氏　　名	渡辺　啓介
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学 位 論 文 題 目	A role of netrin-1 in the dorsal spinal cord formation
論 文 審 査 委 員	主　査　教授　鍋倉　淳一 教授　池中　一裕 助教授　東島　眞一 教授　渡辺　雅彦（北海道大学）

Dorsal root ganglion (DRG) neurons extend axons to the specific targets in the gray matter of the spinal cord, and convey somatic and visceral sensory information from peripheral tissues to the spinal cord. The projections of sensory axons to specific targets are crucial for the accurate perception of and reflex to external sensory information. During development, process of the axonal projections of DRG neurons is tightly regulated. During early embryogenesis, DRG axons enter the spinal cord at the dorsal root entry zone (DREZ) and then grow to the marginal zone longitudinally to form the dorsal funiculus without penetrating the dorsal gray matter. After a few days, proprioceptive afferents, which are involved in the muscle stretch reflex, penetrate the mantle layer and project ventrally through the dorsal layers. Subsequently, thermoceptive and nociceptive cutaneous sensory afferents start to send collaterals into the dorsal mantle layer and terminate in the dorsal-most laminae of the cord. Therefore, projection pattern of DRG axons show delay between formation of the dorsal funiculus in the marginal zone and extension of collaterals into the dorsal mantle layer, called the "waiting period". Based on this growth pattern of DRG axons, it has been presumed that repellant(s) and/or inhibitory cues transiently prevent sensory afferents from penetrating the dorsal spinal cord during the waiting period. In other words, the inhibitory cuesare apparently required for correct patterning of sensory afferents. However, a diffusible guidance molecule(s) involved in sensory axon patterning in the dorsal spinal cord is totally unknown.

Diffusible signals acting at distance, including the netrin, semaphorin and slit families, are important cues for the early guidance of neuronal pathways during development. Netrin-1, from the Sanskrit term "one who guides", is originally identified in the ventral midline of the neural tube as a axon guidance molecule during early embryogenesis; it is a long-range diffusible factor that exerts chemoattractive or chemorepulsive effects for distinct developing neural cells, thus regulating axon outgrowth and cell migration. The attractive effects of netrin appear to be mediated by a netrin receptor, Dcc, whereas Unc5 family receptorsare involved in mediating repulsive actions. Netrin secreted from cells in the floor plate directs many axons to midline. Netrin-1 is also weakly expressed in the developing dorsal spinal cord. However, roles of this dorsally derived netrin-1 remain totally unknown. Here he shows that dorsally derived netrin-1 controls the correct guidance of primary sensory axons. During the waiting period for primary sensory axons, netrin-1 is transiently expressed or upregulated in the dorsal spinal cord, and the absence of *netrin-1* results in aberrant and premature projections of sensory axons, including both cutaneous and proprioceptive afferents, into the dorsal mantle layer without initial growth along the marzinal zone of the dorsal spinal cord. Thus, *netrin-1* deficient mice show loss of the waiting period. He also demonstrates that aberrant projections of DRG axons is not due to the abnormal cell migration or patterning in the dorsal spinal cord, which is reported in the spinal cord development of*Dcc* knockout mice. To examine netrin-1 function on DRG axons, he has applied collagen gel culture experiment. Netrin-1 suppresses axon outgrowth from DRG explants in vitro. However, there did not seem to be a specific effect of netrin-1 on either axon attraction or repulsion. Netrin-1 expressed in the floor plate apparently influences the dorsal spinal cord in early stages. To examine dorsally derived netrin-1 is

important for correct projections of DRG axons, he used *Gli2* mutant mice, which is devoid of the floor plate, and demonstrated that the mutant embryos show normal projections of the DRG axons. Therefore, netrin-1 derived from the dorsal spinal cord, but not the floor plate, is involved in the correct projections of DRG axons. Furthermore, to elucidate on which netrin receptors the projections of sensory afferents are dependent, he analyzed the trajectories of sensory axons in netrin receptor *Dcc* mutant and *Unc5c*^{rcm} mutant mice. *Unc5c*^{rcm} mutant shows abnormal invasion of DRG axons as observed in *netrin-1* mutants, whereas the dorsal funiculus of *Dcc* mutant mice is normally established at early stage These results clearly demonstrate that netrin-1 in the dorsal spinal cord acts as an inhibitory cue for primary sensory axons andthus serves as a critical signal for proper formation of sensory neural networks.

論文の審査結果の要旨

後根神経節細胞の軸索が脊髄背側部に達した後に、"waiting period"と呼ばれる期間を経て、脊髄内外套層の標的細胞への突起伸長を開始する。この緻密な神経回路網の形成の一端を担っている"waiting period"のメカニズムおよび分子背景の解明を目的とした研究論文である。

後根神経節細胞の軸索が脊髄に到達する発生時期に一致して後根進入部位近傍で一過性に発現する Netrin-1 に注目している。*netrin-1* 欠損マウスを用いて感覚神経線維の軸索走行の観察を行った結果、野生型より早期に、また正常後索を形成することなしに脊髄内に異所性に侵入することを明らかにしている。脊髄後索形成異常を示す *netrin-1* 欠損マウスではそれ以外の後根神経節細胞の投射形成異常はなく、Netrin-1 強制発現細胞と後根神経節細胞との共培養系では後根神経節細胞の突起伸長の著しい抑制をもたらすこと、また、Netrin-1 の脊髄腹内側部発現のみ欠損する発現改変動物においては後索形成異常は観察されないこと、また *Unc5c 受容体*突然変異マウスでも感覚神経の異常侵入が認められた。これらの結果から、一過性に発現する脊髄背側部 Netrin-1 が感覚神経 Unc5 受容体を介しての脊髄内侵入を抑制していることが"waiting period"の分子メカニズムであることを示している。

本研究論文は、研究背景、研究の進め方、結果および、結果から得られる考察も十分に検討され、本研究結果の一部は英文雑誌（Development）に掲載受理されている。

今後、脊髄回路形成における waiting period の重要性および分子メカニズムばかりでなく、他の中枢および末梢回路形成の分子メカニズムにも大きな貢献することが期待できる。

以上の結果をもって提出論文は学位に値するものと判定された。