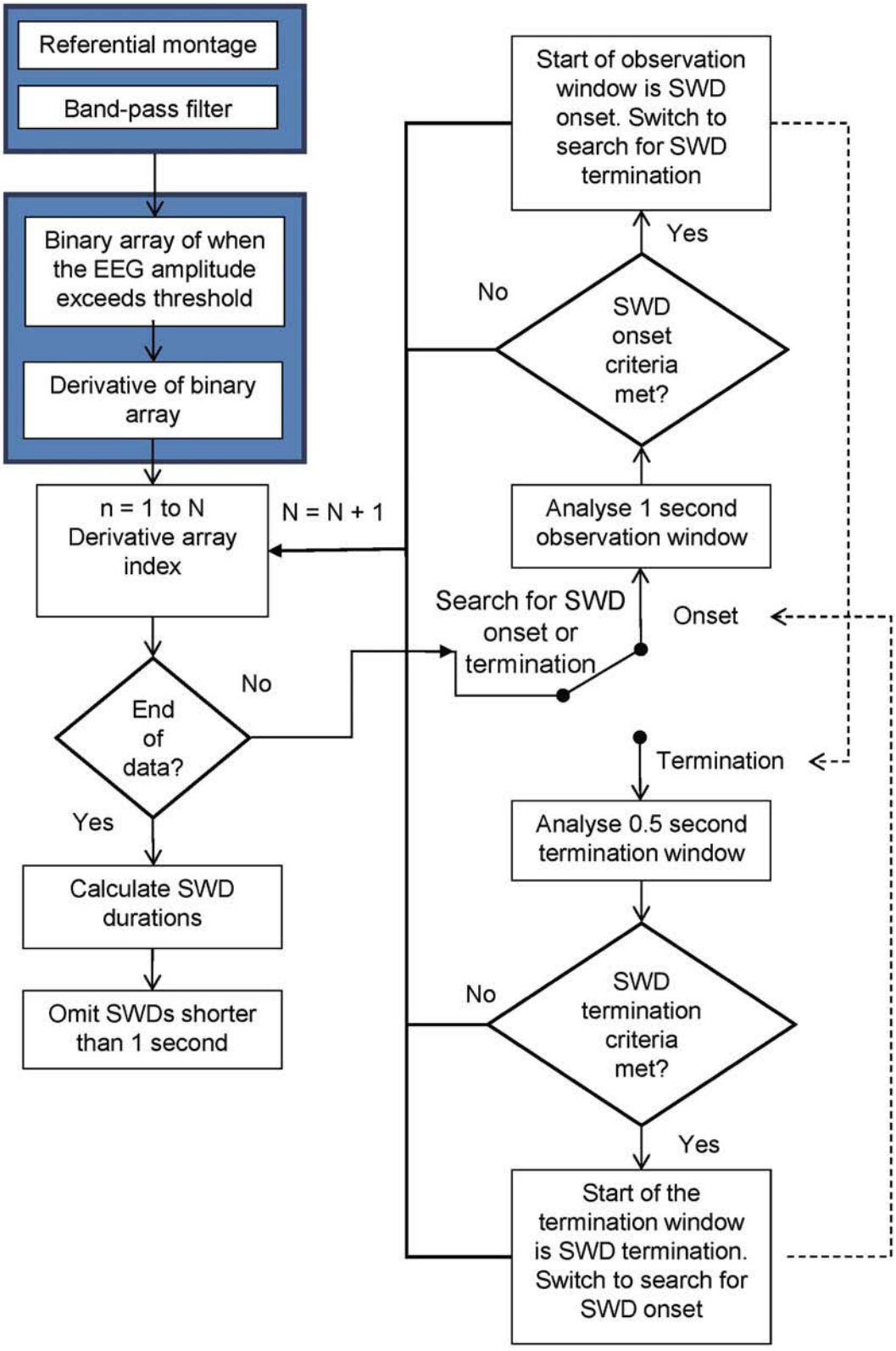

Fig. 4. Flowchart of the semi-automated algorithm.

