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A multi-site array for combined local electrochemistry and electrophysiology in the non-human primate brain

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HIGHLIGHTS

- We present a novel multi-contact recording array and supporting headstage.
- The system allows concurrent *in vivo* electrochemistry and electrophysiology.
- The system is validated using measures of arousal in an awake macaque monkey.
- A detailed protocol for device fabrication by photolithography is provided.

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ABSTRACT

Background: Currently, the primary technique employed in circuit-level study of the brain is electrophysiology, recording local field or action potentials (LFPs or APs). However most communication between neurons is chemical and the relationship between electrical activity within neurons and chemical signaling between them is not well understood *in vivo*, particularly for molecules that signal at least in part by non-synaptic transmission.
New method: We describe a multi-contact array and accompanying head stage circuit that together enable concurrent electrophysiological and electrochemical recording. The array is small (<200 μm) and can be assembled into a device of arbitrary length. It is therefore well-suited for use in all major *in vivo* model systems in neuroscience, including non-human primates where the large brain and need for daily insertion and removal of recording devices places particularly strict demands on design.
Results: We present a protocol for array fabrication. We then show that a device built in the manner described can record LFPs and perform enzyme-based amperometric detection of choline in the awake macaque monkey.
Comparison with existing methods Existing methods allow single mode (electrophysiology or electrochemistry) recording. This system is designed for concurrent, dual-mode recording. It is also the only system designed explicitly to meet the challenges of recording in non-human primates.
Conclusions: Our system offers the possibility for conducting *in vivo* studies in a range of species that examine the relationship between the electrical activity of neurons and their chemical environment, with exquisite spatial and temporal precision.

1. Introduction

The ability to concurrently measure the electrical activity of neurons (action potentials and/or local field potential) and non-synaptic neuromodulatory signals, both on fast timescales, will be central to understanding cortical circuits. Excellent connectivity maps exist for many neural circuits, and in the case of some organisms, such as *Caenorhabditis elegans* we have the full neural circuit diagram. In several organisms, we also have excellent descriptions of the firing responses of individual neurons within those circuits to diverse sensory inputs, and in many cases the patterns or correlated firing across large populations of neurons. In some animal models, we have characterized the responses of neurons during complex cognitive tasks involving selective attention, reward prediction, and quantity estimation. And yet we continue to struggle to

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explain or predict behavior from circuit structure or from momentary neural activity. In part this is because missing from the above descriptions is an understanding of connectivity that is not made *via* synapses, and of signals that modulate rather than generate action potentials. That is, the neuromodulators are largely absent from our current descriptions of the nervous system. This absence is particularly notable in descriptions of the cortical circuits of large mammals, including primates.

Neuromodulators signal key variables related to context and internal state (reviewed by Dayan, 2012; Marder, 2012) and in doing so they profoundly alter the activity of the circuits within which they act. Many neuromodulators operate, at least in part, by non-synaptic means—a signaling mode often referred to as volume transmission (Vizi et al., 2004). In order to understand the interplay between volume transmitted neuromodulators and the activity of the cortical circuits into which they are released we need a means to record both the concentration of various signaling molecules in the extracellular space, and the activity of the nearby neurons. We need to be able to make these recordings in a spatially precise fashion, on timescales relevant to behavior, and in various model systems.

Electrophysiological recording techniques are well developed in neuroscience, and the field of electrochemistry has made exciting inroads into the field in recent years (Burmeister et al., 2000; Parikh et al., 2007, 2004; Park et al., 2011). Concurrent intracranial electrochemical and electrophysiological recording has been possible with separate sensors since the early 1980s (Bickford-Wimer et al., 1991; Hefti and Felix, 1983). However, with the notable exceptions of switched circuits for the measurement of local catecholamine concentration and local electrophysiological activity (Stamford et al., 1993; Takmakov et al., 2011) and a system limited to measurement of low frequency local field potentials (Zhang et al., 2009) electrochemistry and electrophysiology – each of which offers exquisite spatial and temporal precision – have not yet been combined into a single sensor in service of fully concurrent recording of a wide array of extracellular molecules with diverse measures of electrical activity of neurons. In addition to this gap in our capabilities, it is also the case that the excellent electrochemical recording devices that have been designed to detect molecules other than catecholamines (Burmeister et al., 2000) are too large (and therefore destructive of tissue) for use in daily recording in non-human primates.

