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Title: Ventral hippocampal mossy cell hyperactivity degrades dorsal hippocampal mnemonic function via longitudinal projections

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Abstract

The anterior hippocampus of individuals with early psychosis or schizophrenia is hyperactive compared to healthy controls. In rodent models of schizophrenia etiology, the ventral hippocampus, analogous to the human anterior hippocampus, is also hyperactive with effects on extrahippocampal neural circuits that might contribute to positive, negative, and cognitive symptoms. Less is known about how anterior hippocampal hyperactivity might directly influence intrahippocampal function across the structure's longitudinal axis. This question is important for understanding cognitive dysfunction in schizophrenia, which includes deficits attributed to both the anterior and posterior hippocampus. We hypothesized that hyperactivity of ventral hippocampal mossy cells, which send dense longitudinal projections throughout the hippocampal longitudinal axis, may be sufficient to disrupt spatial memory encoding, a dorsal hippocampal-dependent function. Using an intersectional viral strategy, we targeted ventral mossy cells projecting to the dorsal dentate gyrus. *In vivo* fiber photometry revealed these cells were activated during behavior related to context mapping but not during non-exploratory motor behaviors. Anterograde transsynaptic tracing and optogenetic terminal stimulation revealed functional connectivity between ventral mossy cells and dorsal dentate gyrus granule cells. Finally, chemogenetic activation of ventral mossy cells during the encoding phase of an object location memory task impaired retrieval 24 hours later, without effects on locomotion or other exploratory behaviors. These findings suggest that anterior hippocampal hyperactivity may have intrahippocampal consequences to degrade posterior hippocampal function and support future studies engaging this circuit target to mitigate specific cognitive deficits associated with schizophrenia.

INTRODUCTION

Cognitive impairment in schizophrenia (CIIS) independently predicts functional deficits [1-3]. Despite several pharmacological strategies showing strong promise in preclinical and early clinical studies, most compounds have not shown clinically meaningful benefit in large, phase III clinical trials [4]. As such, there are currently no FDA-approved pharmacotherapies for neurocognitive deficits in schizophrenia. While these translational challenges are multifactorial, they suggest that the underlying mechanisms of CIIS are incompletely understood.

Robust, replicable neuroimaging differences between patients and controls that have been studied longitudinally are logical starting points to understand CIIS. Powerful contemporary techniques in rodent models can then be leveraged to probe mechanisms underlying the human neuroimaging differences. Along these lines, anterior hippocampal changes in early psychosis and schizophrenia have been reported by several independent groups, including reduction in volume [5-9] and baseline hyperactivity [5,10-13]. Baseline anterior hippocampal hyperactivity inversely correlated with anterior hippocampal recruitment during a scene processing task in early psychosis subjects, suggesting hyperactivity limits recruitment [10]. Despite these findings however, the exact relationship between aberrant anterior hippocampal activity and deficits in learning and memory remains uncertain [14].

Many rodent models with genetic or developmental insults for modeling schizophrenia etiology exhibit hyperactivity in the ventral hippocampus (vHPC), the rodent analogue of the human anterior hippocampus. The bulk of previous work in these models has focused on how vHPC hyperactivity drives aberrant dopamine neurotransmission outside of the hippocampus (reviewed in [15]). Ventral HPC efferents to other brain structures are also important for cognitive domains that are impaired in patients with schizophrenia, such as spatial working memory, which involves monosynaptic connections between vHPC and medial prefrontal cortex (mPFC) [16,17]. Studies of theta oscillation synchrony between

mPFC, vHPC, and dorsal HPC (dHPC, analogous to the human posterior hippocampus) during a spatial working memory task have illustrated the importance of direct and/or indirect interaction between vHPC and dHPC to support cognitive function [18]. Furthermore, individuals with schizophrenia demonstrate deficits in cognitive domains that are dependent on the posterior hippocampus, such as visuospatial contextual memory [19-22]. Understanding how anterior hippocampal hyperactivity influences posterior hippocampal function is a potentially important mechanism for developing strategies to mitigate CIIS.

