Effects of acoustic stimuli on neuronal activity in the auditory cortex of the rat

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Running title: Sound and neuronal activity in rat auditory cortex

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Summary

Spontaneous activity of cortical neuron exhibits alternative fluctuations of membrane potential consisting of phased depolarization called "up-state" and persistent hyperpolarization called "down-state" during slow wave sleep and anesthesia. Here, we examined the effects of sound stimuli (noise bursts) on neuronal activity by intracellular recording *in vivo* from the rat auditory cortex (AC). Noise bursts increased the average time in the up-state by 0.81±0.65s (range, 0.27 - 1.74 s) related to a 10 s recording duration. The rise times of the spontaneous up-events averaged 69.41± 18.04 ms (range, 40.10 - 119.21 ms), while those of the sound-evoked up-events were significantly shorter ($p<0.001$) averaging only 22.54±8.81 ms (range, 9.31 - 45.74 ms). Sound stimulation did not influence ongoing spontaneous up-events. Our data suggest that a sound stimulus does not interfere with ongoing spontaneous neuronal activity in auditory cortex but can evoke new depolarizations in addition to the spontaneous ones.

Keywords: Auditory cortex, Intracellular recording, Depolarization, Spontaneous Activity, Rhythmic discharges

Introduction

Spontaneous activity of the neocortex including the sensory cortices appears in periodic burst firing of single units and periodic negative waves of local field potentials, and is also reflected in a slow oscillation of the electroencephalogram (EEG) during sleep and anesthesia (Eggermont *et al*. 1993, Amzica and Steriade 1995). The cellular basis of rhythmic cortical events is found in fluctuations of the membrane potential of cortical neurons consisting of short pulsed depolarizations called "up-state" and hyperpolarizations of longer duration called "down-state" (Amzica and Steriade 2002). These rhythmic events are observed not only in cortical neurons *in vivo* but also in cortical slices *in vitro* in which the thalamocortical inputs are absent (Mao *et al*. 2001, Sanchez-Vives and McCormick 2000, Ikegaya *et al*. 2004). This suggests that spontaneous activity originates from local cortical circuits.

In the sensory cortex, prolonged visual stimulation can increase the probability of the up-state in complex neurons of the visual cortex (Anderson *et al*. 2000). Whisker-evoked responses in the somatosensory cortex are dependent on membrane state (i.e. up- or down-state) (Sachdev *et al*. 2004). In the auditory cortex (AC), previous experiments showed spontaneous membrane potential fluctuations and their ion mechanism (Metherate and Ashe 1993). Compared with studies on other cortices (i.e. somatosensory and visual cortex), little is known about the effects of acoustic stimuli on the membrane potential fluctuations of the AC neurons. The aim of the present study was to investigate the effects of acoustic stimuli on spontaneous membrane potential fluctuations in the rat AC using *in vivo* intracellular recording. We found that acoustic stimuli can evoke up-events during the spontaneous hyperpolarization phase (down-state), but have no effect on the membrane potential during the depolarization phase (up-state). In addition,

the level of resting membrane potential appeared to be a major factor that determined the amplitudes of both spontaneous and sound-evoked up-events.

Methods

Experiments were carried out in 45 female Sprague-Dawley rats with body weight ranging from 200 to 250 g. All protocols and procedures were in accordance with Ethics in the Care and Use of Laboratory Animals of China and approved by the Animal Care and Use Committee of the Third Military Medical University.

Animal preparation

All surgeries and experiments were performed under anesthesia by intraperitoneal injection of urethane (ethyl carbamate, 1.5 g/kg). An additional dosage of urethane (0.2 g/kg) was given when rats showed responses to tail pinch. Tracheotomy was performed and the trachea was cannulated in order to maintain smooth breathing during experiments. Then the animal's head was immobilized with a custom-made head clamp by rigidly clamping between the palate and nasal/frontal bones. The head clamp was adjusted to align bregma and lambda points of the skull in one horizontal plane. The rat's body was suspended by hanging up the caudal back. In order to minimize the pressure difference between the body cavities and the skull cavity, the body level usually was adjusted 15—30° higher than the horizontal level (Konopacki *et al.* 2003). By doing this, cortical fluctuation caused by breathing can largely be eliminated. The scalp was incised along the midline and subcutaneous tissue and muscle were removed to expose the right skull. AC of the rat typically lies at the dorsolateral portion of the temporal cortex and is framed by a characteristic blood vessel pattern (Kelly and Sally, 1988). ~~The coordinates of the rat AI is 2.7 to 5.8 mm posterior to bregma and 3.1 to 5.4 mm ventral to bregma (Doron et al. 2002).~~ A large

