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CHOLINERGIC MODULATION OF AUDITORY STEADY-STATE RESPONSE IN THE AUDITORY CORTEX OF THE FREELY MOVING RAT

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Abstract—As disturbance in auditory steady-state response (ASSR) has been consistently found in many neuropsychiatric disorders, such as autism spectrum disorder and schizophrenia, there is considerable interest in the development of translational rat models to elucidate the underlying neural and neurochemical mechanisms involved in ASSR. This is the first study to investigate the effects of the non-selective muscarinic antagonist scopolamine and the cholinesterase inhibitor donepezil (also in combination with scopolamine) on ASSR. We recorded the local field potentials through the chronic microelectrodes implanted in the auditory cortex of freely moving rat. ASSRs were recorded in response to auditory stimuli delivered over a range of frequencies (10–80 Hz) and averaged over 60 trials. We found that a single dose of scopolamine produced a temporal attenuation in response to auditory stimuli; the most attenuation occurred at 40 Hz. Time–frequency analysis revealed deficits in both power and phase-locking to 40 Hz. Donepezil augmented 40-Hz steady-state power and phase-locking. Scopolamine combined with donepezil had an enhanced effect on the phase-locking, but not power of ASSR. These changes induced by cholinergic drugs suggest an involvement of muscarinic neurotransmission in auditory processing and provide a rodent model investigating the neurochemical mechanism of neurophysiological deficits seen in patients.

Key words: auditory cortex, scopolamine, donepezil, local field potential, ASSR, neurophysiological recording.

INTRODUCTION

Networks of sensory cortical neurons can be entrained to periodic auditory stimulation, such as a train of clicks or amplitude modulated tones (Rager, 1998; Oshurkova et al., 2008; Ma et al., 2013). The associated change in the field potential as measured by electroencephalography (EEG) or magnetoencephalography (MEG) is referred to as the auditory steady-state response (ASSR) (Picton et al., 2003; Wilson et al., 2007; Rojas et al., 2011). Human scalp-recorded steady-state oscillations are maximal at 40 Hz, because a simple juxtaposition of middle-latency event-related potentials (ERPs) occurs most effectively at this frequency (Galambos et al., 1981). It is also proposed that the natural resonance frequency of neural population leads to a larger recruitment and a greater response in EEG at 40 Hz (Kwon et al., 1999). Localization of the 40 Hz ASSR has been made to the primary auditory cortices in multiple species (Tsuzuku, 1993; Franowicz and Barth, 1995; Herdman et al., 2002; Kuwada et al., 2002), with contributions from subcortical structures (Tsuzuku, 1993; Steinmann and Gutschalk, 2011).

Several studies have used ASSR to probe neural network function underlying selective auditory attention and found that attention significantly modulated the power of ASSR (Gander et al., 2010). Moreover, the 40-Hz ASSR has been consistently found to be abnormal in many neuropsychiatric disorders, such as tinnitus (Roberts et al., 2015), autism spectrum disorder (Gandal et al., 2010) and schizophrenia (Brenner et al., 2003; Light et al., 2006; Lenz et al., 2011; Gandal et al., 2012). ASSRs to 40-Hz stimulation are reduced in power (magnitude) or phase synchronization (phase consistency across trials) in schizophrenia patients in most (Kwon et al., 1999; Brenner et al., 2003; Light et al., 2006; Spencer et al., 2008; Vierling-Claassen et al., 2008), but not in all studies (Hong et al., 2004; Rass et al., 2012). Nevertheless, ASSR may be a useful biomarker reflecting neurophysiological abnormalities in auditory pathways or cortex (Teale et al., 2008; Brenner et al., 2009). Hence, there has been considerable interest in the development of translational rodent models to elucidate the underlying neural and neurochemical mechanisms involved in ASSR.

The basal forebrain cholinergic system has been described as a neuromodulator system that influences broadly defined behavioral and brain states. Many studies demonstrated that cholinergic actions at both major subtypes of acetylcholine (Ach) receptor

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Abbreviations: AC, auditory cortex; AEP, auditory-evoked potentials; ASSR, auditory steady-state response; EEG, electroencephalography; ITC, inter-trial coherence; LFPs, local field potentials; mAChR, muscarinic; nAChR, nicotinic.

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– nicotinic (nAChR) and muscarinic (mAChR) receptors – regulate the properties of sound-evoked neural response and plasticity in auditory cortex (AC) (Ashe et al., 1989; Chen and Yan, 2007; Liang et al., 2008; Metherate et al., 2012). The basal forebrain cholinergic projections distributed widely to the cortex and thalamic nuclei may be involved in the modulation of ASSR by attention (Sarter et al., 2005; Roberts et al., 2013). Also, cholinergic projections to the subcortical auditory pathway could modulate the ASSR recorded in the cortex (Mellott et al., 2011). However, to date, the effects of cholinergic neurotransmitter on ASSR are less investigated. Nicotine has been shown to augment steady-state responses in healthy human volunteers and rodent models (Thompson et al., 2000; Harkrider and Champlin, 2001). This result is consistent with the notion that nicotinic cholinergic agonists ameliorate sensory deficits in schizophrenia patients (Winterer, 2010; Miwa et al., 2011). Despite alterations in muscarinic signaling appear to underlie the symptoms of schizophrenia (Sarter et al., 2012), no data are available on the roles of muscarinic cholinergic neurotransmission in ASSR.

