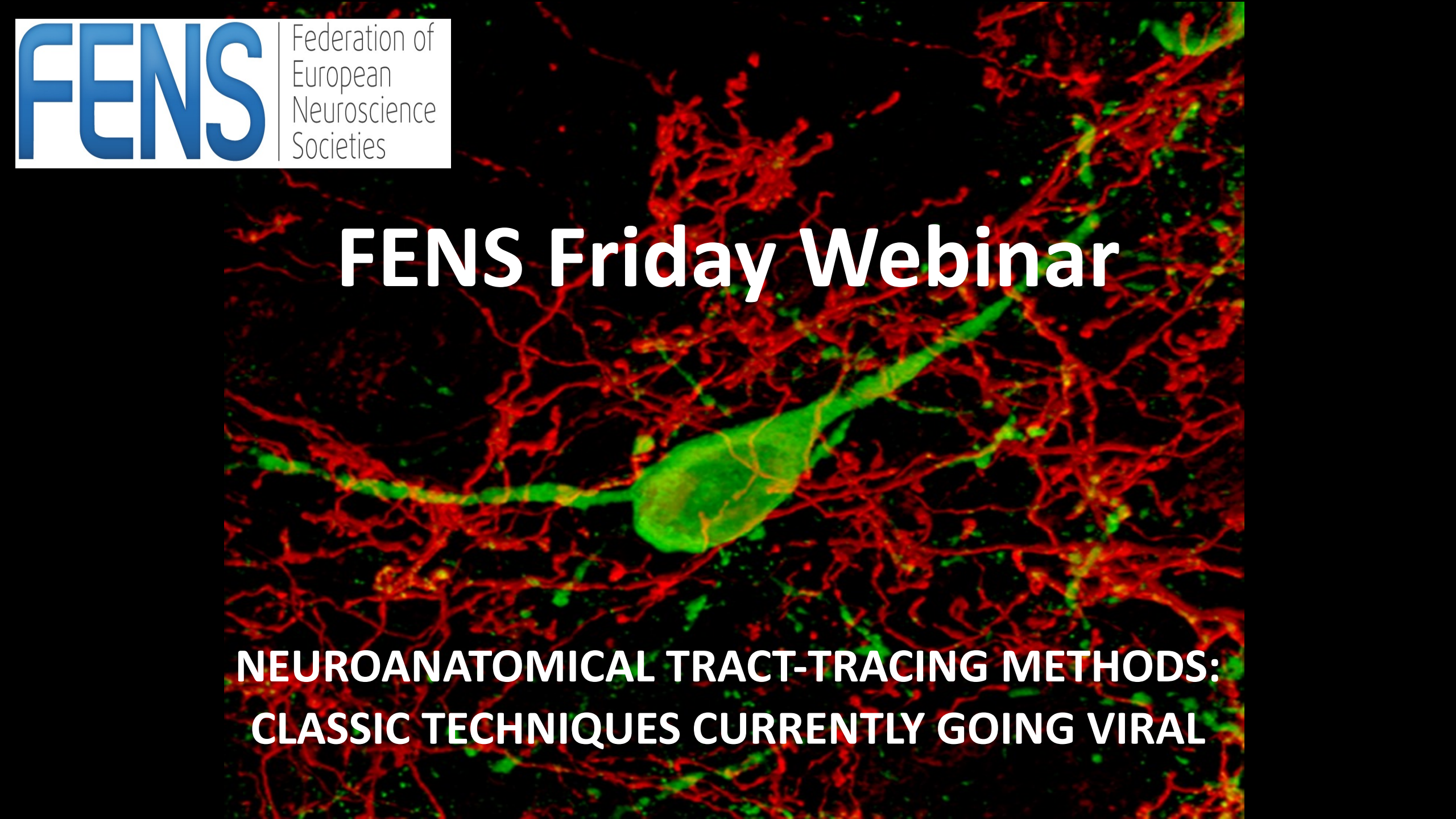
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FENS Friday Webinar
NEUROANATOMICAL TRACT-TRACING METHODS:
CLASSIC TECHNIQUES CURRENTLY GOING VIRAL

FENS Federation of European Neuroscience Societies

FENS FRIDAY WEBINAR – MEET THE SPEAKER

NEUROANATOMICAL TRACT-TRACING METHODS: CLASSIC TECHNIQUES CURRENTLY GOING VIRAL

JOSÉ L. LANCIEGO
(ES)

Dr Lanciego is the head of the Laboratory of Functional Basal Ganglia Neuroanatomy at CIMA-University of Navarra. His current research focuses on gene therapy approaches for neurodegenerative diseases using non-human primate models.

Registration deadline: 16 September 2022

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NEUROANATOMICAL TRACT-TRACING METHODS: CLASSIC TECHNIQUES CURRENTLY GOING VIRAL

ANNA BEYELER
(FR)

Dr Beyeler has been tenured as a principal investigator at the French Institute of Health (INSERM). Dr Beyeler's team lab studies the contribution of circuits of the insular cortex to emotional valence and anxiety and the alteration of those circuits in pre-clinical models of psychiatric disorders.

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NICHOLAS FOSTER
(USA)

Dr Foster joined the laboratory of Hongwei Dong at the University of California (UCLA) to contribute to an investigation that comprehensively mapped the entire corticostriatal pathway from all cortical areas to the dorsal striatum in the adult mouse.