Here we describe a four channel multi-electrode array that modifies the capabilities of an existing multi-channel electrochemical device (Burmeister et al., 2000), combines those capabilities with the ability to record isolated action potentials and/or full spectrum local field potentials, and reduces the device diameter approximately fivefold. We also describe supporting hardware that modifies the capabilities of an existing switched recording mode (Takmakov et al., 2011) to enable concurrent electrochemical and electrophysiological detection. The result is a device that can be used to make repeated measurements of interesting non-synaptic signaling molecules and local neural activity, over time in the awake and behaving non-human primate. We validate this recording device using non-concurrent *in vivo* measurement of extracellular choline concentration (as a reporter for the activity of the cholinergic system) and local field potentials (as a measure of neural activity) in an animal moving naturally between various states of arousal.

2. Materials and methods

2.1. Array fabrication and assembly

The multi-site arrays are made in a two-step process. In the first step (described in Section 2.1.1) ceramic wafers are patterned with recording sites and insulation and are diced into individual "tips". These tips are then wire bonded, assembled into hypodermic tubing and soldered onto connectors (described in Section 2.1.2).

The recording device tips have four contact sites (Fig. 1A). Two sites are 300×15 μm and are designed for electrochemical recording in parallel single-channel or referenced (subtractive or 'sentinel' mode: Burmeister et al., 2000). The other two sites are 15×15 μm and are used for electrophysiological recording. Connecting lines run from these contacts to the distal end of the device tip and terminate in 200×75 μm bonding pads. Patterned scribe lines guide the dicing of ceramic wafers into individual tips.

2.1.1. Two-layer photolithograpy and dicing

One inch square Superstrate 996 polished alumina ceramic wafers (Coorstek, Inc, Golden, CO, USA) are cleaned (10 min with ultrasonic agitation in each of the following: acetone, methanol, isopropanol, and de-ionized water) and dried with a nitrogen gun before spin coating with a ~1.3 μm layer of S1813 photoresist (Shipley Microposit, Marlborough, MA, USA).

After a 1 min pre-bake at 115 °C, the wafers are exposed for 4 s at 11 mW/cm^2 using an MA-6 mask aligner (Karl Suss). The first layer mask (Fig. 1B, top left) was developed using the Layout Editor software package (Juspertor, Germany). The pattern is printed as a dark field mask at 40,000 DPI resolution on Mylar (Fineline Imaging, Colorado Springs, CO, USA). The resulting transparency is taped onto a soda lime glass carrier (Fineline Imaging) for use in the mask aligner.

After developing the photoresist (with Shipley MF319 developer) and conducting a visual examination under 10× magnification to confirm good patterning, the wafers are briefly de-scummed (1 min under oxygen at 200 mT pressure, 200 W power: Technics PE-11B etcher) before metal deposition. Metals are deposited using an AJA ATC Orion DC sputtering system. First, a 5 nm titanium adhesion layer is applied using a gun power of 200 W, under Argon gas at 5 sccm, 3 mT. This is followed by deposition of 250 nm of platinum with a gun power of 300 W, under Argon gas at 5 sccm, 3 mT. Lift-off of the excess metals is achieved in acetone under ultrasonic agitation, after which the wafers are re-cleaned (in acetone, methanol, isopropanol, and water as described above) and dried using a nitrogen gun.

The second layer photoresist is SU-8; a biocompatible insulator. SU-8 2001 (MicroChem Corp, Westborough, MA, USA) is spin-coated over the deposited metals and pre-baked for 2 min at 95 °C. This layer is then exposed for 3.2 s at 11 mW/cm^2 using an MA-6 aligner. The second layer mask (Fig. 1B, bottom right) is a clear field mask printed at 40,000 DPI resolution on Mylar and then taped to a soda line glass carrier.

After exposure, the SU-8 is post-baked on a hotplate for 3 min at 95 °C and then developed (using SU-8 developer, MicroChem) for 60 s with ultrasonic agitation. If visual inspection indicates that the openings in the insulation over the contact sites are too small (or not fully open), the SU-8 is developed further. Once a good result has been achieved (determined by eye using a 10× or 20× objective), the wafer is rinsed in isopropyl alcohol and dried before a final descum (Technics PE-11B etcher; 3 min under oxygen at 200 mT, 200 W).

The wafers are then mounted onto UV-release tape and diced into individual tips with a DISCO DAD 3220 Automated Dicing Saw using a 20 μm electroformed blade running at a speed of 0.2 mm/s. The diced wafers are released by UV exposure and stored in low adhesion (XT) gel-pak carriers (Gel-Pak, Hayward, CA, USA) ready for wire bonding.

2.1.2. Wire-bonding

To each bonding pad on each device tip, a 10 cm length of 99.9% pure 0.002 in. platinum wire with 1× polyimide insulation