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Research paper

Duration sensitivity of neurons in the primary auditory cortex of albino mouse

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ABSTRACT

Many neurons in the central auditory system of a number of species have been found to be sensitive to the duration of sound stimuli. While previous studies have shown that γ-aminobutyric acid (GABA)-ergic inhibitory input is important for duration sensitivity in the inferior colliculus (IC), it is still unknown whether (GABA)-ergic inhibitory input plays an important role in generating duration sensitivity in the cortex. Using free-field sound stimulation and *in vivo* extracellular recording, we investigated duration sensitivity in primary auditory cortical (AI) neurons of the Nembutal anesthetized albino mouse (*Mus musculus, Km*) and examined the effect of the $GABA_A$ receptor antagonist bicuculline on AI neuron duration sensitivity. A total of 63 duration tuning curves were measured in AI neurons. Of these, 44% (28/63) exhibited duration sensitive responses, while 43% (27/63) lacked duration sensitivity. The remaining 13% (8/63) exhibited long-pass properties likely reflecting both duration sensitive and insensitive features. We found that duration sensitive neurons had shorter first spike latency (FSL) and longer firing duration (FD) when stimulated with best duration ($p < 0.05$), while duration insensitive neurons had invariable FSL and FD at different sound durations ($p > 0.05$). Furthermore, 60% (6/10) of duration sensitive neurons and 75% (3/4) long-pass neurons lost duration sensitivity following bicuculline application. Taken together, our results show that cortical neurons in the albino mouse are sensitive to sound duration, and that GABAergic inhibition may play an important role in the formation of *de novo* duration sensitivity in AI. The possible mechanism and behavioral significance of duration sensitivity in AI neurons is discussed.

1. Introduction

Duration is an important temporal feature of acoustic signals (Masterton, 1992; Suga, 2008). Similar to humans who use phoneme duration to recognize speech characteristics (Lehiste et al., 1976; Shannon et al., 1995), animals utilize sound duration sensitivity for sound localization and specie identification (Gooler and Feng, 1992; Casseday et al., 2002). Indeed, duration sensitive neurons have been found in the inferior colliculus (IC) of the central auditory system in a variety of species, and are considered an important adaptation in processing biologically related signals (Gooler and Feng, 1992; Casseday et al., 1994; Fuzessery and Hall, 1999; Jen and Feng, 1999; Luo et al., 2008; Xia et al., 2000; Faure et al., 2003; Mora and Kössl, 2004; Yin et al., 2008).

Numerous studies on duration sensitivity have previously focused on the IC in different animals including mouse (Brand et al., 2000; Xia et al., 2000), with a lack of attention to other important auditory nuclei such as the primary auditory cortex (AI). In addition, previous experiments have also largely ignored the mouse auditory system, tending to focus on other model systems such as the frog (i.e. Narins and Capranica, 1980) and bat (i.e. Casseday et al., 1994). In fact, to date, there is no information about duration processing in the auditory cortex of mouse, a common model system for biomedical research (Fox et al., 2006).

Since the initial description of duration sensitive neurons in the auditory midbrain of frogs (Potter, 1965), the underlying mechanisms of duration sensitive neurons has attracted much attention (Feng et al., 1990). Several mechanistic models of duration

Abbreviations: GABA, γ-aminobutyric acid; AI, primary auditory cortex; FSL, first spike latency; FD, firing duration; IC, inferior colliculus; BF, best frequency; LSL, last spike latency; MT, minimum threshold; PSTH, post-stimulus-time histogram; Bic, bicuculline

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sensitivity have been proposed that either focus on coincidence or anticoincidence mechanisms. While these models explain many of the observed duration sensitive neural response properties, they are, however, still incomplete. For instance, previous studies have shown that the interaction between excitatory and inhibitory inputs play an important role in duration sensitivity in the central auditory system (Jen and Schlegel, 1982; Casseday et al., 1994, 2002; Fuzessery and Hall, 1999; Brand et al., 2000; Faure et al., 2003; Pérez-González et al., 2006; Aubie et al., 2009). GABAergic inhibition can shape echo duration selectivity of IC neurons in echolocating bats (Jen and Wu, 2005; Wu and Jen, 2006), and duration selectivity of most IC neurons in the guinea pig also change during $GABA_A$ receptor blockade (Yin et al., 2008). The source of inhibitory input that may account for duration sensitivity in cortical neurons has however, largely remained unexplored.

Thus, the goal of the present study was to determine whether stimulus duration is represented in the auditory cortex of albino mouse, and if so, whether cortical duration sensitivity reflects duration sensitive inputs from subcortical nuclei or whether it is created *de novo*? To help address these questions we used the $GABA_A$ antagonist bicuculline to evaluate how GABAergic inhibition shapes duration sensitivity in cortex neurons.

For this study, we used pure tones of different durations as a sound probe and combined this with electrophysiological and neuropharmacological methods to measure the duration sensitive neurons in AI. First spike latency (FSL) and firing duration (FD) were used to evaluate the effect of locally infused GABAergic inhibition on AI neuron duration sensitivity.

