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NON-INVASIVE MONITORING OF SPREADING DEPRESSION

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Abstract—Spreading depression (SD), a slow propagating depolarization wave, plays an important role in pathophysiology of different neurological disorders. Yet, research into SD-related disorders has been hampered by the lack of non-invasive recording techniques of SD. Here we compared the manifestations of SD in continuous non-invasive electroencephalogram (EEG) recordings to invasive electrocorticographic (ECoG) recordings in order to obtain further insights into generator structures and electrogenic mechanisms of surface recording of SD. SD was induced by KCl application and simultaneous SD recordings were performed by scalp EEG as well as ECoG electrodes of somatosensory neocortex of rats using a novel homemade EEG amplifier, AgCl recording electrodes, and high chloride conductive gel. Different methods were used to analyze the data; including the spectrogram, bi-spectrogram, pattern distribution, relative spectrum power, and multivariable Gaussian fit analysis. The negative direct current (DC) shifts recorded by scalp electrodes exhibited a high homogeneity to those recorded by ECoG electrodes. Furthermore, this novel method of recording and analysis was able to separate SD recorded by scalp electrodes from non-neuronal DC shifts induced by other potential generators, such as the skin, muscles, arteries, dura, etc. These data suggest a novel application for continuous non-invasive monitoring of DC potential changes, such as SD. Non-invasive monitoring of SD would allow early intervention and improve outcome in SD-related neurological disorders. © **2016 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: brain waves, migraine, stroke, slow wave, epilepsy, ultraslow wave.

INTRODUCTION

Voltage fluctuations generated by ionic currents within the brain are comprised of sustained displacements and slow fluctuations as well as faster waves up to at least 100 Hz superimposed on deviations of the baseline. These bioelectrical activities are comprised of conventional electroencephalogram (EEG) waves and of slow potentials. Recording of slow components of brain activity are often distorted by artifacts generated by the body (e.g. movements of eyes and head, muscle contractions). This has led to the introduction of voltage amplifiers with high- and low-filters that eliminate components below and above given frequencies and thus stabilize the baseline. The EEG band pass is considered between 0.2 and 90 Hz, with the frequencies out of this range being eliminated. The high-pass filter (usually set at 0.5–1 Hz) filters out slow waves or artefacts, and a low-pass filter (usually set at 35–70 Hz; Niedermeyer and da Silva, 2004) eliminate high-frequency fluctuations and power line interferences (Caspers et al., 1980; Caspers, 1993).

A functional unit consisting of neurons, glial cells, and blood–brain barrier, acts as a compound generator of direct current (DC) potentials. Investigation of the slow components of the cortical DC potentials, although often neglected, is a valuable source for understanding of higher brain functions as well as the pathophysiology of different neurological disorders. These slow waves reflect changes in the level of excitation and excitability of the cortical and subcortical structures. DC potentials are correlated with voluntary muscle movement (Cui and Deecke, 1999), state of high alertness (Bachmann, 1984), task performance (Trimmel et al., 2001), motivational state (Hallschmid et al., 2001), behavior (Haider et al., 1981), and several other variables in the healthy human brain.

DC potential displacements have been observed in several pathologic conditions affecting the brain. Epileptiform field potentials, either focal or generalized discharges, are always associated with characteristic deviations of the DC potential in different animal models of epilepsy (Walden and Speckmann, 1988) as well as

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Abbreviations: ATD, analog-to-digital; CMRR, Common Mode Rejection Ratio; DC, direct current; ECoG, electrocorticography; EEG, electroencephalogram; SD, spreading depression.

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in the human epileptic brain (Rodin et al., 2008). A reduction in the oxygenation of the brain evokes negative DC displacements in both *in vivo* and *in vitro* animal models (Speckmann et al., 2011). The same negative DC deflections were reported in the human brain *in vitro* (Schmidinger et al., 1998) and in patients suffering from hypoxic brain injuries (Dreier et al., 2009).