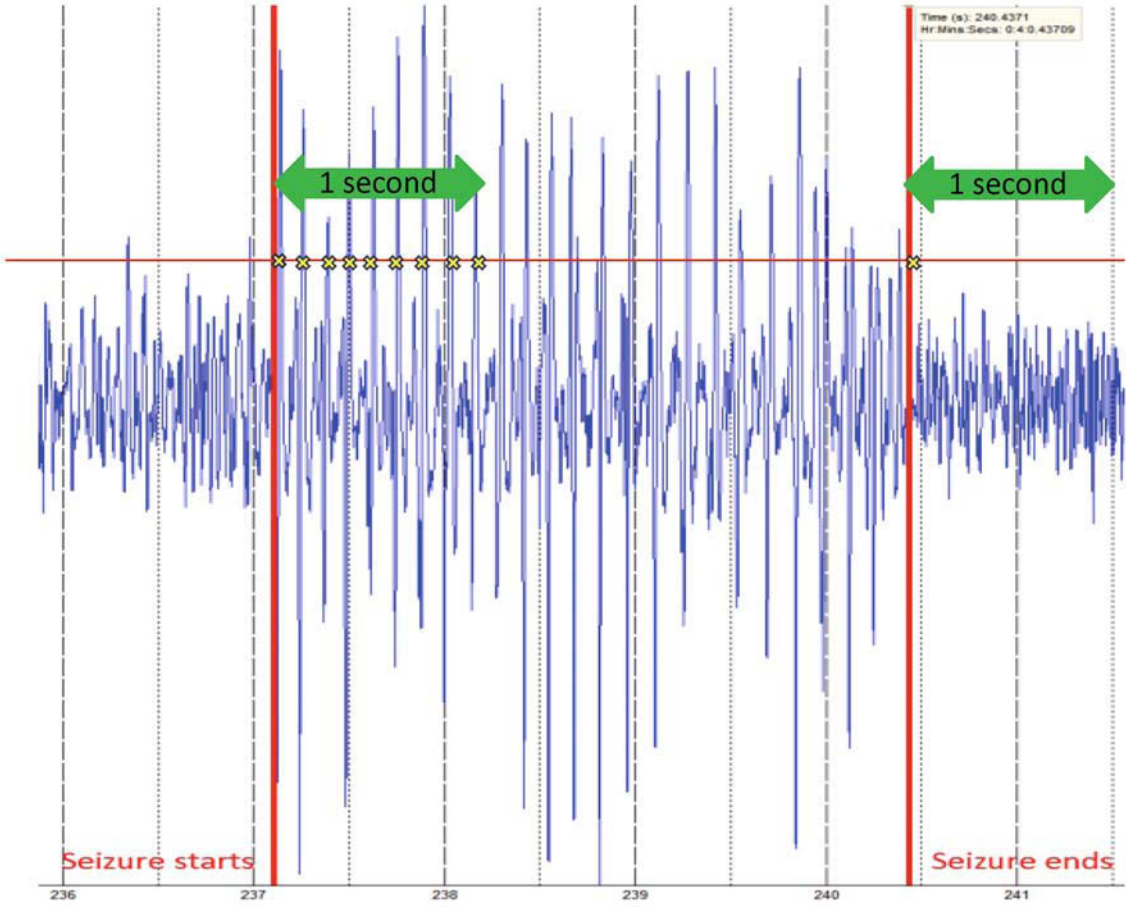

Fig. 5. The process of automatic marking. Horizontal red line, the threshold level. The left green arrow indicates the first 1-s observation window classified as a SWD onset (yellow crosses represent spike rises going above the threshold level), and the right green arrow indicates the first 1-s observation window classified as a SWD termination (yellow cross represents a spike fall). See text for detailed criteria of classification.

of SWDs over 20 min ranged from 40 to 46 and the mean duration of SWDs from 8.9 to 11.4 s for each evaluator and method, with extreme values resulting from the two manual markings (Table 1). The calculated sensitivity, specificity, PPV, and NPV were >91% for all (Table 2).

