Activating Gi signaling in dorsal hippocampal astrocytes prevents memory consolidation

Ryan Thiede, Lisa Taxier, Karyn M. Frick

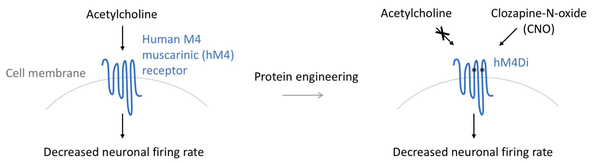
Department of Psychology, University of Wisconsin-Milwaukee, Milwaukee, WI

Introduction

For many years, memory researchers have been investigating the mechanisms that contribute to the formation and consolidation of memory. This work has primarily focused on looking at the functions of neurons in the hippocampus, but recent work suggests there may be other cells involved in the memory process. This data shows that astrocytes are crucial to synaptic functioning, plasticity, and memory consolidation. However, this data has not yet been shown in a living organism, which is what our research will focus on. Our aim **is to determine whether dorsal hippocampal astrocyte activity is necessary for object memory consolidation**. Based on previous research we hypothesize that astrocyte activity in the dorsal hippocampus is required for object memory consolidation.

Methods

Designer receptors exclusively activated by designer drugs (DREADDs)

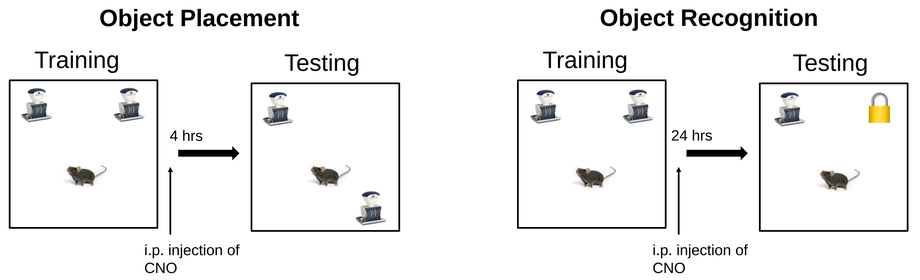


DREADDs are receptors that are virally transduced into cells within a target region, and are exclusively activated by designer drugs. In the present experiment, a control eGFP virus (AAV-GFAP-eGFP, 2x1012 vg/ml, serotype 8) or inhibitory hM4Di virus (pAAV-GFAP-HA-hM4D(Gi)-IRES-mCitrine, 6x1012 vg/ml, serotype 8) was delivered directly into the DH (-1.7 mm AP, ±1.5 mm ML, -2.3 mm DV; 1.2 µl/hemisphere) of OVX mice (n=10/group; viruses purchased from the Duke Univ. School of Medicine Viral Vector Core). Three 0.4 µl injections were delivered per hemisphere at depths of -2.2 mm, -2.1 mm, and -1.9 mm DV. The mCitrine tag of the DREADD virus and the GFP tag of the control virus were visualized in 20 µm sections taken on a cryostat. Sections were mounted on gelatin-subbed slides, and coverslipped using mounting media with DAPI to stain cell nuclei. Sections were imaged on a confocal microscope to verify DREADD expression within the dorsal hippocampus (see Fig 2).

Object Recognition and Object Placement

In object recognition, mice accumulate 30 s investigating 2 identical objects. Immediately after training, mice were injected intraperitoneally with CNO, our DREADD activating ligand. During testing, a familiar object is replaced with a novel object, and mice are again required to accumulate 30 s of investigatory behavior. Mice who remember the familiar object spend more time than chance (15s) with the novel object. OP training is identical to that for OR, but during testing, one object is moved from a familiar location to a novel location. Mice with intact memory for the unmoved object spend more time than chance (15s) with the moved object.

Experimental design of one-trial learning tasks



5 mg/kg CNO, but not 1 mg/kg CNO, impairs object memory

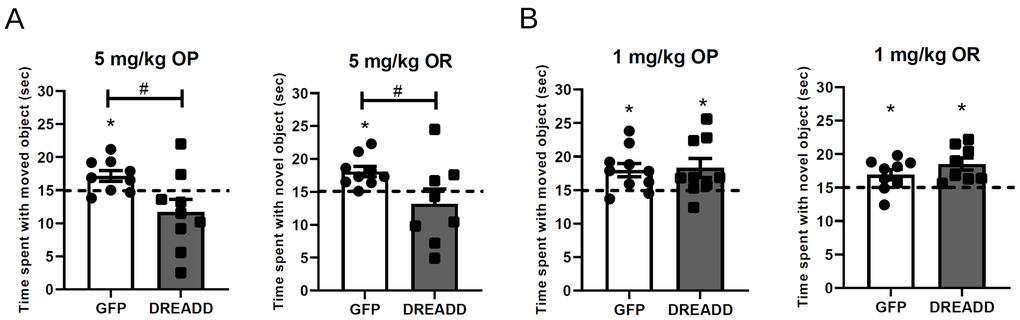


Figure 1. Mice expressing a control viral construct (GFP) spent significantly more time than chance with a moved or novel object after receiving a 5 mg/kg post training ip injection of CNO (dotted line at 15 s, *$p < 0.05$), whereas mice expressing hm4di in hippocampal astrocytes did not (A). By contrast, mice receiving a 1 mg/kg post training ip injection of CNO (B) spent significantly more time than chance with a moved or novel object during testing, regardless of whether they expressed control (GFP) or DREADD virus in the dorsal hippocampus.

Confirmation of DREADD expression in dorsal hippocampal astrocytes

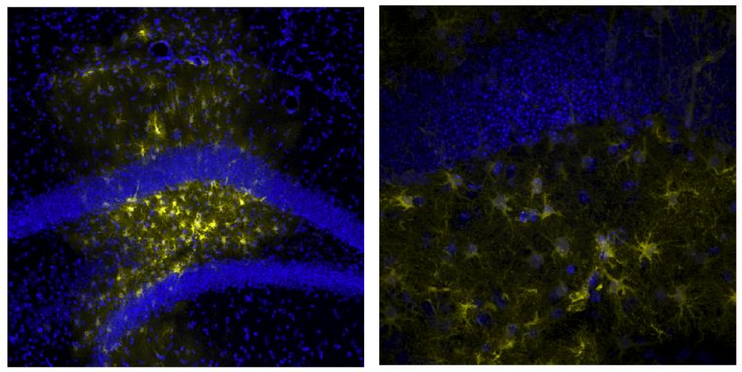


Figure 2. Expression of hM4di virus (yellow) in dorsal hippocampus of ovariectomized mice at 10x magnification (left) and 40x magnification (right). Viral expression appears to be consistently localized to astrocytes, given the lack of mCitrine expression in the neuron-dense layer of the dentate gyrus and the astrocyte-like morphology of mCitrine-expressing cells.

Conclusions

Activating Gi signaling in astrocytes prevents object memory consolidation

Future Directions

We plan to use immunohistochemistry to look for immediate early gene expression, which is upregulated in response to learning, within the dorsal hippocampus. We plan to examine c-fos, because this immediate early gene has been implicated in memory-related processes. We expect that c-fos expression in dorsal hippocampal astrocytes will be elevated in response to learning, suggesting a role for astrocytic activity in learning.

References

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Acknowledgements

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