**Introduction**

Current methods of intact tissue structural analysis are incompatible with most methods of molecular phenotyping, leading to difficulty in gaining a perspective on a tissue which includes both its structural and molecular level information.

The barrier to the visualization of intact systems is the lipid bilayers in biological tissues, which hinder our molecular probing and limit our ability to image the tissue by rendering the tissue opaque. These membranes prevent the diffusion of molecules, preventing what is known as the “tissue barrier” and making it difficult to work on very thin slices of tissue. In order to overcome this barrier, clearing techniques have been developed, but have been limited by their high expense and time consumption.

**Thick Slice CLARITY**

Because of the inherent limitations of thin slice immunohistochemistry (IHC) methods and the cost, both financially and in terms of time, of whole organ/body CLARITY, Xiaochen Lu developed Thick Slice CLARITY in the lab of Lisa Stubbs at the Carl R. Woese Institute for Genomic Biology at the University of Illinois at Urbana-Champaign.

**Thick Slice CLARITY takes the clearing and antibody staining principles of whole organ CLARITY and applies it instead to 200μm thick slices of brain tissue.**

**Fig. 1.** Above, a mouse brain before and after CLARITY technique, by K. Chung and N. Desai, Howard Hughes Medical Institute/Stanford.

After a transcardial perfusion, mouse brains are extracted and fixed in a paraformaldehyde/acrylamide solution. The brains are then sectioned on a vibrating microtome, and the slices incubated at 37°C for three hours, and then polymerized at 37°C for three hours. For Thick Slice CLARITY, rather than doing clearing by electrophoretic tissue clearing (ETC) methods, passive clearing over the course of two to three days in SDS-ETC solution at 37°C is more typical. After the lipids have been cleared from the tissue, the slices may undergo multiple rounds of antibody staining, and then may be mounted and imaged, generally via confocal microscopy.

Thick Slice CLARITY allows for the viewing of regional sections of the brain and analysis at a structural and molecular level. It has proven very useful for the localization of novel neuroactive target gene products.

**Current Work**

Recent research into mouse activity and exercise showed that there is a strong positive correlation between the expression levels of Gpr3, a gene encoding an orphan G-protein-coupled receptor involved in signal transduction, and motivation for voluntary running activity in mice. Because of its density of dopamine receptors, the striatum was identified as a brain region likely to have experienced changes at a molecular level as a result of changed intrinsic exercise motivation.

**The Thick Slice CLARITY technique, used in conjunction with standard fluorescent staining methods and imaging via confocal microscopy, has allowed for the colocalization of GPR3 with neurons in the mouse striatum, verifying the hypothesis of the area’s importance. As seen on in Fig. 3, the small number of cells labeled with GPR3 reinforce standing ideas where Gpr3 is expressed. Similar techniques were employed to localize NR2F1, the product of a deeply conserved autism gene, in neurons in the brain. While previously known to be existent in the human brain, its localization was unknown.**

**Fig. 3.** Above, GPR3 (in green) co-localized with neurons, identified using the pan-neuronal marker NeuN (in red).

**Future Work**

The localization of GPR3 with the CLARITY technique has made future work on the exercise project actionable. It will lead to further localization and other efforts, and contribute to research into the possibility of the use of pharmacological compounds to increase voluntary exercise motivation.

The localization of NR2F1 in neurons was a step towards understanding its position in the brain, and will contribute to research involved in autism and other developmental disabilities.

CLARITY itself is a fairly new technique with a lot of potential, both seen and unseen, for future purposes. The technique has potential for mapping neural circuits and other connectomics work, for classifying and subclassifying neurons, and for protein localization in other projects.

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