area of the skull (about 3 mm×3 mm) was thinned above this region using a reliable dental device (Drill: Strong 90, Saeshin Precision Co. Ltd, Korea; Bur: SSW HP-2, SS White Burs, Inc., Lakewood, NJ, USA) such that a thin, well-polished, transparent, bone membrane remained (Pinault 2005). This procedure helped to easily identify the blood vessel pattern on the temporal cortex and to avoid blood vessels when a hole was opened. A hole of less than 1 mm in diameter was made roughly at the center of this area. The dura was gently incised using a sharp needle mounted on a 1-ml syringe and ending in a miniature hook (made when gently scratching a piece of metal with the tip of the needle). This small-hole surgical technique avoids brain movements by keeping the brain's volume constant within the cranial cavity and does not require additional technical procedures (Pinault 2005). The exposed cortex was kept moist and protected with warm paraffin oil. The surgery and following electrophysiological experiments were performed in an electrically shielded and anechoic chamber. Body temperature of rat was maintained at 37 °C by using a U-shape feedback-controlled heating pad that was placed just beneath the rat body.

Acoustic stimulus

White-noise or tone bursts of 60 ms in duration and 5 ms in rise/decay time were used to evoke auditory responses of cortical neurons. Acoustic signals were digitally synthesized and converted to analog signals by Real-time Processor (RP2, Tucker-Davis Technologies, Alachua, FL, USA). The output amplitude of the sinusoidal waves from the RP2 was set at 20 V peak-to-peaks. The signals were then fed to an attenuator (PA5) and presented by an electrostatic speaker (ES1) via an electrostatic speaker driver (ED1). The speaker was placed 45º to the left of and 10 cm away from rat's left ear. During the experiment, acoustic stimuli were played by BrainWare data acquisition software (Tucker-Davis Technologies). This software also allowed setting the frequency of tone bursts and the attenuation of the PA5. The noise or tone amplitude was

92 expressed as decibel sound pressure level (dB SPL, ref. 20 μPa). The output of the electrostatic
93 speaker was calibrated at the position of the animal's left ear with a condenser microphone
94 (Model 377A01, ***PCB*** *Piezotronics*, NY, USA) and a microphone preamplifier (Model 426B03,
95 ***PCB*** *Piezotronics*). The signal for calibration was not attenuated. Frequency and amplitude of
96 tone bursts were varied manually with BrainWare software.

97 *Recording of spontaneous and tone-evoked activities in the primary auditory cortex*

98 A tungsten electrode of ~2 MΩ tip impedance was first used for extracellular recording, to
99 determine the best frequency and minimum threshold of cortical neurons. The electrode was
100 advanced perpendicularly to the surface of the AC.

101 Tone-evoked responses were commonly observed when the electrode tip was ~500 μm
102 below the cortical surface. Electrical signals were filtered with a bandpass of 0.3-10 kHz and
103 amplified 10,000 times with a RA16 module (Tucker-Davis Technologies). The output signals
104 were monitored on an oscilloscope. A noise burst was delivered at a rate of 1 per second during
105 electrode penetration. Once noise-evoked responses were observed, the best frequency and
106 minimum threshold of the recorded neurons were measured with manual variation of the tone
107 frequency and amplitude. The tungsten electrode was then removed and a sharp glass-pipette
108 filled with 1.0 M potassium acetate was employed for intracellular recording. The tip impedance
109 of the sharp glass electrode ranged between 65 and 90 MΩ (Konopacki *et al.* 2003). The sharp
110 glass electrode was placed at a location adjacent to the penetrating point made by the tungsten
111 electrode and penetrated perpendicularly to the AC surface by a stepping motor at a step width of
112 1 or 2 μm.

113 **Data acquisition and analysis**

Upon penetrating the membrane of a cell, the electrode detected a sharp drop of membrane potential. Neurons showing a resting membrane potential less than -50mV commonly indicated unhealthy cells and were excluded in the present study. Electrical signals were first fed to a wide-band active probe electrometer (intra 767, World Precision Instruments, Sarasota, FL, USA), and then stored on a computer via an analog-to-digital converter (Digidata 1332A; Axon Instruments, Foster City, CA). Data were analyzed off-line using the pClamp 10.0 software (Axon Instruments).

