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学位論文題目	Transient expression of c-kit receptor in the immature projection neurons of the olfactory bulb
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In the mammalian olfactory system, each olfactory neuron expresses only one of about 1,000 different odorant receptor genes. In the olfactory epithelium, the olfactory neurons expressing the same receptor are scattered, but their axons converge into a few topographically fixed glomeruli, the specialized synaptic structures in the olfactory bulb (OB). Consequently, a stereotyped spatial map is constructed on the surface of the OB, in which about 1,800 glomeruli are orderly arranged in mice. The physiological and anatomical studies show that the glomeruli receiving similar odorant molecules are grouped together and form a special domain on the OB.

The information converged in the glomeruli is transmitted to the second-order neurons, mitral and tufted cells in the OB. These neurons project axons caudally and construct the lateral olfactory tract (LOT) on the surface of the ventrolateral telencephalon. The LOT axons eventually sprout collateral branches invading the olfactory cortex and form connections with the third-order neurons. The olfactory cortex consists of morphologically distinct areas such as the anterior olfactory nucleus, the piriform cortex (PC), the olfactory tubercle (OT), the entorhinal cortex and the amygdala. Each second-order neuron projects to the multiple target areas with massive sprouting of collateral branches.

In contrast to the clear rule of the peripheral olfactory projection, the principal of central olfactory projection is obscure. Although a few studies suggest specific targeting of the projections from second-order neurons in the olfactory cortex, there is not a point-to-point topographic relationship between the spatial map on the OB to any of the olfactory cortical areas, as other sensory systems. For example, the retrograde axonal labeling shows that a single cortical region receives inputs from the mitral/tufted cells scattering over the OB. The anterograde axonal tracing also shows that the spatial arrangement in the OB is not reflected in the olfactory cortical areas. Furthermore, there is the evidence that the spatial representation of the OB is already lost in the LOT, in which mitral/tufted cell axons are intermingled randomly regardless of the position of their cell bodies.

In the present study, I demonstrated that c-kit receptor tyrosine kinase is expressed on a fasciculated subset of axons in the LOT at each developmental stage. Their c-kit-expressing cell bodies were distributed in the intermediate zone flanked between the ventricular zone and the mitral cell layer in the OB. BrdU-labeling experiment showed that the newly-differentiated mitral/tufted cells radially migrating in the intermediate zone transiently expressed c-kit so that the expression of c-kit is always fixed in the intermediate zone. These results indicate that mitral/tufted cell axons projecting at the same developmental stage are grouped together and constitute a special assembly within the LOT bundle, regardless of the position of their cell bodies in the OB. The newly elongating c-kit-positive axons usually occupied the ventral surface area in

the LOT. Therefore, there seems to be a developmental gradient in the organization of LOT axons from the dorsal depth to ventral surface. These results together with the previous axonal tracing studies suggest that arrangement of LOT axons is not based on the topographical position of the cell bodies but the developmental status of the axons.

The OT is one of the olfactory cortical areas that receive the late innervation by OB axons. The OT receives a heavier projection from tufted cells the late-born projection neurons in the OB. In later developmental stages, many c-kit-positive axons were observed to project into the OT selectively and directly. This observation further supports that the OT receives the selective projection from the late-born projection neurons in the OB.

In the LOT, c-kit-positive axons were always fasciculated and segregated from c-kit-negative axons. When OB explants were cultured in the dish, c-kit-positive neurites did not fasciculate but randomly mixed with c-kit-negative neurites. Even in the organotypic co-culture, c-kit-positive axons did not choose the superficial part but randomly elongated within the LOT bundle, intermingling with c-kit-negative axons. Thus, the selective fasciculation of c-kit-positive axons in the LOT was not reproduced in culture and might require developmentally ordered projection of LOT axons.

The c-kit is encoded by the *W* locus in mice and the ligand for c-kit is stem cell factor (SCF), which encoded by the *Sl* locus in mice. The interaction between c-kit and SCF is considered to be essential for the development of melanocytes, erythrocytes, mast cells and germ cells, because mutations in either the *W* or *Sl* locus result in serious defects in differentiation of these cells. I examined whether c-kit or SCF was involved in the projection of LOT axons. However, I did not detect any abnormality in the LOT projection of *W/W* or *Sl/Sl*^{d} mutant mice, although it is still possible that more detailed analyses reveal some function of this signaling.

論文の審査結果の要旨

嗅覚回路では，特定のにおいを受容した神経の軸索がまず嗅球の糸球体に集積し，そこから伸びる２次神経の軸索が外側嗅索を通って大脳皮質の様々な領域に投射する．においの性質に関する情報がどのような「論理」で脳に投射されるのかはまだわかっていない．山谷君は，嗅覚系の回路形成過程において，同時期に生み出された神経細胞が，一過的におなじ蛋白質を発現し，軸索束を形成する，という新たな現象を発見した．

山谷君は，嗅球からの投射軸索束の中の一部の軸索だけを認識するモノクローン抗体 H2C7 を用いて解析した．まず，H2C7 抗原分子が受容体型チロシンキナーゼ c-kit であることを同定した．さらに，嗅球内で生み出されるすべての２次神経細胞は，細胞誕生後３日目から４日目にかけて一過的に c-kit を発現することを示した．c-kit の発現時期は，細胞体が嗅球の中間層に移動してくる時期と相関していた．c-kit 陽性軸索は，嗅球全域に由来しているにもかかわらず，嗅索の腹側表面に収斂して軸索束を形成していた．この軸索束の形成は組織培養や嗅球と終脳の組み合わせ培養では見られなかったため，生体内の環境が重要であることが示唆されたが，c-kit のリガンドである SCF を必要としなかった．

神経系の形成過程では，神経細胞集団の中の生成の順序が運命決定や回路形成の情報を担っていることが多い．たとえば，神経幹細胞から生み出される神経細胞は，誕生の順序に従って大脳皮質の特定の層に移動するし，眼の形成過程でも，新たな神経細胞は前後軸に沿って波状に作り出される．山谷君は，嗅覚回路形成においても，嗅球内の異なる場所で同じ時期に生み出された神経細胞が分子的な性質や軸索経路を共有する，という新たな現象を発見した．同時に生まれた神経細胞が軸索経路において軸索束を形成する事は，それらの細胞の投射先に何らかの関係があることを示唆している．また，これらの細胞が発現する c-kit 蛋白質が投射先の選択に関与している可能性もある．山谷君の研究を契機に今後神経細胞の誕生順序にリンクした新たな回路形成の論理が見出される可能性もあり，山谷君の研究はその先駆的発見として高く評価できる．以上の理由で山谷仁志君の論文は博士号授与の要件を満たすと審査員全員一致で判断した．