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Classification of cortical microcircuits based on micro-electrode-array data from slices of rat barrel cortex

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ABSTRACT

The bewildering complexity of cortical microcircuits at the single cell level gives rise to surprisingly robust emergent activity patterns at the level of laminar and columnar local field potentials (LFPs) in response to targeted local stimuli. Here we report the results of our multivariate data-analytic approach based on simultaneous multi-site recordings using micro-electrode-array chips for investigation of the microcircuitary of rat somatosensory (barrel) cortex. We find high repeatability of stimulus-induced responses, and typical spatial distributions of LFP responses to stimuli in supragranular, granular, and infragranular layers, where the last form a particularly distinct class. Population spikes appear to travel with about 33 cm/s from granular to infragranular layers. Responses within barrel related columns have different profiles than those in neighbouring columns to the left or interchangeably to the right. Variations between slices occur, but can be minimized by strictly obeying controlled experimental protocols. Cluster analysis on normalized recordings indicates specific spatial distributions of time series reflecting the location of sources and sinks independent of the stimulus layer. Although the precise correspondences between single cell activity and LFPs are still far from clear, a sophisticated neuroinformatics approach in combination with multi-site LFP recordings in the standardized slice preparation is suitable for comparing normal conditions to genetically or pharmacologically altered situations based on real cortical microcircuitry.

1. Introduction

Cortical microcircuits are complex structures formed by up to 10^6 neurons that are locally interconnected (e.g. Douglas & Martin, 2004; Nelson, 2002). Much is known about individual participating cells including their morphological and electrophysiological properties (Kawaguchi, 1995; Kawaguchi & Kubota, 1996; Larkman & Mason, 1990; Mason & Larkman, 1990), which allows them to be grouped into a varying number of excitatory or inhibitory classes (Markram et al., 2004; Peters & Jones, 1984). In addition, paired and multiple simultaneous recordings from up to a dozen cells have been performed providing important information on the interactions in such elementary circuits (e.g., Lefort, Tomm, Sarria, & Petersen, 2009; for review see Lübke & Feldmeyer, 2007; Silberberg, Grillner, LeBeau, Maex, & Markram, 2005; Thomson & Lamy, 2007). These methodologies, despite their sophistication, come to a limit when we consider the role of microcircuits in larger units such as layers and columns of the cerebral cortex.

Multi-site stimulation and recording techniques bear the promise to help us characterize and understand collective phenomena of neuronal populations in local microcircuits. Both electrical and optical methods can be employed for multi-site stimulation as well as for recording (for review see, e.g. Callaway & Yuste, 2002; Frostig, Xiong, Chen-Bee, Kvasnak, & Stehberg, 2008; Schubert, Kötter, & Staiger, 2007). In recent years the use of standard pre-fabricated planar Micro-Electrode-Array (MEA) chips has become very popular. These chips can be flexibly deployed with different electrical stimulus and recording paradigms with wide application to different types of tissue, and the complete equipment is available at a relatively affordable price. Most experiments using such MEA chips are carried out with cell cultures that can be followed over several stages of development over days or weeks (e.g. Jimbo, Kawana, Parodi, & Torre, 2000; Otto, Görtz, Fleischer, & Siebler, 2003; Tonomura, Moriguchi, Jimbo, & Konishi, 2008). Acute brain slices are seldom used because of the difficulty to position the slices in a reproducible way relative to the geometry of the underlying electrodes as well as to establish

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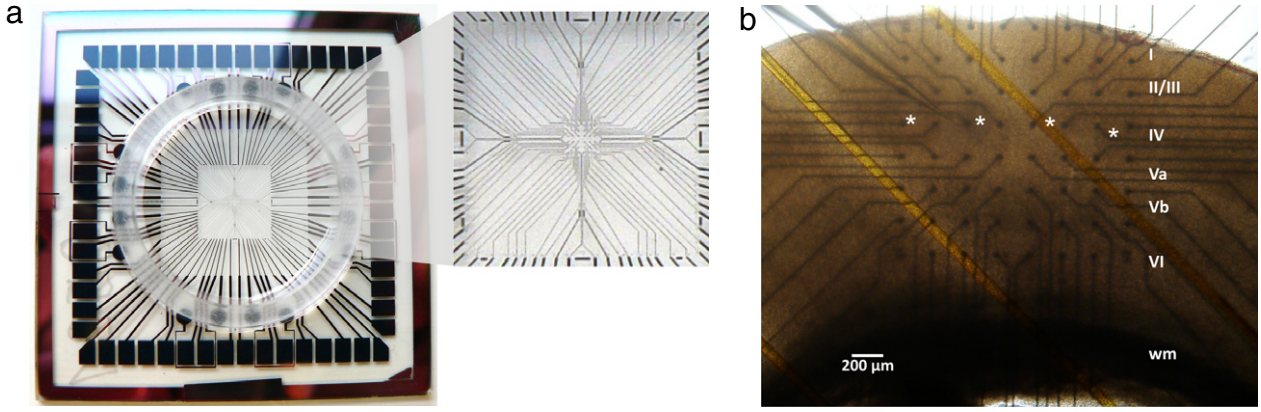

Fig. 1. (a) Photomicrograph of a micro-electrode-array chip (60 electrodes) used for measuring local field potentials in acute cortical slice preparations. Enlarged view illustrates the size of the grid of 60 electrodes (200 µm inter-electrode spacing) in the centre. (b) Photomicrograph of an acute, unstained coronal slice of the rat primary somatosensory cortex mounted on top of a micro-electrode-array chip with a patch clamp recording electrode positioned in the granular layer IV. White asterisks mark the positions of the barrels in layer IV. wm — white matter.

reliable contacts between the tissue and the electrodes (Kopanitsa, Afinowi, & Grant, 2006; Mann, Suckling, Hajos, Greenfield, & Paulsen, 2005; Wirth & Lüscher, 2004).