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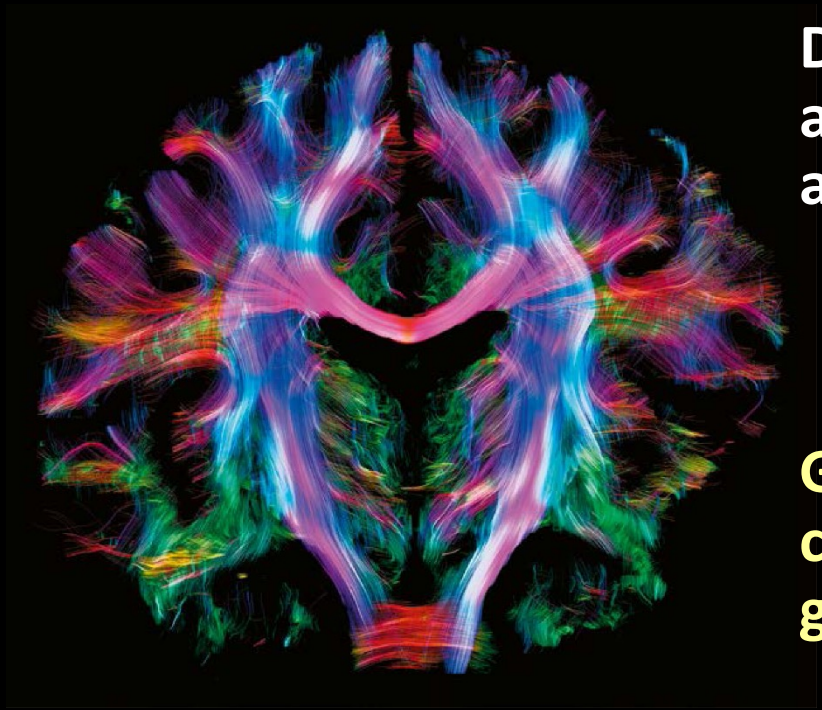
NEUROANATOMICAL TRACT-TRACING METHODS: CLASSIC TECHNIQUES CURRENTLY GOING VIRAL

Neuroanatomical tract-tracing methods remain fundamental for elucidating the complexity of brain circuits

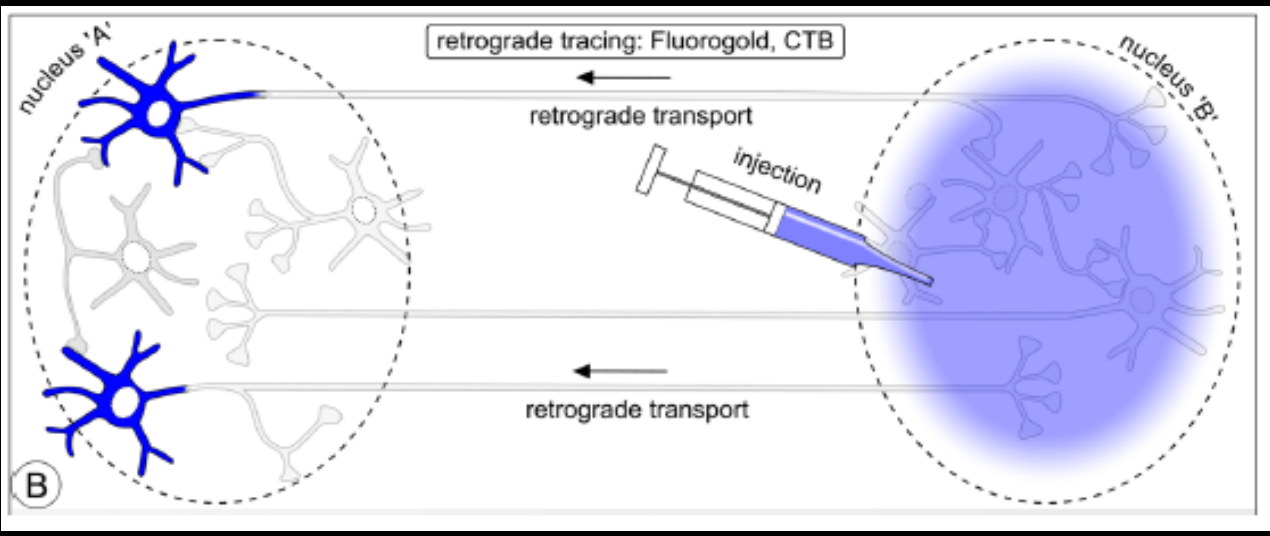


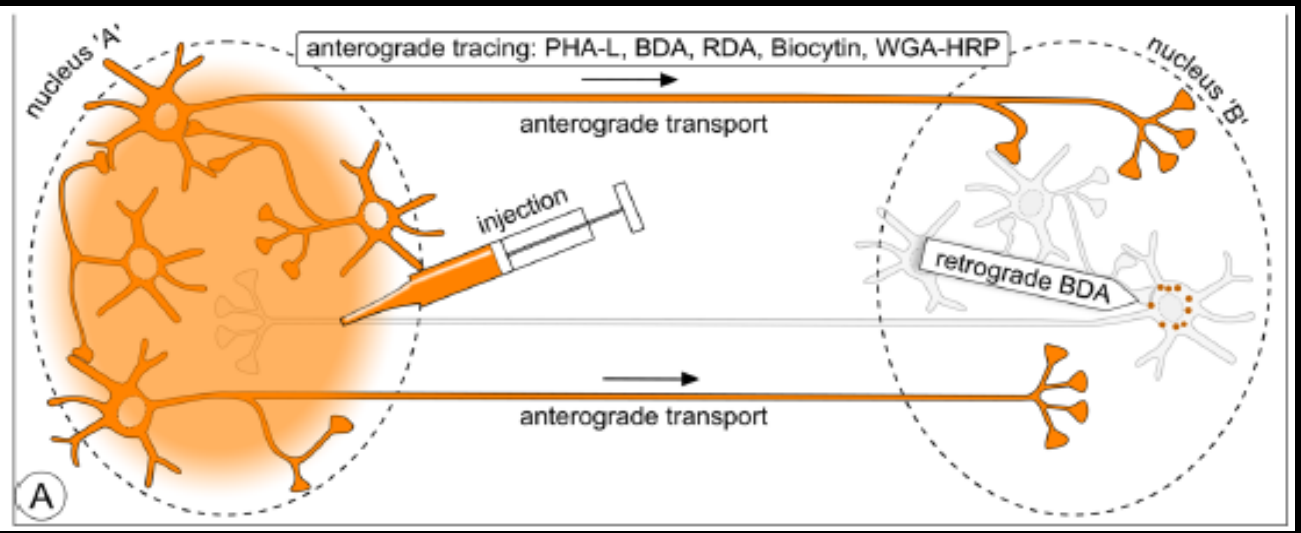
Despite the sophisticated arsenal of tract-tracing tools available at hand, the decision which tracer is best suited for a given tracing experiment still represents a difficult choice.

