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Review

Development and application of novel histochemical tracers for localizing brain connectivity and pathology

Larry C. Schmued

US Food and Drug Administration (FDA), National Center for Toxicological Research (NCTR), Division of Neurotoxicology, 3900 NCTR Rd, Jefferson, AR 72079 United States

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ORIGINAL ARTICLE ABSTRACT

Fluoro-gold: a new fluorescent retrograde axonal tracer with numerous unique properties: A new fluorescent dye, Fluoro-Gold, has been demonstrated to undergo retrograde axonal transport. Its properties include (1) intense fluorescence, (2) extensive filling of dendrites, (3) high resistance to fading, (4) no uptake by intact undamaged fibers of passage, (5) no diffusion from labeled cells, (6) consistent and pure commercial source, (7) wide latitude of survival times and (8) compatibility with all other tested neurohistochemical techniques. © 1986.

Fluoro-Jade C results in ultra high resolution and contrast labeling of degenerating neurons: The causes and effects of neuronal degeneration are of major interest to a wide variety of neuroscientists. Paralleling this growing interest is an increasing number of methods applicable to the detection of neuronal degeneration. The earliest methods employing aniline dyes were methodologically simple, but difficult to interpret due to a lack of staining specificity. In an attempt to circumvent this problem, numerous suppressed silver methods have been introduced. However, these methods are labor intensive, incompatible with most other histochemical procedures and notoriously capricious. In an attempt to develop a tracer with the methodological simplicity and reliability of conventional stains but with the specificity of an ideal suppressed silver preparation, the Fluoro-Jade dyes were developed. Fluoro-Jade C, like its predecessors, Fluoro-Jade and Fluoro-Jade B, was found to stain all degenerating neurons, regardless of specific insult or mechanism of cell death. Therefore, the patterns of neuronal degeneration seen following exposure to either the glutamate agonist, kainic acid, or the inhibitor of mitochondrial respiration, 3-NPA, were the same for all of the Fluoro-Jade dyes. However, there was a qualitative difference in the staining characteristics of the three fluorochromes. Specifically, Fluoro-Jade C exhibited the greatest signal to background ratio, as well as the highest resolution. This translates to a stain of maximal contrast and affinity for degenerating neurons. This makes it ideal for localizing not only degenerating nerve cell bodies, but also distal dendrites, axons and terminals. The dye is highly resistant to fading and is compatible with virtually all histological processing and staining protocols. Triple labeling was accomplished by staining degenerating neurons with Fluoro-Jade C, cell nuclei with DAPI and activated astrocytes with GFAP immunofluoresence. © 2005.

ARTICLE ABSTRACT

The development of novel tracers and associated histochemical methods has always been need driven. One such need was the development of tracers that could be administered to discrete brain regions in vivo to subsequently reveal neuronal connectivity via axonal transport of the tracer. One such compound is Fluoro-Gold (F-G), which can be used to demonstrate retrograde axonal transport. Advantages of this fluorescent tracer include brightness, sensitivity, contrast, stability, permanence and compatibility with multiple labeling studies. It may be applied to resolve either the afferent or efferent connections of brain regions of interest. Another need addressed was for a simple and definitive way to localize degenerating neurons in brain tissue sections. This led to the development of Fluoro-Jade B (FJ-B) and Fluoro-Jade C (FJ-C). Advantages of these fluorescent histochemical tracers include high specificity, resolution, contrast, stability and suitability for use in multiple labeling studies. These methods can be

E-mail address: larry.schmued@fda.hhs.gov

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applied to detect both apoptotic and necrotic neuronal degeneration following a variety of insults including physical trauma, neurodegenerative disease and a wide variety of neurotoxicants.

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Contents

1. Introduction

Over 100 years ago, pioneers in brain science including Nissl, Golgi and Cajal developed and applied histochemical tracers to allow them to resolve the microscopic morphology and pathology of the brain, resulting in profound insights into the structure and function of the nervous system. Although these techniques are still in use today, there has been a continuous evolution in the development of even more sensitive histochemical and specific neurohistochemical tracers. The most successful of these tracers always address a specific need, since a technique is only as strong as its application. With this in mind, this article will take the opportunity to address two specific needs by those who study the anatomy or pathology of the brain.

