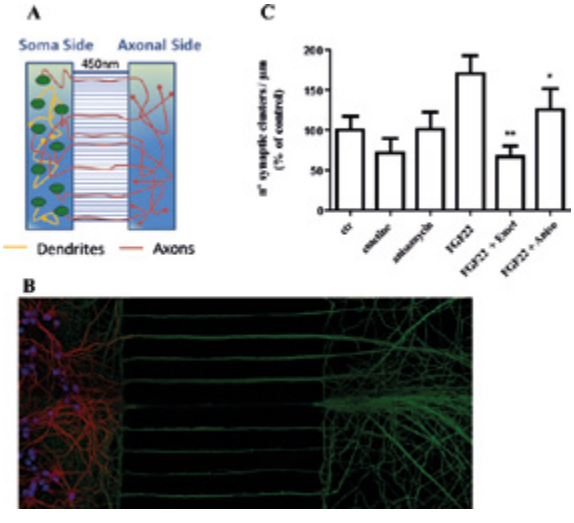
Local protein synthesis is required for synapse formation

Joana R. Pedro[1], Maria Joana Pinto[1], Rui O. Costa[1], Luís Martins[1], Pedro Alves[1,2], Samie R. Jaffrey[3], Ramiro D. Almeida[1,4]

The establishment of synapses requires the formation of a well-structured and functional presynaptic terminal. This process can be regulated by contact with the synaptic partners or by target-derived cues. The canonical model proposes that presynaptic proteins are transported from the cell body in the form of pre-assembled transport vesicles. However, the long distance between the cell body and distal axons is inconsistent with the rapid remodeling of the presynaptic terminal, suggesting that transport cannot be the sole source of new proteins that drive presynaptic differentiation. Therefore, local mechanisms might provide an additional or alternative mechanism to control the formation of new presynaptic sites.

Local mRNA translation controls the pool of proteins present in specific neuronal compartments. This mechanism provides neurons with an incredible ability to regulate dynamic events, such as synaptic plasticity, cue-induced axonal steering, axon turning, axon outgrowth and regeneration. In addition, a diverse set of mRNA species, comprising a variety of protein families, were found in recent axonal microarray screens, but the role of such a diverse array of transcripts is still elusive.

To address these questions we decided to investigate if synapse formation is dependent on local protein synthesis. We started by culturing vertebrate neurons in microfluidic devices (Fig.1A). Specific properties of these devices allow physical and fluidic isolation between both microenvironments (Fig.1B). Thus, microfluidic chambers are well suited to study the intra-axonal mechanisms that regulate synapse formation. We found that synaptogenic cues activate mRNA translation pathways in axons. In addition, we observed that adding protein synthesis inhibitors specifically to axons (but not to cell bodies) blocks the formation of synaptic clusters. Using live cell imaging we also found that a translation reporter is locally synthesized in axons during stimuli-induced synapse formation. Finally, axonal delocalization of axonal mRNA in an in-vitro neuron-muscle co-culture impairs synapse formation. Overall, our results show that axonal mRNA translation is required for synapse formation (Fig.1C) and highlight a functional of this process in nervous system development.



1 — CNC – Center for Neuroscience and Cell Biology, University of Coimbra
2 — IEC – Instituto da Educação e Cidadania, Mamarrosa
3 — Weill Medical College of Cornell University, New York
4 — Department of Medical Sciences & iBiMED, University of Aveiro

FIGURE 1
Microfluidic chambers as tool to study neuronal development and regeneration.
(A) Schematic diagram of microfluidic chambers. (B) Rat embryonic hippocampal neurons were cultured in microfluidic chambers and immunostained against an axonal marker (green), a dendritic marker (red), the nuclei are labelled in blue. The neurons are plated in the somal compartment (right) and as they develop axons extend into the axonal compartment (left). Due to the fluidic and physical characteristics of these devices, no cell bodies or dendrites cross into the opposite compartment allowing the study of axonal-specific events. (C) FGF22, a synaptogenic cue, added specifically to the axonal compartment induces the formation of new synapses and this process is dependent on local mRNA translation.