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Differential thresholds of local field potentials and unit discharges in rat auditory cortex

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Abstract

Thresholds for responses to tone bursts in the primary auditory field of pentobarbital-anesthetized rat are significantly lower for local field potentials (5.60 dB ± 1.76 S.E.M.) than for multiple unit discharges (17.80 dB ± 3.17), recorded simultaneously from the same microelectrode. The characteristic frequencies (CFs) of local field potentials provide a good 'predictive' estimate of unit CFs at their higher thresholds. The findings are consistent with the view that local field potentials in the auditory cortex reflect summed synaptic potentials rather than cellular discharges.

Key words: Tone stimulation; Frequency tuning; Synaptic potentials

1. Introduction

Early studies of the functional organization of the primary auditory cortex (AI) employed local field potentials (LFPs), which revealed cortical tonotopic organization (Woolsey and Walzl, 1942). Subsequently, the recording of unit discharges became pre-eminent, both replicating tonotopic organization and expanding inquiry to other aspects of cortical processing. Recently, there has been a revival of interest in the use of LFPs, for studies of the functional organization of the auditory cortex (Barth and Di, 1990), representation of stimulus parameters (Di and Barth, 1993; Ohl et al., 2000) and for long-term recording in waking animals (Galván et al., 2001).

LFPs and unit discharges are thought to reflect related, but different cellular processes. While discharges are known to be action potentials, LFPs in the cerebral cortex have been attributed to synaptic activity on the basis of intracellular recording (e.g., Creutzfeldt et al., 1966). Based on current source density (CSD) analysis, it is thought that excitatory postsynaptic potentials (EPSPs) make the major contribution to LFPs in the cerebral cortex (reviewed in Mitzdorf, 1985). Although LFPs and unit discharges both have been used to study stimulus processing and functional plasticity in the AI, their relationship has not been well studied. Simultaneous recordings of LFPs and unit discharges from the same microelectrode in AI indicate that unit discharges occur on the initial slope of LFPs (Konig et al., 1972). LFPs and units exhibit similar dynamics as stimulus parameters are varied. LFP magnitude and the rate of unit discharge increase directly with level of click stimulation (Wolpaw, 1979) and both have similar response profiles to changes in the rate of click presentation (Eggermont and Smith, 1995). One study has examined simultaneous LFP and unit response to tones (Eggermont, 1996). Several response parameters were found to be highly similar, e.g., range of frequency response and best frequency at threshold (i.e., characteristic frequency (CF)), while LFPs exhibited a larger bandwidth. Of particular interest, thresholds were found to be the same for LFPs and units. However, as LFPs represent synaptic potentials, they might be expected to have lower thresholds than unit discharges. We report here a re-

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Abbreviations: AI, primary auditory cortex; CF, characteristic frequency; EPSP, excitatory postsynaptic potential; LFP, local field potential

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examination of the relationship between LFPs and unit responses to tones at threshold. Some of these findings have been reported in abstract (Galván et al., 1997).

2. Materials and methods

Adult male Sprague–Dawley rats ($n = 5$; 270–470 g) were studied acutely while under general anesthesia (sodium pentobarbital, 45 mg/kg; diazepam, 9 mg/kg; atropine methyl nitrate, 0.3 mg/kg). After placement in a stereotaxic instrument, an acrylic pedestal was built on the skull and bolted to a frame and the ear bars were removed. Pure tone stimuli (Wavetek digital synthesizer controlled by a minicomputer) were delivered to an Aiwa speaker, calibrated with a B&K #4134 condenser microphone; the speaker was placed at the entrance to the external auditory meatus contralateral to the recording sites to replicate the protocol used in studies with waking, unanesthetized animals (for details see Galván et al., 2001). After a craniotomy, AI was located by its tonotopic organization, as revealed in unit recordings in the rat (Sally and Kelly, 1988). We used a roving surface electrode which yielded tonotopic organization with LFP recordings, as first described by Tunturi (1944) in the dog.

Neuronal activity (tungsten microelectrode 2–3 MΩ) was amplified (Dagan #2400, Minneapolis, MN, USA, 1.0–3000 Hz), LFPs and unit discharges were separated by differential bandpass filtering (Acoustic Devices) (LFPs, 1–100 Hz; units, 0.3–3.0 kHz, 12 dB/octave roll-off). Recordings were obtained from sites below the reversal of the LFP from surface positivity to depth negativity (average recording depth = 597 μm, ± S.E.M. = 63.1). Tuning functions were determined (20 repetitions of a sequence of 11 isointensity ascending frequencies, in either 0.5 octave ($n = 7$) or 0.25 octave ($n = 2$) frequency steps; tone duration = 100 ms, rise/fall time = 5 ms, inter-tone interval = 800 ms, 1.5 s inter-sequence interval, frequency range = 0.97–30.0 kHz, 0–80 dB SPL, 10 dB steps).

