学位論文内容の要旨

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学 位 論 文 題 名

Characterization of nigrostriatal dopaminergic appositions across striatal cell types and regions using AAV1-mediated trans-neuronal labeling

（AAV1 を用いた順行性神経間標識による黒質線条体ドーパミン作動性軸索の接触様式における細胞型および領域特異性の解析）

[Background and Objective]
Dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc) are essential for movement, motivation, and learning. Their degeneration causes the characteristic motor and cognitive symptoms of Parkinson's disease, making the nigrostriatal pathway a key model for studying DA regulation. Each SNc neuron forms an extensive axonal arbor that spreads across the dorsal striatum, releasing DA from thousands of varicosities to influence diverse neuronal populations. This widespread axonal organization raises a central question: how can DA achieve selective modulation within such a broad network?
The dorsal striatum consists of two functional territories—the dorsomedial striatum (DMS) supporting goal-directed and the dorsolateral striatum (DLS) supporting habitual behavior. Its neuronal composition includes two major classes of projection neurons, the direct- and indirect-pathway medium spiny neurons (dMSNs and iMSNs), as well as several types of interneurons, which play distinct roles in shaping striatal output. Previous studies have revealed that distinct SNc subpopulations preferentially project to these subregions, suggesting that DA transmission may be spatially and functionally organized. However, the structural basis of this organization—specifically, which types of striatal neurons receive DA input and how these differ between regions—remains largely unknown.
To address this issue, I first tested whether adeno-associated virus serotype 1 (AAV1)–mediated anterograde trans-neuronal labeling, by which pre- and post-synaptic neurons are simultaneously labeled, can be applied to the DA nigrostriatal system. Because AAV1 is known to exhibit limited retrograde transport in some projection systems, I focused on striatal neurons whose axons do not directly project to the SNc. Specifically, one of the principal inhibitory interneurons, parvalbumin-positive interneurons (PV-INs), the main modulatory interneurons, choline acetyltransferase-positive interneurons (CINs), and iMSNs were analyzed. It is known that iMSNs can be defined by pre-proenkephalin (PPE-iMSNs). This design minimized potential retrograde transfer and allowed us to evaluate AAV1's suitability for mapping neuromodulatory circuits. Based on this methodological validation, I then investigated whether SNc-derived DA axons form organized, cell-type-specific, and region-dependent appositional patterns within the dorsal striatum. Clarifying this organization is critical for understanding how DA regulates striatal microcircuits across distinct regions and neuronal types that underlie goal-directed and habitual behaviors.
[Materials and Methods]
AAV1-Cre was injected into the SNc to induce anterograde trans-neuronal expression of Cre, while Cre-dependent fluorescent reporters were injected into the striatum to label Cre-expressing neurons using wild type C57BL/6 male mice. This approach enabled simultaneous visualization of presynaptic SNc DA neurons and postsynaptic elements in the striatal neurons. The DA nature of labeled axons was confirmed by DA transporter (DAT) immunofluorescence and the absence of cholinergic, glutamatergic, or GABAergic terminal markers. Based on these labeling results, I next identified the striatal neuron types expressing Cre and analyzed their morphological and connectional features in detail.
High-resolution confocal microscopy was used to obtain image stacks of labeled striatal cells and DA axons, followed by three-dimensional reconstruction. Putative appositions (PAPs) were defined as DA varicosities directly contacting the soma or dendritic surface in 3D space. For each neuron, I quantified the number of PAPs,

the number of axons forming PAPs, and the soma-to-PAPs distance. Morphometric parameters such as soma volume, total dendritic length, and number of primary dendrites were also compared among neuron types. Statistical analyses were performed using ANOVA with appropriate post hoc tests to evaluate differences across neuron types and regions

[Results]

AAV1-Cre injection into the SNc, combined with Cre-dependent reporters in the striatum, successfully induced Cre expression in postsynaptic striatal neurons, demonstrating that AAV1 can undergo anterograde trans-neuronal transfer in the DA pathway. Immunofluorescence confirmed that nearly all labeled axons were dopaminergic (DAT-positive) and not cholinergic, glutamatergic, or GABAergic.