Goal: To provide novice users with some advice when choosing a neuroanatomical tracer that best applies to a given experimental design.

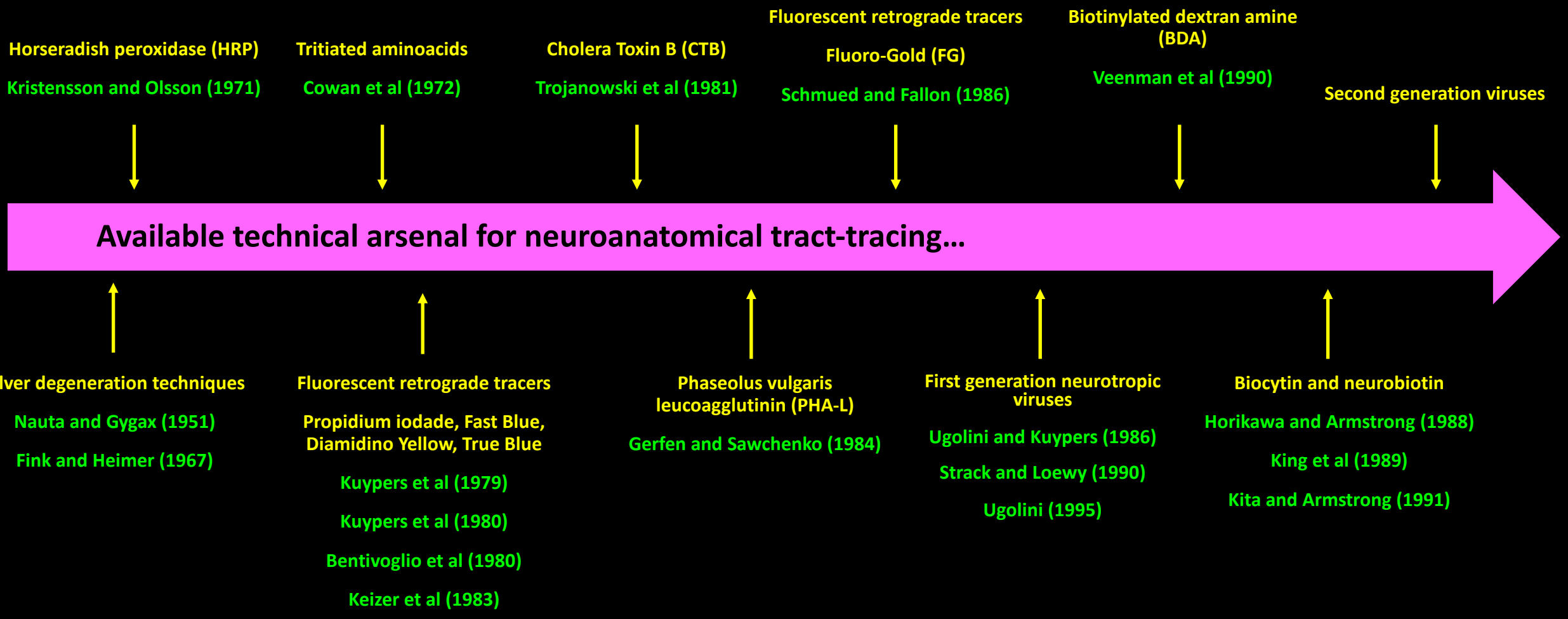
Foundations of neuroanatomical tract-tracing:

Neuroanatomical tracers are substances that, when injected into a given brain area, are taken up by neuronal processes and transported either from axon terminals to parent cell bodies (retrograde tracers) or from neuronal somata towards axon terminals (anterograde tracers). Sometimes tracers are transported into both directions (bi-directional tracers).

