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Recording the adult zebrafish cerebral field potential during pentylenetetrazole seizures

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ABSTRACT

Although the zebrafish is increasingly used as a model organism to study epilepsy, no standard electrophysiological technique for recording electrographic seizures in adult fish exists. The purpose of this paper is to introduce a readily implementable technique for recording pentylenetetrazole seizures in the adult zebrafish. We find that we can consistently record a high quality field potential over the zebrafish cerebrum using an amplification of 5000 V/V and bandpass filtering at corner frequencies of 1.6 and 16 Hz. The cerebral field potential recordings show consistent features in the baseline, pre-seizure, seizure and post-seizure time periods that can be easily recognized by visual inspection as is the case with human and rodent electroencephalogram. Furthermore, numerical analysis of the field potential at the time of seizure onset reveals an increase in the total power, bandwidth and peak frequency in the power spectrum, as is also the case with human and rodent electroencephalogram. The techniques presented herein stand to advance the utility of the adult zebrafish in the study of epilepsy by affording an equivalent to the electroencephalogram used in mammalian models and human patients.

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1. Introduction

A seizure is a sustained and synchronous elevation in brain electrical activity that can result in loss of consciousness and injury. Epilepsy is the condition of having recurrent and unprovoked seizures. Up to 0.5% of the general population has epilepsy, or an estimated 60 million people worldwide (Hauser et al., 1991). Recurrent seizures result in higher rates of mortality (Zielinski, 1974), cognitive impairment (Aldenkamp, 2006), and psychosocial dysfunction (Shorvon, 2007) and cost the US economy over $12 billion annually (Begley et al., 1994).

The zebrafish (Danio rerio) is a model vertebrate organism for many human diseases (Lieschke and Currie, 2007) including cardiovascular disease (Dahme et al., 2009), cancer (Amatruda and Patton, 2008; Payne and Look, 2009), motor neuron disease (Beattie et al., 2007) and epilepsy. The zebrafish epilepsy model is widely accepted as a screening tool for new antiepileptic drugs (Berghmans et al., 2007) and has also been used in mutagenesis studies to screen for genes that promote either susceptibility or resistance to seizures (Baraban et al., 2007; Hortopan et al., 2010). Genetic mutations associated with known human epilepsy syndromes have also been successfully implemented in transgenic zebrafish (Bassuk et al., 2008; DiBella et al., 2009). In fact, Teng et al. (2010) successfully implemented a morpholino knockdown of leucine-rich glioma inactivated 1 (LGI1) gene expression that shows a reduced threshold for seizures. Mutations in the LGI1 gene are associated with a form of human autosomal dominant epilepsy (Michelucci et al., 2003; Ottman et al., 2004). But thus far these transgenic animals have been used mainly to study the effects of the epileptogenic genes on development and not on brain electrical activity or seizure threshold. While techniques have been described for recording electrographic seizures in zebrafish larvae (Baraban et al., 2005), no techniques to our knowledge are available for use in adult fish. Epilepsy syndromes are often classified primarily on abnormalities of brain electrical activity as measured by the electroencephalogram (EEG). Thus, having an EEG equivalent for the adult zebrafish would advance the role of the zebrafish in the study of epilepsy considerably.

The purpose of this study is to describe a technique for recording the field potential over the zebrafish cerebrum and to determine if it can be used for the electrographic evaluation of seizures in the adult zebrafish. More specifically, we detail the construction of a readily implementable amplifier and filter bank and provide a characterization of its gain vs. frequency transfer characteristics. We also provide a detailed method for constructing and characterizing a

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suitable set of recording electrodes. We then use this apparatus to characterize pentylenetetrazole (PTZ) seizures in adult zebrafish in terms of both visual appearance and power spectral content. Taken as a whole, this study establishes a set of tools and baseline measurements for the electrographic study of seizures in the adult zebrafish.

2. Materials and methods

2.1. Zebrafish care and maintenance

Zebrafish were maintained in The Ohio State University's zebrafish core facility. All studies used adult (9–14 month) laboratory strain WIK zebrafish. Fish were fed to satiety twice daily on weekdays, once daily on weekends. All experiments were conducted greater than 2 h after a weekday morning feeding. All studies in this paper were approved by The Ohio State University's Institutional Animal Care and Use Committee (IACUC Protocol # 2010A0196).

2.2. Anesthesia and euthanasia

Prior to electrode insertion fish were anesthetized with eugenol (clove oil extract) obtained at a local pharmacy. Eugenol induces anesthesia and analgesia by blocking fast voltage gated sodium channels in the cortex and in the dorsal horn cells respectively (Cho et al., 2008). Eugenol also has an additional analgesic effect on both central and peripheral pain circuits by activation of the transient receptor potential vanilloid subtype 1 (TRPV1) (Vriens et al., 2009). Anesthesia was induced with 15 ppm eugenol and maintained with 7.5 ppm eugenol as described by Grush et al. (2004). Anesthesia induction was deemed adequate when the fish lost posture and rolled to the side. Swim attempts in the recording chamber were taken to be signs of distress and prompted increased eugenol delivery during experiments. At the end of each experiment individual fish were euthanized by emersion in tricaine (MS-222; Sigma, 160 μg/ml) until all respiratory movements had ceased for 3 min.

