

Peptidergic innervation of the olfactory bulb: a sleep/wake-regulatory route through the nose

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Olfactory epithelium receptor neurons in the nasal cavity, which are exposed to the external environment, reach the olfactory bulb (OB), representing a direct port of entry to the brain. Through retrograde axonal transport, pathogens, toxins and misfolded proteins can reach brain cell groups which innervate the OB and result in functional alterations. Indeed, influenza virus nasal instillation was found to target brainstem and hypothalamic cell groups and result in narcoleptic-like sleep/wake changes [1]. These cell groups included the wake-promoting orexin (OX)-containing neurons, and the sleep-promoting melanin-concentrating hormone (MCH)-containing neurons [1]. Orexinergic innervation of the OB has been reported, but OX and MCH neurons innervating the OB have never been visualized. OX immunoreactivity in the mouse olfactory receptor neurons has been ascribed to the olfactory mucosa. Sources of input to the OB have been studied [2] before the discovery of OX in 1998. Orexinergic innervation of the prefrontal cortex is instead well established. Aim of this study was to reveal OX- and MCH-containing neurons projecting to the OB. Unilateral injections of the retrograde fluorescent tracer Fluoro-Gold (FG) confined to the OB of adult mice were combined with immunophenotyping and quantitative analysis of retrogradely labeled neurons. The findings were compared with those obtained after FG injections in the prefrontal cortex. Following FG injections in the OB, labeled neurons were found in the ipsilateral lateral hypothalamus, and included intermingled OX-A- or MCH-immunoreactive cells. About 8% of orexinergic neurons were labeled when the tracer was confined to the OB. This proportion increased ($13 \pm 2.49\%$) in cases in which a faint halo of tracer diffusion to the lateral portion of the prefrontal cortex was observed. Preliminary data indicate retrograde labeling from the OB of almost 15% of MCH-containing neurons. The findings demonstrate that OX and MCH neurons reach the OB directly, thus providing to environmental agents a route to sleep/wake-regulatory nodes via the nasal cavity.

References

- [1] Tesoriero et al. (2016) H1N1 influenza virus induces narcolepsy-like sleep disruption and targets sleep-wake regulatory neurons in mice. *PNAS*; 113:E368-E377.
- [2] Shipley et al. (1984) The connections of the mouse olfactory bulb: a study using orthograde and retrograde transport of wheat germ agglutinin conjugated to horseradish peroxidase *Brain Res Bull*; 12:669-688.

Keywords

Orexin/hypocretin; MCH; connectomics; olfactory bulb; sleep.