Spreading depression (SD), a depolarization wave that propagates at 3–5 mm/min in the neuronal tissues, has been shown to play a crucial role in the aura phase of migraine, cerebrovascular diseases, and epilepsy (Gorji, 2001). It has been shown that SD is a correlate of the aura phase in migraine (Hadjikhani et al., 2001), a predictor of poor functional outcome in cerebrovascular diseases and traumatic brain injury (Hartings et al., 2011) as well as a risk factor for late post-hemorrhagic seizures (Dreier et al., 2012). Electrocorticography (ECoG) as well as deep electrodes have been used for most of SD recording in animal and human brain. Non-invasive recording of DC potentials and SD by magnetoencephalography have been reported (Bowyer et al., 2012). Using conventional EEG, investigations on generator structures and mechanisms of DC potentials meet with several difficulties. Non-invasive recording of DC field potentials raises the issue whether and to what extent DC field potentials recorded from the scalp represent the changes in cortical and subcortical DC potentials (Tomita-Gotoh and Hayashida, 1996). Some studies indicate that negative DC potentials recorded non-invasively at the surface of the scalp during SD do not always reflect the activation state of the brain (Lehmenkühler et al., 1999). A main problem is that sustained or slowly changing DC potentials picked up from the scalp can originate from a variety of generators, such as the skin, muscles, arteries and dura (Caspers et al., 1984). Using a novel method, the aim of this study was to compare the SD waves recorded from the scalp and ECoG in order to provide further insights into generator structures and electrogenic mechanisms of these negative DC potentials.

EXPERIMENTAL PROCEDURES

All experiments were conducted in accordance to the guiding principles for the care and use of animals in the University of Münster, Germany (50.0835.2.0/A-18/2006), by the Animal Care Committee of University of British Columbia, and Shefa Neuroscience Research Center, Tehran, Iran. Twenty adult Wistar rats (210–400 g) were housed individually under controlled environmental conditions (12-h light/dark cycle) with food and water available *ad libitum*. The animals were anesthetized with intraperitoneal injection of chloral hydrate (350 mg/kg Sigma–Aldrich) and the head of each rat is placed in a stereotaxic instrument (Stoelting Instruments, Wood Dale, IL, USA). This dose of chloral hydrate provides sufficient deep anesthesia for surgical procedures (Field et al., 1993). Soft tissues of the skull at the incision and at contact points were anesthetized using local application of 0.5% lidocaine hydrochloride. Additional supplemental doses of lidocaine and chloral hydrate were applied when necessary (Koulchitsky et al., 2012).

Stainless steel guide cannula and three Ag/AgCl recording electrodes (2–3 mm apart) were implanted above the somatosensory neocortex. Three electrodes were positioned next to the cannula, which were designated ECoG, scalp (EEG) 1, and scalp (EEG) 2. Craniotomy exposed the surface of the neocortex for insertion of cannula (used for application of KCl) and a further 2–3 mm posteriorly, for an Ag–AgCl electrode to record surface DC potentials after drilling small holes in the skull and removing the dura (ECoG electrodes; Ag/AgCl ball on a flexible silver wire, 1 mm diameter). Scalp recordings electrodes (Ag/AgCl disk-recording electrodes, 2 mm diameter) were positioned posterior to ECoG electrode on the depilated skin. An AgCl reference electrode was implanted in the nasal cavity.

Recording of negative DC potentials

EEG amplifier. Electrodes were connected to a novel purpose built AC/DC EEG amplifier. For design of this novel amplifier several points were taken into consideration. EEG noise reduction was the main challenging part in designing the amplifier. Other challenges encountered during the design of this amplifier were: (*i*) amplification of the large amplitude range (20 μV–30 mV), (*ii*) the amplifier saturation caused by electrode and tissue due to elimination of the high-pass filter, (*iii*) the achievement of a high Common Mode Rejection Ratio (CMRR) in low ADIF (differential gain), and (*iv*) control of the electrode potential stability during DC recording of bioelectrical activities. Various known pathological DC potentials can be produced in the brain, such as a DC potential shift during seizure activities (amplitude up to 500 μV and duration up to 16 s) or SD (the amplitude of 2–30 mV and duration of 30–120 s). On the other hand, in AC recording mode, the frequency of interest is typically in the 0.5–70-Hz range with an amplitude range of 20–600 μV. Therefore, the AC/DC amplifier should amplify the bioelectrical signals across these boundaries in order to be capable of both AC and DC EEG data recording. In order to eliminate the common mode artifacts, a high CMRR of >100 dB with low absolute values of differential gain were used.

As the potentials of the electrode–electrolyte-tissue interface do not change symmetrically, currents in the input circuit must be kept as low as possible to minimize slow drift artifacts. Moreover, the electrode impedance is higher in the slow frequencies; therefore, the amplifier input impedance should be higher than 1 GΩ to minimize this effect. The control and measurement of electrode potential stability are other important steps toward obtaining stable DC recordings. In this novel AC/DC EEG amplifier, CMRR >110 db with differential gains of 20 for the preamplifier and 10 for the amplifier, and sensitivity of 20 μV–40 mV were considered. The input impedance is >10 GΩ, bias currents of 10 pA maximum, the low-frequency cutoff is <0.015 Hz, and a driven right leg circuit (DRL) was used as a connection