The purpose of the present study was to examine the effects of the mAChR antagonist scopolamine and the cholinesterase inhibitor donepezil (also in combination with scopolamine) on ASSR. We recorded the local field potentials (LFPs) through the chronic electrodes implanted in the primary auditory cortex, as the freely moving rats were passively listening to a click-train stimulation. The power and phase-locking of stimulus-evoked response were evaluated at LFP levels.

EXPERIMENTAL PROCEDURES

Subjects

All animal experiments were carried out in strict accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996. The protocol for animal handling and the treatment procedures were approved by the China Medical University Animal Care and Use Committee. All surgery was performed under anesthesia, and all efforts were made to minimize the number of animals used and their suffering. Adult male Wistar rats weighing 250–350 g (8–10 weeks old) at the beginning of the experiments were used. Animals came from our own colony housed in a humidity-controlled (50–55%) and temperature-controlled (22–24 °C) facility on a 12-h light/dark cycle (lights on at 7:30 A.M.) with access to food and water *ad libitum*.

Surgical preparation and electrode implantation

The animal was anesthetized by an initial injection of chloral hydrate (330–350 mg/kg, 5%, i.p.) supplemented by additional doses (usually 100 mg/kg once per hour). Temperature was monitored rectally and maintained at 37 °C using a feedback-controlled blanket. After placing the animal in a stereotaxic frame (SR-5R, Narishige, Tokyo, Japan), the cranium was exposed, four small holes were drilled over the parietal bone and fine jeweler's screws were inserted to serve as an anchor for a metal head-post holder that was cemented to the skull with dental acrylic. A craniotomy (2×1.5 mm) was performed above the left AC according to the coordinates of the Paxinos and Watson brain atlas: 3–7 mm posterior of the bregma and 3–5 mm lateral to the bregma (Paxinos and Watson, 1986). The dura above the AC was removed under binocular control. We then implanted a microwire array consisting of 4 (2×2) formvar-insulated 50-μm-diameter nichrome wires (part #762000; A-M Systems, Carlsborg, WA, USA). The tip impedance of each wire was around 0.5 MΩ at 1 kHz. The distance of two neighbor electrodes was approximately 300 μm. A silver wire, used as ground, was inserted between the temporal bone and the dura mater on the contralateral side. The microwire array was mounted on a custom-built holder that was lowered stepwise with a pulse motor-driven manipulator (SM-20, Narishige, Tokyo, Japan). Wires were inserted into the cortex until the tips of the electrodes were 0.5–0.8 mm below the dura, while viewing through a microscope and listening to an audio monitor of the recorded signal. The craniotomy was then filled with SILASTIC, a silicone elastomer (World Precision Instruments, Sarasota, Florida, USA) and sealed using dental acrylic. After the cement had completely hardened, the spare parts of wires and the connector were fixed on the skull by dental acrylic. At the end of surgery, an antibiotic (Cefuroxime, Zinacef injection, Glaxosmithkline, Brentford, UK) was administered systematically (30 mg/kg, i.p.). Animals were then housed in a medal grid cage for 1–2 weeks of postoperative recovery. All recording experiments were performed between 6 and 12 PM. This ensured that the animals were in their active phase and minimized the possibility that they would fall asleep during a test session, which was always passive and hence did not require a behavioral response. The animals had unlimited access to food and water.

Auditory stimulus

Auditory clicks (rectangular pulse of 0.2 ms duration) at a variable rate (10, 20, 40 and 80 cycles/s; 0.5 s train duration) were generated by custom-built programs under MATLAB (The Mathworks, Nantic, MA, USA) environment and delivered via an earphone (NW-STUDIO PRO W; Ninewave, Japan), which was attached on the cement platform implanted on the rat's skull during the surgery. The placement of the earphone was adjusted to 1 cm from the ear canal contralateral to the recording side. Auditory stimulations were delivered at a nominal sound pressure of 55 dB measured by placing a sound pressure meter (Brüel & Kjaer 1/2 inch condenser microphone with a pre-amplifier 2669) 1 cm in front of the earphone. In each session, the click-trains at different repetition rates were randomly interleaved and repeated 60 times with inter-train-intervals of 2–4 s.

Data acquisition

The microwire output was connected to a multi-channel preamplifier (RA16PA; TDT, Alachua, FL, USA) using a flexible, low-noise cable. The output of the preamplifier