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Research Paper

# Strain-specific differences in the development of neuronal excitability in the mouse ventral nucleus of the trapezoid body

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ABSTRACT

This investigation compared the development of neuronal excitability in the ventral nucleus of the trapezoid body (VNTB) between two strains of mice with differing progression rates for age-related hearing loss. In contrast to CBA/Ca (CBA) mice, the C57BL/6J (C57) strain are subject to hearing loss from a younger age and are more prone to damage from sound over-exposure. Higher firing rates in the medial olivocochlear system (MOC) are associated with protection from loud sounds and these cells are located in the VNTB. We postulated that reduced neuronal firing of the MOC in C57 mice could contribute to hearing loss in this strain by reducing efferent protection. Whole cell patch clamp was used to compare the electrical properties of VNTB neurons from the two strains initially in two age groups: before and after hearing onset at ~ P9 and ~P16, respectively. Prior to hearing onset VNTB neurons electrophysiological properties were identical in both strains, but started to diverge after hearing onset. One week after hearing onset VNTB neurons of C57 mice had larger amplitude action potentials but in contrast to CBA mice, their waveform failed to accelerate with increasing age, consistent with the faster inactivation of voltage-gated potassium currents in C57 VNTB neurons. The lower frequency action potential firing of C57 VNTB neurons at P16 was maintained to P28, indicating that this change was not a developmental delay. We conclude that C57 VNTB neurons fire at lower frequencies than in the CBA strain, supporting the hypothesis that reduced MOC firing could contribute to the greater hearing loss of the C57 strain.



## 1. Introduction

Age related hearing loss is the most common sensory deficit (US National Institute on Deafness and Other Communication Disorders, http://www.nidcd.nih.gov/health/statistics/hearing.html, accessed 25/11/16) with multiple and complex underlying mechanisms. Studies of hearing and hearing loss in mice have identified many genes which are associated with deafness or a predisposition to hearing loss (Lenz and Avraham, 2011; Moser et al., 2013; Richardson et al., 2011; Steel and Kros, 2001). The mouse strain C57BL/6J (C57) has long been studied as a model of accelerated hearing loss (Mikaelian et al., 1974; Mikaelian, 1979; Henry and Chole, 1980). C57 mice show raised thresholds to high frequency sound from 1 to 3 months of age, with near complete degeneration (especially in high frequency regions) of inner and outer hair cells by 26 months (Mikaelian, 1979; Spongr et al., 1997; Sonntag et al., 2009). A hypomorphic allele for the hair cell stereociliary tip-link protein cadherin23 (*Cdh23*$^{Ahl}$) was identified as a major causative factor in the C57's rapid progression of hearing loss (Johnson et al., 1997; Noben-Trauth et al., 2003). Hair cell death in the C57 occurs via B-cell lymphoma 2 homologous antagonist killer mediated apoptosis (Someya et al., 2009) but the mechanism linking the *Cdh23*$^{Ahl}$ mutation with apoptotic pathways is unclear. In addition to hair cell death, the C57 mice also exhibit changes in neuronal characteristics in central auditory structures (Willott et al., 1992, 1994; Willott and Bross, 1996; O'Neill et al., 1997; Zettel et al., 2007; Sonntag et al., 2009) though it is unclear whether this is secondary to the loss of peripheral thresholds.

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However, there is clear evidence of neurological dysfunction in the medial olivocochlear efferent system (MOC) in the C57 that precedes measurable loss of peripheral thresholds, and may predispose the ear to hearing loss (Maison and Liberman, 2000; Frisina et al., 2007). The MOC forms the descending arm of an efferent feedback loop which tunes the sensitivity of the cochlea according to the level of incident sound (for a schematic see Liberman and Guinan, 1998). MOC activity reduces the sensitivity of the cochlea by acting directly on the outer hair cells (OHCs). This serves to increase the dynamic range of the auditory nerve during persistent background noise (Galambos, 1956; Dewson, 1967). MOC efferents inhibit OHCs via nicotinic receptors coupled to calcium-activated potassium channels (Elgoyhen et al., 1994). $K_{Ca}$ hyperpolarizes the OHCs, decreasing their voltage-dependent length change in response to incident sound and reducing the force they lend to boost the basilar membrane motion as part of the 'cochlear amplifier' (Zha et al., 2012). Most MOC efferent neurons in mice have their cell bodies in the ventral nucleus of the trapezoid body (VNTB), which is within the brainstem superior olivary complex (Brown and Levine, 2008) and they are driven by multipolar neurons in the ventral cochlear nucleus (de Venecia et al., 2005; Darrow et al., 2012). Besides MOC neurons, the VNTB contains non-olivocochlear neurons that project to other auditory regions in the brainstem (Warr and Beck, 1996). Frisina et al. (2007) demonstrated that the MOC efferent system of the C57 was less effective than that of the CBA strain. This deficit is present from an early age, prior to any detectable pathology or significant reduction of hearing thresholds in the C57. This suggests the hypothesis that a MOC deficit within the C57 strain could be due to central changes, for example by altered excitability of the VNTB. Here we compare the electrophysiological characteristics of neurons in the VNTB in pre- and post-hearing CBA and C57 mice, to test whether strain-specific change in excitability of the VNTB could help explain the differing strengths of the MOC reflex. Our results show slower APs and reduced firing rates which support this hypothesis.

