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Quantitative proteomics reveals olfactory input-dependent alterations in the mouse olfactory bulb proteome

Hao-Long Zeng[a], *Xiaoping Rao*[b], *Lei-Ke Zhang*[a], *Xiaolu Zhao*[a], *Wei-Ping Zhang*[a], *Jie Wang*[b], *Fuqiang Xu*[b,*], *Lin Guo*[a,**]

[a]*State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, China*
[b]*State Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Wuhan, China*

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ABSTRACT

Olfactory sensory information is processed and integrated by circuits within the olfactory bulb (OB) before being sent to the olfactory cortex. In the mammalian OB, neural activity driven by external stimuli can lead to experience-dependent changes in structures and functions. In this study, quantitative proteomics techniques were employed to study proteome-wide changes in the OB under four levels of neural activity (from low to high): devoid of peripheral input (using a transgenic model), wild-type control, and short-term and long-term odor exposures. Our results revealed that proteins related to various processes were altered in the OBs of odor-deprived and odor-stimulated mice compared to the wild-type controls. These changes induced by odor stimulation were quite different from those induced by a deficit in peripheral olfactory inputs. Detailed analysis demonstrated that metabolic process and synaptic transmission were the most commonly altered pathways and that the effects of peripheral deprivation were more profound. Our comparative proteomics analysis indicated that olfactory deprivation and odor exposure lead to different alterations in the OB proteome, which provides new clues about the mechanisms underlying the olfactory deprivation- or odor stimulation-induced plasticity of OB function and organization.

Biological significance
By combining quantitative proteomics, bioinformatics and WB/IHC analysis, this study reports the results of the first comparative study on proteome-wide changes in the olfactory bulb under different levels of olfactory input. Odor deprivation and stimulation induced proteomic changes clearly demonstrate significant metabolic shifts and alterations on synaptic transmission. This quantitative system biology study leads to a new level of

Abbreviations: OB, olfactory bulb; GCLs, granule cell layers; EPLs, external plexiform layers; TH, tyrosine hydroxylase; OCNCX, olfactory cyclic nucleotide-gated channel subunit 1 knockout; LT, long-term odor stimulation; ST, short-term odor stimulation; GO, gene ontology; SVC, synaptic vesicle cycle; WB, Western blot; IHC, immunohistochemistry.

* *Correspondence to:* F. Xu, Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Wuhan 430071, China. Tel.: +86 27 87197091; fax: +86 27 87199543.
** *Correspondence to:* L. Guo, College of Life Sciences, Wuhan University, Wuhan 430072, China. Tel.: +86 27 68753800; fax: +86 27 68753797.
E-mail addresses: fuqiang.xu@wipm.ac.cn (F. Xu), guol@whu.edu.cn (L. Guo).

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understanding in the development of olfactory bulb plasticity induced by odor deprivation or stimulation, and provides many new clues for the olfactory bulb related functional studies.

1. Introduction

In all living things, from bacteria to mammals, detecting chemicals in the environment is critical for survival. This is particularly true for higher eukaryotes, as nearly 4% of the genomes of these organisms are involved in olfactory function [1]. Olfactory system is linked to many important biological functions, including mating success, predator–prey balance, food preferences, orientation, social interactions, and mother care [2]. In the mammalian main olfactory system, olfactory sensory neurons (OSNs) in the epithelium detect odor molecules via the large family of G protein-coupled receptors (GPCRs) and send information to the olfactory bulb (OB) via the projection of axons that terminate at the dendrites of second-order interneurons and output neurons (mitral/tufted cells) in glomeruli [3,4]. Although the odorant receptors comprise the largest family of GPCRs, each OSN expresses only one GPCR, and the OSNs that express the same type of receptors converge into one or two glomeruli in the OB. These precise axon connections enable the about 1000 different types of receptors to be sorted into 1800 glomeruli in the mouse OB [5].