While indirect pathways might link vHPC and dHPC [23,24], these regions are anatomically connected through direct, intrahippocampal longitudinal circuits. Here we focus on vHPC mossy cells (vMCs), which are glutamatergic neurons with cell bodies in the dentate gyrus (DG) hilus [25,26]. Ventral MCs send long-distance excitatory projections to both ipsi- and contralateral DG, resulting in dense connectivity of the entire longitudinal hippocampal axis preferentially in the ventral to dorsal direction [27-30]. Dorsal MCs (dMCs) are highly sensitive to spatial context [31-33], and a recent study reported that inhibition of dMCs impaired spatial memory encoding but not retrieval [34]. Inhibition of vMCs also impaired spatial memory encoding [34], a surprising finding given the reliance on the dHPC in spatial memory [35-39]. Understanding how vMCs transmit information across the HPC is highly relevant for *in vivo* anterior HPC hyperactivity in patients with schizophrenia, since MCs are sensitive to even low levels of activity throughout the HPC [40]. Based on this, we hypothesized that aberrant activation of the dHPC by the unique longitudinal projections of vMCs to dDG may also impair dHPC function and degrade spatial encoding. We tested this hypothesis using *in vivo* calcium imaging from vMCs to correlate their activity with behavior related to contextual mapping, and chemogenetics to selectively model hyperactivity in dDG-projecting vMCs during an object location memory task that is dependent on dHPC function.

MATERIALS AND METHODS

Additional details are available in the Supplementary Materials and Methods.

Animals

Wildtype CD-1 male and female mice (age at delivery: six-eight weeks) were purchased from Charles River Laboratories (Wilmington, MA) and acclimated to the vivarium for at least one week prior to any procedure. All mice were group housed except for cannula-implanted mice, which were single housed. Wildtype C57BL/6J male mice (age at delivery: six-seven weeks) were purchased from Jackson Laboratory (Bar Harbor, ME) and used as intruders during resident-intruder social interactions. Mice were housed under standard conditions with a 12 h light–dark cycle (lights on at 6:00 am) with food and water available ad libitum. The Vanderbilt Institutional Animal Care and Use Committee approved all procedures.

Drugs

Clozapine N-oxide (CNO) for systemic administration was provided by the NIMH Chemical Synthesis and Drug Supply Program, dissolved in 0.5% dimethyl sulfoxide in saline, and injected intraperitoneally (i.p.) 30 mins prior to start of behavioral assay. For slice electrophysiology, CNO was purchased from Hello Bio (Princeton, NJ).

Adeno-associated viruses

Adeno-associated virus particles (AAVs) used, purchased from Addgene (Watertown, MA), were AAV.pgk.Cre, pAAV.Syn.Flex.GCaMP6f.WPRE.SV40 [41], pAAV-hSyn-DIO-mCherry, pAAV-hSyn-DIO-hM3D(Gq) [42], pENN.AAV.hSyn.Cre.WPRE.hGH, and pAAV-EF1a-double floxed-hChR2(H134R)-mCherry-WPRE-HGHpA.

Stereotaxic surgery and viral infusion

Under inhaled isoflurane anesthesia, mice were mounted in a stereotaxic frame, and AAV (0.2 – 0.4 µL) was infused at a rate of 50 nL/min using a 2 µL syringe (Hamilton, Reno, NV). The syringe was left in place for an additional 3 min to reduce backflow. Fiber photometry cannulas were targeted to the same coordinates as ventral DG hilus and cemented in place with C&B-Metabond (Parkell, Edgewood, NY). Mice were housed for at least three weeks prior to experimentation to allow for viral expression. Targeting was confirmed by fluorescence microscopy. Mice with either unilateral or bilateral vMC expression were included in behavioral analyses due to extensive bilateral projections.

Fluorescent immunostaining, microscopy, and cell counting

Primary antibodies used were mouse anti-calretinin (MAB1568, Millipore, Burlington, MA) and rabbit anti-cFos (226 003, Synaptic Systems, Goettingen, Germany), both diluted 1:1000. Widefield fluorescent images were acquired with an AF6000 LX fluorescent microscopy system (Leica, Buffalo Grove, IL) equipped with an HCX PL FLUOTAR 10x objective (NA = 0.30). Confocal images were acquired with an LSM 710 META Inverted microscope (Zeiss, White Plains, NY) equipped with a 20x Plan-Apochromat objective (NA = 0.8). Cell counting was performed blinded from confocal images.

