

COMMENTARY ON: 3D DISTRIBUTION OF TYROSINE HYDROXYLASE, VASOPRESSIN AND OXYTOCIN NEURONS IN THE TRANSPARENT POSTNATAL MOUSE BRAIN. GODEFROY D, DOMINICI C, HARDIN-POUZET H, ANOUAR Y, MELIK-PARSADANIANTZ S, ROSTENE W, REAUX-LE GOAZIGO A. *J NEUROENDOCRINOL* 2017; e12551.

Michelle Carroll and Melissa J Chee

Department of Neuroscience, Carleton University, Ottawa ON Canada

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In the past decade, systematic improvements in whole tissue clearing and staining has enabled the visualization of single cells or entire fiber pathways within the three-dimensional space of an intact brain. This achievement is made possible by technological improvements for i) tissue clearance, which renders the brain tissue transparent, and ii) automated whole brain imaging, which enables fast, unbiased histological analysis. Godefroy *et al.* (2017) used the iDISCO+ technique for clearance of mouse brains then labelled tyrosine hydroxylase (TH), oxytocin (OXT) and arginine vasopressin (AVP) neurons. Subsequently, they applied light sheet microscopy to determine the distribution of these neurons in three-dimensional space (1).

iDISCO+ is a descendant of the 3DISCO^a and iDISCO^b clearing method. Both 3DISCO (2) and iDISCO (3) use tetrahydrofuran to produce completely clear brain tissue but the clearing and dehydration steps involved will typically shrink or deform the brain. iDISCO+ delivers a completely cleared brain while minimizing tissue shrinkage by using a combination of methanol and dichloromethane (4). The resulting transparent brain then undergoes immunostaining established by iDISCO. While the introduction of 3DISCO made significant improvements to preserve the fluorescence of native proteins, these concerns became immaterial to the iDISCO method because it allows for immunolabeling after brain clearance. iDISCO allowed antibodies to penetrate thick tissues and uses photostable Alexa Fluor dyes so that the brain can be imaged multiple times without photobleaching (3); thus its application is limited only by antibody availability. iDISCO+ capitalizes on this to achieve a completely cleared and transparent brain that is amenable to immunolabeling but without consequence to brain cytoarchitecture or morphology (4). However, Godefroy *et al.* did note that the chemical treatments involved may reduce the effectiveness of some antibodies shown to work on other brain tissues.

Using the brains of postnatal day 5 (P5) mice, Godefroy *et al.* used iDISCO+ to clear the brain and immunolabel TH, OXT and AVP neurons and their fiber pathways. They then used light sheet fluorescent microscopy to visualize the distribution of these neuronal populations in 3D. One of the main goals of this study is to quantify these neurons in the P5 mouse brain and/or form comparisons to the rat brain. Furthermore, the authors used iDISCO+ to provide new information on the 3D structure and somatic volume of the labeled cells. Between the mouse and rat, Godefroy *et al.* found that the pattern of TH-immunoreactivity is similar. In contrast

there is a prominent interspecies difference for OXT- and AVP-immunoreactivity. The authors confirmed the localization of OXT and AVP cell bodies to the supraoptic nucleus (SON) and paraventricular nucleus (PVN). However, they noted that there is dense labeling of both these cell types along the basoventral region of the hypothalamus that is not seen in the rat. The authors also noted that both these cell types are loosely localized and not restricted to the boundaries of the accessory nucleus, which may represent cells that are still migrating in the P5 brain. With regards to the difference between the distribution of OXT and AVP cells in the P5 brain, Godefroy *et al.* found more OXT than AVP neurons in the dorsal part of the SON and PVN. In addition, consistent with the delayed maturation of OXT neurons relative to AVP neurons, Godefroy *et al.* used iDISCO+ to show that the volume of OXT neurons is less than AVP cells at P5.

The allowance of iDISCO+ to determine cell volume poses intriguing new possibilities in disease research (4) by allowing a comparison of cells in the diseased and healthy state. This may enhance our knowledge on the pathology of several different mental afflictions. The iDISCO+ clearing method can be easily adapted by research labs as it is simple, quick and does not require expensive equipment to implement. When combined with the appropriate imaging abilities, it may lead to new information on the three-dimensional representation and distribution of many neurotransmitter systems. Therefore, it may greatly enhance knowledge in the fields of neuroendocrinology, neuroanatomy and others.

^a Three-dimensional imaging of solvent-cleared organs (3DISCO)

^b Immunolabeling-enabled 3DISCO (iDISCO)

References:

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