Journal of Neuroscience Methods 267 (2016) 14–20

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Second spatial derivative analysis of cortical surface potentials recorded in cat primary auditory cortex using thin film surface arrays: Comparisons with multi-unit data

James B. Fallon[a,b,c,*], Sam Irving[a], Satinderpall S. Pannu[d], Angela C. Tooker[d], Andrew K. Wise[a,b,c], Robert K. Shepherd[a,c], Dexter R.F. Irvine[a]

[a] Bionics Institute, Melbourne, Victoria, Australia
[b] Department of Otolaryngology, University of Melbourne, Melbourne, Victoria, Australia
[c] Medical Bionics Department, University of Melbourne, Melbourne, Victoria, Australia
[d] Lawrence Livermore National Laboratory, Livermore, CA, United States

HIGHLIGHTS

- A conformable 2-dimension thin-file electrode array is described.
- A method for improved spatial resolution of local field potentials is described.
- Results obtained with the new method are in agreement with multi-unit data.

ARTICLE INFO

Article history:
Received 21 October 2015
Received in revised form 4 April 2016
Accepted 5 April 2016
Available online 6 April 2016

Keywords:
Local field potential
Cochlear implant
Cortical plasticity
Neural prosthesis
Sensorineural hearing loss

ABSTRACT

Background: Current source density analysis of recordings from penetrating electrode arrays has traditionally been used to examine the layer-specific cortical activation and plastic changes associated with changed afferent input. We report on a related analysis, the second spatial derivative (SSD) of surface local field potentials (LFPs) recorded using custom designed thin-film polyimide substrate arrays.
Results: SSD analysis of tone-evoked LFPs generated from the auditory cortex under the recording array demonstrated a stereotypical single local minimum, often flanked by maxima on both the caudal and rostral sides. In contrast, tone-pips at frequencies not represented in the region under the array, but known (on the basis of normal tonotopic organization) to be represented caudal to the recording array, had a more complex pattern of many sources and sinks.
Comparison with existing methods: Compared to traditional analysis of LFPs, SSD analysis produced a tonotopic map that was more similar to that obtained with multi-unit recordings in a normal-hearing animal. Additionally, the statistically significant decrease in the number of acoustically responsive cortical locations in partially deafened cats following 6 months of cochlear implant use compared to unstimulated cases observed with multi-unit data ($p = 0.04$) was also observed with SSD analysis ($p = 0.02$), but was not apparent using traditional analysis of LFPs ($p = 0.6$).
Conclusions: SSD analysis of surface LFPs from the thin-film array provides a rapid and robust method for examining the spatial distribution of cortical activity with improved spatial resolution compared to more traditional LFP recordings.

1. Introduction

Current source density (CSD) analysis has been applied to a wide range of neocortical and other brain structures for over 50 years (Mitzdorf, 1985), and was first used to analyse recordings from auditory cortex by Müller-Preuss and Mitzdorf (1984). More recently, CSD analysis has been used in a number of studies investigating the plastic changes that occur in the auditory cortex as

---

Abbreviations: ABR, auditory brainstem response; AI, primary auditory cortex; ICES, intra-cochlear electrical stimulation; SEM, standard error of the mean.

[*] Corresponding author at: Bionics Institute, 384-388 Albert Street, East Melbourne, Victoria 3002, Australia. Fax: +61 3 9667 7518.
E-mail addresses: jfallon@bionicsinstitute.org, james.fallon@ieee.org (J.B. Fallon).

http://dx.doi.org/10.1016/j.jneumeth.2016.04.004
0165-0270/

a result of long-term profound deafness and/or chronic intracochlear electrical stimulation (Klinke et al., 1999; Kral et al., 2005; Middlebrooks, 2008; Schroeder et al., 2001).

By taking the second spatial derivative (SSD) of a series of local field potentials (LFPs) generated by the superposition of synaptic events (Cottaris and Elfar, 2009), CSD analysis allows identification of the laminar sources of currents, based on a characteristic pattern of current sources and sinks. CSD analysis of cortical activity presumes the LFPs are dominated by signals from within a single cortical column (Mitzdorf, 1985), and changes that occur in parameters encoded across multiple cortical columns, such as tonotopic organization, consequently cannot be investigated with CSD. However, surface LFPs are well suited to studying these phenomena. They were used in the first demonstrations of the tonotopic organization of primary auditory cortex (AI) (Woolsey and Walzl, 1942), and have been used to investigate the propagation of 'travelling' waves of activity across the cortex (Kral et al., 2009). Such studies have typically utilized sequential recordings from small (1 mm in diameter) silver or platinum-ball macroelectrodes, although they can also utilize microelectrode recordings from the cortical surface (e.g., Kral et al., 2009) or the middle cortical layers (e.g., Norena and Eggermont, 2002). The need to average responses to multiple presentations of the same stimuli to extract the small (10–100 μV) LFP from background activity, and to move the electrode to successive recording sites, makes these experiments extremely time-consuming. When recordings are made with macroelectrodes, the size of the LFP can also be influenced by the variable contact of the electrode with the cortical surface over the duration of the recordings. A final issue with standard LFP recording is that LFPs are a mixture of locally generated potentials and potentials volume conducted from sites up to 1 cm away (Eggermont et al., 2011; Gaucher et al., 2012; Kajikawa and Schroeder, 2011; Norena and Eggermont, 2002).

