Corrigendum: A Student’s Guide to Neural Circuit Tracing

Christine Saleeba1,2†, Bowen Dempsey3†, Sheng Le1, Ann Goodchild1 and Simon McMullan1*1

1 Neurobiology of Vital Systems Node, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, NSW, Australia, 2 The School of Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol, United Kingdom, 3 CNRS, Hindbrain Integrative Neurobiology Laboratory, Neuroscience Paris-Saclay Institute (Neuro-PSI), Université Paris-Saclay, Gif-sur-Yvette, France

Keywords: neuroanatomy, viral tracers, anterograde tracer, retrograde tracers, synaptic contacts, connectome analysis

A Corrigendum on
A Student’s Guide to Neural Circuit Tracing
by Saleeba, C., Dempsey, B., Le, S., Goodchild, A., and McMullan, S. (2019). Front. Neurosci. 13:897. doi: 10.3389/fnins.2019.00897

In the original article, there was an error: one section of our review considers reagents traditionally considered to be anterograde tracers (i.e., fluorescent or antigenic substances that are taken up by neuronal cell bodies at the site of application and transported to the synaptic terminals). The original text read:

Emerging in the mid-1980s, dextran-based tracers, particularly biotinylated dextran amine (BDA), were rapidly adopted and remain one of the most widely used conventional anterograde tracers (Glover et al., 1986; Brandt and Apkarian, 1992; Veenman et al., 1992; Wouterlood et al., 2014). BDA enters injured neurons at the injection site, undergoes rapid anterograde transport and spreads evenly throughout the entire neuron, resulting in a Golgi-like level of staining detail (Köbbert et al., 2000; Lanciego and Wouterlood, 2011; Wouterlood et al., 2014). Interestingly, while 10 kDa BDA travels mostly anterogradely, the 3 kDa form is a retrograde tracer (Reiner et al., 2000; Lanciego and Wouterlood, 2011). Like CTb, fluorophore-labeled dextran amine variants are now widely used instead of biotinylated versions that require histological processing for visualization, and a number of authors have used tetramethylrhodamine-conjugated dextran for juxtacellular labeling during electrophysiological recordings (Noseda et al., 2010; Dempsey et al., 2015).

Limitations of Conventional Tracers

Despite their ongoing popularity, the major limitations of conventional tracers are worthy of consideration:

(1) Conventional tracers are taken up by fibers of passage (Dado et al., 1990; Chen and Aston-Jones, 1995; Conte et al., 2009), which can lead to incorrect identification of projections. [Notably, canine adenovirus (CAV) can also be taken up by fibers of passage (Schwarz et al., 2015)].

(2) The spread of many conventional tracers around the injection site results in intense and diffuse labeling that may reflect deposition in the extracellular matrix or take-up by neurons or glia. Such non-specific labeling makes it difficult to reliably identify labeled neurons within ~1 mm of the injection site. Thus the historical use of conventional tracers has probably overemphasized the relative significance of distant inputs/outputs compared to those originating from local interneurons; contemporary connectomic studies indicate that long-distance projections are relatively rare compared to short-distance connections (Oh et al., 2014; Henriksen et al., 2016; van den Heuvel et al., 2016; Dempsey et al., 2017).
(3) Tracer uptake relies predominantly on sugars that are located on the glycocalyx of most, if not all, neurons, or on common mechanisms such as endocytosis. Consequently, restricted uptake by functionally or neurochemically/genetically homogeneous neuronal populations is not possible.

(4) The direction of axonal transport is often not exclusive, which complicates circuit analysis; for example, CTb, perhaps the mostly widely used “retrograde” tracer, is also an efficient anterograde tracer (Luppi et al., 1987; Angelucci et al., 1996; Noseda et al., 2010).

The authors were contacted by Professor Joel Glover, who first described the use of dextran amines as neuronal tracers in the 1980s and expressed concern that we had inadvertently perpetuated a myth regarding the directional sensitivity of these tracers.

The amendment to the article clarifies the bidirectional nature of dextran amine transport.

A correction has been made to the Anterograde and Retrograde Tracers section, subsection Conventional (Mainly) Anterograde Tracers;

Emerging in the mid-1980s, dextran-amines (DAs) were rapidly adopted and remain widely used as conventional axonal tracers (Gimlich and Braun, 1985; Glover et al., 1986; Brandt and Apkarian, 1992; Veenman et al., 1992; Wouterlood et al., 2014). DAs enter injured neurons at the injection site and spread evenly throughout the entire neuron via diffusion, resulting in a GoGi-like level of staining detail (Glover et al., 1986; Fritzsch, 1993; Glover, 1995; Köbbert et al., 2000; Lanciego and Wouterlood, 2011; Wouterlood et al., 2014).

Despite the common perception that DAs are preferential anterograde tracers, many studies indicate bidirectional travel (Schmued et al., 1990; Fritzsch, 1993; Glover, 1995; Zhang et al., 2017), including the original description of their axonal transport by Glover et al. (1986). Their retrograde capabilities have been exploited both for conventional tracing (Silvertsen et al., 2014, 2016; Lunde et al., 2019) and for delivery of calcium-sensitive indicators for optical recording of neurons selected by axonal trajectory (O’Donovan et al., 1993; McPherson et al., 1997).

There is a perception that the molecular weight of DA-conjugates contributes to their directional selectivity, with smaller molecules exhibiting superior performance as a retrograde tracer (Reiner et al., 2000; Lanciego and Wouterlood, 2011). However, the influence, if any, of molecular weight on directional specificity is probably overstated, and may instead reflect differences in speed of transport, which is distinctly faster for smaller compounds (Fritzsch, 1993), combined with differences in volume of synaptic terminals compared to cell bodies (Joel C Glover, personal communication).