Among the Cre-expressing neurons, immunostaining identified three major classes: PV-INs, CINs, and PPE-iMSNs. Morphometric data supported these findings: CINs (8 neurons in DMS, 6 in DLS) had the largest somata (1235.69 ± 392.55μm³ in DMS) and longest mean dendrites (186.17 ± 148.23μm in DMS), PV-INs (7 in DMS, 4 in DLS) had more primary dendrites (6.43 ± 1.90 in DMS) but smaller soma volume (575.35 ± 220.44 μm³ in DMS), and PPE-iMSNs (5 in DMS, 4 in DLS) exhibited small soma with spiny dendrites, consistent with previous reports. Confocal imaging showed multiple DA appositions on somata and dendrites of these neurons, validating AAV1-based labeling for visualizing pre- and postsynaptic components.

Quantitative analyses showed that DA appositions were broadly distributed but followed distinct organized patterns across neuron types and regions. Interneurons generally received more proximal appositions to their somata, whereas PPE-iMSNs exhibited more distal dendritic appositions. CINs received the highest number of somatic PAPs per neuron (10.3 ± 4.7 in DMS and 6.2 ± 3.9 in DLS), followed by PV-INs (7.8 ± 3.1 and 5.4 ± 2.8) and PPE-iMSNs (3.2 ± 1.8 and 2.5 ± 1.4). The proportion of axons forming somatic appositions was significantly higher in DMS CINs than in DLS CINs ($p < 0.05$). Regarding spatial distribution, the average soma-to-PAP distance was shortest in CINs (24.8 ± 9.3 μm), intermediate in PV-INs (31.4 ± 10.7 μm), and longest in PPE-iMSNs (46.8 ± 12.5 μm in the DMS; 72.3 ± 15.1 μm in the DLS).

[Discussion]

This study demonstrates that AAV1 can serve as a reliable anterograde trans-neuronal tracer in the nigrostriatal DA system. By focusing on interneurons and iMSNs that lack reciprocal projections, I minimized the possibility of retrograde artifacts and confirmed that the labeling primarily reflected anterograde transfer. Methodologically, my results extend AAV1's applicability to neuromodulatory systems beyond fast neurotransmitter pathways.

I demonstrated both cell-type- and region-specific heterogeneity in DA innervation within the striatum. Interneurons, particularly CINs and PV-INs, received denser and more proximal DA appositions than PPE-iMSNs. The strong somatic contacts on CINs may explain their stronger DA-dependent inhibition, while PV-INs may integrate DA input to fine-tune inhibitory timing within striatal microcircuits. In contrast, the distal contacts on PPE-iMSNs may influence dendritic processing, and thereby modulate indirect pathway activity.

The regional differences between the DMS and DLS suggest that DA signaling is spatially adapted to distinct functional roles. The DMS, associated with goal-directed learning, showed more proximal appositions, while the DLS, related to habitual behavior, exhibited broader dendritic distributions. These anatomical gradients provide structural support for differential DA-dependent regulation across striatal circuits.

[Conclusion]

I established that AAV1-mediated anterograde trans-neuronal labeling can be effectively applied to the nigrostriatal DA pathway. Using this validated approach, I identified structured, non-random patterns of DA innervation characterized by cell-type- and region-dependent organization. PV-INs and CINs received more proximal and frequent contacts, whereas PPE-iMSNs showed more distal appositions. These findings demonstrate that DA innervation within the striatum is organized in a structured, cell-type- and region-specific manner rather than by random diffusion. The methodological advancement also provides a powerful framework for future studies investigating circuit-specific DA regulation and its disruption in neurological disorders such as Parkinson's disease. The present study relied solely on light microscopy observation and did not provide direct evidence of ultrastructural or functional connectivity. Future investigations combining AAV1 tracing with electrophysiological, ultrastructural, or behavioral analyses will be essential to validate functional relevance and elucidate how these organized DA connections shape circuit activity in health and diseased states.