The present study describes a method for simultaneous recording of LFPs from a large number of surface recording sites, and a method of analyzing those potentials derived from CSD analysis. The method utilizes a custom designed thin-film polyimide substrate electrode array that was developed to be flexible enough to follow the undulating surface of the primary auditory cortex (AI) in the cat, and allow simultaneous recordings at 32 sites. Analysis of the data by taking the SSD of the LFPs across the major caudal–rostral tonotopic axis of the cat AI effectively 'sharpens' the LFP recordings to allow a more detailed analysis of the underlying activity. These procedures were validated by comparing the tonotopic organization of AI as determined by standard LFP analysis and SSD analysis to that obtained with multi-unit (MU) recordings in a normal-hearing cat. Additionally, the responsiveness of the cortex in two groups of partially deafened cats was assessed, revealing a difference in the acoustic responsiveness of AI in cats that had received chronic intracochlear electrical stimulation compared to partially deafened, unstimulated controls. This difference, which was evident in the MU data and was also in accord with previously reported MU data (Fallon et al., 2009b), was not evident using the standard simpler analysis of the LFPs.

2. Materials and methods

2.1. Recording electrode arrays

Details of the fabrication of these types of arrays have been reported previously (Tooker et al., 2012), and will only be summarized here. The electrode arrays were fabricated using multiple layers of polyimide and metal. The fabrication process began by depositing the first layer of polyimide. A first layer of trace metal (gold) was deposited and patterned, followed by a second layer of polyimide. Interconnection vias were etched into this second layer of polyimide using oxygen plasma. A second layer of trace metal (gold) was then deposited and patterned. The electrode metal (platinum) was next deposited and patterned. A final layer of polyimide was deposited and openings for the electrodes were etched using oxygen plasma.

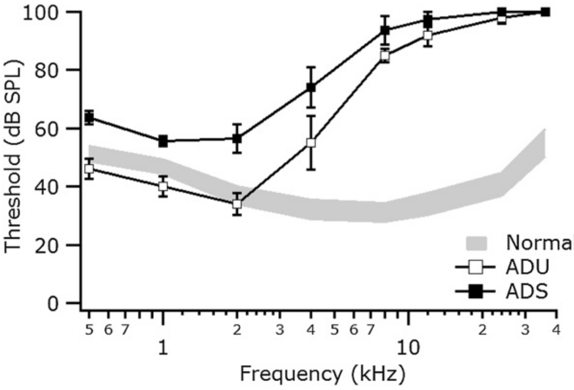

Fig. 1. Auditory brainstem response audiograms for the adult partially deafened unstimulated (ADU) and adult deafened stimulated (ADS) animals at the time of the acute electrophysiological experiment. Values are mean (±standard error of the mean). Grey area indicates the 95% confidence range for normal-hearing animals ($n = 40$ ears).

The novel array geometry described in this study was specifically designed to follow the undulating surface of AI in the cat by utilizing 8 separate fingers, each with 4 electrodes, for a total of 32 electrodes. The electrodes were 200 μm in diameter, with 800 μm center-to-center spacing. The resulting array allowed coverage of the majority of the surface accessible portion of the cat AI (Fig. 2A). Surface tension attracted the array to the cortical surface and the flexible fingers follow the contours of the cortex ensuring all electrodes made good electrical contact with the cortical surface.

2.2. Experimental subjects

Fourteen healthy adult cats with otoscopically normal tympanic membranes and normal hearing were used in the present study. Hearing status was determined using auditory brainstem response (ABR) recordings using standard procedures (Irving et al., 2014), with normal hearing defined as a click-ABR threshold <32 dB peak equivalent sound pressure level (SPL). All procedures were in accordance with Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and with the Guidelines laid down by the National Institutes of Health in the US regarding the care and use of animals for experimental procedures, and were approved by the Royal Victorian Eye and Ear Hospital Animal Research and Ethics Committee.

2.3. Deafening procedure

Thirteen cats were administered a daily subcutaneous (s.c.) injection of kanamycin sulfate (200 mg/kg) for 17 days (Irving et al., 2014), which preferentially damages the high-frequency basal region of the cochlea. Tone-evoked ABRs were used to determine the degree of hearing loss achieved. Additional daily kanamycin injections were continued until a satisfactory high-frequency hearing loss (>60 dB HL at frequencies >8 kHz) had been achieved. Mean ABR audiograms for the two partially deafened groups, along with data for a large sample ($n = 40$ ears) of normal-hearing cats, are shown in Fig. 1. Thresholds are similar to normal for frequencies below 2 kHz, but increase progressively up to 10 kHz, and at higher frequencies are at or above our maximum intensity of 100 dB SPL.