Cognition and memory result from communication between specific populations of brain cells. In an area of the brain called the medial entorhinal cortex (MEC), representations of location that are crucial for spatial cognition and memory are generated by neurons called grid cells. The individual activity of these cells can be decoded into activity fields that form a hexagonal grid spanning the environment. The information about spatial location is also encoded by the activity of the MEC network as a whole. The spatial information code requires synaptic input from a structure called the medial septum (MS). Yet, we know very little about how different projections from the MS influence different neuronal populations in the MEC. We combined optogenetic tools, to selectively activate inputs from the MS, with state of the art in vitro and in vivo electrophysiological recordings to identify and characterize their targets in the MEC. The research advanced our understanding of cellular and circuit mechanisms for spatial cognition and memory. It has also shone a light on a potential new treatment strategy for epilepsy that involves stimulating MS projections with optogenetics. Alfredo Gonzalez-Sulser was recently awarded a £250000 from Epilepsy Research United Kingdom in order to pursue this avenue of research.

The overarching hypothesis of the Marie Curie Fellowship was that GABAergic neurons in the MS control the timing of network activity in the MEC through actions on specific neuronal populations. In order to test this we worked on three specific aims:

Aim 1 - To test the hypothesis that parvalbumin GABAergic axons from the MS target specific lamina and cell types. Here, we originally proposed to utilize genetically modified mice to specifically target the GABAergic parvalbumin MS population and express a fluorescent protein at the synapses of the output of these neurons through the use of a virus targeting the synaptic protein synaptophysin. Our results showed few axons emerging from this population targeting the MEC. Instead we quantified whether the projections from unspecific populations of the MS target specific region lamina in the MEC. Indeed we found that layers II and V in MEC show higher axonal densities. This result, along with the objective of Aim 2 was published by our group in December 2014 (Gonzalez-Sulser et al. 2014, Figure 1).

**Fig 1.** Labeling of axonal projections from MS to MEC. ***A***, Diagram illustrating AAV injection site in theMSand labelled axonal projections (red) to the MEC.

***B***, ChR2-mCherry-expressing cells (red) in a horizontal section containing the MS and also labelled with NeuroTrace (green). ***C***, Horizontal section of the MEC, with cell bodies labelled with NeuroTrace (top left), MS axons expressing ChR2-mCherry (top right), and merged image (bottom left). Normalized mCherry fluorescence quantification reveals differences between layers (bottom right; \**p* < 0.05, Tukey’s test). Scale bars, 200 µm.

 

Aim 2 - To test the hypothesis that activation of MS GABAergic neurons causes laminar and cell-type specific patterns of activation in the MEC. In this objective we proposed to use in vitro electrophysiological techniques in combination with optogenetics to record inputs from the MS to specific MEC layers and cell types. We showed in Gonzalez-Sulser et al., 2014 that the GABAergic projection from the MS to the MEC specifically targets GABAergic interneurons in the MEC across all layers. We also tested the glutamatergic projection from the MS to the MEC and we found that inputs to the MEC were not layer or cell type specific, and was also not as prevalent as the GABAergic input (Figures 2 and 3).



**Fig 2.** Responses to activation of MS fibers across different MEC cell types. ***A***, Examples of averaged (*n*\_5) IPSPs and EPSPs evoked by photostimulation (blue bar) of ChR2-positive MS axons recorded at holding potentials on either side of the chloride reversal potential. No stimulation (gray),GABAAR (red), and iGluR (blue) blockade traces are also shown.***B***, Representative responses of fast spiking interneurons, low threshold spiking interneurons, non-stellate pyramidal cells,, and stellate cells to injection of positive and negative current steps. The cells are the same as in ***A***. ***C***, Streptavidin (red) staining of biocytinfilled cells recorded in ***A*** and ***B***. ChR2-venus MS axons are in green. Inset displays 40x image of dendritic regions indicated in the main image, showing the absence and presence of spines in inhibitory and excitatory neurons, respectively. Scale bar, 50 µm.



**Fig 3.** MS GABAergic fibers target FS and LTS interneurons in MEC. A, Fraction of neurons

of each cell type responding to photostimulation in MEC. Numbers indicate responding cells and

total number of recorded cells. B, Cumulative probability plots of IPSP amplitude, latency, rise, and decay time constants for responsive inhibitory neurons. C, Fraction of FS and LTS interneurons in each layer receiving GABAergic input from the MS.

Aim 3 - To test the hypothesis that specific activation of PV neurons in the MS-DB modifies network activity in the MEC in behaving animals. Here we are currently using in vivo extracellular recordings in combination with optogenetics while animals perform a task in a virtual reality environment. We are progressing with this series of experiments. Animals are able to perform a navigation task on the virtual reality systems and we were able to perform optogenetic modulation of brain cells during the task for the first time recently.

We hope in aim 3 to test the influence of GABAergic projections from the MS to the MEC while animals are behaving. Although the parvalbumin cell type does not strongly project to the MEC, we are testing projections from the MS to the MEC through anatomical studies from other subtypes of GABAergic cells. We hope to see whether we can modify the rhythmic activity of MEC neurons by optogentically changing the activity of these MS projections. We will also test whether by modulating these projections we can influence how the mice navigate the virtual environment.

These data and published results have improved our understanding of the cognitive representation of space in the MEC. These data have implications on how spatial information is computed in these structures. Furthermore, the projections from the MS to the MEC are damaged in Alzheimer ’s disease in both humans and animal models. Also, since the MS targets the hippocampal formation, of which the MEC is a part of, our data has implications for epilepsy research since the hippocampal formation is the initiation site for many types of seizures that are not treatable with drugs. Alfredo Gonzalez-Sulser has secured funding to continue with epilepsy research involving the MS. Our experiments are also advancing European Excellence and Innovation as we improving both training with already established technologies, such as the slice multi-electrode array that helped us acquire pilot data for these studies and also our cutting edge virtual reality system, for which we are developing behavioural protocols that will improve its utilization in order to improve our knowledge of the nervous system. Furthermore, by understanding these neuronal computations we believe our data will be of benefit to the research community that is both making computer models of these circuits and researchers working on improving map and navigation software.

**Reference:** Gonzalez-Sulser, A., Parthier, D., Candela, A., McClure, C., Pastoll, H., Garden, D., Surmeli, G., and Nolan, M.F. (2014). GABAergic projections from the medial septum selectively inhibit interneurons in the medial entorhinal cortex. The Journal of Neuroscience,34, 16739-16743