Data are expressed as mean ± standard deviation (SD). Student's *t*-test was used to examine the significance between two sets of data, using 95% as the confidence level ($p<0.05$)

Results

We successfully recorded stable activities of 50 neurons sampled from the primary auditory cortices of 45 animals. The depths from which neurons were sampled ranged from 256 μm to 815 μm below the brain surface. Neurons' best frequencies and minimum thresholds measured with extracellular recording ranged from 5.4 kHz to 34.6 kHz and from 7.8 dB SPL to 41.2 dB SPL respectively (average 17.7±8.6 dB SPL).

All sampled neurons exhibited spontaneous alteration of membrane potential, on average, switching between down-state (-72.4 ± 6.9 mV) and up-state (-58.6 ± 4.6 mV) (Fig. 1A). The spontaneous switching of the membrane potential occurred at a rate of 0.54 ± 0.36 Hz. The duration of spontaneous up-events lasted for an average of 0.64 ± 0.32 s (n = 50 cells). Sound evoked up-events occurred when the noise burst with 50 dB sound press level (SPL) was presented during the spontaneous down-state (Fig. 1B, Fig. 3); the duration of noise-evoked up-events lasted for an average of 0.38 ± 0.21s (n = 50 cells). To better demonstrate the effects of the

136 acoustic stimuli on spontaneous activity, the membrane potentials at each time point are
137 presented as frequency histograms from the 10 s recording segments shown in Fig. 1A and B
138 (action potentials excluded). These will be called all-point histograms, which consistent with
139 those used in analyzing data from single-channel recordings (Stern *et al.* 1997). The result is
140 shown in Fig. 1C for spontaneous- (black line) and sound-evoked activity (gray line). The
141 histograms from both spontaneous and noise-evoked activity showed clear differences in the
142 durations at the resting potential levels, with a smaller peak (shorter duration) in the noise-evoked
143 case. In addition, membrane potentials above the resting potential occurred generally for longer
144 durations in the noise-evoked case compared to the spontaneous case. This is quantified for 10 s
145 recording segments from all 50 neurons in Fig. 1D, which shows that the average time spent in
146 the up-state mode increased by 0.81 ± 0.65 s (range, 0.27 - 1.74 s) from the spontaneous to the
147 noise-evoked case.

148 Figure 2 shows typical recordings of spontaneous (Fig. 2A) and noise-evoked up-events
149 (Fig. 2B). The rise phases of the up-events are shown time-expanded by a factor of two in Fig.
150 2Ab and 2Bb. The rise times for the spontaneous events ranged from 40.10 to 119.21 ms,
151 averaging 69.41 ± 18.04 ms. The rise times for the sound-evoked events were significantly
152 shorter ($p<0.001$). They ranged from 9.31 to 45.74 ms, averaging 22.54 ± 8.81 ms.

153 Since a sound stimulus can increase the time spent in the up-state (Fig. 1D), we wanted to
154 know how the sound stimulus affected the spontaneous up-state. We compared two cases: noise
155 bursts occurring coincidently with spontaneous up-states or in intervals between spontaneous up-
156 states. Fig. 3A shows a typical recording of spontaneous fluctuations of the membrane potential
157 of an AC neuron. When the noise bursts were repeatedly presented at a rate of 1/s, they evoked

158 up-events during the down-state of the membrane potential, but had no effect on the membrane
159 potential during the spontaneous up-state (Fig. 3B).

160 Finally, we found that the relative amplitudes between the actual resting potentials and the
161 membrane potentials of the up-states were related to the level of the resting potentials in the same
162 way for both the spontaneous and the sound-evoked up-states. The correlations with regression
163 lines are shown in Fig. 4A for spontaneous ($r=0.79$, $p<0.001$; $n = 50$) and in Fig. 4B for noise-
164 evoked ($r=0.74$, $p<0.001$; $n = 50$) up-states. This result indicates that the cortical neurons show
165 the same average amount of depolarization spontaneously and in response to noise bursts, the
166 magnitude of the depolarization depending only on the level of the resting potential.

