Publishable Summary

Project Objectives

The entorhinal cortex and hippocampus are high-end cortices that are required for the formation of new episodic memories (memories of what happened along with when and where it happened). Despite the fact that the entorhinal cortex and hippocampus are multiple synapses removed from the sensory periphery and the fact that episodic memory is a complex phenomen, recordings of single cells in the two structures have found that the cells have strikingly simple response properties. Most of the cells in hippocampus only respond to a small region of the environment, earning them the name “place cells.” In the enotrohinal cortex, four key cell types have been described: border cells, head direction cells, grid cells and conjuctive cells that have grid and directional properties. The collective activity of the cells in the two regions is thought to provide the animal with a map of the environment suitable for spatial navigation and more generally provide a spatial matrix onto which non-spatial elements of an episode can be overlaid. However, exactly how these cells interact with one another remains an open question. For example, both grid cells and border cells could conceivable generate place selective neurons in the hippocampus. Mapping the connectivity and understanding the contribution of each cell type requires methods for specifically addressing each cell type in isolation.

One of the great advantages of using transgenic technologies in neuroscience is the ability to gain access to individual cell types in the brain. With this genetic access, we can take advantage of the wealth of tools that are now available for tracing the connectivity and perturbing the function. In this project, we have been using a transgenic mouse line that expresses nearly exclusively in cells in layer II of the medial entorhinal cortex (MECII). We have been using this mouse line to address two main questions 1) which functional cell types in MECII project to the hippocampus? and 2) what is the impact of silencing those neurons on other the firing of cells in other layers?

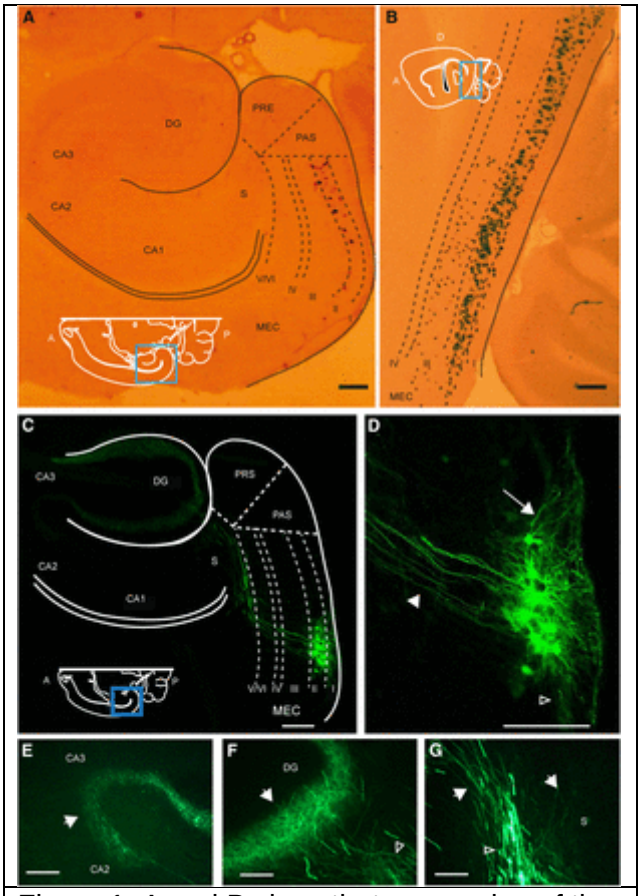

Figure 1. A and B show that expression of the transgene is largely limited to MECII. C-D shows viral labelling of the transgenic neurons, which allowed us to see axon terminations in the dentate gyrus and CA3 regions of the hippocampus (from Rowland et al., 2013)