## 2. Materials and methods

Animal experiments were performed in accordance with the UK Animals (Scientific Procedures) Acts of 1986. Mice sacrificed between P8 and P18 were taken from colonies housed at the University of Leicester (UK): C57BL/6J colony founders were purchased from Jackson Laboratories (UK) and supplemented from Charles River (UK). CBA/Ca colony founders were from Harlan (UK). Animals aged P8-10 and P15-P18 from both strains were killed by decapitation (age groups henceforth referred to by their median as 'P9' and 'P16' groups, respectively). Three older C57 mice (P26-P30, henceforth 'P28' group) were also used and were decapitated under isoflurane anaesthesia. They were housed at the Ludwig Maximilians University (Munich, Germany) with procedures reviewed and approved by the Bavarian district government (TVV AZ: 55.2-1-54-2532-38-13), performed in accordance with European Communities Council Directive (2010/63/EU). The brain was rapidly removed while submerged in a frozen slush of low sodium artificial cerebral spinal fluid (aCSF, contents in mM: 2.5 KCl, 10 glucose, 250 sucrose, 1.25 $NaH_2PO_4$, 0.5 ascorbic acid, 26 $NaHCO_3$, 4 $MgCl_2$, 0.1 $CaCl_2$). Transverse 150 or 200 μm slices containing the superior olivary complex were taken as previously described (Barnes-Davies and Forsythe, 1995) using a DTK-1000 Microslicer (Dosaka, Kyoto, Japan) and transferred to a recovery chamber containing recording aCSF (composition, mM: 125 NaCl, 2.5 KCl, 10 glucose, 1.25 $NaH_2PO_4$, 2 sodium pyruvate, 3 myo-inositol, 0.5 ascorbic acid, 26 $NaHCO_3$, 1 $MgCl_2$, 2 $CaCl_2$), maintained at 37 °C by placing the chamber in a water bath. Slices were incubated for 45–60 min prior to recording, bubbled with 95% $O_2$, 5% $CO_2$ (to maintain pH 7.4). After this time, the slice chamber was removed from the bath and slices were maintained at room temperature for up to 6 h before recording. One slice from which recordings were to be made was transferred to a Peltier controlled environmental chamber (35 °C, perfusion with recording aCSF bubbled with 95% $O_2$, 5% $CO_2$ at 1 ml $min^{-1}$) on the stage of a Zeiss Axioskop microscope. Cells were visualised (63× NA 0.9 Zeiss Achroplan water immersion objective) using differential interference contrast optics. A charge coupled device (KP-M2RP, Hitachi) using WinTV software (Hauppauge Computer Works) was used for image acquisition. Thick walled borosilicate glass capillaries (GC150F-7.5, Harvard Apparatus Ltd, Edenbridge, UK) were pulled to form patch pipettes (resistance 2.5–5 MΩ), using a two-stage vertical puller (PC10; Narishige). Pipette solution contained (in mM): 97.5 potassium gluconate, 32.5 KCl, 10 HEPES, 5 EGTA, 1 $MgCl_2$, 5 sodium phosphocreatine (pH adjusted to 7.2 with KOH, osmolarity was around 280 mOsm).

Sampling was performed at 50kHz using an analogue to digital converter (Digidata 1322a, Molecular Devices) and filtered at 10kHz. Most recordings were made using a Multiclamp 700B amplifier using Clampex 9.2 (Molecular Devices, Sunnyvale, CA, USA). Some P9 CBA recordings were made with an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA, USA). Additional recordings were also made from visually identified VNTB neurons of P26-P30 C57 mice (Olympus BX51WI microscope) using an EPC10/2 HEKA amplifier, sampling at 50 kHz and filtering between 2.9 and 10 kHz, maintained at 37 °C by an inline feedback temperature controller and heated stage (TC344B, Warner Instruments, Hamden, CT, USA). Pipettes had an open tip resistance of around 3 MΩ, pulled using a DMZ Universal puller (Zeitz). The junction potential was calculated to be 11.4 mV using JPCalc software, and the presented voltages adjusted offline by −11 mV to account for this. The data presented here were collected from 6 P9 CBA, 8 P16 CBA, 6 P9 C57, 7 P16 C57 and 3 P28 C57 animals. Neurons were excluded from analysis if the resting potential was more positive than −50 mV or if the current required to hold at −70 mV was greater than −500pA, since this represented a large non-specific leak and potential intracellular calcium over-load. For voltage clamp recordings and current-voltage relationships, series resistances (Rs) were less than 10 MΩ, compensated by 70%. For comparison of smaller, low-voltage activated currents (steps to −21 mV) shown in Fig. 4, recordings with Rs up to 14 MΩ compensated by 70% were included. Mean holding currents for IV data: P9 CBA: 188.5 ± 52.6pA (n = 17), P16 CBA: 107.7 ± 58.3pA (n = 15), P9 C57 60.1 ± 57.2pA (n = 20), P16: 141.69 ± 43.54pA (n = 28). Holding currents for current clamp data were less than 100pA.

### 2.1. Data analysis and statistics

Data were analysed in Clampfit version 10.2.018 (Molecular Devices, CA, USA), Matlab (Mathworks, MA, USA) and Stimfit (Guzman et al., 2014). Statistical analysis was performed using SigmaPlot 13 (Systat Software, Inc.) or Origin (OriginLab, MA, USA). Figures were assembled in CorelDraw. Statistical comparisons were made between age and strain groups with *t*-test or ANOVA with Student Newman Keuls (SNK) post-hoc test if normally distributed (following Shapiro-Wilk (SW) test for normality), or Mann-Whitney rank sum or Kruskal-Wallis ANOVA on ranks (KW) with Dunn's post-hoc test, if SW failed. A significance threshold of $p < 0.05$ was used. Values are reported in text as mean ± standard error of the mean (SEM) unless stated otherwise. N-values refer to total number of neurons, from at least 3 animals unless otherwise indicated. Voltage thresholds were identified by determining the point at which the greatest change was observed in the first derivative of the voltage trace. This is a measure of the greatest change in the rate of change from one sample to the next.