Neuroscience Letters 541 (2013) 173–178

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Odor enrichment sculpts the abundance of olfactory bulb mitral cells

Melissa Cavallin Johnson[a,*], K.C. Biju[b,1], Joshua Hoffman[b,2], Debra Ann Fadool[b,c]

[a] Department of Biology, University of West Georgia, Carrollton, GA, United States
[b] Department of Biological Science, The Florida State University, Tallahassee, FL, United States
[c] Program in Neuroscience and Molecular Biophysics, The Florida State University, Tallahassee, FL, United States

HIGHLIGHTS

- Early postnatal Kv1.3-null mice have a high density of mitral cells in the olfactory bulb.
- Enrichment with olfactory or trigeminal stimuli reduced mitral cell number.
- Mitral cell area is decreased with olfactory and increased with trigeminal stimulation.

ARTICLE INFO

Article history:
Received 28 November 2012
Received in revised form 8 February 2013
Accepted 9 February 2013

Keywords:
Kv1.3
Plasticity
Odor enrichment
Trigeminal

ABSTRACT

Mitral cells are the primary output cell from the olfactory bulb conveying olfactory sensory information to higher cortical areas. Gene-targeted deletion of the *Shaker* potassium channel Kv1.3 alters voltage-dependence and inactivation kinetics of mitral cell current properties, which contribute to the "Super-smeller" phenotype observed in Kv1.3-null mice. The goal of the current study was to determine if morphology and density are influenced by mitral cell excitability, olfactory environment, and stage of development. Wildtype (WT) and Kv1.3-null (KO) mice were exposed to a single odorant (peppermint or citralva) for 30 days. Under unstimulated conditions, postnatal day 20 KO mice had more mitral cells than their WT counterparts, but no difference in cell size. Odor-enrichment with peppermint, an olfactory and trigeminal stimulus, decreased the number of mitral cells in three month and one year old mice of both genotypes. Mitral cell density was most sensitive to odor-stimulation in three month WT mice. Enrichment at the same age with citralva, a purely olfactory stimulus, decreased cell density regardless of genotype. There were no significant changes in cell body shape in response to citralva exposure, but the cell area was greater in WT mice and selectively greater in the ventral region of the OB in KO mice. This suggests that trigeminal or olfactory stimulation may modify mitral cell area and density while not impacting cell body shape. Mitral cell density can therefore be modulated by the voltage and sensory environment to alter information processing or olfactory perception.

Published by Elsevier Ireland Ltd.

1. Introduction

Olfactory sensory information is transported by olfactory sensory neurons (OSNs) that synapse with mitral cells in the olfactory bulb (OB), which in turn relay the information to the piriform cortex where olfactory perception occurs [2]. The majority (60–80%) of the outward current in mitral cells is carried by the *Shaker* voltage-gated potassium channel, Kv1.3 to regulate the resting membrane potential and cell excitability [1,7]. Mitral cells recorded from Kv1.3-null (KO) mice exhibit altered biophysical properties [7,14] and notable anatomical changes [1] that underlie an observed "Super-smeller" phenotype, in which the KO mice have an increased ability to detect and discriminate odors [8]. Mitral cell responses to odors are plastic and can be modified based on environmental cues such as reward learning [6]. Passive stimulation with odorants enhances olfactory discrimination and memory [11,17] Odor enrichment also accelerates the refinement of olfactory maps [8,12], while increasing the number of inhibitory interneurons in the OB by decreasing cell death in the glomerular and granule cell layers [10,23,24]. Exposure to cyclohexanone or deodorized air for two months decreases mitral cell

Abbreviations: KO, Kv1.3-null (−/−); MOE, main olfactory epithelium; OB, olfactory bulb; OSN, olfactory sensory neuron; PBS, phosphate buffered saline; SEM, standard error of the mean; WT, wildtype.

* Corresponding author at: University of West Georgia, Department of Biology, 1601 Maple Street, Carrollton, GA 30118, United States. Tel.: +1 678 839 4043; fax: +1 678 839 6548.
E-mail address: mcavalli@westga.edu (M.C. Johnson).
[1] Current address: Department of Medicine, University of Texas Health Science Center, South Texas Veterans Health Care System, Audie L Murphy Division, San Antonio, TX 78229, United States.
[2] Current address: Vanderbilt Vision Research Center, 111 21st Ave. South, 525 Wilson Hall, Nashville, TN 37203, United States.

0304-3940/$ – see front matter. Published by Elsevier Ireland Ltd.
http://dx.doi.org/10.1016/j.neulet.2013.02.027

size in rats when compared to control animals exposed to normal laboratory air, and this effect is age dependent [9,15,16]. It is not clear how voltage-gated activity of the mitral cell influences these enrichment-induced responses. Previous studies indicate that voltage-gated activity influences anatomical changes at the level of the OSN in an odor-receptor specific manner [4]. It is plausible that combined odor-enrichment and voltage-gated activity may concomitantly induce changes in mitral cell numbers.

We proposed that chronic odor enrichment under conditions of a highly sensitive olfactory system, as in our KO "Super-smeller mice", would result in anatomical changes at the level of the mitral cell. Mitral cell density (number), area, and shape were compared between WT and KO mice in response to an olfactory stimulus, citralva, and in response to peppermint, which stimulates the olfactory and trigeminal systems [5]. We chose to use peppermint in addition to a purely olfactory stimulus to parallel our previous studies comparing WT and KO mice [7]. There are very few studies that have accessed OB structure and function in response to trigeminal stimuli [19,23]. We also chose to analyze different developmental ages (P20, 3 mo, and 1 yr) because these time points showed the most morphological plasticity in OSNs [4]. Previous work has demonstrated that OSNs are highly plastic, and anatomical changes are influenced by changes in mitral cell activity in KO mice [1] and in response to odor enrichment [4].

