Sparse Labeling in the Mouse Cerebellar Circuit Using Adeno-Associated Virus Constructs

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Rationale
T, Nakamura KC, Matsuda W, Kaneko T. 2011. Exclusive and common targets of IP/ml. C) A red nucleus neuron after injection of AAV.CreERT2 and 4-

Background & Rationale
Regional cerebellar circuitry has been well characterized by classic imaging techniques. However, due to the density and morphological complexity of neurons, special sparse labeling techniques are often required for detailed investigation of neuronal structure on a single cell level. Sparse labeling in vivo has historically been cumbersome, requiring time consuming electrophysiological experiments or complicated staining procedures inappropriate for tracing long axons. Modern genetic techniques have allowed improved visualization and analysis of single cells by taking advantage of Cre recombinase (Cre) and fluorescent proteins, but they still require multiplex visualization methods or viruses requiring high levels of biosafety precautions

Our goal was to develop a novel method for labeling and reconstructing <10 neurons per hemisphere in the cerebellum using convenient, low-toxicity adeno-associated virus (AAV) constructs.

Results

Both AAV.Cre and AAV.CreERT2 methods resulted in sparse label, but there are gaps in dendrite traces for all of the cells labeled. (Figure 4)

- Although AAV.Cre concentrations of 10^3 IP/ml and 10^4 IP/ml were both successful, further
- Mice are currently in the infecation period to validate 10^5 IP/ml. Wu SH. 2014. Genetically targeted binary labeling of retinal neurons. J ONE 4(11):e7859

Low signal
Both IP/ml and TREES toolbox. (Figure 6)

Results (continued)

- Neurameric allows manual reconstruction of neurons by building out segments of user-defined cylinders. This method effectively eliminated noise for better reconstruction of dimly labeled cells. (Figure 7)

Conclusions and Future Directions
• AAV.Cre and AAV.CreERT2 methods both resulted in sparse label, but dim signal led to incomplete filling of neuronal arbors.
• Neurameric, and manual reconstruction in general, is most effective at producing models in these conditions where signal and noise intensity values overlap.
• The AAV-Cre method should be preferred over AAV-CreERT2 for future studies as it constitutes one less step and yields similar results.
• Future attempts should alter incubation time, reporter mouse line, or virus type to improve label strength and obtain complete neuronal morphology.

References & Acknowledgements

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