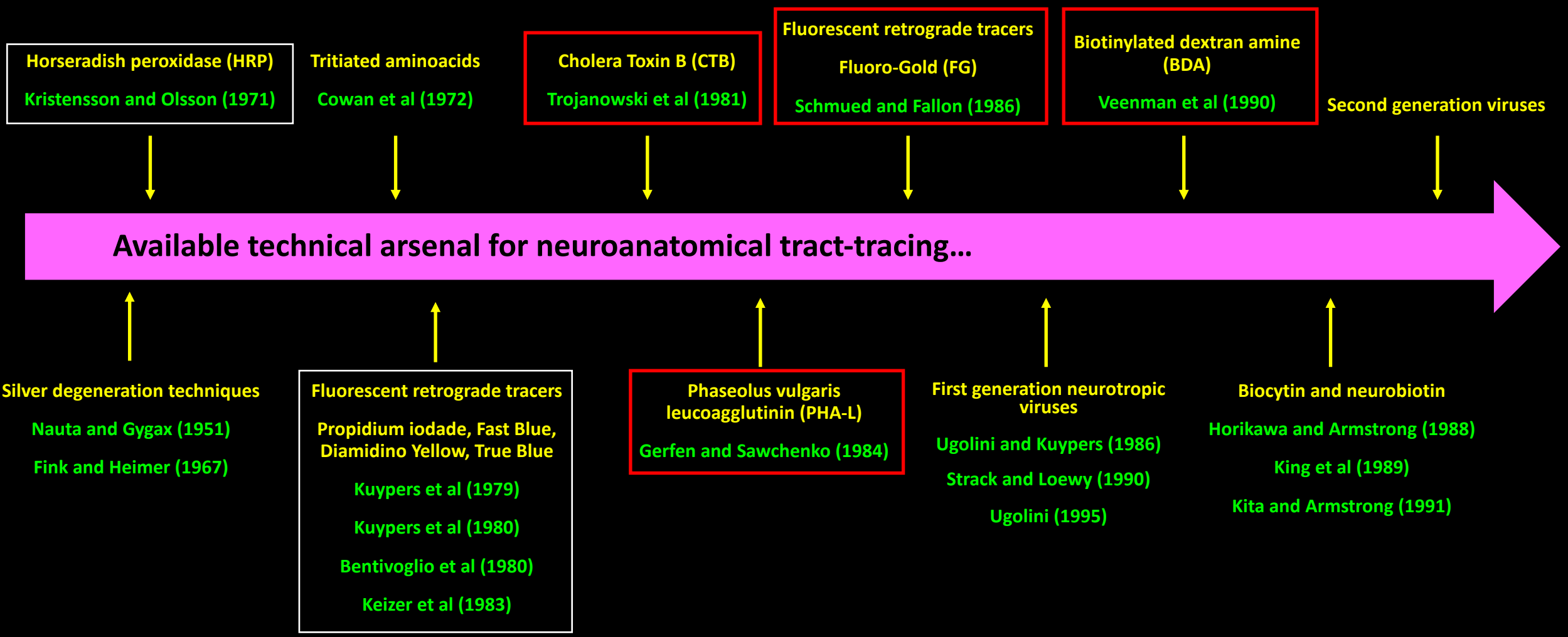




a brief historical perspective…



a brief historical perspective…



Classic & most widely used neuroanatomical tracers:

Cholera Toxin B

- Very good retrograde tracer
- Reliable antibody available
- Fluorescent conjugates available
- Pressure & iontophoretic deliveries
- Very good choice for large injection sites
- Some degree of anterograde tracing expected
- Works fine in all animal species tested

Fluoro-Gold

- Very good retrograde tracer
- Reliable antibody available
- Fluorescent signal resistant to fading
- Pressure & iontophoretic deliveries
- Works better when dissolved in 0.5M cacodylate buffer
- 100% retrograde performance
- Some concerns with FG when used in macaques

BDA 10 Kd

- Very good anterograde tracer
- No need for antibody-based detection
- Best suited for combination with other tracers
- Pressure & iontophoretic deliveries
- Some degree of retrograde tracing expected (particularly when pressure-delivered)
- Another variant available (BDA 3Kd)
- Very good for ultrastructural studies

PHA-L

- Very good anterograde tracer
- Performance sometimes capricious
- Only iontophoretic deliveries
- Lack of retrograde transport
- Second-choice anterograde tracer

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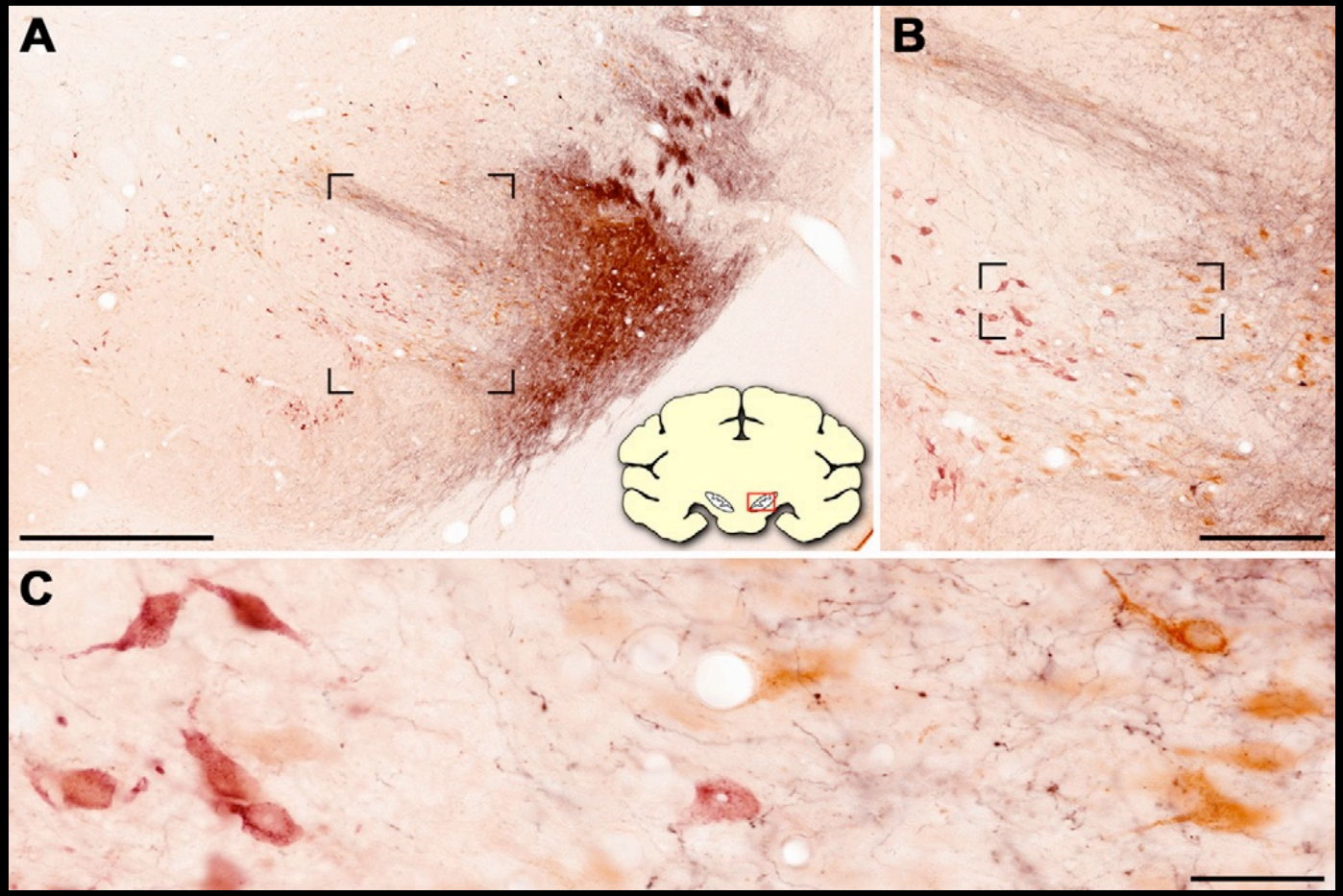
However, brain complexity often requires the use of multiple tract-tracing paradigms (this representing another trouble)…

