Hyperactivity of ventral hippocampal mossy cells degrades dorsal hippocampal mnemonic function via longitudinal projections

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INTRODUCTION

- The *anterior hippocampus* of individuals with early psychosis or schizophrenia is hyperactive,[1] as is the ventral hippocampus in many rodent models for schizophrenia risk.[2]
- Anterior hyperactivity is associated with cognitive deficits and impaired hippocampal recruitment or habituation during cognitive tasks.[3,4] However, *causal* relationships between anterior hippocampal activity and deficits in learning and memory remains unclear.
- Mossy cells of the ventral dentate gyrus densely project in the hippocampal long axis, targeting both dorsal dentate gyrus granule cells and inhibitory interneurons.[5] Inhibition of ventral mossy cells impairs long-term spatial learning and memory.[6]
- Mossy cells are responsive to stimulation throughout hippocampal subfields,[7] and thus may be suited to detect hyperactivity in areas where it originates such as CA1.[1]
- **Here we quantified ventral mossy cell activity *in vivo* during environmental exploration, characterized their connectivity with target dorsal granule cells, and tested effects of hyperactivation on long-term spatial memory involving dorsal hippocampus.**

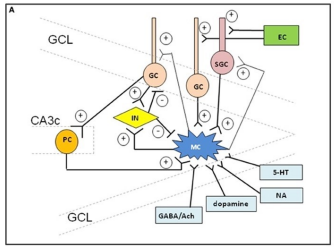

Figure 1. Schematic of local neural circuitry of the dentate gyrus as well as afferent and efferent projections. Abbreviations: ACh, acetylcholine; EC, entorhinal cortex; GC, granule cell; GCL, granule cell layer; IN, interneuron; MC, mossy cell; NA, noradrenaline; PC, pyramidal cell; SGC, semilunar granule cell; 5-HT, serotonin. Reproduced from [6] under Creative Commons license 3.0.

KEY METHODS SUMMARY

- **Animals:** Adult male and female CD-1 (Charles River), with *ad lib* access to food and water. Studies were approved by VU IACUC.
- **Viruses:** rg-AAV-pgk-Cre, AAV1-Syn-Flex-GCaMP6f, AAV8-hSyn-DIO-hM3D(Gq)-mCherry, AAV8-hSyn-DIO-mCherry, AAV8-EF1a-double floxed-hChR2(H134R)-mCherry (all from Addgene).
- **Stereotactic infusion coordinates:** Dorsal dentate gyrus: AP: -1.94, ML: 1.20, DV: -2.50; Ventral dentate gyrus: AP: -3.40, ML: 3.0, DV: -3.50. At least 3 weeks was allowed for viral expression.
- **Fiber photometry:** Performed as mice explored home cage with lid off in novel recording room. Sampled at 100 Hz using FP console (Doric) with 405 nm and 465 nm LEDs time-locked with video. $\Delta F/F_0$ was calculated for 405 nm and 465 nm signals and corrected GCaMP6f signal calculated by subtraction, followed by 2 Hz low-pass filtering.
- **Behavior**
  - General: Mice were habituated to the testing room (~200 lux) for at least one hr before testing. All testing was between 0900 and 1700.
  - Open field test: 10 mins in a 61 x 61 cm arena. Distance traveled, time in arena center, and center entries quantified using ANY-maze.
  - Object location memory: *Training session*: After 6 d habituation to arena containing spatial cues on wall, mice were placed in arena with two identical glass bottles and allowed to explore for 10 mins. *Testing session*: 24 hrs later, mice were given 5 mins to explore the arena with one bottle in same location and other bottle in novel location (novel side was counterbalanced across mice). Discrimination index (DI) = (time exploring novel object – familiar object)/(total time exploring both objects).

