Supplementary Information

Supplementary Methods.

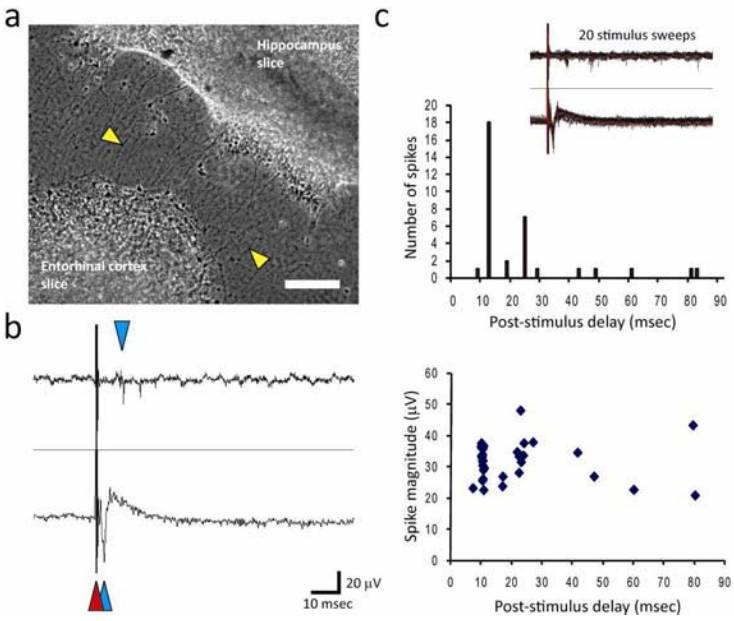
The entorhinal cortex-hippocampus co-cultures shown in Supplementary Figure 1 were created using a modification of the roller-tube method[1]. Briefly, poly-lysine coated coverslips were coated with a collagen gel (rat tail collagen, extracted in our laboratory). Slices of mouse hippocampus and entorhinal cortex were placed on the same coverslip, separated by a distance of 2 mm. Cover slips were placed into 6-well dishes, which were then filled with just enough medium to cover the top of the slice. Cultures were placed into a standard tissue culture incubator (5% CO_2 humidified atmosphere, at 37º C) onto a rocker platform operating at ten cycles per hour.

Electrical responses were evoked with a bipolar stimulating electrode (twisted tungsten wire) placed into entorhinal cortex. Single biphasic current pulses (200-400 μA) of 100 μs duration were applied with a stimulus isolator, and neuronal response was recorded with tungsten microelectrodes placed in CA1 of the hippocampal slice.

Co-cultures in Supplementary Figure 2 were carried out as described in the main Methods section.

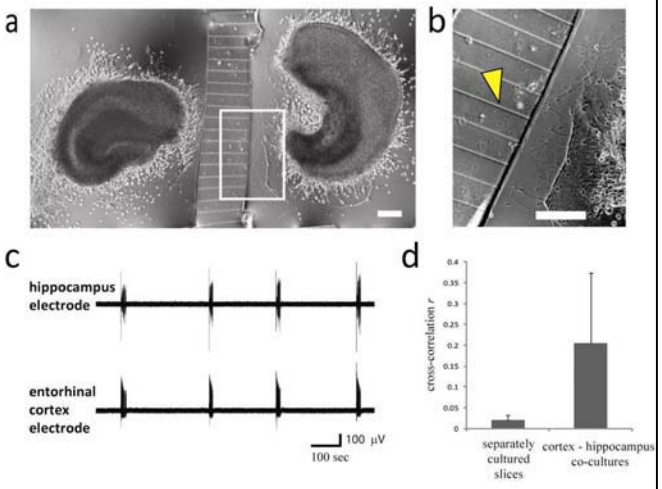
Supplementary Results.

We have carried out co-culture experiments where hippocampus and entorhinal cortex slices have been placed in close physical proximity on the same substrate after the subiculum has been removed. The cortical and the hippocampal slices extend neurites, which can be observed to enter the neighboring slice (Supplementary Fig. 1a). Electrophysiological experiments showed that the stimulus delivered to the entorhninal cortex slice evoked field potentials in the hippocampal slice after a 10-20 msec delay (Supplementary Fig. 1b, c). These recordings confirmed that the extended neurites allow the propagation of depolarization and form functional synapses with the target neurons in the other slice.



Supplementary Fig. 1. Entorhinal cortex and hippocampal co-cultures form functional connections, (a) hippocampus and cortex slices form multiple inter-slice connections, 7 DIV, phase microscopy, scale bar represents a distance of 500 μm, (b) voltage pulses (red triangle) were delivered to the entorhinal electrode, and extracellular potentials (blue triangles) recorded with microelectrodes in the entorhinal and hippocampus slices. A delayed spike can be seen in the hippocampus, indicating a functional connection between the slices. (c) evoked spikes for 20 stimulation sweeps, shown for 100 ms post-stimulation. In 18 out of 20 sweeps, a spike was detected with a delay of 10-11 ms relative to the stimulus. A second group of spikes can also be observed at 22-27 ms delay.

We have also carried entorhinal cortex-hippocampus co-cultures in PDMS mini-wells with connecting microchannels, to verify that our method is generally applicable and not restricted to CA1 axon sprouting. We placed slices of rat hippocampus and entorhinal cortex in the configuration shown in Supplementary Figure 2a, to approximate their positions in the intact brain. After three days in vitro (3 DIV), axons were extended by neurons in the entorhinal slice, and began to enter microchannels (Supplementary Fig. 2a, b). After 14 DIV, spontaneous activity in hippocampal and entorhinal slices in the presence of picrotoxin and CGP became highly correlated, showing evidence of functional synapses formed by axons extending from one slice to the other through the microchannels (Supplementary Fig. 2c, d).



Supplementary Fig. 2. Activity is synchronized in entorhinal-hippocampal co-cultures via axons growing through the microchannel fluid barrier. (a) Phase micrograph showing hippocampus (left) and entorhinal cortex (right) slices in PDMS miniwells. Area bounded by the white box is shown at higher magnification in (b), where a dense network of neurites can be seen extending beyond the perimeter of the entorhinal slice. Some long neurites (likely axons) enter the microchannels by 3 DIV, yellow arrow. (c) Bursts recorded in co-cultured slices showed a high degree of synchrony by 14 DIV. (d) Average cross-correlation coefficients *r* are plotted for co-cultures (n = 2 co-cultures) and separately cultured slices (n = 2 pairs of separate slices).

1. B. H. Gahwiler, *J. Neurosci. Methods*, 1981, **4**, 329-342.