2. Materials and methods

2.1. Ethics statement

This work has been carried out in accordance with *EC Directive 86/609/EEC for animal experiments*, American Veterinary Medical Association (AVMA) approved methods, and guidelines set by the National Institutes of Health. This manuscript also adheres to the *Uniform Requirements for manuscripts submitted to Biomedical Journals*.

2.2. Solutions

All salts and other reagents were purchased from Sigma Aldrich (St. Louis, MO) or Fisher Scientific (Atlanta, GA).

2.3. Animals

All mice were housed at the Florida State University vivarium in accordance with the institutional requirements for animal care. Kv1.3-null mice (KO) were a generous gift from Drs. Leonard Kaczmarek and Richard Flavell (Yale University, New Haven, CT, USA) and were generated by excision of the Kv1.3 promoter region and one-third of the 5′ coding region in a C57BL6/J background [25]. Male and female wildtype (C57BL6/J; WT) and KO mice were maintained under a standard 12/12 h light/dark cycle with *ad libitum* access to 5001 Purina Rodent chow. Following weaning, all mice were housed individually in conventional style rodent cages; room air circulation was standardized at 19 changes/h.

2.4. Odor enrichment

Odor enrichment protocols have been described previously [4,22]. Briefly, three month old and one year old WT and KO mice were exposed to cotton swabs soaked with 200 μl peppermint extract (1:1000; McCormick and Co., Inc., Hunt Valley, MD.). A separate group of three month old WT and KO mice were exposed to cotton swabs soaked with 200 μl citralva (1:1000; Intercontinental Fragrances catalog # RM0429, Houston, TX). Each odorant was presented five sessions per day for 30 days. Cotton swabs were introduced to the testing cage for five 10-min trials separated by a 10-min recovery interval.

2.5. Histochemistry and image analysis

At the completion of the odor enrichment protocol, mice were sacrificed with a lethal dose of pentobarbital and perfused transcardially with phosphate buffered saline (PBS) followed by 4% paraformaldehyde. The OBs were processed as previously [1] and then stained with 0.05% cresyl violet (Fisher Scientific). A Zeiss Axiovert S100 microscope (Jena, Germany) equipped with Hoffman optics was used at 40× magnification to acquire images of the mitral cell layer (Zeiss Axiocam;Oberkochen, Germany) from a randomly chosen side of each OB section. All sections along the rostral–caudal axis from each animal were measured (NIH Image J Software). Mitral/tufted cells chosen for quantification resided along a 250 μm line within a 25,000 μm^2 box for each of four regions within the OB (Fig. 1A). Mitral cell area was normalized to the sample area of the region of the OB containing the mitral cell (Fig. 1A). Cell shape was determined by using a circularity scale of 0–1.00, with 1.00 being a perfect circle, which has been used to describe triangular shaped cells such as pyramidal cells [21] and mitral cells in the accessory olfactory bulb [20]. Data for mitral cell populations were collected by randomly drawing a box with an area of 25,000 μm^2 in each of the dorsal, ventral, lateral, and medial regions of the OB being analyzed (Fig. 1A). Only mitral cells with a prominent nucleus within this defined region were counted and measured, which eliminated duplicating measurements for the same cell across serial sections. All data are presented as the mean ± standard error of the mean (SEM). Due to the size of the measured population and capability of the statistical software (GraphPad Prism, La Jolla, CA), 30 measurements per treatment were randomly analyzed through selection of a random number generator. Data contained in Fig. 1 are compared by anatomical region using a Student's *t*-test and a Bonferroni correction for multiple comparisons for increased stringency ($\alpha \leq 0.0125$). Data contained in Figs. 2 and 3 are analyzed within region using a randomized two-way analysis of variance (ANOVA) with genotype × odor stimulation as factors. Significant main or interactive effects were determined by Bonferroni's post hoc test with adjusted error rate for number of comparisons ($\alpha \leq 0.0125$).

3. Results

3.1. Gene-targeted deletion of Kv1.3 increases the number of mitral cells in the lateral, medial, and ventral regions of the early postnatal OB

Because Kv1.3 is expressed by mitral cells and targeted deletion is known to affect mitral cell function and olfactory behavior, we wondered if there were also concomitant changes in the morphology of the mitral cell in the "Super-smeller" mice. Mitral cell area and density were analyzed from four regions (dorsal, lateral, medial, ventral; 25,000 μm^2 each) on coronal sections of OBs from P20 aged WT and KO mice (Fig. 1A). The mitral cell area was not significantly different across genotype for any sampled region (Fig. 1B–D). However, there was a 1.5 fold increase in the number of mitral cells in the lateral, medial, and ventral regions of KO mice vs. WT mice (Fig. 1B, C, and E). This increase in cell number was only observed in early postnatal mice and was not present by age three months to one year (non-odor stimulated controls, Figs. 2 and 3). This suggests that the increased frequency of action potentials and shorter interspike interval observed in mitral cells in KO mice [7] modifies early mitral cell development by either proliferation or survival, which changes with age.