In other words, which tracers are better suited for their combined use?

- Whenever possible, all involved tracers should be transported unidirectionally (either anterograde or retrograde)
- Only tracers with similar nature should be combined together (avoid combining fluorescent and non-fluorescent tracers)
- Only tracers requiring similar survival times should be used (avoiding multiple surgical sessions)
- Available antibodies for tracer detection should be specific and do not cross-react with each other.
- It is desirable that the detection of all involved tracers can be accomplished both by immunoperoxidase (permanent stains) and by immunofluorescence (confocal microscopy)
- Ultrastructural correlate often is a desirable outcome (tracer detection should be compatible with EM processing)

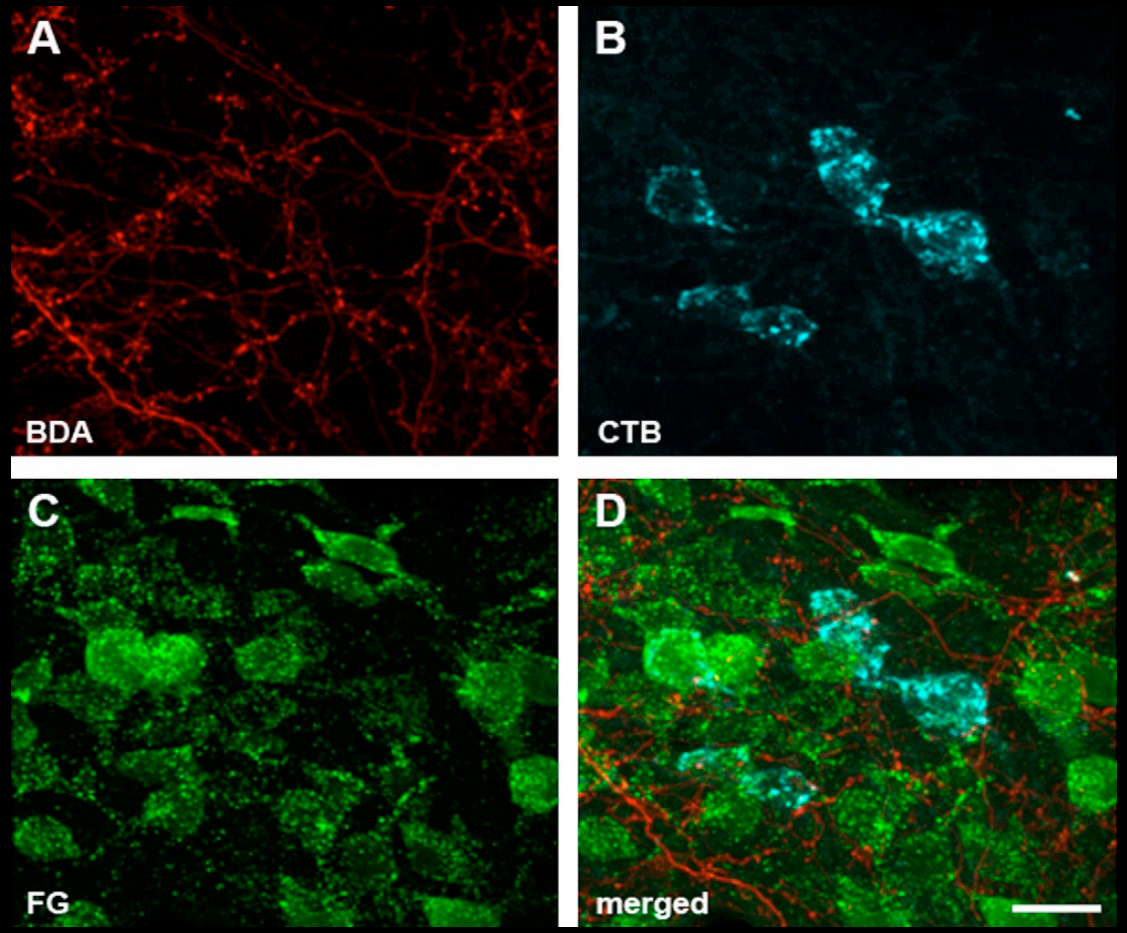
Multiple tract-tracing paradigms:

Example 1: Dual retrograde tracing with CTB and FG combined with BDA anterograde tracing