DISCUSSION

The algorithm was demonstrated to have good sensitivity, specificity, PPV, and NPV for detecting SWDs with durations ≥1 s. The study was not randomized, as the EEG data were always analyzed manually by the investigators first and then by automatic detection. This was done so that the investigators' manual analysis would not be biased by the automatic detection results.

The characteristics of the SWDs of GAERS used to build the algorithm were slightly modified from Maurescaux *et al.* (1992)[3]. Indeed only SWDs of ≥1 s duration were detected and the reported SWD frequency range of 7 to 11 Hz was extended to include SWDs ranging from 5 to 13 Hz. The larger frequency range provided tolerance as, for example, spikes can sometimes be below threshold resulting in a lower spike count.

Twenty-minute recording was used for the analysis as this was the duration reported in the original paper describing the EEG of GAERS (Marescaux *et al*., 1992)[3]. Comparisons were made between the manual EEG recording evaluations by the two researchers to establish a baseline range of sensitivity, specificity, PPV and NPV. Comparison between the manual and that investigator's

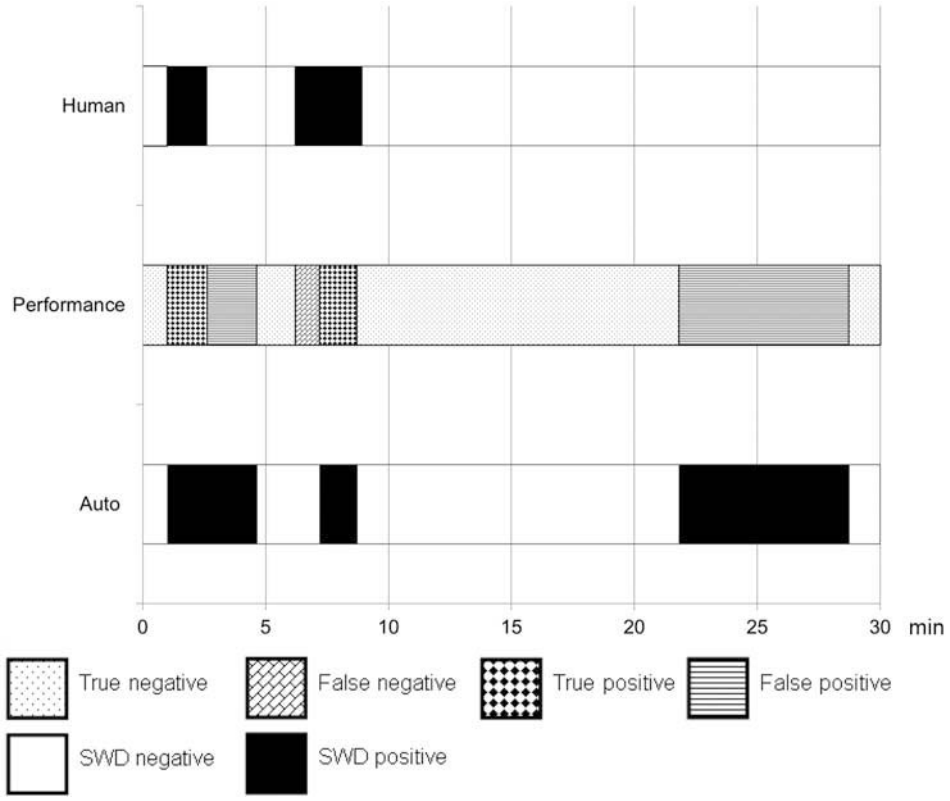

Fig. 6. Performance metrics: SWD negative, EEG not showing spike-and-wave discharges (SWDs) as evaluated by the researcher (human) or the algorithm (auto); SWD positive, EEG showing SWDs as evaluated by the researcher (human) and the algorithm (auto); true negative, neither human nor auto detected SWDs; false negative, human-detected SWDs whereas auto did not; true positive, both human and auto detected SWDs; false positive, human did not detect SWDs whereas auto did.

Table 1. Median number and mean duration of spike-and-wave discharges (SWDs)

	Investigator A	Automatic A	Investigator B	Automatic B
Median number of SWDs	40 (30–56)	41 (29–58)	46 (37–63)	42 (29–54)
Mean duration of SWD (s)	11.4 (±2.9)	10.7 (±2.9)	8.9 (±2.8)	10.2 (±2.8)

Automatic A and B represent the semi-automated algorithm run by investigators A and B. Median (min–max), mean (± SD).

