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Microfabricated solid-state ion-selective electrode probe for measuring potassium in the living rodent brain: Compatibility with DC-EEG recordings to study spreading depression

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ABSTRACT

There is considerable need in neuroscience research to reliably measure concentrations of extracellular ions in the living brain as the dynamics of ion levels increasingly is considered to play a key role in the pathophysiology of many brain disorders. Unfortunately, most probes currently used for direct measurement of extracellular ion concentrations in living brain tissue in experimental animals have considerable limitations. They are custom-made glass capillary ion-selective microelectrodes that are fragile, time-consuming to prepare, and can practically only be used to measure at one depth and one location in the brain. We here present the design and fabrication of a novel type of miniaturized probe that can simultaneously measure, in the brain of a living animal, multiple parameters relevant to the neurological phenomenon of spreading depression (SD) at multiple depths and locations. SD is characterized by a slowly propagating wave of initial neuronal and glial cell depolarization that is followed by depression of activity. SD is accompanied by a massive redistribution of ions, including K^+, between intracellular and extracellular compartments and is considered important in brain diseases such as stroke, traumatic brain injury, and migraine with aura. This work focuses on the functionalization of fabricated probes for measuring changes in extracellular K^+ concentration (and its associated changes in neuronal activity) using an ion-selective potentiometric sensor. We show that the choice of conductive polymer and the method of electro-deposition is critical to obtain low cross-sensitivity for pH and O_2. The sensor's response to K^+ is approximately linear between 2 and 40 mM with a voltage-response of 39 mV/log[K^+]. Proof-of-principle *in vivo* brain recordings were performed in the cortex of a wild-type mouse during induction of cortical SD. We reliably measured the theoretically expected rise and fall of brain tissue K^+ levels during a locally induced cortical SD. We envisage that our probe can be of great use to reliably measure K^+, without being affected by pH or O_2 changes, in the living brain and be widely applicable in neuroscience research.

1. Introduction

Our knowledge of pathological mechanisms of brain diseases has greatly improved over the last decades, for instance by experimentally comparing the diseased and healthy situation. There remains, however, a considerable technical challenge to reliably measure in the living brain *acute* changes, such as changes in the level of extracellular ions over a short time-span, that are relevant to disease pathophysiology. One important example is spreading depression (SD) which has been implicated in a number of important brain diseases, such as stroke, traumatic brain injury and migraine with aura [1,2]. SD is characterized by a slowly propagating wave of initial spreading depolarization of neurons and glial cells in cerebral grey matter that is accompanied by a massive ion redistribution, neuronal swelling, and a subsequent depression of neuronal and glial activity. Apart from the changes in ionic activity, SD is accompanied by large changes in direct-current coupled voltage recording [1]. Insight particularly in the dynamics of changes in ion levels during the initiation phase SD will likely increase our understanding of the underlying disease mechanisms and may aid the development of therapeutic strategies for these diseases.

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Apart from some studies in humans [2], most insight in characteristics of SD has come from invasive brain recordings in experimental animal models; mostly rats and mice [1]. SD events are associated with marked changes in the brain's extracellular ion composition and pH [1,3,4]. The extracellular brain K^+ level normally is around 3–5 mM and increases by one order of magnitude during a SD event, while the extracellular Cl^- level, which normally is around 110–120 mM, drops with one order of magnitude [1]. In addition to changes in K^+ and Cl^- levels, SD events, at least in rats, are associated with profound changes in brain pH, which can drop by 0.2–0.5 pH unit from a basal pH of around 7.2 [3]. These changes in ion levels typically occur in the second-timescale as the SD waves travel through the brain tissue with a speed of around 3 mm/min [1,4].

Most of the currently used probes for measuring extracellular ion levels in living brain tissue in experimental animals consist of custom-made glass capillary ion-selective microelectrodes that are fragile and time-consuming to prepare. In addition, such microelectrodes are impractical for measurements at more than one depth and one location in the brain. Depending on the research question, but certainly for investigating SD waves, it can be necessary to combine measurements of ion levels with DC-potential recordings at multiple locations in the brain (for instance to monitor the spread of SD waves). Glass microelectrodes are not ideally suited for this purpose given the space restrictions in acute brain recordings, especially in a small animal such as a mouse.

We set out to fabricate and test a miniaturized probe that is optimized to measure critical parameters (*i.e.* K^+ level, pH, but also changes in DC potential and neuronal single or multi-unit activity) relevant to a SD event in the brain of a mouse. We deemed miniaturization essential to obtain localized information in a small brain and focused on mice, as they are increasingly used in neuroscience research mainly because of the various transgenic approaches that allow modification of the mouse genome to generate meaningful genetic models of neurological diseases such as migraine [5]. Although there are multiple relevant compounds to measure in relation to a SD event in addition to K^+ and pH, such as extracellular Cl^- or Ca^{2+} ions, and neurotransmitters such as glutamate or GABA [1,3,4], for this paper, we focused exclusively on the development of a specific K^+-sensing solid state electrode that allows acute K^+ level recordings in the brain of a living rodent.

