IMAGING NEURONAL SYNAPSES TO UNDERSTAND MEMORY IMPAIRMENT IN NEURODEGENERATION

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Alzheimer's disease (AD) is the most common form of dementia. The protein tau is associated with AD and has been shown to impair synaptic function [1,2] though the mechanisms involved are not yet well understood. In this study we employed TIRFM, dSTORM, ExM, and AFM to understand the compounding aspects of tau dysfunction, and this report highlights the complementary information obtained by these methods.

Synapses are difficult to locate in live cells due to a lack of appropriate fluorescent probes. To address this, we have developed a convenient biofunctionalisation method, simplified from [3], to bind synapses to coverglass, bringing them into the evanescent region of a TIRF microscope. Since this method uses live cells, we can track tau through synapses. Fig 1b shows patterned biofunctionalised protein on cover glass, allowing synapses to be easily located on a grid. This also allows us to image synapses with super-resolution dSTORM and quantify the amount of tau in synapses. As an alternative, we used Expansion microscopy (ExM) to view synapses with tau.

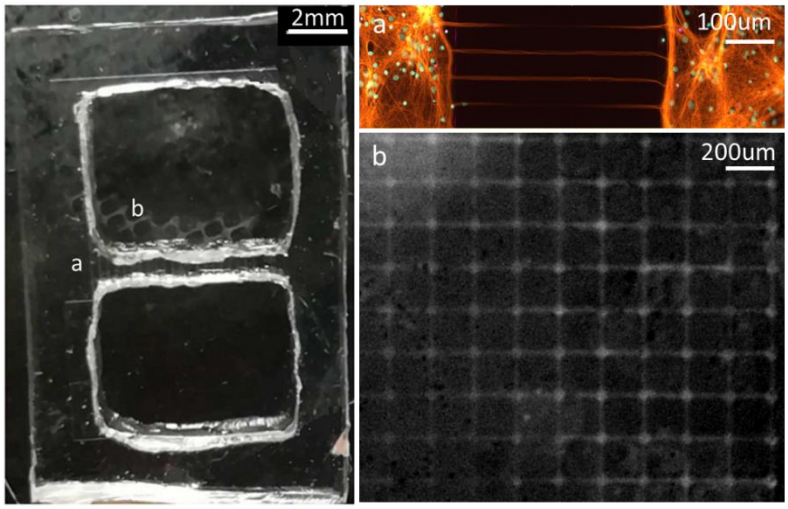

Figure 1. A custom-designed microfluidic culture device with (a) two neuronal compartments connected via microchannels. Colin Hockings & Lianne Roode. (b) biofunctionalised proteins for TIRF microscopy.

Finally, we took mechanical measurements using AFM. To measure how tau affects stiffness of synapses, we used AFM on live cells in different calcium conditions that may influence synaptic activity.

This project highlights complementary techniques which provide a full understanding of tau's effect on synaptic activity. A novel biofunctionalisation method allows tracking of live-cell pathways or can be combined with super-resolution microscopy to further increase resolution, or ExM can be used as an alternative approach. Mechanical measurements are used in parallel as a physical read-out of the effect of tau in synapses, as detected from optical methods.

[1] Wu et al., (2016) Nat Neurosci. 19(8): 1085–1092.
[2] Tai et al., (2012) Am J Pathol. 181(4): 1426–1435.
[3] Czöndör et al., (2013) Nature Comms. 4:2252.