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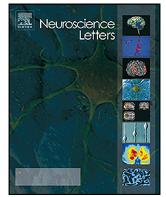

Research paper

Anatomical and molecular consequences of Unilateral Naris Closure on two populations of olfactory sensory neurons expressing defined odorant receptors

Adrien Molinas, Imad Aoudé, Vanessa Soubeyre, Bassim Tazir [1], Hervé Cadiou [2], Xavier Grosmaitre *

Centre des Sciences du Goût et de l'Alimentation, CNRS, INRA, Univ. Bourgogne Franche-Comté, F-21000 Dijon, France

HIGHLIGHTS

- The density of MOR23 and M71 olfactory neurons is affected differently by UNO.
- UNO modulates transduction pathway transcripts levels at the cellular level.
- Effects of UNO will have consequences on odorant coding properties of OSNs.

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ABSTRACT

Mammalian olfactory sensory neurons (OSNs), the primary elements of the olfactory system, are located in the olfactory epithelium lining the nasal cavity. Exposed to the environment, their lifespan is short. Consequently, OSNs are regularly regenerated and several reports show that activity strongly modulates their development and regeneration: the peripheral olfactory system can adjust to the amount of stimulus through compensatory mechanisms. Unilateral naris occlusion (UNO) was frequently used to investigate this mechanism at the entire epithelium level. However, there is little data regarding the effects of UNO at the cellular level, especially on individual neuronal populations expressing a defined odorant receptor. Here, using UNO during the first three postnatal weeks, we analyzed the anatomical and molecular consequences of sensory deprivation in OSNs populations expressing the MOR23 and M71 receptors. The density of MOR23-expressing neurons is decreased in the closed side while UNO does not affect the density of M71-expressing neurons. Using Real Time qPCR on isolated neurons, we observed that UNO modulates the transcript levels for transduction pathway proteins (odorant receptors, CNGA2, PDE1c). The transcripts modulated by UNO will differ between populations depending on the receptor expressed. These results suggest that sensory deprivation will have different effects on different OSNs' populations. As a consequence, early experience will shape the functional properties of OSNs differently depending on the type of odorant receptor they express.

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1. Introduction

Olfactory sensory neurons (OSNs) represent the first step of the olfactory system. Located in the olfactory mucosa lining the nasal cavity, they convert the chemical information (i.e. odorant molecules) into an electrical signal that is sent to the brain. The diversity of odorant molecules detected is extremely vast thanks to a very large olfactory receptors' (ORs) repertoire. ORs are G protein-coupled receptors and the vast majority of OSNs rely on the cAMP transduction pathway [33]. This pathway involves activation of olfactory-specific G-protein (Golf), adenylyl cyclase type III (ACIII), and cyclic nucleotide-gated (CNG) channel [20]. In the

* Corresponding author at: Centre des Sciences du Goût et de l'Alimentation, UMR 6265 CNRS – Université de Bourgogne-Franche-Comté – UMR1324 INRA, 9E boulevard Jeanne D'Arc, 21000 Dijon, France.
E-mail address: xavier.grosmaitre@u-bourgogne.fr (X. Grosmaitre).
[1] Present address: Max Planck Research Unit for Neurogenetics, Max-von-Laue-Straße 3, D-60438 Frankfurt am Main, Germany.
[2] Present address: Team molecular determinants of pain, Institut des Neurosciences Cellulaires et Intégratives (INCI), CNRS UPR3212, 5 Rue Blaise Pascal, F-67084 Strasbourg, France.

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mouse, the repertoire of ORs is encoded by >1000 genes [5]. Each individual OSN expresses only one allele of a single OR [8,21,27]. The expression of each OR is restricted to broad, partially overlapping, zones in the olfactory epithelium (OE) [22,24,29,31,35]. Within a zone, different populations of ORs show a multitude of spatial expression patterns, from even antero-posterior distribution to localized peak distribution [4]. The zonal patterning of ORs in the OE is maintained during normal or lesion-induced neurogenesis [18,23]. However, the effects of experience, especially sensory deprivation on the spatial repartition or density of OSNs within a particular zone is unknown.

Exposed to the environment, the olfactory epithelium is continuously regenerated throughout life. OSNs regenerate from dividing basal cells in order to maintain the epithelial homeostasis [for review,see 26]. This continuous regeneration makes the olfactory system prone to activity-dependent development and modulation. To investigate the role of activity-dependent modulation, Unilateral Naris Occlusion (UNO) has been widely used. It induces modifications of sensory inputs into the two nostrils: the closed side deprived of airflow and odor stimulation, while the open side, being the only source of air during breathing, experiences greater strain than the normal airflow and odorant stimulation. UNO has been shown to cause structural, molecular and functional changes in the MOE [1,2,11,12,14,15,17,30,36–38], as well as in higher structures [9][for review see 9]. More specifically, the MOE's activity is modulated by UNO: the closed side showed increased amplitude and slower kinetics in odorant-induced electroolfactogram (EOG) signals as compared with the open side [36]. UNO also alters OR gene expression in both the closed and open sides [11,32] while odorant receptor gene expression is diversely modulated depending on the OR [38]. At the cellular level, UNO induces individual modulation of odorants' induced activity in single OSNs [17]. Since individual OSN's activity is modulated, it is essential to measure molecular changes at the cellular level: does experience modulate gene expression in individual cells and consequently modify functional characteristics of OSNs?