Electrophysiology

To validate DREADD activation of vMCs, mice were rapidly decapitated under deep isoflurane anesthesia and 300-µm horizontal sections containing the vDG were prepared in *N*-methyl-D-glucamine cutting solution and recorded in artificial cerebrospinal fluid. Hilar MCs were identified by mCherry expression, patched to obtain whole cell access, and voltage-clamped at -75 mV while 10 µM CNO was perfused in the bath. The mean of the holding current was quantified before and after CNO perfusion using pClamp 10.4 software (Axon Instruments, Union City, CA). For optogenetic activation of vMC terminals in dDG, brain slices were prepared as previously described [43,44]. Whole-cell patch-clamp recordings were made from dDG granule cells and slices were continuously superfused with ACSF.

Current-clamp mode was used to record action potentials. 473 nm blue laser light (5 ms pulse duration, 20 Hz, 1 s duration) was delivered through a fiberoptic cable to stimulate channelrhodopsin-expressing vMC axon terminals. In both sets of experiments, recordings were acquired with a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA), filtered at 2 kHz and digitized at 10 kHz.

Behavioral studies

General

Mice were habituated to the dimly lit testing room for at least one hr before testing. Testing occurred between 0900 and 1700. Experimental and control groups were tested on the same days and all tests were videotaped. Vehicle or CNO was injected i.p. 30 mins prior to the start of behavioral assays.

Open field test

Mice were placed into the center of a 61 cm by 61 cm square arena for 10 mins. Distance traveled, time spent in arena center, and number of center entries were quantified using ANY-maze version 6.1 (Stoelting, Wood Dale, IL). Rearing was quantified manually by an observer blind to treatment group.

Object location memory test

Performed largely as described previously [45]. Mice were handled for five days, followed by five mins of habituation daily in an empty open field containing spatial cues on the wall for six days (final two days of handling were combined with habituation). The next day, mice underwent an encoding session during which they were placed in the same arena now containing two identical 500 mL glass bottles and allowed to explore the arena for 10 mins. Bottles were cleaned with dilute alcohol solution between mice. 24 hrs later, mice were returned to the same arena for a 5 min retrieval session where one bottle remained in the same location and the other bottle was moved to a novel location, counterbalanced across mice. Time

spent exploring each bottle was quantified from video by blinded observers. Only time during which mice directly explored the object and were not rearing on hindlegs was counted [45]. Discrimination index (DI) was quantified as (time spent exploring novel object – familiar object)/(total time spent exploring both objects). Mice demonstrating an initial side preference during the encoding session (DI > 0.20) [34] were removed from the analysis (n = 2 mCherry, 3 hM3Dq).

cFos expression

Following return to the vivarium for at least overnight, mice were habituated to the testing room for >2 hours, administered CNO or vehicle i.p., and transcardially perfused 90 minutes later.

Resident-intruder test for fiber photometry

To enable comparison of vMC activity between an exploratory and non-exploratory behavior both involving abrupt locomotor onset, we performed fiber photometry while mice were undergoing resident-intruder tests. Because mice perform exploratory rearing and offensive aggression in these assays, this design enabled within-subject, within-session comparison. Resident-intruder tests were performed similarly to that previously described [46]. To start the assay, a C57BL/6J mouse was introduced to the home cage of the photometry cannula-implanted single housed CD-1 male and allowed to interact for >10 mins.

Fiber photometry

Photometry was performed from male CD-1 mice implanted with photometry cannulas (Doric Lenses, Quebec, Canada) using Doric Lenses system controlled by Doric Neuroscience Studio (DNS) version 5.3.

Video recording was time-locked with photometry signal. Signal processing was performed by DNS photometry analyzer. $\Delta F/F_0$ was calculated for the 405 nm and 465 nm channels independently using a least mean square fit, and then $\Delta F/F_0$ (405 nm) subtracted from $\Delta F/F_0$ (465 nm) to yield a corrected trace that was subsequently lowpass filtered at 2 Hz [47] to yield the final bulk calcium signal. This signal was Z-normalized across the entire social interaction. Behavior in videos was manually annotated for exploratory rearing (centered around apogee) and offensive attack onset. Data analysis was performed using custom written scripts in Matlab R2019a (MathWorks, Natick, MA).