167 **Discussion**

168 Although there is clear diversity in the architecture and function of different cortical areas
169 such as prefrontal cortex and sensory cortices, spontaneous activity is commonly observed in
170 quiescence and appears in similar fashion (Eggermont *et al*. 1993; Amzica and Steriade, 1995). In
171 natural sleep or under anesthesia, spontaneous discharges occur typically with temporal
172 regularity. Specifically, this temporal regularity is periodic, showing up in rhythmic spike bursts
173 of single units, negative-going waves of local field potentials, and slow oscillations of local EEG
174 waves at a rate of less than 1 Hz (Steriade *et al.* 1993). Both *in vivo* and *in vitro* studies have
175 revealed that the spontaneous activity of the cortex is based on the cyclic switching of the activity
176 state of cortical networks (Mao *et al*. 2001, Shu *et al*. 2003, Ikegaya *et al*. 2004). In agreement
177 with previous studies in other sensory cortices, our data show that periodic switching of
178 membrane potentials between two states was found in all sampled AC neurons at a rate of 0.54 ±
179 0.36 Hz, without exception. In most cases, depolarization of neuronal membrane potential
180 resulted in burst firing. Such low-frequency spontaneous activity of the cortex may be generated

in and propagated over cortical intrinsic circuits, although the exact location of the generator is as yet unknown (Steriade *et al*. 1993, Sanchez-Vives and McCormick 2000).

Our results indicate that an acoustic stimulus can induce up-state events during time intervals of spontaneous down-states, leading to an overall increased time of up-state. This increase was not due to the increase of the duration of single up-events but rather the increase of the number of the up-events induced by the sound stimuli. Since bistable fluctuations of the membrane potential arise largely from synaptic activity (Stern *et al*.1997, 1998; Stevens and Zador 1998), sensory stimulation may not act independently on individual cortical cells, but seems to interact at the network level with the mechanism generating synchronized fluctuations of membrane potentials within a neuronal populations (Anderson *et al*. 2000).

For individual cortical neurons, excitatory synapses arising from intracortical connections are greater in number than synapses from thalamocortical projections (Ahmed *et al*. 1994, Douglas *et al*. 1995) whereas the latter are greater in strength (Gil *et al*. 1999. Stratford *et al*. 1996). This suggests that the activity of individual cortical neurons could potentially be impacted in different ways by activities of both intracortical and thalamocortical fibers. In our study, the rise time was significantly shorter in sound-evoked up-events. This difference could result from stronger thalamocortical compared to spontaneous intracortical synaptic activity.

It is noticeable that acoustic stimulation induced depolarization of cortical neurons only when delivered in between the spontaneous up-states (Fig. 3). These findings are in general agreement with previous reports from the somatosensory cortex *in vivo* (Petersen *et al*. 2003) and the thalamocortical brain slice (Watson *et al*. 2008). These studies demonstrated that sensory or electrical stimulation of the thalamus did not alter ongoing cortical activity during the up-state.

This indicates that neural computation or integration of sensory information shares similar mechanisms across different sensory cortices. Underlying mechanism may be related to the increased membrane conductance during the up-state when membrane potentials reach the firing-threshold level. ~~This can also be evidenced in the present study that almost all spontaneous up events accompany with action potentials.~~

Our data showed that the amplitudes of both spontaneous and sound-evoked up-events were related linearly to the levels of resting membrane potentials: the lower the resting membrane potential, the larger the depolarization amplitude (Fig. 4). This phenomenon was also reported in previous studies both *in vivo* and *in vitro* (Amzica and Steriade 1995, Petersen *et al*. 2003, Thomson 1986) and was regarded as depending on NMDA-mediated depolarization (Thomson 1986).

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Figure Legend

Fig.1. Spontaneous (A) and noise-evoked activity (B) of the same AC neuron. In B, the timing of the noise bursts is indicated on the trace below the recording. C. All-points histogram showing the amount of time spent at any given membrane potentials for the recordings shown at A (black) and B (gray). Histograms do not include the action potentials. D. Average time from a 10 s measuring period of membrane potentials from all 50 neurons in an up-state due to spontaneous (black) or sound-evoked (gray) conditions

Fig.2. Rise time of spontaneous and noise-evoked up-events. A. Typical recording of spontaneous up events (Aa), which is shown time-expanded by a factor of two in the lower panel (Ab). B. Same as in A, but for noise-evoked up events.

Fig.3. A. Typical recording of spontaneous activity from an AC neuron. B. Noise bursts have no effect on the neuron during the up-state (indicated by triangles under the stimulus trace), but can evoke responses during the down-state (indicated by asterisks under the stimulus trace). Spikes are truncated in both recordings.

