Atypical subiculum pyramidal neurons participating in a specialized spatial memory circuit

Background and Importance: The impairment of spatial memory, as exhibited by Alzheimer's disease, can prove devastating. Consequently, determining the underlying neural mechanisms of spatial memory is invaluable to both fundamental and translational neuroscience[1]. A brain region termed the subiculum has been shown to contain cells that represent spatial landmarks[2,3], and published work from our lab has recently discovered a sparse, molecularly distinct pyramidal cell subtype that occupies the deepest layer of the subiculum[4]. Follow-up preliminary data that I have acquired (Fig.1) illustrates this deep subtype differs markedly from classical subiculum pyramidal cells, and seems ideally poised to convey landmark-based spatial information.

Objectives: Characterize this pyramidal cell subtype and its associated circuits, as well as investigate causal contributions of these anomalous cells to spatial memory.

Hypothesis: Deep subiculum pyramidal cells participate in a specialized spatial memory circuit, providing landmark information to downstream spatially tuned cells.

Preliminary data: We have generated a new transgenic mouse line (Ly6g6e-IRES-cre) that allows for selective access to these deep cells. Leveraging Ly6g6e mice along with viral tracing tools, I have validated subtype-specific access and demonstrated that these cells form a distinct population of excitatory neurons (Fig.1A) with an atypical dendritic morphology (Fig.1B,C). Moreover, these cells form a dedicated projection to the anterior thalamic nuclei (ATN) (Fig.1D), a key brain region for landmark-based spatial navigation[5,6,7].

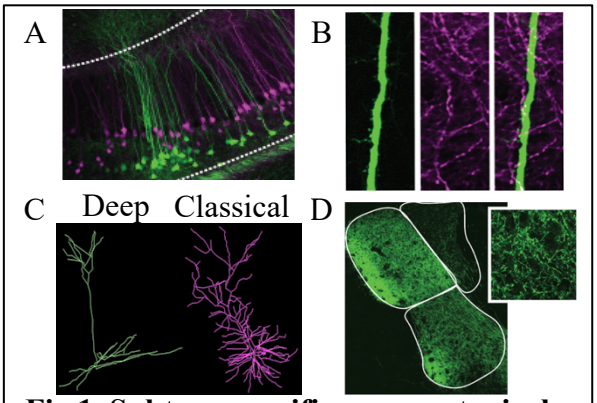

Fig.1. Subtype-specific access, atypical dendritic morphology, and dedicated projections A. Viral labeling of deep subiculum pyramidal cells (green) and classical pyramidal cells (magenta) in dorsal subiculum. B. Expansion of dendrites. C. Dendritic reconstructions of deep (green) and classical (magenta) subiculum neurons, from Mouselight Neuron Browser database D. Viral labeling of axon terminals in the ATN, with expansion of axon terminals

Experimental Approach/Methods:

Circuit Mapping: Mouse models and viral tracing tools will be utilized to map the circuits of this deep subiculum cell population. Inputs to the deep pyramidal cells will be assessed by injection of modified rabies virus of different colors into the ATN and another downstream target of the subiculum[8]. With helper viruses injected into the subiculum, the modified rabies virus will retrogradely label cells projecting to the downstream regions (i.e. deep cells to ATN), as well as cells providing input to these populations (i.e. projections to deep cells). This will enable us to observe inputs to deep cells versus classical pyramidal cells, as they will be labeled by different fluorophores.

Behavioral correlates: Ly6g6e transgenic mice and chemogenetic tools will be used to selectively manipulate this deep subiculum cell population during spatial memory behavior paradigms. hM4D DREADDs (Designer receptors exclusively activated by designer drugs) will be delivered bilaterally via cre-dependent viral injection to the subiculum in order to selectively silence neuronal activity of deep pyramidal cells in Ly6g6e mice[9]. After allowing time for viral expression, animals will be habituated and trained in object recognition and object location memory paradigms[10]. Animals will receive either a systemic injection of the DREADD-activating ligand or a vehicle injection (i.e. no ligand) 30 minutes before the training session to inhibit memory encoding. Mice will then be tested after 24 hours, and video recordings will be subsequently analyzed for behavioral changes correlating to landmark recognition.