Statistical analysis

To compare two groups, unpaired t tests with Welch's correction or paired t tests were used, as appropriate. To compare three or more groups, one- or two-way ANOVA with Sidak's multiple comparisons test was used. One-sample t tests were used to compare experimental to hypothetical means. $p < 0.05$ was considered significant and all tests were two-tailed. Statistical analyses were performed in Prism 9 (Graphpad, San Diego, CA). Error bars depict standard error of the mean (s.e.m.).

RESULTS

Intersectional targeting of vMCs projecting to the dHPC

We first sought to develop an intersectional targeting strategy to express proteins of interest in vMCs that project to the dHPC using wildtype mice. While Cre lines have been used to target MCs previously (D2-Cre [48] and Crlr-Cre [49]), Cre expression occurs also in non-MCs of the HPC [50], and it is not known whether there exists heterogeneity amongst MCs in their distant projections. We exploited the well-organized distant bilateral axonal projection of vMCs to the inner molecular layer of the dDG [27,29,51-53] (**Figure 1a-b**). Retrograde-AAV-pgk-Cre was unilaterally infused into dDG and Cre-dependent GCaMP6f was infused into the contralateral vDG hilus. This resulted in the expression of GCaMP6f in

large ventral hilar cells, with a band of GCaMP6f+ projections to the contralateral vDG and bilateral dDG inner molecular layers (**Figure 1c**). Local inner molecular layer projection was present but weaker, consistent with previous studies [51].

Dorsal and vMCs differ in their expression of the calcium binding protein calretinin, which is only expressed in vMCs (**Supplementary Figure 1**) [29,30,54]. Since vDG borders vary depending on how they are defined [55], we tested whether the targeted neurons expressed calretinin and could be considered vMCs. In the hilus, we found ~87% of GCaMP6f+ neurons were also calretinin+, while ~68% of calretinin+ neurons were GCaMP6f+, with variable but low levels of GCaMP6f off-target expression in neighboring vDG granule cells and vCA3 pyramidal neurons (n = 4 mice; **Figure 1d, e**). These data demonstrate this viral strategy is selective and efficient for vMCs projecting to the dHPC.

Dorsal HPC-projecting vMCs are activated during behavior related to mapping the environment

We first sought to assess the activity pattern of vMCs *in vivo* in behaving mice, as this understanding will help us interpret how vHPC and vMC hyperactivity might influence dHPC-dependent mnemonic function. Previous work using cFos as a marker of active cells has shown in rats that vMCs are more active than dMCs at baseline, and in mice vMC activity is upregulated in response to novel stimuli and contexts [56]. One limitation of cFos is that it cannot distinguish between increased vMC activity due to greater motor behavior and arousal in a novel environment versus activity specifically related to mapping and encoding information about the environment. If vMCs do contribute to environmental encoding, then perturbing this system with hyperactivity might also degrade spatial encoding. To temporally isolate environmental encoding, we used fiber photometry [57,58] to record bulk GCaMP6f calcium activity in vMCs (**Figure 2a, b**) as a proxy for neuronal firing during exploratory rearing events. Rearing on hind legs rapidly changes the mouse's vantage point and multisensory inputs from local cues to more global environmental landmarks [59,60], and is instantaneously associated with marked changes in HPC

dynamics, especially in the DG [61]. Importantly, unlike recording behavior during introduction to a novel environment or in the presence of a novel object, the rearing apogee can be time locked to photometry signals. To disentangle whether vMC activity during rearing was nonspecifically due to motor output, we compared activity during rearing with activity during offensive aggression toward a novel conspecific, a naturalistic social behavior that, like rearing, involves an abrupt increase in motor output but unlike rearing is not exploratory since the mice have already thoroughly investigated each other prior to attack. Rearing was consistently associated with increased vMC activity within (**Figure 2c**, 46 rearing events in one mouse) and across (**Figure 2d**, 158 rearing events|5 mice) mice. In contrast, there was no consistent change in vMC activity associated with the onset of offensive attack behavior in the same mice during the same behavioral session (**Figure 2e**, 50 attacks|5 mice). Ventral MC activity was significantly greater during rearing compared to attack ($t(4) = 4.02$, $p = 0.016$) (**Figure 2f**). These experiments suggest that vMCs projecting to the dHPC are activated during behaviors related to contextual mapping but not non-selectively activated by any motivated locomotor action.

