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Automated analysis of brain activity for seizure detection in zebrafish models of epilepsy

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HIGHLIGHTS

- Algorithm to detect seizures in local field potentials recorded in zebrafish larvae.
- Support vector machine classification of preselected high energy segments.
- Validation both on a chemically induced seizure model and a genetic epilepsy model.
- Significant difference in number of seizures between epileptic and control groups.
- Replacement of cumbersome manual analysis to enable high-throughput studies.

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ABSTRACT

Background: Epilepsy is a chronic neurological condition, with over 30% of cases unresponsive to treatment. Zebrafish larvae show great potential to serve as an animal model of epilepsy in drug discovery. Thanks to their high fecundity and relatively low cost, they are amenable to high-throughput screening. However, the assessment of seizure occurrences in zebrafish larvae remains a bottleneck, as visual analysis is subjective and time-consuming.
New method: For the first time, we present an automated algorithm to detect epileptic discharges in single-channel local field potential (LFP) recordings in zebrafish. First, candidate seizure segments are selected based on their energy and length. Afterwards, discriminative features are extracted from each segment. Using a labeled dataset, a support vector machine (SVM) classifier is trained to learn an optimal feature mapping. Finally, this SVM classifier is used to detect seizure segments in new signals.
Results: We tested the proposed algorithm both in a chemically-induced seizure model and a genetic epilepsy model. In both cases, the algorithm delivered similar results to visual analysis and found a significant difference in number of seizures between the epileptic and control group.
Comparison with existing methods: Direct comparison with multichannel techniques or methods developed for different animal models is not feasible. Nevertheless, a literature review shows that our algorithm outperforms state-of-the-art techniques in terms of accuracy, precision and specificity, while maintaining a reasonable sensitivity.
Conclusion: Our seizure detection system is a generic, time-saving and objective method to analyze zebrafish LPF, which can replace visual analysis and facilitate true high-throughput studies.

1. Introduction

Epilepsy is a collective term for a wide spectrum of neurological disorders, characterized by sudden, recurrent seizure episodes. Seizures may cause various kinds of severe clinical symptoms, loss of consciousness, injury, and even death. In approximately one third (Engel, 1996) of the 65 million cases worldwide (Thurman

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et al., 2011), the occurrence of seizures cannot be controlled with anticonvulsant drugs. These patients continue to have seizures and consequently experience a serious negative impact on their quality of life. Therefore, there are continued efforts towards developing better medications to treat epilepsy.

Zebrafish models of epilepsy are gaining popularity in drug discovery research. Their small size, high fecundity and optical transparency enable easy observation under basic stereomicroscope and high-throughput screening (Berghmans et al., 2007). Moreover, the close homology of their genome to the human one makes them an appropriate model for studying pathophysiology of human epilepsies. Different genetic manipulations (Hortopan et al., 2010) as well as various chemical treatment options (Pham et al., 2016) can be applied to induce epilepsy or epileptic seizure-like behavior in zebrafish larvae in order to model different aspects and subtypes of epilepsy.

Common to all epilepsies, seizures occur due to an abnormal, excessive and synchronous activity of large neuronal populations. As such, seizures can be observed on electrophysiological recordings such as invasive or non-invasive local field potential (LFP) measurements, being a counterpart of electroencephalogram (EEG) performed in humans. Indeed, these techniques serve as a gold standard for epilepsy diagnosis. In the current context, LFP recordings for monitoring seizure activity can be used to validate novel zebrafish epilepsy models, as well as to evaluate the efficacy of new drug candidates.

Manual analysis of EEG/LFP recordings is time-consuming and prone to subjectivity. Alternatively, automated analysis techniques can save time and provide an objective assessment of seizure occurrences. Although the electrical patterns generated during an epileptic seizure in humans, rodents and zebrafish can be described by very similar dynamical models and share some fundamental characteristics (Jirsa et al., 2014), they are observed at very different spatiotemporal scales. Seizure patterns and brain activity of different sources appear different depending on the recording equipment (e.g. invasive or non-invasive) due to volume conduction. Finally, equipment as well as recording conditions determine the types of artifact and noise superimposed on brain signals. Therefore, existing seizure detection algorithms cannot be applied directly; nevertheless, some of their features can be adapted to the current problem.

