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Hearing Research 201 (2005) 145–155
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Disrupted tonotopy of the auditory cortex in mice lacking M_1 muscarinic acetylcholine receptor

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Received 19 August 2004; accepted 15 October 2004
Available online 21 November 2004

Abstract

Sensory cortices have multiple and distinct functional maps that systematically represent environmental information. Development of these maps is precisely controlled by a number of intrinsic and extrinsic factors. Cortical cholinergic regulation is a crucial factor for normal cortical morphogenesis. In this study, we test the role of the M_1 muscarinic acetylcholine receptor, the main muscarinic receptor subtype in the neocortex in the development of tonotopic maps in the auditory cortex. Mice lacking M_1 receptors have normal hearing sensitivity but exhibit disrupted tonotopic organization and frequency tuning in the auditory cortex. In contrast, tonotopic organization and frequency tuning remain normal in the auditory midbrain. In addition, cortical layer IV neurons of M_1 mutants exhibit significantly shorter or sparser dendrites compared to neurons of wildtype mice. In summary, our data suggest that the M_1 receptor appears to be critical for the refinement or normal maturation of cortical tonotopy that is guided by thalamocortical inputs during early development.

Keywords: Auditory cortex; Tonotopy; Frequency tuning, Muscarinic receptor; Cholinergic; Development; Mouse

1. Introduction

Functional maps of sensory cortices, such as the tonotopic organization in the auditory cortex and the retinotopic organization in the visual cortex, are quickly established after the arrival of the thalamocortical axons during early development. The functional organization is initiated by spontaneous thalamocortical activity and then is refined and reshaped by sensory-driven thalamocortical activity (Wiesel and Hubel, 1963; Schmidt et al., 1999; Yuste and Sur, 1999; Sharma et al., 2000; Zhang et al., 2001, 2002; Pallas, 2001; Chang and Merzenich, 2003; Yan, 2003). A major event that occurs soon after the ingrowth of thalamocortical afferents is the arrival of cholinergic afferents from the cholinergic

Abbreviations: AAF: anterior auditory cortex; ABR: auditory brainstem response; AC: auditory cortex; AI: primary auditory cortex; AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; BF: best frequency; FA-scan: frequency-amplitude scanning (frequency-scan plus amplitude-scan); IC: inferior colliculus; M_1: muscarinic acetylcholine receptors subunit one; mAChR: muscarinic acetylcholine receptor; MT: minimum threshold; NMDA: *N*-methy-D-aspartate; PST: peristimulus time histograms; PSTC: cumulative PST; TI: tonotopy index; WT: wildtype

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doi:10.1016/j.heares.2004.10.003

basal forebrain (Molnár et al., 1998; Mechawar and Descarries, 2001). Cortical cholinergic activity is highest during the most dynamic period of cortical maturation and synapse formation (Robertson et al., 1991; Hohmann and Berger-Sweeney, 1998).

What is the biological significance of this early cholinergic innervation of the neocortex? Morphological studies show that neonatal ablation of the cholinergic basal forebrain causes numerous deficits in the pattern of cortical architecture (Hohmann and Berger-Sweeney, 1998). This suggests that the cortical cholinergic system plays a substantial but still largely undefined role in the development of cortical function during early life. Our goal is to understand how the cortical cholinergic system contributes to the development of the precise functional organizations of sensory cortices.

Muscarinic acetylcholine receptors (mAChRs) are critical to cortical plasticity in both the neonatal and adult cortex. For example, cortical application of the muscarinic receptor antagonists atropine or scopolamine decreases or abolishes the frequency-specific plasticity of the auditory cortex when evoked by basal forebrain stimulation paired with a sound (Bakin and Weinberger, 1996; Weinberger, 1998, 2003; Miasnikov et al., 2001; Ji and Suga, 2003). The M_1 subtype is the most abundant muscarinic receptor in the neocortex (Levey, 1993; Hohmann et al., 1995). Distribution of M_1 receptor displays a dynamic laminar pattern; M_1 receptors mostly concentrate in layer IV of the cortex during early development and are distributed in superficial and deep layers during adulthood (Prusky and Cyander, 1990; Liu et al., 1994). Administration of M_1 selective antagonists can prevent acetylcholine-mediated facilitation in the adult auditory cortex as well as suppress experience-dependent plasticity in neonatal visual cortex (Metherate et al., 1990; Gu and Singer, 1993).

We hypothesized that the refinement and maturation of tonotopic organization requires regulation by M_1 mAChR during early development. Our studies demonstrate that M_1 mAChR knockout mice exhibit disorganized frequency maps of the auditory cortex as a result of deficits in frequency tuning of the cortical neurons and disruptions to the cytoarchitecture in layer IV of the auditory cortex.

2. Materials and methods

2.1. Animal models

M_1 mAChR-deficient mutants with a deletion of the *Chrm1* gene were generated at the University of Washington in Seattle. Previous studies have shown that the M_1 mAChR-deficient mice exhibit no change in the level or pattern of expression of other mAChR subtypes in the brain and exhibit no obvious differences compared to wildtype littermates (Hamilton et al., 1997). The mice used in this study were from a line that had been backcrossed a minimum of five times against C57BL/6. Previous studies of the visual cortex have demonstrated that the neocortex of the mouse appears to achieve maturity at four to five weeks following birth (Gordon and Stryker, 1996; Leuba et al., 1978). Therefore, we analyzed 15 M_1 null mutant (−/−) and 13 wildtype (+/+) female mice at ages six to eight weeks. The body weight of these mice ranged from 16.3 to 22.9 g. There was no difference in their physical characteristics. M_1 mutants and wildtypes were reared in the same acoustic environments at the University of Washington and shipped to Calgary at age five weeks. Experiments were begun within one week of their arrival. In all instances, the experimenter was blinded to genotype.

2.2. Surgical procedures

All protocols and procedures followed institutional guidelines (protocol M02034, University of Calgary). Animals were anesthetized with a mixture of ketamine (120 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.) and then fixed in a custom-made head holder by rigidly clamping between the palate and nasal/frontal bones. Bregma and lambda points of the skull were aligned to the same horizontal plane by adjusting the mouth bar. The scalp covering the left part of the skull was removed. For the cortical mapping experiments, the skull and dura were then carefully removed with a drill and needle in order to expose the left temporal cortex. The exposed cortex was continuously treated with saline to prevent the tissue from drying. For the midbrain mapping experiments, the skull and dura above the inferior colliculus were removed. After surgery, the animal was placed in a soundproof chamber for electrophysiological experiments. The animal's body temperature was kept at a constant 37 °C throughout the experiment with the use of a feedback controlled heating pad. The anesthetic status was examined approximately every 40 min by clamping the animal's tail with forceps. When the animal showed any response to the tail clamping, additional dosages of ketamine (25 mg/kg) and xylazine (1 mg/kg) were injected to maintain the anesthetic level (Yan and Ehret, 2002).

2.3. Acoustic stimulation

Pure tone bursts (60 ms duration with 5 ms rise and fall times) were used as acoustic stimuli. Acoustic signals were first synthesized through a wave generator (TG6) by SigGen software and played back by BrainWare software (Tucker–Davis Tech., FL). Digital signals were then converted into analog sinusoidal waves through a digit-analog converter (DA3) and an anti-alias filter (FT6). The output amplitude of the sinusoidal waves