Table 2. Sensitivity, specificity, positive predictive value (PPV), and negative predicted value (NPV)

	Investigator A *versus* Automatic A	Investigator B *versus* Automatic B	Investigator A *versus* investigator B
Sensitivity	0.96 ± 0.02	0.95 ± 0.03	0.91 ± 0.05
Specificity	0.96 ± 0.03	0.97 ± 0.02	0.98 ± 0.02
PPV	0.94 ± 0.04	0.94 ± 0.05	0.97 ± 0.03
NPV	0.97 ± 0.01	0.97 ± 0.02	0.95 ± 0.03

Automatic A and B represent the semi-automated algorithm run by investigators A and B. Mean ± SD.

evaluation using the semi-automated SWD detection algorithm resulted in a higher sensitivity when compared to baseline (95% to 96% *versus* 91%) while retaining a high specificity (96%–97%). These results are comparable to the performance of other high-quality detection algorithms for SWDs in rodents[11-13]. In these three publications, the analyses were based on transforming the EEG from the time domain to the frequency domain to quantify changes. The methodology reported by Ovchinnikov *et al*. 2010, only detected the onset of SWDs (evaluating the number of leading edges without taking the length of the detector event into consideration) and was based on wavelet analysis[11]. The methodology reported by Van Hese *et al*. 2009 was based on spectral and variant analysis and that of Sitnikova *et al*. 2009 on spectral and wavelet analysis[12, 13]. In contrast, the methodology described in the present study analyzes the EEG in the time domain, mimicking manual marking, while decreasing the subjectivity and allowing the results of the EEG analysis to be more reproducible. In consequence, it provides a good alternative for researchers. Nelson *et al*. 2011 developed an SWD detection system in GAERS based on time-series analysis, gauging changes in amplitude and/or frequency[14]. This system only allowed the detection of the start of the SWD in order to trigger therapy. Subsequent EEG analyses were performed manually.

The median number of SWDs over 20 min and the mean duration of one SWD were similar in the different evaluations, with the results of the manual recordings showing the extreme values. This could indicate that the semi-automated algorithm provides more accurate results than manual marking. The frequency of SWDs (~2/min) was slightly higher than that described in the original paper (1.5/min)[3]. This is easily explained by the fact that the seizure activity in GAERS is colony- and age-dependent[3].

Although spontaneous SWDs start and end abruptly on a normal background EEG and are quite easy to isolate, the EEG patterns seen during sleep make it more difficult to differentiate the beginning and end of an SWD. During the present experiments, rats were stimulated when seen to be sleeping, to improve seizure detection.

Although not all of the manual analyses were timed, it took the researchers on average 15 min to analyze one EEG recording. In comparison, it took the computer 95–100 s to analyze one EEG recording using the algorithm. If we consider that some studies can include >400 EEG recordings (3 groups of 6 rats undergoing 3 recordings a week for 8 weeks), the use of the algorithm can avoid 88 h of laborious work. The computer used in this study was certainly more powerful than average, but subsequent analysis using a laptop computer resulted in similar durations.

The system requires manual intervention to select the best EEG channel/electrode and set an amplitude threshold for SWDs. These steps may cause inter-individual variability in seizure detection; however, the use of the present algorithm is still an improvement in inter-individual variation compared to manual marking. Also, the manual intervention is relatively short and allows control of the quality of the EEG recording.

Another limitation of the study is the absence of an automatic artefact-rejection step. Nonetheless, the performance of the algorithm was as expected from such a system and the manual channel-selection allowed rejection of recordings that included too many artefacts.

The semi-automatic SWD detector algorithm described here allowed analysis of the EEG of GAERs with sensitivity, specificity, PPV, and NPV >94% compared to manual analysis. The use of this algorithm would reduce the time necessary to analyze such data and the subjectivity of the results. as well as providing a good performance alternative for researchers without an engineering background.

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