LFP threshold was determined for the frequency-tuned N1 component (20–30 ms latency; Galván et al., 2001) by independent visual inspection of averaged records by two of the authors, each of whom was blind to the judgements of the other. Threshold was defined as the lowest intensity with a clear LFP, provided that the CF also elicited larger LFPs at all levels above threshold. Multiple unit activity was voltage discriminated (signal:noise ≈ 3:1) and peristimulus time histograms were constructed using 10 ms bins. Evoked discharges were calculated by subtracting background activity from spikes occurring during responsive portions of tone duration. The criterion for unit threshold was the lowest intensity with a time-locked response on at least a quarter (5/20) of the tone presentations, based on inspection of raster displays, provided that this criterion was also met for all higher stimulus levels, validated by quantitative records of latency within a time window of 10–30 ms after stimulus onset. If more than one frequency met this criterion, the CF was assigned to the frequency that elicited the largest response. All procedures were performed in accordance with the University of California Irvine Animal Research Committee and NIH Animal Welfare guidelines.

3. Results

Data were obtained from nine recording sites in five animals. An example of LFPs and corresponding unit responses is given in Fig. 1A. The LFP threshold was 10 dB. In contrast, units exhibited no responses at this stimulus level, but did reach threshold at 20 dB. Analysis of all paired recordings ($n = 9$) revealed that the mean threshold for LFPs was 5.6 dB (± 1.76 S.E.M.) vs. 17.8 dB (± 3.17 S.E.M.) for unit responses: the average difference was 12.2 dB (± 3.24 S.E.M.) (Fig. 1B). The difference is statistically significant (t-test, $t_8 = 3.77$, $P < 0.01$).

Given the lower LFP thresholds, it was of interest to determine the extent to which the CFs of LFPs provided information about the CFs of units. Therefore, the correlation between LFP and unit CFs (at their respective thresholds) was determined. The correlation coefficient was 0.86 (Fig. 1C). This indicates that knowledge of the CFs of LFPs provides a good 'predictor' of the unit CFs that will be obtained at higher stimulus levels.

4. Discussion

The findings indicate that LFPs have lower thresholds than unit discharges recorded simultaneously from the same microelectrode, in contrast to Eggermont (1996) who reported no significant difference in thresholds. Methodological differences might account for the discrepancy. We used pentobarbital anesthesia in the rat, vs. ketamine in the cat. One cannot rule out species differences, although the basis for this factor is unclear. Differential effects of anesthesia might contribute to the differences in findings. Pentobarbital potentiates synaptic inhibition (e.g., Nicoll, 1972). CSD analysis indicates that the depth negativity of LFPs (such as that recorded in this study) reflects predominantly EPSPs (reviewed in Mitzdorf, 1985). Consistent with this interpretation, pharmacological block of inhibition in the rat cortex by application of the γ-aminobutyric acid-A receptor antagonist bicuculline methiodide does not alter the