Impact: Defining this anomolous subiculum cell population and its behavioral correlates will provide a powerful understanding of the cell-type-specific mechanisms of spatial learning and memory. This will benefit basic neuroscience by providing a means of identifying and causally manipulating these circuits, which in turn will inform our understanding of deficits of spatial memory (e.g., as in Alzheimer's disease).

Bibliography

1. O'Keefe, J. & Nadel, L. *The hippocampus as a cognitive map*. (Clarendon Press; Oxford University Press, 1978).
2. Brotons-Mas, J. R., Schaffelhofer, S., Guger, C., O'Mara, S. M. & Sanchez-Vives, M. V. Heterogeneous spatial representation by different subpopulations of neurons in the subiculum. *Neuroscience* **343**, 174-189, doi:10.1016/j.neuroscience.2016.11.042 (2017).
3. O'Mara, S. M., Sanchez-Vives, M. V., Brotons-Mas, J. R. & O'Hare, E. Roles for the subiculum in spatial information processing, memory, motivation and the temporal control of behaviour. *Prog Neuropsychopharmacol Biol Psychiatry* **33**, 782-790, doi:10.1016/j.pnpbp.2009.03.040 (2009).
4. Cembrowski, M. S. *et al.* Dissociable Structural and Functional Hippocampal Outputs via Distinct Subiculum Cell Classes. *Cell* **173**, 1280-1292 e1218, doi:10.1016/j.cell.2018.03.031 (2018).
5. Tsanov, Marian, Ehsan Chah, Seralynne D. Vann, Richard B. Reilly, Jonathan T. Erichsen, John P. Aggleton, and Shane M. O'Mara. Theta-Modulated Head Direction Cells in the Rat Anterior Thalamus. *Journal of Neuroscience* **31**, 9489–9502. doi:10.1523/JNEUROSCI.0353-11.2011 (2011).
6. Warburton, E. C., Baird, A., Morgan, A., Muir, J. L., Aggleton, J. P. The Conjoint Importance of the Hippocampus and Anterior Thalamic Nuclei for Allocentric Spatial Learning: Evidence ftom a Disconnection Study in the Rat. *The Journal of Neuroscience* **21**, 7323-7330, doi: 0.1523/JNEUROSCI.21-18-07323.2001 (2001).
7. Nelson, A. J. D., Kinnavane, L., Amin, E., O'Mara, S. M. & Aggleton, J. P. Deconstructing the Direct Reciprocal Hippocampal-Anterior Thalamic Pathways for Spatial Learning. *J Neurosci* **40**, 6978-6990, doi:10.1523/JNEUROSCI.0874-20.2020 (2020).
8. Cembrowski, M. S. & Spruston, N. Heterogeneity within classical cell types is the rule: lessons from hippocampal pyramidal neurons. *Nat Rev Neurosci* **20**, 193-204, doi:10.1038/s41583-019-0125-5 (2019).
9. Roth, Bryan L. DREADDs for Neuroscientists. *Neuron*, **89**, 683-694, doi:10.1016/j.neuron.2016.01.040 (2016).
10. Vogel-Ciernia, A. & Wood, M. A. Examining object location and object recognition memory in mice. *Curr Protoc Neurosci* **69**, 8 31 31-17, doi:10.1002/0471142301.ns0831s69 (2014).
11. Aggleton, John P., and Kat Christiansen. *The Subiculum: The Heart of the Extended Hippocampal System*. *Progress in Brain Research*. 1st ed. Vol. 219. Elsevier B.V. doi:10.1016/bs.pbr.2015.03.003 (2015).
12. Aggleton, J. P. and Nelson, A. J. D. Why do lesions in the rodent anterior thalamic nuclei cause such severe spatial defecits? *Neuroscience and Biobehavioral Reviews* **54**, 131-144, doi:10.1016/j.neubiorev.2014.08.013 (2015)
13. Hulse, B. K. & Jayaraman, V. Mechanisms Underlying the Neural Computation of Head Direction. *Annu Rev Neurosci* **43**, 31-54, doi:10.1146/annurev-neuro-072116-031516 (2020).