—Photogravure—

Recombinant Adeno-associated Virus-mediated Gene Delivery to the Central Nervous System

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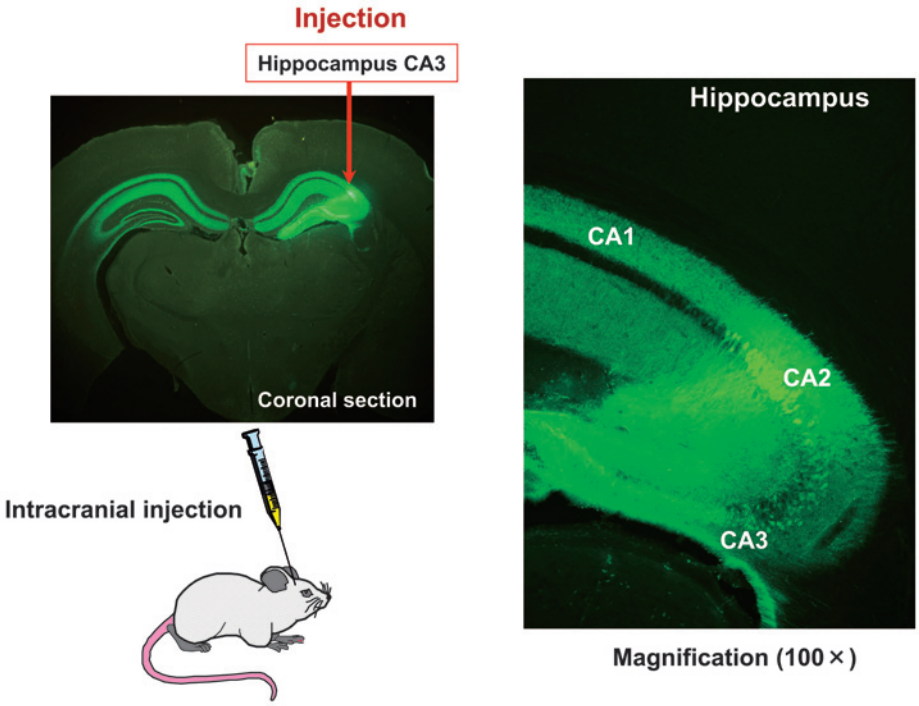

Fig. 1

Recombinant adeno-associated virus (rAAV) is capable of highly efficient gene transfer and long-term expression of transgenes in nondividing cells. Accordingly rAAV is a useful vector system for gene therapy of neurological diseases. We are examining the feasibility of AAV-mediated gene therapy of metachromatic leukodystrophy (MLD), which is a lysosomal storage disease caused by the deficiency of arylsulfatase A (ASA) and is characterized by deposition of sulfatide throughout the brain. In the present study, we generated AAV serotype1-based vectors expressing green fluorescent protein (GFP) (AAV1-GFP) or ASA (AAV1-ASA). When AAV1-GFP was stereotactically injected into the CA3 region of the hippocampus in BL6 mice, cells strongly positive for GFP were detected in the area of injection (**Fig. 1**). In addition, GFP signals were seen throughout the hippocampus even on the side opposite the injection, indicating efficient axonal transport of intracellular GFP molecules to the contralateral hemisphere. Next, AAV1-ASA was injected into the hippocampus of ASA-knockout mice (MLD mice). Detailed immunohistochemical examination revealed that ASA molecules were detected mainly in neuronal cells and localized in the perinuclear area (**Fig. 2**). Levels of ASA activity and the

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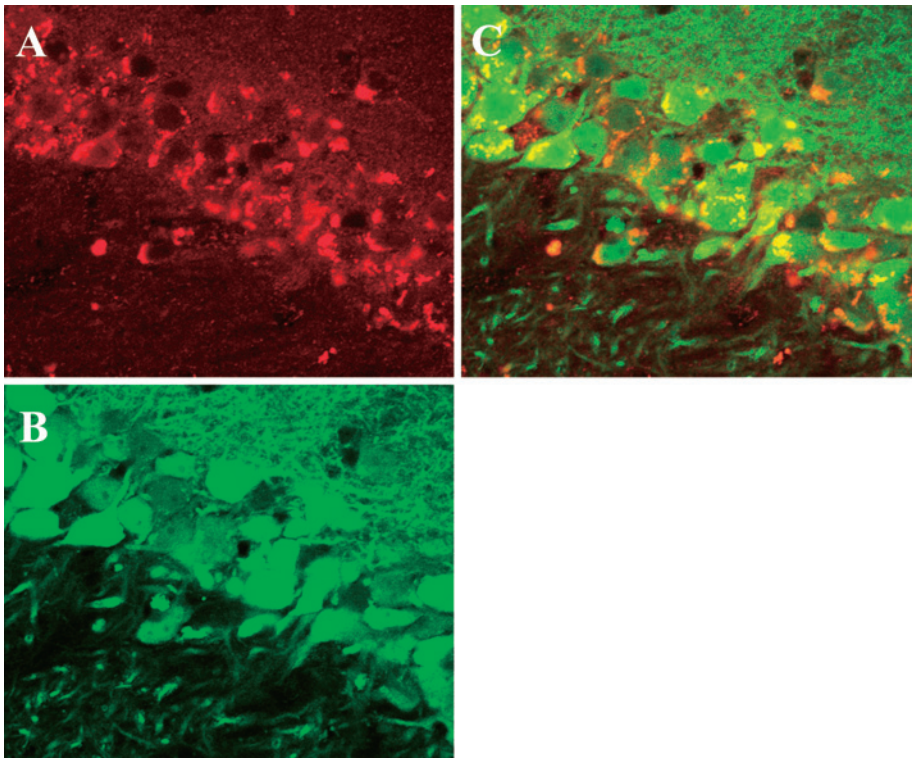

Fig. 2

extent of ASA distribution could be significantly enhanced by co-expression of formylglycine-generating enzyme, a recently identified activator for sulfatase. These results demonstrate that direct injection of AAV1 vector into the brain is a highly promising approach for gene therapy of MLD.

Fig. 1 Distribution of GFP in the brain after direct injection of AAV1 vector into the hippocampus. GFP signals were detected in the hippocampus bilaterally.

Fig. 2 Localization of ASA in neuronal cells after AAV-mediated expression. Confocal microscopic examination shows that ASA is localized in the perinuclear area. (A) ASA immunostaining; (B) GFP signal; (C) Merged picture.

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