2.3. Electrode and recording chamber manufacture

As shown in Fig. 1A two identical segments of 5 mil (0.005″) 316 alloy enamel coated stainless steel wire (California Fine Wire Company, Grover Beach, CA) stripped of insulation along 0.5 mm of the distal tip served as recording electrodes. The proximal ends of the recording electrodes were also stripped of insulation and fitted into small segments of 1/32″ inner diameter brass tubing (K&S Industries, Chicago, IL #05035). Segments of Kynar-insulated stainless steel wire (Radio Shack, Fort Worth, TX #278-502) were then used to connect the recording electrodes to the head-stage amplifier that is affixed to the recording chamber. This electrode configuration has been shown to average all potentials along the stripped section of wire and to result in reduced amounts of high frequency noise (Banks et al., 1995; Robinson, 1968; Cooley and Vanderwolf, 1978). The exposed metal was then coated with a brush on epoxy (Gardner Bender, Milwaukee, WI—#LTB-400) for additional electrical insulation.

As shown in Fig. 1B, following induction of anesthesia, fish were mounted into the recording chamber by pinning the lower lip and the body posterior to the gills to a bed of modeling clay using "horseshoe"-shaped segments of wire. The recording electrodes were then introduced over the cerebrum on either side of the forebrain by direct skull puncture using established landmarks (Rupp et al., 1996) for anatomical reference. The electrodes were then affixed to a bridge to provide mechanical stabilization. The net effect was to record the potential difference across the forebrain as shown in Fig. 1C.

The recording chamber was machined out of a 1 in. section of Acetyl block (Small Parts Inc., Plainfield, IL). The bridge was machined from a section of 1/4″ Acetyl rod in such a fashion so as to lock in place and to hold the recording electrodes by force of friction. Machining was done using a Sherline (Vista, CA—#3580) vertical milling column affixed to a Sherline (#4400) lathe bed. The entire setup was inside a metal screen Faraday cage (not shown). Tank water with 7.5 ppm eugenol was then added so as to submerge the fish up to the level of the eye. The amplifier headstage was seated in a groove precisely machined into the side of the recording chamber. To be discussed below, we used a high-input impedance, high gain differential head-stage amplifier so strictly speaking it was not necessary for the fish and recording chamber to be grounded as each electrode would register approximately the same ambient noise. Nonetheless, we did ground the recording chamber by way of a wire that runs up through the bottom of the chamber to contact the fish and recording solution. So long as the bare tips of the recording wires did not contact the fish, skin or skull, we did not register a "flat-line" signal.

2.4. Amplifier and filter-bank manufacture

A prior characterization of goldfish EEG by Schade and Weiler (1959) indicates that the potential we are trying to measure is along the order of 100 μV and that the frequency range of interest is 3–14 Hz. Our target output voltage was ±0.5 V, which necessitated a gain of 5000 V/V. Using such a high gain imposes the risk of saturating the analog-to-digital converter (ADC) if the DC offset is too high with respect to the peak to peak signal amplitude. To reduce the risk of ADC saturation, we chose a high pass frequency of 1.6 Hz to provide rejection of any DC signal component without distorting the 3 Hz signal component. The high gain also increased the risk of 60-cycle and ambient high frequency noise contamination. We chose a low pass frequency of 16 Hz to reject this high frequency noise without distorting the 14 Hz band.

Fig. 2 shows a schematic representation of the amplifier and filtering circuit. The circuit was inspired by and manufactured according to methods presented by Land et al. (2001). As shown in the figure, an INA129 bioinstrumentation amplifier (Burr-Brown, Texas Instruments, Dallas, TX) served as the head-stage amplifier. Low noise BiFET operational amplifiers (TL082, Radio Shack, Fort Worth, TX) were configured to provide further amplification and filtering. A Burr-Brown Model 503A DC power source was used to power the circuit using rail voltages of ±15 V. All other electrical components were obtained from a local electronics supplier.

As shown in Fig. 2, the INA129 was configured to produce a head stage gain of 50 V/V. A second stage with two identical sub-stages produced an additional gain of 100 V/V and two poles of band-pass filtering between 1.6 and 16 Hz. A third stage consisting of 4 identical sub-stages provided 4 additional poles of band-pass filtering (1.6–16 Hz) with a uniform gain of 1 V/V. The circuit as a whole produced a net gain of 5000 V/V with 6 poles of band-pass filtering at corner frequencies of 1.6 and 16 Hz.

2.5. Recording of cerebral field potential during PTZ seizures

The output of the recording apparatus was input to a Measurement Computing Corporation (MCC—Norton, MA) USB-1208HS-4AO data acquisition device linked to a laptop computer by a USB cable. MCC's Tracer DAQ® program was then run in "strip-chart" recording mode sampling at 50 Hz, well in excess of the 32 Hz that would be required to eliminate aliasing in accordance with the Shannon–Nyquist sampling theorem (Nyquist, 1928; Shannon, 1949). Individual data files were stored electronically on the laptop for future analysis.