Like CTb, fluorophore-labeled dextran amine variants are now widely used instead of or in addition to biotinylated versions that require histological processing for visualization, and we and others have used tetramethylrhodamine-conjugated dextran for juxtacellular labeling during electrophysiological recordings (Noseda et al., 2010; Dempsey et al., 2017).

**Limitations of Conventional Tracers**

Despite their ongoing popularity, the major limitations of conventional tracers are worthy of consideration:

1. Conventional tracers can be taken up by fibers of passage (Dado et al., 1990; Chen and Aston-Jones, 1995; Conte et al., 2009), which can lead to incorrect identification of projections. [Notably, canine adenovirus (CAV) can also be taken up by fibers of passage (Schwarz et al., 2015)].

2. The spread of many conventional tracers around the injection site results in intense and diffuse labeling that may reflect deposition in the extracellular matrix or take-up by neurons or glia. Such non-specific labeling makes it difficult to reliably identify labeled neurons within ~1 mm of the injection site. Thus the historical use of conventional tracers has probably overemphasized the relative significance of distant inputs/outputs compared to those originating from local interneurons; contemporary connectomic studies indicate that long-distance projections are relatively rare compared to short-distance connections (Oh et al., 2014; Henriksen et al., 2016; van den Heuvel et al., 2016; Dempsey et al., 2017).

3. Tracer uptake relies predominantly on sugars that are located on the glycocalyx of most, if not all, neurons, or on common mechanisms such as endocytosis. Consequently, restricted uptake by functionally or neurochemically/genetically homogeneous neuronal populations is not possible.

4. The direction of axonal transport is rarely exclusive, which complicates circuit analysis; the archetypal retrograde and anterograde tracers, CTb and BDA respectively, both label axons traveling in the “wrong” direction (Luppi et al., 1987; Schmued et al., 1990; Fritzsch, 1993; Glover, 1995; Angelucci et al., 1996; Noseda et al., 2010; Zhang et al., 2017)."

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

**REFERENCES**

Angelucci, A., Cascii, F., and Sur, M. (1996). Anterograde axonal tracing with the subunit B of cholera toxin: a highly sensitive immunohistochemical protocol for revealing fine axonal morphology in adult and neonatal brains. J. Neurosci. Methods 65, 101–112. doi: 10.1016/0165-0270(95)00155-7

Brandt, H. M., and Apkarian, A. V. (1992). Biotin-dextran: a sensitive anterograde tracer for neuroanatomical studies in rat and monkey. J. Neurosci. Methods 45, 35–40. doi: 10.1016/0165-0270(92)90041-b

Chen, S., and Aston-Jones, G. (1995). Evidence that cholera toxin B subunit (CTb) can be avidly taken up and transported by fibers of passage. Brain Res. 674, 107–111. doi: 10.1016/0006-8993(95)00020-q

Conte, W. L., Kamishina, H., and Reep, R. L. (2009). Multiple neuroanatomical tract-tracing using fluorescent Alexa Fluor conjugates of cholera toxin subunit B in rats. Nat. Protoc. 4, 1157–1166. doi: 10.1038/nprot.2009.93

Dado, R. J., Burstein, R., Cliffer, K. D., and Giesler, G. J. Jr. (1990). Evidence that fluoro-gold can be transported avidly through fibers of passage. Brain Res. 533, 329–333. doi: 10.1016/0006-8993(90)91358-n
Dempsey, B., Le, S., Turner, A., Bokiniec, P., Ramadas, R., Bjaalie, J. G., et al. (2017). Mapping and analysis of the connectome of sympathetic premotor neurons in the rostral ventrolateral medulla of the rat using a volumetric brain atlas. Front. Neural Circ. 11:9. doi: 10.3389/fncir.2017.00009

Dempsey, B., Turner, A. J., Le, S., Sun, Q. J., Bou Farah, L., Allen, A. M., et al. (2015). Recording, labeling, and transfection of single neurons in deep brain structures. Physiol. Rep. 3:e12246. doi: 10.14834/phy2.12246

Fritsch, B. (1993). Fast axonal diffusion of 3000 molecular weight dextran amines. J. Neurosci. Methods 50, 95–103.

Gimlich, R. L., and Wouterlood, F. G. (2011). A half century of experimental neuroanatomical tracing. J. Neurosci. Methods 20, 345–361. doi: 10.1016/j.jneumeth.2014.06.001

Lanciego, J. L., and Wouterlood, F. G. (2011). An improved method to label cells with tetramethylrhodamine-dextran amine through thalamocortical and corticothalamic pathways. Anterograde and retrograde tracing with high molecular weight biotinylated dextran amines as an anterograde tracer for single- and double-labeling studies. J. Neurosci. Methods 41, 239–254. doi: 10.1016/j.jneumeth.2014.06.001

van den Heuvel, M. P., Bullmore, E. T., and Sporns, O. (2016). Comparative connectomics. Trends Cogn. Sci. 20, 345–361. doi: 10.1016/j.tics.2016.03.001

Veenman, C. L., Reiner, A., and Honig, M. G. (1992). Biotinylated dextran amine as an anterograde tracer for single- and double-labeling studies. J. Neurosci. Methods 41, 239–254. doi: 10.1016/0165-0270(92)90089-v

Wouterlood, F. G., Bloem, B., Mansvelder, H. D., Luchici, A., and Deisseroth, K. (2014). A fourth generation of neuroanatomical tracing techniques: exploiting the offspring of genetic engineering. J. Neurosci. Methods 235, 331–348. doi: 10.1016/j.jneumeth.2014.07.021

Copyright © 2020 Saleeba, Dempsey, Le, Goodchild and McMullan. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.