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Review

A half century of experimental neuroanatomical tracing

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ABSTRACT

Most of our current understanding of brain function and dysfunction has its firm base in what is so elegantly called the 'anatomical substrate', i.e. the anatomical, histological, and histochemical domains within the large knowledge envelope called 'neuroscience' that further includes physiological, pharmacological, neurochemical, behavioral, genetical and clinical domains. This review focuses mainly on the anatomical domain in neuroscience. To a large degree neuroanatomical tract-tracing methods have paved the way in this domain. Over the past few decades, a great number of neuroanatomical tracers have been added to the technical arsenal to fulfill almost any experimental demand. Despite this sophisticated arsenal, the decision which tracer is best suited for a given tracing experiment still represents a difficult choice. Although this review is obviously not intended to provide the last word in the tract-tracing field, we provide a survey of the available tracing methods including some of their roots. We further summarize our experience with neuroanatomical tracers, in an attempt to provide the novice user with some advice to help this person to select the most appropriate criteria to choose a tracer that best applies to a given experimental design.

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1. Introduction

Even the brain of the most diminutive vertebrate appears to us from a strictly anatomical point of view as an incomprehensibly large collection of neurons with sets of equally incomprehensibly complicated interactions. Special about this remarkable central organ is that large groups of neurons act together to collect and analyze sensory and visceral information arriving from the periphery via cranial and spinal nerves. These neurons process the information, integrate and take care of storing the outcome for later use. Simultaneously they formulate an appropriate response, for instance a motor act, autonomous response or, as is so essentially human, a thought, logical reasoning or a creative idea.

Attempts to unravel the anatomy of the brain, spinal cord and peripheral nerves have resulted so far in huge amounts of descriptive and experimental data and a wealth of creative integrative ideas. von Waldeyer-Hartz (1891) was the first to propagate the idea that the nervous system is made up of discrete individual cells, and together with Foster and Sherrington's (1897) notion of the synapse as the physiological site of information transfer the intellectual framework had been established that supports neuroanatomical tracing as we know it today. Giant steps in unraveling neuronal connectivity were made by Santiago Ramón y Cajal who pioneered the art of neuronal network visualization with the then newly discovered Golgi silver impregnation technique. Techniques like these create light absorbing inorganic deposits, 'dyes' that accumulate inside neurons or diffuse through cell processes in post mortem tissue, such in contrast to labels that are taken up and transported in living neurons. In this review we keep this distinction between purely physical diffusion of dyes in essentially fixed material and the spread of label in living cells via active physiological transport processes. Ramón y Cajal was the first who described neuron types in the brain systematically, based on the morphological features of their cell bodies, dendrites and axons (although he considered dendritic spines as artifacts). His great scientific legacy was published in the two-volume 'Système nerveux de l 'Homme et des Vertébrés in 1909–1911. In Ramón y Cajal's age, experimental lesioning of large myelinated tracts was already an established yet coarse method of tract-tracing (Waller, 1850). Building upon Waller's legacy a whole subdiscipline blossomed in experimental neuroanatomical tract tracing, supported in its heyday by a most powerful tool, the selective silver staining method originally developed by Nauta (1952) and refined by Fink and Heimer (1967). Only in the second half of the 20th century techniques became available, starting with the groundbreaking discovery by Kristensson and Olsson (1971a,b) that intrinsic cellular transport mechanisms in the live subject can be used to deliver original labels that identify the origin, course and termination of axons of groups or individual neurons. Combined with immunohistochemical, genetic and neurophysiological techniques, our knowledge about the wiring of neuron populations thus exploded. A picture of 'brain' is emerging in which the neurons maintain a multitude of synaptic and nonsynaptic relationships in order to fulfill incredibly complex and dynamic tasks. We consider the brain with awe: an incomprehensibly large collection of neurons with sets of incomprehensibly complicated anatomical and chemicophysiological interactions. This review tries to provide an oversight of the main categories of techniques in neuroanatomical tracing and the state of the art after the first 10 years of the 21st century.

2. Tracing based on transport in living neurons

In the living subject, macromolecules are continuously manufactured by the cellular molecular machinery, transported inside cells or sent packed or unpacked across cell membranes into the extracellular space. Macromolecules can be taken up from the extracellular space, transported to the cell body and metabolized there, or stored for later use. As this happens everywhere in the body the nervous system is no exception to this rule. Thus, neurons may take up small or big macromolecules anywhere along their outer membrane, for instance at or close to their peripheral axon terminals or their motor end plates. Molecules may enter the cell's interior via receptor-mediated uptake, or in a 'mass process' by vesicular endocytosis. Following internalization these molecules are transported to the perikaryon for further metabolic processing, storage or disposal. The transport from the periphery back to the cell body is called *retrograde* transport. Tracing methods that employ this class of transport are called retrograde tracing methods. Conversely, macromolecules can be taken up by the perikaryon and dendrites and transported centrifugally, along their axon towards the periphery. This kind of transport is called *anterograde* transport, and it forms the basis of so-called anterograde tracing techniques. We will keep this distinction throughout this review, although most tracers are to a certain degree transported bidirectionally with one direction of transport prevailing.

2.1. Anterograde tracing with biotinylated dextran amine (BDA)

Glover et al. (1986) introduced dextran amine conjugated with selected fluorescent dyes, fluorescein and rhodamine, as new neuroanatomical tract tracing macromolecules. Previously, dextrans conjugated to fluorescein isothiocyanate (dextran-FITC), had been applied to study vascular permeability in the peripheral nervous system (Olsson et al., 1975; Hultström et al., 1983). Glover et al. (1986) portrayed the fluorescing dextrans basically as retrograde tracers in the CNS. Their novel application generated a wave of interest in the use of dextran-based fluorescent tracers, several of which appeared to be transported predominantly in the anterograde direction (Nance and Burns, 1990; Schmued et al., 1990; Fritszch and Wilm, 1990; Chang, 1991). The first to report on the superior quality of biotinylated dextran amine (BDA) as an anterograde neuroanatomical tracer were Veenman et al. (1992). Dextran molecules, dextran amines and their conjugates are taken up via an unknown mechanism by dendrites and neuronal cell bodies and mainly transported in the anterograde direction (Reiner et al., 2000; Reiner and Honig, 2006). We will deal here further with BDA 10 kDa which is the most outspoken anterograde tracer of the family and maybe for this reason overwhelmingly applied.