Ventral MCs functionally target dDG granule cells

Electron microscopy has revealed that most vMC axonal synaptic targets in the dHPC are on spines of granule cell dendrites in the inner molecular layer [51,62]. However, there is limited direct evidence of the functionality of these synapses [63,64], which would be necessary for vHPC hyperactivity to directly influence dHPC mnemonic function. We tested this question using AAV1 as an anterograde, transsynaptic tracer at functional synapses, which has been shown in a variety of neuronal circuits [65,66]. We infused AAV1-hSyn-Cre unilaterally into the ventral hilus to serve as the seed region and infused Cre-dependent mCherry (AAV-DIO-mCherry) into either ipsilateral or contralateral dDG to report Cre recombination (**Figure 3a**). We first demonstrated that AAV-DIO-mCherry did not express mCherry in the absence of ventral hilar AAV1-Cre, suggesting that any detectable flurophore is due to Cre recombination and not viral leak expression (**Figure 3b, left**). The addition of AAV1-Cre in either the

contralateral (**Figure 3b, middle**) or ipsilateral (**Figure 3b, right**) ventral hilus resulted in distinct staining of granule cells in both the inferior and superior blades of the dorsal DG, along with sparse labeling of dorsal hilar neurons. We then used a complementary method to physiologically verify functional connection using optogenetic terminal stimulation in slices. We expressed channelrhodopsin in dHPC-projecting vMCs and performed whole-cell patch-clamp recordings from dDG granule cells while stimulating channelrhodopsin+ terminals in the inner molecular layer using 473 nm blue light at 20 Hz (**Supplementary Figure 2**). Consistent with previous reports in slice preparations in which MCs and granule cells were within the *same* transverse hippocampal plane [67,68], action potentials were readily detected upon depolarization of granule cells membrane potential to approximately -60 mV. Taken together, these data support functional, monosynaptic connectivity between vMCs and dDG granule cells.

Chemogenetic activation of vMCs activates dDG granule cells and impairs object location memory encoding

We next aimed to directly model hyperactivation of vMCs and test whether vMC hyperactivation influences a behavior dependent on the dHPC: fine-grained spatial learning [35-39]. We expressed the G_q-coupled Designer Receptor Exclusively Activated by Designer Drugs (DREADD) construct hM3Dq-mCherry [42] or control mCherry in dHPC-projecting bilateral vMCs (**Figure 4a,b**). Whole cell recordings from vMCs expressing hM3Dq (n = 4 cells|3 mice) demonstrated a significant inward current after bath application of the DREADD-ligand 10 μM CNO, whereas mCherry control cells (n = 3 cells|2 mice) were not responsive to CNO ($t(5) = 4.24$, $p = 0.0068$) (**Figure 4c**). We next sought to determine an appropriate dosage of CNO for *in vivo* behavioral experiments. Ninety minutes after systemic injection, we observed low levels of cFos in vMCs expressing mCherry (n = 3 mice) or hM3Dq (n = 4 mice) after vehicle treatment or in mCherry-expressing vMCs (n = 4 mice) after 10 mg/kg CNO treatment (**Figure 4d**). cFos expression was robustly increased in hM3Dq-expressing vMCs (n = 5 mice) by 10 mg/kg CNO treatment, as revealed by a significant treatment x virus interaction in a two-way ANOVA ($F(1,12) =$

33.38, $p < 10^{-4}$; hM3Dq + CNO vs. all other groups: $p < 10^{-4}$, Sidak post-test). However, hM3Dq + CNO-treated mice also demonstrated dense cFos expression in neighboring granule cells, CA3, and CA1 neurons despite their not expressing hM3Dq, suggestive of overly high levels of activation and possible seizure activity. Indeed, careful review of recorded behavior revealed myoclonic jerks in a subset of mice (data not shown). Reducing CNO dosage to 1 mg/kg maintained robust vMC activation in hM3Dq-expressing MCs (n = 3 mice) compared to mCherry-expressing vMCs (n = 3 mice) ($t(2.98) = 5.58$, $p = 0.012$) (**Figure 4e**) without dense hyperactivation of neighboring granule or pyramidal neurons. Consistent with excitatory connectivity *in vivo* between vMCs and dDG granule cells, 1 mg/kg CNO also significantly increased cFos expression in dHPC granule cells ($t(3.77)=3.21$, $p = 0.036$) (**Figure 4f**). We asked how activation of dDG granule cells by chemogenetic activation of vMCs compared to novelty-induced activation of vMCs [56]. Exposing mice to a novel context (n = 5) increased dDG cFos expression compared to home cage controls (n = 5), however this increase did not reach significance threshold ($t(7.49) = 2.01$, $p = 0.08$) (**Supplementary Figure 3**). Thus, naturalistic activators of vMCs such as rearing (**Figure 2**) and novelty [56] likely convey information to the dDG, but may differ from processes that hyperactivate the vHPC in which vMC to dDG projections lead to clear hyperactivation of granule cells. We chose 1 mg/kg CNO to model the consequences of vMC hyperactivation on dHPC-dependent learning.