Triple immunoperoxidase detection

(substantia nigra nonhuman primate)

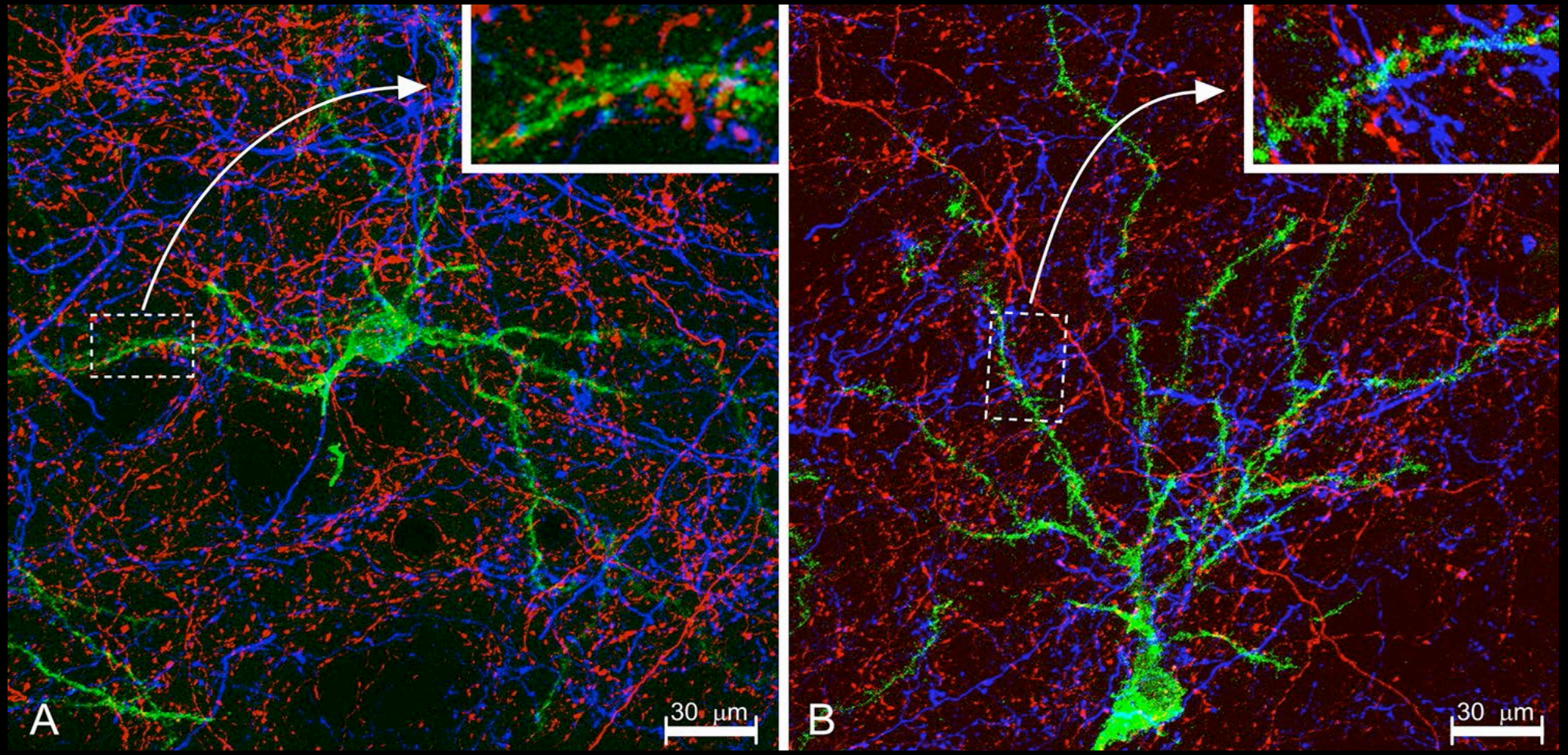


Triple immunofluorescent detection

(striatum rodent)

Multiple tract-tracing paradigms:

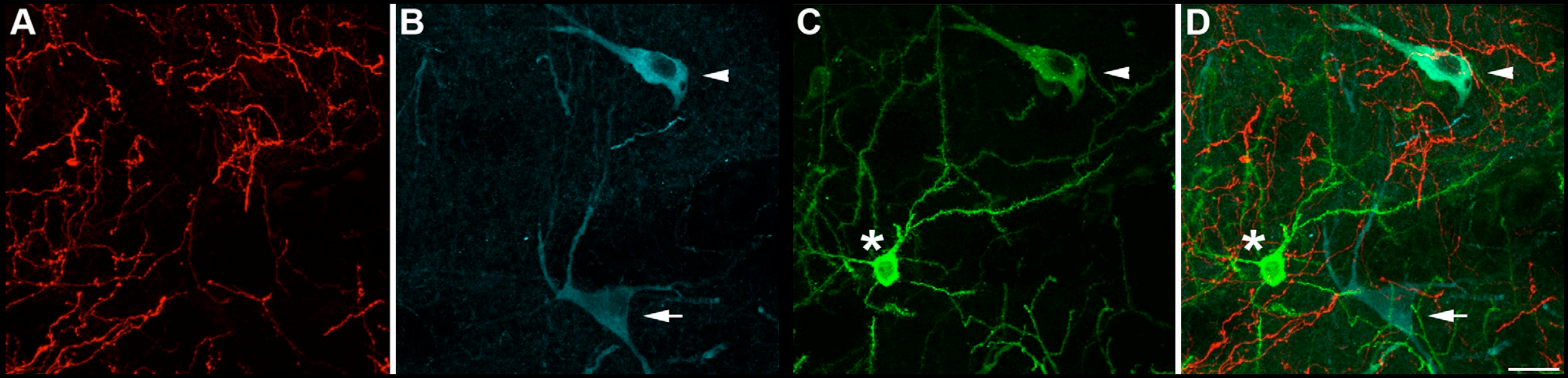
Example 2: Retrograde tracing with Rabies virus combined with dual anterograde tracing (BDA + PHA-L)