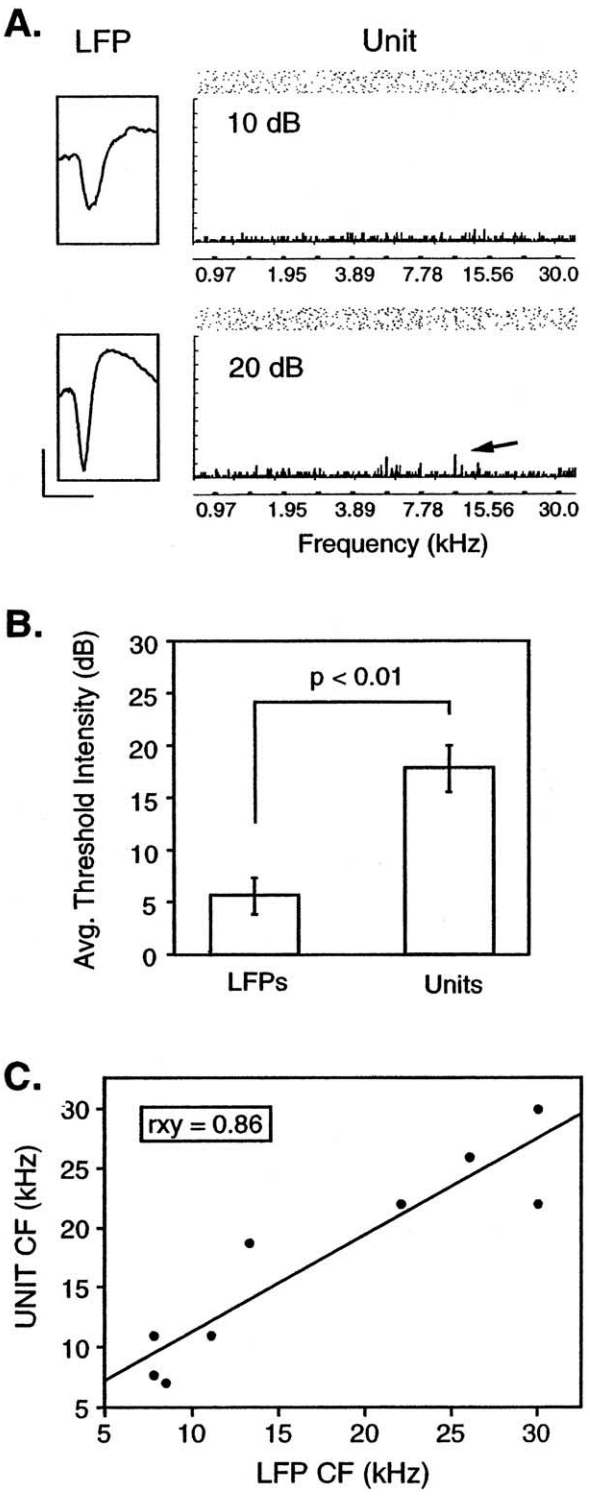

Fig. 1. (A) An example of average LFPs and unit responses (peristimulus time histograms) at their thresholds. Note that an LFP response is present at 10 dB. However, the unit response does not appear until 20 dB (arrow). The LFP threshold was at 7.78 kHz. The CF for unit discharge was 11.00 kHz; discharge also reached threshold, but fewer spikes, at 5.50 kHz and exhibited below-criterion responses at 7.78 kHz. LFP calibration bar = 50 ms and 250 μV; polarity, negative is down. The ordinate scale for the peristimulus time histograms is five spikes per division. (B) Mean thresholds for LFPs and unit discharges ($n = 9$). (C) Scattergram and best linear fit for CFs at LFP threshold vs. unit discharge CFs at their higher threshold.
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pattern of the CSD distribution in motor cortex (Aroniadou and Keller, 1993). Simultaneous intracellular and extracellular recordings in AI of the rat also support the conclusion that LFPs predominantly reflect summed EPSPs (Metherate and Ashe, 1993). Nonetheless, the possible contribution of inhibitory postsynaptic potentials (IPSPs) has not been definitively excluded. Any increased contribution of IPSPs under pentobarbital might reduce the probability of spikes, and therefore might increase spike threshold. Another difference between the two studies is that we employed multiple unit recordings whereas Eggermont recorded single unit waveforms. However, this seems unlikely to account for the higher unit versus LFP threshold because the unit having the lowest threshold would still be detected (South and Weinberger, 1995).

Perhaps the most important difference between the reports concerns the methods for determining threshold. Both experiments used voltage detectors to accept unit activity for recording and analysis. Eggermont selected thresholds based on visual judgements of stimulus time-locked raster displays. We required that responses occur on at least five of 20 stimulus presentations. It seems unlikely that we were less sensitive in detecting unit responses, because 5/20 is not a stringent criterion and we verified judgements based on rasters by examining latency printouts. It is possible that we used different voltage criteria for detecting unit waveforms. If our voltages were higher, then small amplitude unit discharges could have been excluded, but the resultant findings would be that larger amplitude waveforms have higher thresholds. However, amplitude is dependent to a large degree on distance of a cell from the electrode tip and distance is not known to affect threshold.

The difference in conclusions could well be a function of different criteria for determining LFP threshold. Eggermont detected LFPs by setting a voltage criterion of two standard deviations greater than background (EEG) activity. These level crossings yielded raster displays that were also visually judged to be time-locked, or not, to tone bursts. This criterion may have excluded small amplitude LFPs. In contrast, the present study used averaging which is more likely to detect small LFPs that are time-locked to tone bursts. Thus, it seems possible that this difference in LFP detection criteria is responsible for the lower LFP thresholds obtained in the present experiment.

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