As the initial site involved in the processing of olfactory information in the brain, the OB exhibits a multi-layer cellular architecture. The glomerular modules interact with each other through neuronal circuits via local interneurons: periglomerular cells, short axon cells and granule cells. Periglomerular cells and short axon cells modulate intro- or interglomerular signal processing, contributing to lateral inhibitory/excitatory circuits that regulate functional specificity in small populations of glomeruli [5–7]. Granule cells, which do not have axons, extend a few short neurites toward deeper parts of the granule cell layers (GCLs) and one large dendrite toward the external plexiform layers (EPLs), where it branches extensively [8]. The interneurons are thought to modulate the activity of mitral and tufted cells, which are the principal neurons in mammalian olfactory bulb, to optimize discrimination of external olfactory stimuli by sharpening the odor representation by these projection neurons [8–10].

In addition to the complicated architecture of the OB, its neuronal circuitry is characterized by high adaptability, as it is one of the few structures in the mammalian nervous central system in which there is a continuous supply of newly generated neurons [11]. This neural activity is mainly driven by external stimuli, which can lead to experience-dependent morpho-functional changes in adult circuits, thus allowing ongoing integration of new and different smells [12]. Previous studies have suggested that sensory experiences such as olfactory enrichment or deprivation influence OB organization and function [13–15]. A particularly striking example of this phenomenon is provided by the rapid down-regulation of the catecholamine biosynthetic enzyme tyrosine hydroxylase (TH) in dopamine neurons intrinsic to the OB that has been observed in many odorant deprivation studies employing unilateral naris closure in neonatal rats [16,17]. In addition to the effects on OB neural transmission, energy demands in OB could also be affected by external stimuli energy, as odor information processing elicits high energy demands on activated glomeruli [18,19].

Olfactory dysfunctions or anosmia are common symptoms associated with many neurological diseases, such as Alzheimer's disease, Parkinson's disease and major depression. The biochemical and molecular mechanisms linking the olfactory system, and especially the OB, to these neurological diseases are not clear [20,21]. Studies on OB function and organization are traditionally conducted following odor stimulation or olfactory deprivation by means of a hypothesis-driven approach focusing on biochemical changes in one or a few pathways [22]. However, because olfactory function/dysfunction involves the interactions of multiple cellular pathways (synaptic transmission, metabolic process, transcription regulation etc.), it is necessary to study the olfactory function/dysfunction from a global and integrative point of view.

To identify proteins associated with olfactory function, in this study, we employed quantitative proteomic techniques to study proteome-wide alterations in the OB under four neural activity levels, from low to high: devoid of peripheral input (using a transgenic model), a wild-type control, and short-term and long-term odor exposures. Through stable isotope dimethyl labeling coupled with high-resolution nano-liquid chromatography–mass spectrometry (LC–MS), we were able to generate large-scale quantitative proteomics data from ex vivo OBs. Western blot (WB) and immunohistochemistry (IHC) analyses were performed on selected proteins, providing additional evidence validating the MS-based quantification results. Our data revealed that proteins related to various processes were altered in the OBs of odor-deprived and odor-stimulated mice compared with wild-type mice, and the changes induced by odor stimulation (both short and long terms) were quite different from those induced by deficits in peripheral olfactory inputs. Bioinformatics analysis demonstrated that the synaptic transmission and metabolic process are the most commonly altered pathways under all three conditions and that the effects of peripheral deprivation are more profound. Our functional study by magnetic resonance spectroscopy (MRS) using a ^{13}C isotopic labeled glucose further validated our conjecture. Our quantitative proteomic analysis may provide new clues about the mechanism underlying the peripheral olfactory input-dependent plasticity of OB organization, leading to a new level of understanding of OB function.

2. Materials and methods

2.1. Animals

Olfactory cyclic nucleotide-gated channel subunit 1 knockout (OCNCX) mice are a well-defined model of olfactory deprivation. The olfactory cyclic nucleotide-gated ion channel is necessary for the olfactory sensory nerve to generate odor-induced action potentials, rendering the OCNCX mice essentially anosmic [22].