Fig.4. The relative amplitude of both spontaneous (A) and sound-evoked (B) up events (y-axis) varies linearly with the value of the resting membrane potential (x-axis). Equations of the respective regression lines are indicated

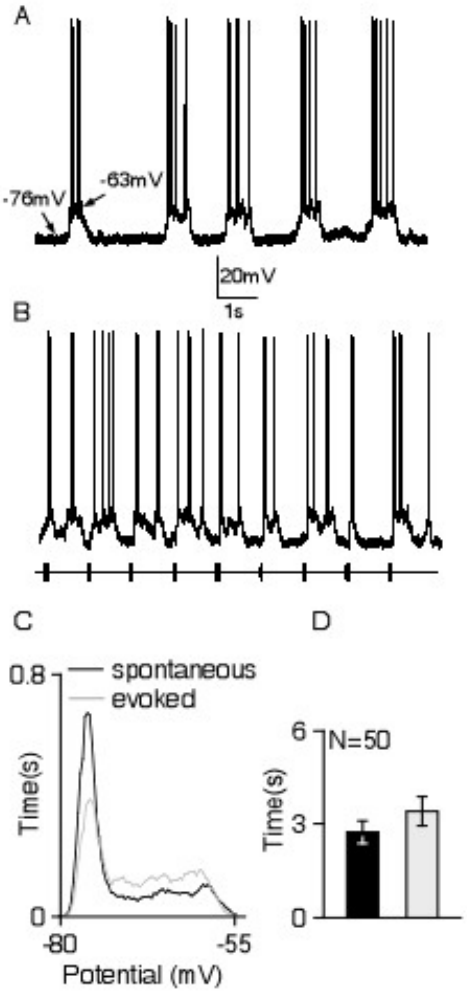

Fig. 1

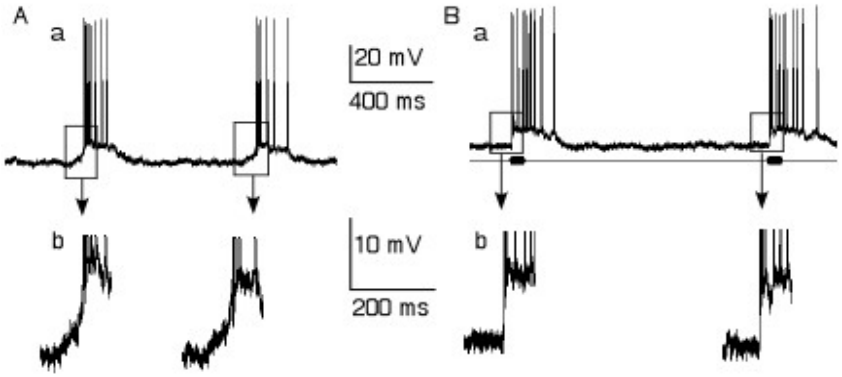

Fig. 2

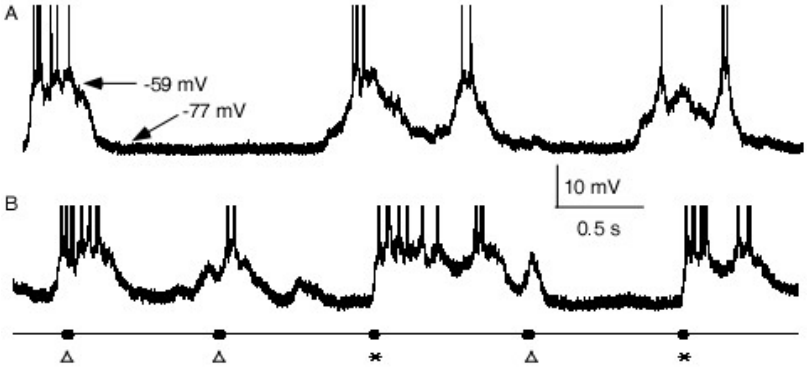

Fig. 3

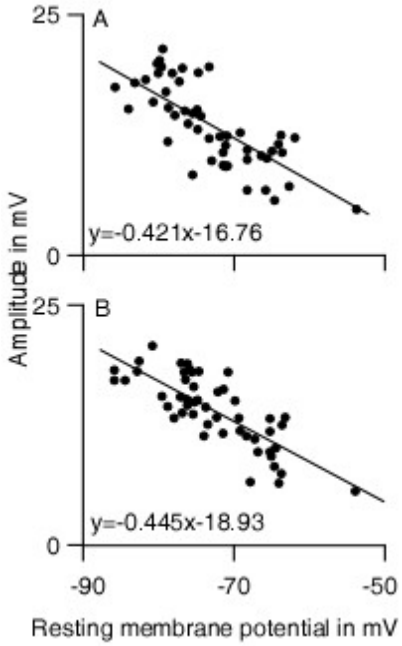

Fig. 4