Automated human epileptic seizure detection algorithms have a very extensive literature, the first published works dating back to the early '80s (Gotman, 1982). Typical algorithms start with a feature extraction step, where the EEG is characterized by certain linear or non-linear metrics in the time, frequency or time-frequency domain, on the level of the EEG channels or extracted sources (Alotaiby et al., 2014; Acharya et al., 2013). Subsequently, automated decision is made based on calculated features using criteria such as knowledge-based rules, neural networks or other classification techniques (Tzallas et al., 2017). Due to the fact that seizure patterns of different patient groups and even individual patients can be very different, algorithms are dedicated either to adults (Fürbass et al., 2012) or neonatal patients (Deburchgraeve et al., 2008), or work in a patient-specific manner (Hunyadi et al., 2012). The use of automated seizure detection techniques in animal models of epilepsy is scarce, although a few promising approaches to detect seizures on intracranial recordings in rodents have been published (Bergstrom et al., 2013; Amal et al., 2013). Recently, a long-term, non-invasive platform for electrophysiological monitoring of zebrafish larva (Hong et al., 2016) was introduced, including an automated seizure monitoring option. To our knowledge, this is the only existing automated LPF scoring system developed for zebrafish larvae to date. One of the two main features of the algorithm is based on measuring the correlation among the multichannel electrode signals. Considering that many electrophysiological recording systems measure a single channel, this approach is not widely applicable.

We propose a novel seizure detection algorithm based on single-channel LFP recorded from zebrafish larva. Different zebrafish models of epilepsy can produce seizures with very different length, morphology, and occurrence rate. Therefore, it is challenging to develop an algorithm, which can reliably detect seizures from various models. We propose a universal pipeline based on a preselection of candidate events, a discriminative feature set and machine learning. A model-specific classifier is automatically trained by feeding training LFP data from a specific model to the learning machine, which will be suitable to detect seizures from the same epilepsy model. We illustrate the applicability of our proposed method for a chemically-induced seizure model and a genetic epilepsy model, i.e. respectively the convulsant pentylenetetrazole (PTZ) and the homozygous *scn1Lab* mutant (Sourbron et al., 2017). However, after applying proper training data, this approach can also be used on any other model.

In Section 2.1 we discuss our measurement setup and the LFP datasets, which were used in this study. Then, in Section 2.2 we present the criteria and the protocol for manual labeling of the data based on visual analysis, and the characteristics of typical events observed in LFP recordings. In Section 2.3 we explain the technical details of the proposed seizure detection algorithm, while Section 2.4 presents the metrics used to evaluate the performance of our technique. In Section 2.5 we discuss the possibility to manually adapt the seizure detection system if necessary. Subsequently, our results are presented; regarding the outcome of the manual labeling (Section 3.1), the optimal algorithmic choices based on the training data (Section 3.2); and the final results obtained on the test data (Sections 3.3). Finally, Section 4 is dedicated to the critical discussion of our study.

2. Materials and methods

2.1. Data collection

2.1.1. Zebrafish husbandry

Adult zebrafish (*Danio rerio*) of the AB strain were maintained at 28.5 °C on a 14-h light/10-h dark cycle under standard aquaculture conditions, and fertilized eggs were collected via natural spawning. Embryos were raised in Danieau's medium (1.5 mM HEPES, pH 7.6, 17.4 mM NaCl, 0.21 mM KCl, 0.12 mM $MgSO_4$ and 0.18 mM $Ca(NO_3)_2$) in an incubator on a 14-h light/10-h dark cycle at 28.5 °C. All zebrafish experiments were approved by the Ethics Committee of the University of Leuven (Ethische Commissie van de KU Leuven, approval number 061/2013 and 154/2015) and by the Belgian Federal Department of Public Health, Food Safety and Environment (Federale Overheidsdienst Volksgezondheid, Veiligheid van de Voedselketen en Leefmileu, approval number LA1210199).

2.1.2. Chemically-induced seizure model and genetic epilepsy models in zebrafish

1. Chemical model

7 dpf wild-type zebrafish larvae (AB) were incubated 2 h with 1% DMSO in a 100 μl volume of Danieau's medium, whereafter either 100 μl of Danieau's medium (hereafter referred to as VHC group), or 100 μl of 40 mM PTZ (hereafter referred to as PTZ group) was added for 15 min to obtain a 20 mM working concentration.

2. Genetic models

Two different genetic models were used: 5 dpf morpholino (MO)-injected larvae (transient knockdown of a previously validated epilepsy-causing gene (Schubert et al., 2014)) and 7 dpf *scn1Lab* mutant zebrafish larvae (having point mutation M1208R leading to a loss-of-function of the *nav1.1Lb* channel (Sourbron