2. Materials and methods

2.1. Surgical procedures and animal preparations

Experiments were performed on eleven healthy adult Kunming mice (*Mus musculus Km*) (4–5 weeks, 20–25 g, body weight, both sexes). As described previously (Wang et al., 2007; Qi et al., 2013), the flat head of a 1.8 cm nail was glued onto the exposed skull of each Nembutal anesthetized (60–90 mg/kg b. w.) mouse with acrylic glue and dental cement. After securing the mouse to an aluminum plate inside a sound-proof room (temperature 28–30 °C), its head was immobilized by fixing the shank of the nail into a brass rod with a set of screws. The head of the mouse was oriented with the eye-nostril line pointed to 0° in azimuth and 0° in elevation with respect to frontal auditory space. A small hole (diameter: 200–500 μm) was drilled in the skull above the auditory cortex (Paxinos and Franklin, 2001) for orthogonal insertion of 2 M NaCl glass pipette electrodes (impedance: 5–10 MΩ) to record sound-evoked responses. A silver-wire electrode was placed on the temporal muscles. Recording depth was read from the scale of a hydraulic drive (Model 640, David-Kopf Instruments, Tujunga, CA, USA). Each mouse was used in one to three recording sessions on separate days and each recording session typically lasted for 2–6 h. Mice were maintained under light anesthesia. While this generally reduced the response of a given neuron, it did not change the response type (see "*Effects of general anesthesia*" in discussion). The experiments were conducted with the approval of the Institutional Animal Care and Use Committee of Central China Normal University, Wuhan, Hubei, People's Republic of China. All experiment procedures were performed strictly in accordance with the guidelines published in the NIH Guide for the care and use of Laboratory Animals.

2.2. Acoustic stimulus paradigm

The acoustic stimulation system consisted of a function generator (GFG-8016G, Good Will Instrument Co., Ltd. Bayan Lepas, Penang, Malaysia), a tone burst generator (homemade), an attenuator (LAT-45, Leader, Kohokuku, Yokohama, Japan), an ultrasonic sound power amplifier (homemade), and a loud speaker (AKG model CK 50, 1.5 cm in diameter,1.2 g, frequency response 1–70 kHz at ±9 dB). During the experiment, a 40 ms tone burst at 20–30 dB above threshold was used (stimulus repetition rate: 1 Hz) to search for sound responsive AI neurons. We located AI by recording the best frequency (BF) at multiple points and compared this with the frequency distribution topology of auditory cortex in mouse (Linden et al., 2003; Luo and Yan, 2013). Pure tone bursts at BF (duration: 5, 10, 20, 40, 80, 140 or 200 ms; intensity: 10 dB above minimum threshold) were pseudo-randomly chosen and delivered at 1 Hz to measure the duration tuning curve of each AI neuron. The loud speaker was fixed at 0° in elevation and at 60° contra lateral to the middle axis of the recording site in azimuth, and its output intensity was calibrated with a 1/4 inch microphone (B&K4939, Narum, Denmark) placed at the mouse's pinna using a measuring amplifier (B&K 2610, Narum, Denmark). Sound intensity was expressed in dB SPL referenced to 20 μPa root mean square.

2.3. Recording and bicuculline application

When an AI neuron sound-stimulated response was isolated, its BF and minimum threshold (MT) were audio-visually measured by systematically changing the frequency and intensity of sound pulses. The sound frequency that elicited the neuronal response at the lowest intensity was defined as the BF. The threshold at the BF was defined as the MT. At the MT, the neuron responded with 50% probability to BF pulses. The duration tuning curve was plotted by the combinations of duration and spike counts to 32 stimuli at each duration. The duration tuning curves measured for these neurons can be described as band-, short-, long- and all-pass using the same criterion in our previous studies (Luo et al., 2008; Wang et al., 2010). The duration corresponding to 50% of the maximum spike count was defined as the cut-off duration. A short-pass (SP) duration sensitive response (Fig. 1A) consisted of an activity pattern in which the spike count obtained for short durations was high, but with increasing duration, dropped to <50% of the peak value at durations above the cut-off duration. In a band-pass (BP) duration sensitive response, one duration yielded maximal spike counts (best duration, BD), and responses dropped to <50% of the peak value beyond the cut-off durations at higher or lower ends. All-pass (AP) neurons were defined as those for which the spike counts at all durations never varied by >50% of the peak value (Fig. 1D). As such, band-pass and short-pass neurons were considered duration sensitive neurons while all-pass neurons were not. Long-pass (LP) responses had high spike counts with longer sound stimuli, but the spike counts with lower sound stimuli were <50% of peak value. The response to sound duration higher than the cut-off duration did not vary more than 20%. Given that LP neurons' duration sensitivity may be mediated by either insufficient energy integration or neural inhibition, we characterized LP neurons separately and tested the duration sensitivity at different intensity levels above threshold.

Duration tuning curves were measured before and during bicuculline application. Piggy-back multi-barrel electrodes were constructed for the iontophoretic application of bicuculline (Havey and Caspary, 1980; Lu et al., 1998; Wang et al., 2007). Briefly, a three-barrel electrode (tip: 10–15 μm) was "piggy-backed" on a 2 M NaCl single-barrel electrode (tip: less than 1 μm, used for recording). The tip of the single-barrel electrode was extended about 10 μm beyond the tip of the three-barrel electrode. One barrel of the three-barrel electrode was filled with bicuculline methiodide (10 mM in 0.16 M NaCl, pH 3.0; Sigma), used for ion