RESULTS

Intersectional targeting of ventral mossy cells

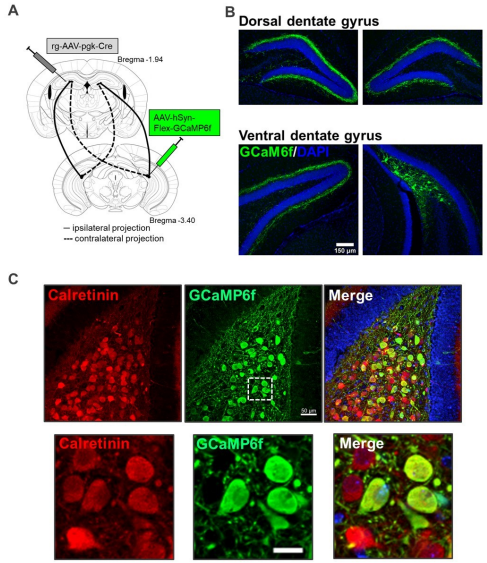

Figure 2. Intersectional targeting of longitudinal ventral mossy cell projections. A To target ventral mossy cells (vMCs) specifically, retrograde-AAV-pgk-Cre was infused into dorsal dentate gyrus (dDG) inner molecular layer, and AAV-hSyn-Flex-GCaMP6f was infused into contralateral vDG hilus, enabling recombination only in vMCs that project to dDG. **B** Fluorescence microscopy following targeting strategy shown in (A) reveals GCaMP6f is expressed in vMC somata with organized projections to contralateral vDG and bilateral dDG. **C** GCaMP6f from targeting strategy in (A) is highly colocalized with calretinin, a marker for ventral but not dorsal MCs in mice.

Fiber photometry from ventral mossy cells during exploratory behavior

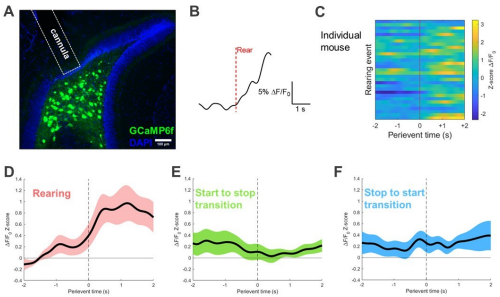

Figure 3. *In vivo* fiber photometry reveals ventral mossy cells are selectively activated by exploratory rearing. A GCaMP6f expression in ventral mossy cells (vMCs) and fiber optic cannula placement. **B** Example photometry trace surrounding an exploratory rearing event. **C** $\Delta F/F_0$ Z-scores of rearing events aligned to time of initial rearing apogee in an individual mouse. Events are ordered chronologically during the recording. **D** $\Delta F/F_0$ Z-scores are aligned to time of rearing apogee (perievent time = 0), averaged within mouse, then averaged across mice (68 rearing events, 5 mice). Black line: mean $\Delta F/F_0$ Z-score, pink: SEM. **E, F** To test whether vMCs were activated by changes in motor behavior, $\Delta F/F_0$ Z-scores for the same 5 mice from (D) during the same behavioral session were aligned to time when mice transitioned between XY-plane horizontal movement and no horizontal movement (E, "Start to stop", 98 transitions), or between no horizontal movement and XY-plane horizontal movement (F, "Stop to start", 98 transitions). Black line: mean ΔF/F0 Z-score, color shading: SEM.

RESULTS

Functional connectivity between ventral mossy cells and dorsal dentate gyrus granule cells

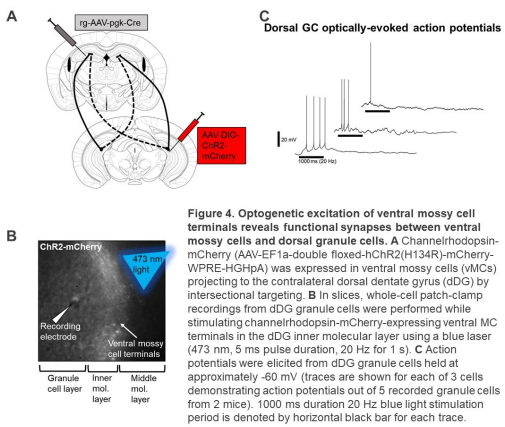

Figure 4. Optogenetic excitation of ventral mossy cell terminals reveals functional synapses between ventral mossy cells and dorsal granule cells. A Channelrhodopsin-mCherry (AAV-EF1a-double floxed-hChR2(H134R)-mCherry-WPRE-HGHpA) was expressed in ventral mossy cells (vMCs) projecting to the contralateral dorsal dentate gyrus (dDG) by intersectional targeting. **B** In slices, whole-cell patch-clamp recordings from dDG granule cells were performed while stimulating channelrhodopsin-mCherry-expressing ventral MC terminals in the dDG inner molecular layer using a blue laser (473 nm, 5 ms pulse duration, 20 Hz for 1 s). **C** Action potentials were elicited from dDG granule cells held at approximately -60 mV (traces are shown for each of 3 cells demonstrating action potentials out of 5 recorded granule cells from 2 mice). 1000 ms duration 20 Hz blue light stimulation period is denoted by horizontal black bar for each trace.