Triple immunofluorescent detection *(striatum, rodent)*

Multiple tract-tracing paradigms:

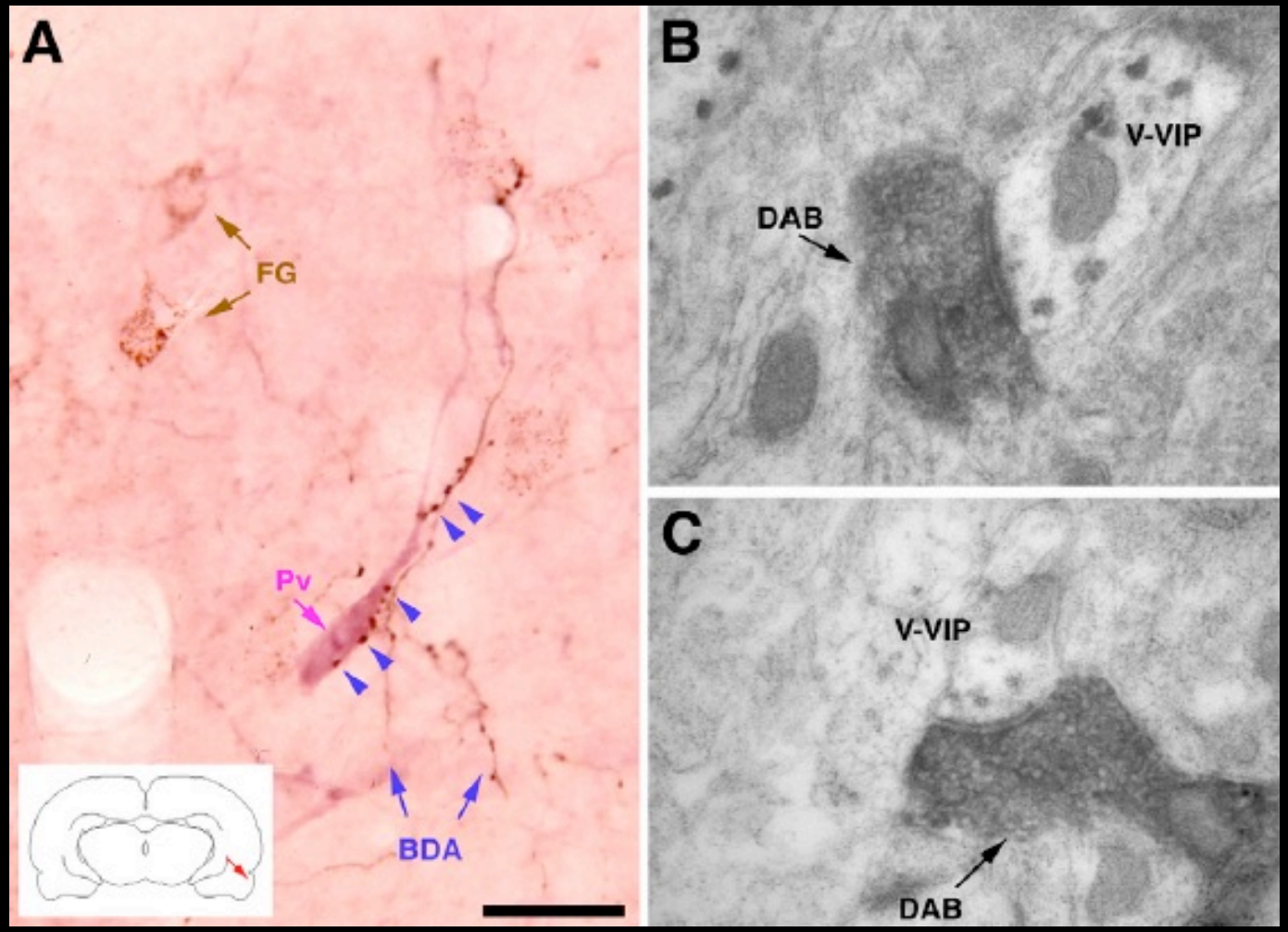
Example 3: Trans-synaptic tracing with Rabies virus combined with BDA anterograde tracing and ChAT IHC



Triple immunofluorescent detection *(striatum, rodent)*

Multiple tract-tracing paradigms:

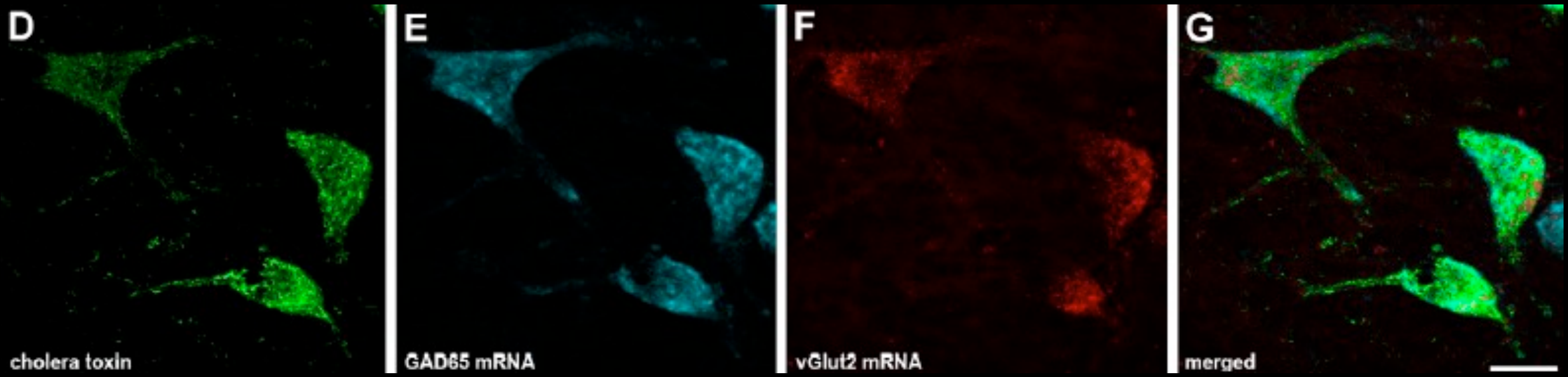
Example 4: Retrograde tracing with FG combined with BDA anterograde tracing and Parvalbumin IHC