The first need we addressed was for improved axonal tract tracing methods for resolving neuronal connectivity. Early studies of brain circuitry typically involved staining tissue sections of lesioned brains with a suppressed silver method (Nauta and Gygax, 1954) which allowed the axons of degenerating neurons to be followed to their terminal destination. Subsequently, direct tracer methodologies that do not require the killing of nerve cells grew in popularity, such as anterograde transport demonstrated by autoradiographic localization of tritiated amino acids (Cowan et al., 1972), the histochemical detection of enzymes such as HRP (Kristensson and Olsson, 1971), or lectins such as WGA or PHA (Gerfen and Sawchenko, 1984). Limitations of the autoradiographic method include long development times and relatively low resolution. Limitations of the enzyme or lectin histochemical tract tracing methods include their labor intensiveness and difficulties when using in multiple labeling studies. Most of these problems were circumvented with the advent of the earliest direct retrogradely transported fluorescent tracers (Catsman-Berrevoets et al., 1980) such as True Blue and Fast Blue. The primary limitations associated with these prototypical fluorescent axonal tracers were their relatively weak fluorescence, their propensity to diffuse from the labeled structure along with a tendency to fade rapidly under ultraviolet light excitation.

The strategy used to develop an improved fluorescent retrogradely transported axonal tracer was based largely on structure-activity predictions. Since True Blue and Fast Blue are both essentially bis-amidino-indole compounds, we investigated the possibility that the highly basic amidino groups are primarily responsible for the axonal transport properties, while the indole conferred the fluorescent properties. To test this theory we synthesized a novel diamidino compound containing a hydroxyl-trans-stilbene fluorophore. The resulting molecule was called Fluoro-Gold, due to its yellow fluorescent emission at neutral pH. It was subsequently shown to result in the very bright, stable and high resolution labeling of neurons with projections to the tracer injected brain region (Schmued et al., 2005) (Fig. 1). As this tracer appears yellow under UV illumination and is highly resistant to diffusing or fading, it is ideal for combining with other red or green fluorophores in multiple labeling studies. It has been used to investigate neuronal projections in a wide variety of brain systems and species. Antibodies have been made to F-G, allowing connections to be localized at the electron microscopic level as well. We initially developed the tracer for resolving the connectivity and cytochemistry of the basal forebrain (Schmued, 1994). These connectional studies employed F-G in conjunction with another fluorescent axonal tract tracer that we also developed called Fluoro-Ruby (F-R) (Schmued et al., 1990). The primary difference between these two fluorescent axonal tract tracers is that generally F-G is employed to demonstrate retrograde axonal transport, while F-R is typically used to demonstrate anterograde axonal transport. A combined approach can therefore be used to infer neuronal circuitry by injecting F-R into structures with potential axonal projections to a brain region of interest, while F-G is injected into possible efferent projection sites of the region of interest. Circuitry can thus be inferred by looking for F-R labeled axon terminals in association with F-G labeled cells and dendrites (Fig. 2). It is also possible to use F-G and another retrograde axonally transported tracer such as propidium iodide (van der Kooy and Steinbusch, 1980) with each being injected into different brain regions to demonstrate collateral axonal projections of identified neurons (Figs. 3 and 4).

Although we typically describe F-G as undergoing retrograde axonal transport and F-R as undergoing anterograde axonal transport, the distinction is not quite that absolute. For example,

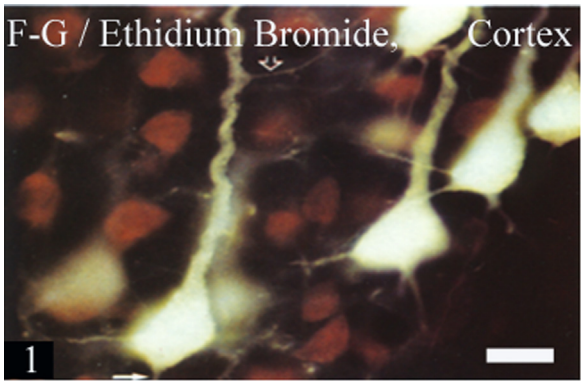

Fig. 1. Illustrates a single pyramidal neuron (yellow) in layer V sensory-motor cortex of the rat following injection of the tracer into the ventral horn of the cervical spinal cord. Neurons that do not share projections to the spinal cord appear red following counterstaining with ethidium bromide. Arrow at bottom indicates axon hillock while arrow head at top indicates secondary dendrite. Combined UV / green light excitation, mag bar = 10 μm.