Summary of main results

Results of our anatomical study showed that the cells the express the transgene are also cells that project to the dentate gyrus and CA3 region of the hippocampus, and therefore likely contribute to the firing of hippocampal place cells (Rowland et al., 2013; figure 1). Recent evidence suggests that only the stellate cells, not pyramidal cells, in MECII project to the dentate gyrus or CA3 and pyramidal neurons receive stronger cholinergic inputs, it is therefore possible that the two populations of cells have different functional properties as well (Ray et al., 2014; Kitamura et al., 2014). Our current work is exploring the functional identity of the transgenic cells as well as some additional molecular characterization of the cells. To characterize which cells express the transgene, we expressed the optogenetic silencer

ArchT in the same population of cells and used multiple tetrodes to record the activity as the animals explored a 1m x 1m box. We first ran a baseline session to determine the functional characteristics of the cell and then pulsed green light via an optical fiber implanted with the tetrodes on for 10 seconds and then off for 30 seconds and repeated that approximately 45 times. Because only the transgenic neurons should be immediately and robustly silenced, we can use this method to identify which neurons are transgenic. Our data show that the population of transgenic neurons contains border cells and grid cells, but not head direction cells or fast-spiking inhibitory interneurons (figure 2). Coupled with our ongoing molecular characterization of the transgenic line, these data should provide strong evidence that border cells and grid cells of layer II project to the dentate gyrus and CA3 region of the hippocampus.

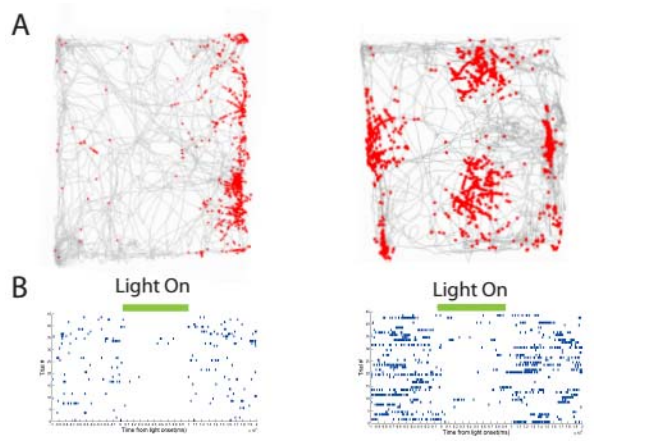

Figure 2. Silencing of a border cell (left) and grid cell (right) using the light-induced switch ArchT expressed specifically in MECII. Images in A show the trajectory of the mouse in grey and the firing of the cell as red dots over a 30 minute exploratory session without any optical inactivation. Cells were recorded from two different mice. Raster plots in B show the response of the same two cells to repeated pulses of green light. The rapid and pronounced silencing of these cells in response to the light indicates that they are transgenic.

We are also using this line of mice to ask whether inactivating cells in MECII influences the firing of cells in other layers. To test this we ran separate sessions in the same animals as above but this time with continuous illumination of the cells for 30 minutes (long enough for the animal to explore the box). Here the results have been more equivocal. Our preliminary data suggested that the inactivation did not noticeably alter the firing of non-transgenic neurons. We are continuing to explore new strategies for inactivating larger populations of cells, but for now we can conclude that the network is surprisingly robust to perturbations.

Expected final results and potential impact

The brain is an immensely complicated processing system comprised of many anatomically and functionally defined cell-types. Within the MEC and hippocampus, however, there are a surprisingly small set of functional cell-types that are readily distinguishable from one another. This affords a unique opportunity to understand how the cells are connected and interact with one another to facilitate spatial navigation and the formation of new memories, the two well-known functions of the entorhinal cortex and hippocampus. We have sought to understand whether the grid cells and border cells of layer II of the medial entorhinal cortex project to the hippocampus, which is assumed in most recent models of place field formation in the hippocampus. Our results indicate that the population of layer II projection neurons includes both grid cells and border cells. These data are extremely valuable for understanding how the entorhinal cortex and hippocampus interact to form spatial maps and memories of episodes. We expect to have the study ready for publication by the end of the year.