Triple immunoperoxidase detection *(entorhinal cortex, rodent)*

“Functional” tract-tracing paradigms:

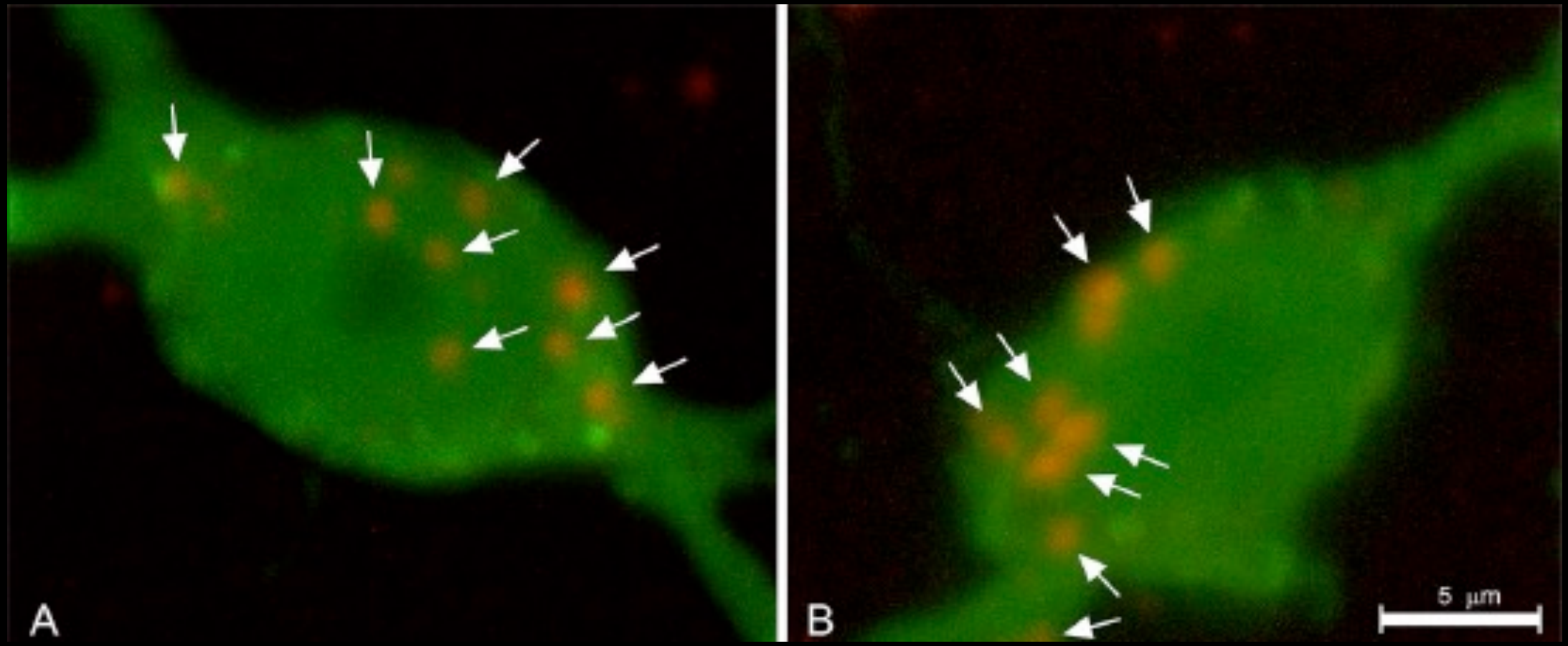
Example 5: Retrograde tracing with CTB combined with dual FISH for GAD65 and vGlut2



Triple immunofluorescent detection *(internal division globus pallidus, nonhuman primate)*

“Functional” tract-tracing paradigms:

Example 6: Retrograde tracing with BDA combined with in situ proximity ligation assay (D1-D2 receptor heteromers)



Dual immunofluorescent detection *(putamen nucleus, nonhuman primate)*

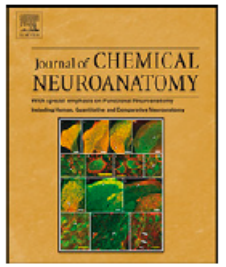
Some suggestions for further reading…

Journal of Chemical Neuroanatomy 42 (2011) 157–183

Contents lists available at ScienceDirect

Journal of Chemical Neuroanatomy

journal homepage: www.elsevier.com/locate/jchemneu



Review

A half century of experimental neuroanatomical tracing

José L. Lanciego [a], Floris G. Wouterlood [b,*]

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Brain Structure and Function (2020) 225:1193–1224
https://doi.org/10.1007/s00429-020-02041-6

REVIEW

Neuroanatomical tract-tracing techniques that did go viral

Jose L. Lanciego[1,2,3] · Floris G. Wouterlood[4]


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Thanks!