Solid-state ion-selective electrodes (ISEs) are popular for use as probes because of their ease of maintenance, sensitivity, miniaturizability, and fast response [6–11]. Solid-state ISEs are usually composed of a stack of materials, starting with the electrode material, a conducting polymer, and an ion-selective membrane composed of an ionophore with a polymeric matrix and ionic additives to achieve Donnan exclusion. The conducting polymer can also be mixed with the ion-selective membrane, but the conducting polymer might have an interaction with the solution by itself, thereby resulting in unwanted potentiometric responses from the sensor. The conducting polymer acts as a transducer between the electrons from the electrode and the ions entering the ion-selective membrane. Many different conducting polymers that are based on *e.g.* polypyrroles, polythiophenes or polyanilines have been used in solid-state ISEs [7] of which polypyrrole and poly(3,4-ethylenedioxythiophene) (PEDOT) seem most popular [11]. Also a large number of different ionophores have been used in solid-state ISEs; for pH-sensitivity alone more than 37 different types have been published [9,11]. The choice of ionophore depends highly on the desired sensitivity, acidity of the environment, the duration of the response, and the long-term stability. Sensitivity to physicochemical factors could impose limitations on the use of polymer electrodes in the application of recordings of, for instance, SD events. With respect to SD, but this may also be relevant to other applications, main factors of concern are changes in concentrations of main electrolytes such as Cl^- as well as changes in O_2 concentration and pH that are known to be associated with a spreading depolarization and SD [1,12]. Apart from the choice of conducting polymer, in order to be used in invasive intracranial recordings, the mechanical properties for the polymer electrodes need to be taken into account. Intracranial electrodes must have mechanical strength for (deep) impalements in brain tissue, and should be miniaturized to the micrometer scale to prevent unnecessary damage to brain tissue due to electrode insertions.

In this paper the development of a solid-state ISE for measuring K^+ levels (and associated changes in neuronal activity) during a SD in the mouse cortex is presented. We show that the choice of conducting polymer is critical for a reliable response, due to the pH sensitivity of the conducting polymer itself. Another important role of the conducting polymer is to shield the electrode from the solution to prevent drift of the ISE during changes in O_2 concentration (as they occur during a SD). We show that it is important to use an electro-deposition method based on alternating currents to form a well-adhered and well-electropolymerized layer of conducting polymer. While the ultimate goal for future work is to develop a probe that allows combined K^+, DC-potential and pH measurements using a single probe, we here show a first proof-of-principle for *in vivo* K^+ measurements using K^+-sensitive electrodes. We show that our probe can be used to record acute, transient changes in K^+ levels during a SD in the brain cortex of a living mouse.

2. Materials and methods

2.1. Probe and electrode design

Initial ion-selective electrodes (ISE) characterization measurements were performed on-chip with flat electrode arrays, of which each electrode ranges in size from 30 μm × 30 μm to 50 μm × 50 μm. Arrays consist of 15–39 electrodes. The total electrode area varies between 5.4E−3 and 0.039 cm^2. The design is shown in Fig. 1. The chip consists of a Pyrex wafer of 500 μm thickness with electrodes that are patterned in 100 nm thick platinum with a 20 nm thick tantalum adhesion layer. Two large platinum counter electrodes in the shape of a semi-circle are included for convenience. Finally, the electrode leads are insulated with a patterned 200 nm thick parylene layer.

In vivo brain K^+ measurements (see below) were conducted with microfabricated probes, as depicted in Fig. 2. The probe has two shafts, separated by 2 mm, which can be inserted in the brain for simultaneous measurements at two locations, and are fabricated in a 525 μm thick silicon wafer. For the proof-of-principle *in vivo* measurements, only one of the two shafts was used for recordings.

After thermal oxidation, 200 nm thick platinum electrodes are deposited that serve as a template for the sensors. Using plasma-enhanced chemical vapor deposition SiO_2 is deposited to insulate the leads of the platinum electrodes. Backside lithography is performed and with Deep Reactive Ion Etching (DRIE) the probe shafts are patterned and etched back to a thickness of approximately 100 μm. Finally, the substrate is transferred to a carrier wafer and a second DRIE step is performed to pattern the probes and release them from the silicon wafer. Fig. 2 summarizes the fabrication results; a High Resolution Scanning Electron Microscope (HRSEM) image of a probe shaft is shown (Fig. 2A) together with a probe mounted on a Printed Circuit Board (PCB; Fig. 2B) and two photomicrographs of a probe tip and functionalized sensor (Fig. 2C and D).