Live imaging of synapse engulfment by microglia *in vitro*

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Synaptic pruning is fundamental for the refinement of synaptic connections. Accumulating reports have suggested that the brain-resident immune cells microglia are the executioner of synaptic pruning. Major skepticism to this idea is based on the fact that no report has successfully captured an actual moment of synaptic pruning by microglia both *in vivo* and *in vitro*. Here, we investigated whether and how microglia prune synapses from living neurons, using a live imaging system for neuron-glia cocultures. Firstly, we prepared the general culture system of dispersed microglia on glass dishes but failed to reproduce the ramified shape of microglia that is observed *in vivo*. Because the morphology of microglia is relevant to their function, we needed a proper *in vitro* system in which the ramified shape of microglia is maintained. For this purpose, we cocultured microglia with astrocytes and neurons so that the surrounding milieu was similar to *in vivo* conditions. Under these experimental conditions, microglia now exhibited ramified-like morphology. To time-lapse image these microglia, the glial cocultures were prepared from CX3CR1^{GFP/+} mice whose microglia express GFP. Neurons were transfected with synaptophysin-RFP after added to the glial cocultures. Using this coculture system, we observed a translocation of RFP-labeled synaptophysin from the axons of living neurons to GFP-labeled microglial processes. We are now investigating whether neuronal activity regulates synaptic pruning by pharmacological modulations. Our experimental system is expected to clarify the processes and mechanisms of synaptic pruning by microglia.