Chemogenetic activation of ventral hilar mossy cells increases activity of dorsal dentate gyrus granule cells

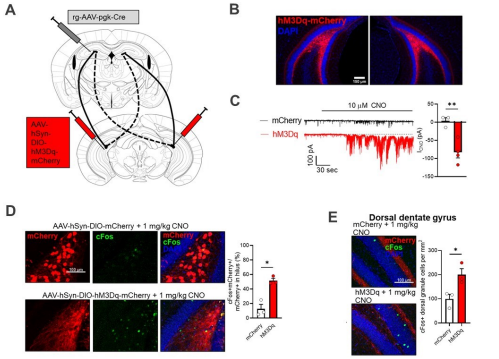

Figure 5. Chemogenetic activation of ventral mossy cells activates dorsal granule cells. A To express hM3Dq-mCherry or mCherry in ventral mossy cells (vMCs), retrograde-AAV-pgk-Cre was unilaterally infused into dorsal dentate gyrus (dDG) and AAV-hSyn-DIO-hM3D(Gq)-mCherry or AAV-hSyn-DIO-mCherry was bilaterally infused into vDG hilus. **B** Bilateral vMC targeting. **C** Whole cell recordings from vMCs expressing either mCherry (N = 3 cells from 2 mice) or hM3Dq-mCherry (N = 4 cells from 3 mice) revealed hM3Dq+ neurons showed significantly greater mean inward current after bath application of 10 micromolar clozapine N-oxide (CNO) than control mCherry+ neurons. **p = 0.0068. **D** Mice with vMCs expressing mCherry or hM3Dq-mCherry were administered vehicle or 1 mg/kg CNO i.p., then perfused 90 mins later and immunostained for cFos (N = 3 mice per group). *p = 0.012. **E** Dorsal DG granule cell cFos was also significantly upregulated in mice with vMC expression of hM3Dq-mCherry treated with 1 mg/kg CNO as compared to mice with vMC expression of mCherry (N = 3 mice per group). *p = 0.036.

RESULTS

Effects of ventral mossy cell activation on spatial learning and memory, locomotion, and exploration

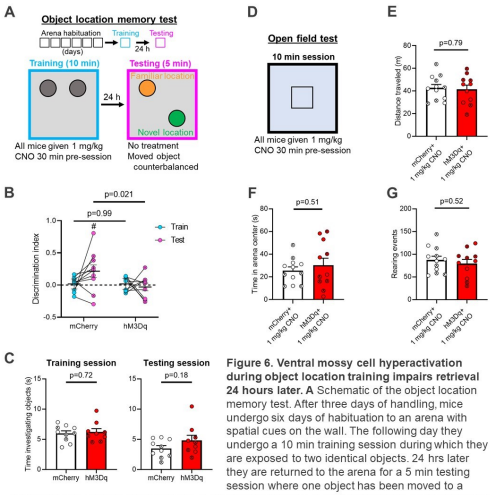

Figure 6. Ventral mossy cell hyperactivation during object location training impairs retrieval 24 hours later. A Schematic of the object location memory test. After three days of handling, mice undergo six days of habituation to an arena with spatial cues on the wall. The following day they undergo a 10 min training session during which they are exposed to two identical objects. 24 hrs later they are returned to the arena for a 5 min testing session where one object has been moved to a novel location and the other remains in the familiar location. 1 mg/kg CNO was administered 30 mins before the training session only. **B** CNO administration during training impaired object location memory in mice expressing hM3Dq in vMCs (N = 10 mCherry, N = 9 hM3Dq). #p = 0.028, one-sample t test versus DI = 0. **C** Total time spent investigating both objects did not significantly differ between the mCherry (N = 10) or hM3Dq (N = 9)-expressing mice during the training (left) or testing (right) session. **D-G** Mice were later tested in a 10 min open field test in an arena with a novel floor surface 30 mins after 1 mg/kg CNO (n = 12 mCherry, n = 11 hM3Dq) (D). There was no significant difference between mCherry and hM3Dq-expressing mice in total distance travelled (E), time spent in arena center (F), or number of rearing events (G). For (B-C) and (E-G), individual data points containing "X" represent female mice.

CONCLUSIONS AND FUTURE DIRECTIONS

These data suggest that ventral MC activation can directly excite dorsal granule cells and interfere with dorsal DG function, supporting future study of their *in vivo* activity in animal models for schizophrenia featuring ventral hyperactivity

REFERENCES

1) Schobel, *et al.* Neuron, 2013.
2) Kätzel, *et al.* Frontiers in Pharmacology, 2020.
3) McHugo, *et al.* American Journal of Psychiatry, 2019.
4) Tregellas, *et al.* American Journal of Psychiatry, 2014.
5) Scharfman, Nature Reviews Neuroscience, 2016.
6) Bui, *et al.* Science, 2018.
7) Scharfman and Schwartzkroin, J Neurosci., 1988.

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