



# Ciliary neurotrophic factor (CNTF) in the olfactory system of rats and mice

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## Summary

The rodent olfactory system is a regarded model for the relationship between neurotrophic factors, their receptors, and their compound influence on the notable lifelong neuroplasticity occurring in this sensory system. It was known that high amounts of ciliary neurotrophic factor (CNTF), a hematopoietic cytokine, can be found in the olfactory bulb. In the awarded work, a detailed cellular characterization of CNTF-localization in the olfactory system was obtained. The results demonstrated CNTF-immunoreactivity in olfactory ensheathing cells, newborn interneurons in the olfactory bulb, and in a subpopulation of mature olfactory sensory neurons in the olfactory epithelium. Three-dimensional reconstructions of CNTF-immunoreactive axonal bulbar projections of these neurons revealed an ordered bilaterally symmetric pattern. This finding implies a potential connection between neuronal CNTF-expression in the olfactory epithelium and olfactory information processing.

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The olfactory system is the functional basis for odour recognition in metazoans. The sense of smell was the first sensory system that evolved and its constituent molecular and anatomical components are highly conserved throughout phylogeny.

Olfactory sensory neurons (OSN), the primary sensory neurons of the olfactory epithelium, are the only neurons that are exposed to the external

environment. Noxious airborne compounds and infections can cause massive degeneration of OSN. Throughout the lifetime of an individual, asymmetric divisions of neuronal precursor cells in the epithelium give rise to new OSN, thereby counterbalancing neuron destruction and preventing loss of the sense of smell. Furthermore, all mammalian species display divisions of neuronal stem cells in the subventricular zone of the lateral ventricle, which maintain a lifelong delivery of interneurons to the olfactory bulb. Both types of newly generated neurons need to be integrated into existing neuronal networks of the olfactory system. Such integration encompasses highly regulated processes

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including neuronal differentiation, axonal pathfinding and synaptogenesis. Additionally, neuroplasticity is required for the enhancement of individual synaptic connections in the context of olfactory learning processes.

Neurotrophic factors are essential regulators of neuroplastic processes. Ciliary neurotrophic factor (CNTF) is one of numerous neurotrophic factors that are constitutively highly expressed in the olfactory system. A role of the factor in olfactory neuroplasticity was postulated, but has remained elusive yet. To date, CNTF has been localized in peripheral and central glial cells. Approximately 3% of the human population lack expression of functional CNTF, and recent studies suggest a possible connection between CNTF-deficiency and the etiology of neurodegenerative diseases (e.g. multiple sclerosis, motoneuron diseases). Hence, CNTF has attracted much attention in the past years because of both its neurobiological and possible clinical importance.

Since it is easily accessible to experimental manipulation, the peripheral olfactory system is ideally suited to elucidate possible roles of CNTF in neuroplasticity. A necessary basis for such efforts is the exact knowledge of the cellular and subcellular localization of the factor. Since previous immunolocalization efforts had returned ambiguous results, perhaps owing to compromised specificity of CNTF antibodies under individual reaction conditions, the first objective of the current study was to optimize immunoreaction conditions in order to specify strict identification criteria for CNTF-immunoreactive (CNTF-ir) structures and to obtain an unequivocal CNTF-localization. A combinatorial panel of different tissue fixation protocols, immunohistochemical methods and anti-CNTF-antibodies was tested in rat and wildtype-mice specimen, and the immunoreactivity patterns were compared. Immunoreactions on tissue from CNTF-knockout mice and reactions using CNTF-antisera preadsorbed with recombinant CNTF served as controls.

Thereafter, the identity of CNTF-ir cells in the olfactory system was determined using different marker antibodies in double immunolocalization experiments and immunoelectron microscopy. In the olfactory bulb, CNTF-localization could be demonstrated in the cytoplasm and nuclei of olfactory glial cells (ensheathing cells) of the olfactory and accessory olfactory nerves and in astrocytes of the lateral olfactory tract. These results are of importance for further studies about possible roles of CNTF in axon growth-promoting features of ensheathing cells and their therapeutic use to bridge defects in the CNS. Additionally, individual polysialylated neural cell adhesion mo-

lecule-positive cells displayed CNTF-immunoreactivity (CNTF-Ir). This finding is the first indication of a possible function of CNTF in the terminal differentiation of newly generated interneurons in the olfactory bulb.

In the olfactory epithelium, individual intensely CNTF-ir cells were observed. Double immunofluorescence labeling documented that these cells represented a small subpopulation of mature OSN, and immunoelectron microscopy showed that CNTF-ir axons established synaptic contacts typical for OSN axons with secondary neurons in olfactory glomeruli.

The second part of the study was designed to further characterize the group of OSNs displaying intense CNTF-Ir. First, we tested whether CNTF-Ir could be associated with a specific and short phase in the life cycle of OSN, in particular an early phase of apoptotic or necrotic cell death. However, double labeling for CNTF and apoptotic markers, and CNTF-immunoreactions at different time points after light chemical lesions of the olfactory epithelium failed to demonstrate increased CNTF-Ir in OSN displaying signs of apoptotic or necrotic death.

The second hypothesis regarding the identity of the CNTF-ir subpopulation of OSNs was based on the fact that OSN express only one specific olfactory receptor protein (ORP) from a pool of several hundred genes in rodents. Each ORP binds to odorant epitopes with high affinity. Therefore, several hundred neuronal subpopulations with differently tuned sensory specificity exist in the olfactory epithelium. A striking feature of all OSN possessing the same ORP is the convergence of their axons onto few specific olfactory glomeruli in the ipsilateral olfactory bulb. The position of these target glomeruli in both olfactory bulbs is bilaterally symmetric. Odour information emanating from airborne chemical compounds, which stimulate different olfactory neuronal subpopulations, are thereby converted into spatial odour maps of activated olfactory glomeruli whose positions are bilaterally symmetric in one animal. To investigate whether CNTF-ir OSN are restricted to certain neuronal subpopulations of the same odour specificity a method was established to identify target glomeruli of CNTF-ir OSN axons. Serial sections of anti-CNTF-immunostained olfactory bulb pairs of female and male adult rats and mice were photographed and digitized. Images were then software-processed to reconstruct three-dimensional bulb pair models, in which spatial positions of glomeruli that receive CNTF-ir olfactory axons were marked. This method enabled us to demonstrate that indeed the majority of CNTF-ir glomeruli are bilaterally

symmetrically located. In addition, the position of these glomeruli exhibited an interindividual similarity. The region where glomeruli innervated by CNTF-ir olfactory axons are mostly located – the ventrolateral olfactory bulb – is known to process urine odours. Quantitative analyses of CNTF-ir glomeruli indicated similar numbers in both olfactory bulbs in the same animal, but different counts interindividually. The findings suggest an association of neuronal sensory specificity and CNTF-Ir. Interindividual differences indicate activity-dependence of CNTF-Ir in individual OSN, implicating the factor in olfactory learning processes.

In summary, the current dissertation revealed the localization of the neurotrophic factor CNTF in

the peripheral olfactory pathway by means of classical anatomical–histological techniques and newly developed software-aided 3D-reconstruction of histologically processed tissue sections. The results yielded further hypotheses regarding the functional relationship of CNTF with olfactory information processing.

#### **Further reading**

- Asan, et al., 2003. *Neuroscience* 120 (1), 99–112.  
Langenhan, et al., 2005. *Neuroscience* 134 (4), 1179–1194.