Here we report successful multi-site recordings from acute slices of rat somatosensory (barrel) cortex using standard commercial MEA equipment. The spatiotemporal characteristics of stimulus-induced local field potentials (LFPs) fit with our earlier observations using other methods such as flash-release of caged glutamate with individual glass electrodes (Kötter, Staiger, Zilles, & Luhmann, 1998) or one-dimensional arrays of tungsten wires (Staiger, Kötter, Zilles, & Luhmann, 2000). More importantly we have been able to standardize both the experiments and the subsequent analysis such that rapid and informative comparisons can now be made between changing conditions for the same slice (e.g. bath applications of drugs) or between slices from different animals (e.g. with genetic modifications or drug treatment). In this study we investigated the following aspects:

- reliability of responses on repeated stimulation of the same site;
- characteristic response patterns to stimulation of different cortical layers;
- stimulation within the same vs. a neighbouring column;
- special spatiotemporal features, e.g. wave propagation;
- reproducibility of results in separate slice experiments.

We conclude that the current experimental and analysis methods are capable of reliably identifying major biological features in LFP and population spike data, which will enable us to use this protocol for a variety of experiments involving normal, as well as chemically or genetically altered brain slices.

2. Materials and methods

2.1. Slice preparation

All experiments were approved by the Committee for Animal Experiments of the Royal Dutch Academy of Science and the University Medical Centre Nijmegen, The Netherlands. Efforts were made to minimize animal suffering, and to reduce the number of animals used. Enflurane anaesthetised Wistar rats (postnatal days 21–23) were decapitated and blocks of brain tissue containing the barrel cortex were quickly removed from the skull and placed in ice-cold artificial cerebrospinal fluid (ACSF). The ACSF contained (in mM) 124 NaCl, 1.25 NaH_2PO_4, 3 KCl, 1 $CaCl_2$, 4 $MgCl_2$, 26 $NaHCO_3$ and 10 glucose and was superfused with a 95% O_2 and 5% CO_2 gas mixture (carbogen). The tissue block was glued to the platform of a vibratome (Microm HM 650 V, Microm, Walldorf, Germany) and thalamocortical slices of 300–400 µm thickness were prepared in ice-cold ACSF as described by Agmon and Connors (1991). After slice preparation, the slices were incubated in ACSF at 35 °C for at least 1 h. Individual slices were then transferred to the multi-electrode-array (MEA) recording chamber placed in a fixed stage upright microscope (Model BX51WI, Olympus Europe GmbH, Hamburg, Germany). A 4x objective (UplanFLN, Olympus Europe GmbH, Hamburg, Germany) was used to visualize the barrel cortex, which was clearly identified as a band of alternating dark and light patches in layer (L) 4. Only slices in which at least two barrels could visually be recognized were used for analysis. In the recording chamber, the slices were submerged in ACSF at a flow rate of 12.5 ml/min at room temperature. To improve signal transduction in the slice, the ACSF used for the perfusion differed from the ACSF used for the slice preparation and contained (in mM) 124 NaCl, 1.25 NaH_2PO_4, 3 KCl, 1.6 $CaCl_2$, 1.8 $MgCl_2$, 26 $NaHCO_3$ and 10 glucose and was superfused with carbogen.

2.2. Multi-electrode-arrays

We used a standard 60 electrodes Micro-Electrode-Array (MEA) chip (Multichannel Systems, Reutlingen, Germany) for all experiments. This chip contains 60 TiN electrodes of 30 µm diameter spaced at 200 µm. Fig. 1(a) shows how the electrodes are placed in an eight-by-eight grid with the four corners left empty. The MEA electrodes are embedded in a glass plate with a ring glued on top so that the recording chamber can take the slice bathed in ACSF.

The slice was positioned in such a way that the MEA electrodes were placed underneath a set of barrel related columns of the somatosensory cortex. More precisely, after visualizing the barrel cortex, we opted for having one row of electrodes co-aligned with each of the cortical layers as well as, vertically, with the middle of the barrel related columns. Fig. 1(b) shows a magnification of a prepared slice during such an experiment taken with an infrared camera (IR Camera Vx55, Till photonics GmbH, Gräfelfing, Germany) to enable linking of electrodes to distinct layers and columns within the barrel cortex for analysis purposes. This alignment made a precise stimulation of different layers and columns possible. Slices were held in position as well as pressed onto the MEA electrodes by Teflon-coated harp grids (ALA, Geneva, United States) that were positioned on top of them. Because this grid alone already improved the contact between slice and MEA