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A fourth generation of neuroanatomical tracing techniques: Exploiting the offspring of genetic engineering

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HIGHLIGHTS

- BDA tracing combined with neurochemical fingerprinting.
- PV-cre mice and ChAT-cre mice.
- AAV virus with eYFP construct to force neurochemically specific neurons to express fluorescent protein.
- This virus as an agent to "switch on the light in cells" and reveal fibers.
- Location of neurotransmission-related substance in nerve fibers in the CNS transgenic mice expressing GFP in small populations of neurochemically specific neurons.
- Outlook for 4th generation neuroanatomical tracing techniques.

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ABSTRACT

The first three generations of neuroanatomical tract-tracing methods include, respectively, techniques exploiting degeneration, retrograde cellular transport and anterograde cellular transport. This paper reviews the most recent development in third-generation tracing, i.e., neurochemical fingerprinting based on BDA tracing, and continues with an emerging tracing technique called here 'selective fluorescent protein expression' that in our view belongs to an entirely new 'fourth-generation' class. Tracing techniques in this class lean on gene expression technology designed to 'label' projections exclusively originating from neurons expressing a very specific molecular phenotype. Genetically engineered mice that express cre-recombinase in a neurochemically specific neuronal population receive into a brain locus of interest an injection of an adeno-associated virus (AAV) carrying a double-floxed promoter-eYFP DNA sequence. After transfection this sequence is expressed only in neurons metabolizing recombinase protein. These particular neurons promptly start manufacturing the fluorescent protein which then accumulates and labels to full detail all the neuronal processes, including fibers and terminal arborizations. All other neurons remain optically 'dark'. The AAV is not replicated by the neurons, prohibiting intracerebral spread of 'infection'. The essence is that the fiber projections of discrete subpopulations of neurochemically specific neurons can be traced in full detail. One condition is that the transgenic mouse strain is recombinase-perfect.

We illustrate selective fluorescent protein expression in parvalbumin-cre (PV-cre) mice and choline acetyltransferase-cre (ChAT-cre) mice. In addition we compare this novel tracing technique with observations in brains of native PV mice and ChAT-GFP mice. We include a note on tracing techniques using viruses.

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Contents

1. Introduction

1.1. Three generations of neuroanatomical tracing techniques

The current concept of a 'brain' as an information processing organ has its roots in the achievements of past generations of scientists. In this respect, von Waldeyer, Sherrington and Ramon y Cajal deserve mentioning. Waldeyer conceived the idea that the nervous system is made up of discrete cells (von Waldeyer-Hartz, 1891). Sherrington conceived the concept of the synapse as the physiological site of exchange of information between neurons (Foster and Sherrington, 1897). A convincing and literally solid anatomical substrate for Waldeyer's neurons and Sherrington's synapses was precipitated by Cajal through brilliant application of the newly discovered Golgi silver impregnation technique (Ramon y Cajal, 1909–1911).

Neuroanatomical tracing methods in the days of von Waldeyer, Sherrington and Cajal included knife- or electrode based, physical techniques that induce degeneration in fiber tracts after a lesion (Waller, 1850). The breakdown of myelinated fibers can be made visible by heavy metal staining (Marchi and Algeri, 1885) and that of myelinated and unmyelinated fibers by silver affinity staining procedures (Nauta, 1952; Fink and Heimer, 1967). The researcher de facto interprets pathological processes induced by severing fibers from their parent cell bodies, in terms of connectivity. As these techniques lean on pathological changes we consider them as first-generation.

The second generation of tracing techniques appeared in the second half of the 20th century, exploiting intrinsic, in vivo retrograde cellular transport in neurons (Kristensson and Olsson, 1971a,b; LaVail and LaVail, 1972). An enzyme or some fluorescent tracer substance is introduced in a downstream location relative to the neurons to be studied, resulting in uptake by axon terminals and sometimes by damaged fibers. Uptake is followed by transport back to the cell bodies of the neurons where accumulation or metabolic degradation occurs. Usually the site of injection of the tracer is visible and, far away, the cell bodies of the neurons that project to that injection site. The pathways taken by the fibers remain invisible.

The third generation of tracing techniques encompasses techniques that capitalize on the transport system carrying macromolecules from the cell body to the axon terminals. These are the anterograde tracing techniques. The autoradiographic tracing method that exploits the uptake of radioactive amino acids, incorporation into proteins and subsequent transport (cf. Cowan et al., 1972) can be called in retrospect an early and exotic third-generation technique. Two big advantages of anterograde tracing techniques are that the involved fiber pathways become fully visible and that they supply an enormous amount of cellular detail.

It should be mentioned at this point that most current tracing substances are not exclusively transported in one direction, but to a variable degree also in the opposite direction. Only the aforementioned autoradiographic tracing method is considered virtually 100% anterograde (Hendrickson, 1982).

Today, most tracing techniques listed in research papers belong to the second and third generation: retrograde transport of horseradish peroxidase, fluorescent or nonfluorescent macromolecules, nanoparticles, toxins and virus particles, and anterograde transport of lectins and macromolecules. The most widely applied third-generation technique (BDA tracing; BDA stands for biotinylated dextran amine) will be given a short review, echoing in part a review on the state of the art of neuroanatomical tracing published recently (Lanciego and Wouterlood, 2011). We pay attention to a refinement of anterograde tracing, i.e., neurochemical fingerprinting of the BDA labeled fibers and boutons.