Developing novel techniques for primate neural network analyses by retrograde gene transfer with viral vectors

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To understand the mechanisms of human brain functions, it is essential to analyze neural network functions of the primate brains. Viral vectors have been utilized for characterizing their architecture and clarifying the function of a given pathway. In the present study, novel techniques for primate neural network analyses by retrograde gene transfer with lentiviral vectors and rabies virus (RV) vectors were developed. In Chapter 2, the suitability among various pseudotyped lentiviral vectors for pathway-selective gene manipulation was compared. In Chapter 3, the architecture of cortico-basal ganglia (BG) loop circuits was investigated by employing dual retrograde transneuronal labeling with RV vectors carrying the gene of different fluorescent proteins.

Chapter 2: Lentiviral vectors pseudotyped with fusion glycoproteins for pathway-selective gene manipulation via retrograde transfer

Background

Pseudotyped lentiviral vectors give access to pathway-selective gene manipulation via retrograde transfer. Two types of such lentiviral vectors have been developed. One is the so-called NeuRet vector pseudotyped with fusion glycoprotein type C (FuG-C), which preferentially transduces neurons. Recently, it has been demonstrated in mice that the use of a novel type of fusion glycoprotein (FuG-E) results in increased retrograde gene transfer relative to the parental FuG-C fusion glycoprotein. The other is the so-called HiRet vector pseudotyped with fusion glycoprotein type B2 (FuG-B2), which permits

gene transfer into both neurons and glial cells at the injection site. Although these vectors have been applied in many studies investigating neural network functions, it remains unclear which vector is more appropriate for retrograde gene delivery in the brain.

Materials and Methods

In Section 2.1, the property of the FuG-E pseudotype in the primate brain was compared with that of the FuG-C pseudotype by injecting these vectors into the striatum of macaque monkeys. The pattern and efficiency of transgene expression of the FuG-E and FuG-C pseudotyped lentiviral vectors in the striatal input system were compared. In Section 2.2, to compare the suitability of the FuG-B2 vs. FuG-E vectors for retrograde gene transfer in the brain, both vectors were injected into the striatum in macaque monkeys, common marmosets, and rats. The gene transfer efficiency of these vectors was evaluated in the same way as the comparison of the lentiviral vectors pseudotyped with FuG-E or FuG-C. Immune/inflammatory response around injection sites was also examined by detecting microglial and lymphocytic infiltration.

Results

The FuG-E pseudotype displays not only neuronal specificity around the injection site, but also higher gene transfer efficiency than the FuG-C pseudotype in the macaque brain as well as in the mouse brain. It was also revealed that retrograde gene delivery of the FuG-E pseudotype was equal to or greater than that of the FuG-B2 pseudotype. Furthermore, inflammation characterized by microglial and lymphocytic infiltration occurred when the FuG-B2 pseudotype, but not the FuG-E pseudotype, was injected into the primate brains.

Conclusion

It is indicated that the NeuRet vector pseudotyped with FuG-E is more suitable than the

FuG-C and FuG-B2 pseudotyped vectors in primate and rodent brains.

Chapter 3: Rabies virus vectors carrying fluorescence genes for multi-colored retrograde transneuronal labeling

Background

The cortico-BG loops can be classified into motor, oculomotor, prefrontal, and limbic loops. In the motor loop, there are multiple subloops that originate in various motor-related areas. Although a number of prior works have suggested that these subloops may exhibit a complex pattern of information processing, i.e., parallel vs. integrated fashion, it is still unclear how motor signals derived from distinct areas are organized along the loop circuits. In the present study, dual retrograde transneuronal labeling with RV vectors carrying the gene of different fluorescent proteins was employed to investigate the distribution patterns of origin in the BG of multisynaptic projections to the dorsal or ventral premotor cortex (PMd, PMv).

Materials and Methods

To investigate the architecture of multisynaptic projections from the BG to the PMd and PMv, RV vectors carrying the genes of GFP or RFP gene were separately injected into the forelimb regions of the PMd and PMv. The survival period after the dual RV vector injections was adjusted to allow either the second-order or the third-order neuron labeling across two or three synapses, respectively. The distribution patterns of labeled neurons in each nucleus were examined by histological analyses.

Result

It has been shown that there are double-labeled neurons sending outputs from the BG to both the PMd and the PMv. This suggests that the PMd- and PMv-BG loops are not

completely closed and interact at least partly with each other. The distribution patterns and ratio of the number of labeled neurons in each nucleus characterized the anatomical basis of interaction of information processing between PMv- and PMd-basal ganglia loop circuits.

Conclusion

The results of the present study will provide a novel mechanism underlying motor information processing in the PMd-BG and PMv-BG loop circuits.