To address this question, we report here the consequences of UNO on two populations of OSNs' expressing defined odorant receptors: MOR23 and M71. Using gene-targeted mice expressing the GFP under the promoter of these receptors, we compared the density of GFP labeled neurons in open, closed and control mice. For the molecular consequences at the cellular level, we harvested dissociated GFP labeled cells and quantify the mRNA levels for transduction pathway proteins. We conclude that experience induced plasticity will have different effects on different OSNs' population.

2. Material and methods

2.1. Animals

Gene targeted MOR23-IRES-tauGFP mice and M71-IRES-tauGFP were used to monitor respectively the MOR23 and M71 neurons [3,34]. Animals were allowed access to food (A04 diet, Safe, France) and water ad libitum and were kept on a 12 h light/dark cycle, with a 22 °C constant temperature. All experiments were carried out in accordance with the European Union Directive (Dir. 2010/63/UE) and resulting French regulations. Accordingly, all experimental protocols were approved by the Université de Bourgogne ethic committee.

2.2. Naris occlusion

On the second day after birth, mice were anesthetized by hypothermia. Unilateral naris occlusion (UNO) was performed by cauterization using a low temperature cauterizer (Fine Science Tools, Foster City, CA, USA). Pups were then placed on a heating plate until they recover from anesthesia. Lidocain 5% was deposited on the scar before returning the pup to their home cage. Mice (males and females) were examined 3 weeks later by visual inspection under a stereo microscope. Only mice with successful occlusion were used for further analysis, with age-matched untreated mice as control.

2.3. Tissue harvesting

On postnatal day 21, mice were deeply anesthetized by injection of ketamine HCl and xylazine (150 mg/kg and 10 mg/kg body weight, respectively), and then decapitated. The head was immersed in cold ACSF solution, which contained (in mM): NaCl 124, KCl 3, $MgSO_4$ 1.3, $CaCl_2$ 2, $NaHCO_3$ 26, NaH_2PO_4 1.25, glucose 15; pH 7.6 and 305 mOsm. The olfactory mucosa attached to the nasal septum and to the dorsal recess were rapidly removed: mucosa from each nostril was used either for qPCR analysis or flat mount preparation. Both sides from each animal were not necessarily used for the same experiments: i.e. for cell dissociation only one side per mouse was used, the other side was either used for flat mount or discarded.

2.4. Flat mount preparation and OSNs counting

These experiments were implemented as described in [6]. Briefly, harvested epithelia were fixed with PFA 4% in PBS, mounted, and visualized using a GFP filter (BP460-490) equipped upright microscope. Images covering the entire epithelium were taken and assembled to reconstitute the whole tissue in a unique picture. The neuronal density was then calculated as the ratio of the number of GFP+ neurons against the surface identified as the area including all visible GFP+ neurons.

2.5. Dissociation and cell collection for molecular biology

These experiments were carried out as published earlier [6]. Briefly, olfactory epithelia were enzymatically and mechanically dissociated; then cells were allowed to settle and attach to polyethylenimine coated glass and washed. Single GFP cells were collected under fluorescent light with borosilicate glass pipette. Collected OSNs were placed in 3 μl lysis and stabilization buffer prior to storage at −80 °C. 1 μl of medium is collected as negative control.

For preparation of cDNA, all processes were carried out in one tube to minimize sample loss. Samples were treated with 0.2U Shrimp Dnase and heated to 37 °C for 10 min. Reverse transcription (RT) was carried out using 115U of Superscript III reverse transcriptase (Invitrogen) and random hexamers.

qPCR were performed as previously published [6]. The following gene-specific primers were designed with Primer express 3.0 software and their specificities analyzed using Primer-Blast: *Olfr16:* forward: 5′ CTTGTTGGTTTGTGGGTCTTTAGG 3′, reverse: 5′ CAAAAGGGCAAATGGAACATG 3′; *Olfr151*: foward: 5′ CCATCTGTCAACCCTTGCTTTAT 3′; reverse: 5′ TCAACATGAGGCCAGTCTCAAT 3′; *ACIII:* forward: 5′ AGGCGACTGTTACTACTGCATCTG 3′, reverse: 5′ ACGTACGAGATGGCCTCTACCA 3′; *Cnga2*: forward: 5′ GGCACCAAAAAGAAATTTGAACTG 3′, reverse: 5′ CAGGCTCTGGCTACCAACAGA 3′; *Pde1c: forward:* 5′ GTCCCAGCGTCGTGATTAGC 3′, reverse: 5′ TCATGACATCTCGAGCAAGTCTTT 3′; *Gapdh:* forward: 5′ CCTGGAGAAACCTGCCAAGTAT 3′, reverse: 5′ CTGTTGAAGTCGCAGGAGACAA 3′

The relative expression levels of genes of interest were analyzed using $2^{-\Delta\Delta Ct}$ method by normalization with GAPDH gene expres-

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