We next tested how vMC chemogenetic activation affects fine-grained spatial encoding using an object location memory task, which permits temporal separation of encoding and retrieval [45]. We focused on encoding because vMC inhibition impaired encoding in previous studies [34]. In this assay, mice investigate two identical objects within an arena containing spatial cues (encoding phase), and 24 hrs later return to the arena where one object was moved (novel location) and the other remains in place (familiar location). Mice with intact spatial memory spend more time investigating the object in the novel location. Unmanipulated group-housed CD-1 mice showed robust object location memory (**Supplementary**

Figure 4). To determine whether vMC hyperactivation influences object location encoding, we treated mice expressing vMC hM3Dq or mCherry with 1 mg/kg CNO 30 mins prior to the encoding session. Mice received no treatment prior to the retrieval session 24 hrs later (**Figure 5a)**. Two-way ANOVA for discrimination index (DI) between the object locations revealed a significant session x virus interaction (n = 10 mCherry mice, n = 9 hM3Dq mice, $F(1,17) = 7.64$, $p = 0.013$). Sidak post-hoc tests comparing DI between hM3Dq- and mCherry-expressing mice revealed no significant DI difference during the encoding session ($t(34) = 0.74$, $p = 0.71$) but a significant DI difference during the retrieval session ($t(34) = 2.76$, $p = 0.018$). The total amount of time spent investigating the objects did not significantly differ between virus groups during encoding ($t(15.65) = 0.37$, $p = 0.72$) or retrieval ($t(12.65) = 1.40$, $p = 0.18$) sessions (**Figure 5c**). In a 10 min open field test using an arena with a novel floor (n = 12 mCherry mice, n = 11 hM3Dq mice) (**Figure 5d**) there were no significant differences in total distance traveled ($t(19.74) = 0.27$, $p = 0.79$) or time in the arena center ($t(15.13) = 0.68$, $p = 0.51$) between virus groups (**Figure 5e, f**). The total number of rearing events also did not differ between groups ($t(20.31) = 0.66$, $p = 0.52$) (**Figure 5g**). Taken together, these data suggest that hyperactivation of vMCs impairs fine-grained location encoding but does not significantly change exploration of the environment or gross motor output.

DISCUSSION

Here we used intersectional viral techniques to target, record from, and hyperactivate vMCs that densely project to the dHPC. Our goal was to interrogate this specific intrahippocampal projection that is anatomically well-suited to distribute localized vHPC (anterior HPC in humans) hyperactivity associated with schizophrenia to disrupt cognitive functions reliant on the dHPC. We found that 1) vMCs *in vivo* are activated during behavior related to contextual mapping but not indiscriminately activated by motivated motor behaviors, 2) vMCs make functional synapses with dDG granule cells, 3) chemogenetic activation of vMCs activates dDG granule cells *in vivo*, and 4) chemogenetic activation of vMCs impairs spatial memory encoding without significantly altering exploratory behaviors. Interpreted together, we conclude

first that vMC activity observed by fiber photometry during rearing is more likely related to a role in contextual encoding facilitated by the rearing behavior acutely changing the sensory context, whereas vMC activity does not directly drive the rearing or other exploratory locomotor behavior. Second, because fine-grained spatial encoding is considerably insensitive to vHPC impairments [36], our data suggest that chemogenetic hyperactivation of vMCs impairs spatial encoding by aberrantly activating dDG granule cells. While future studies are needed to determine whether MCs themselves are involved in CIIS, there is post-mortem evidence for cellular and biochemical changes in human hippocampal area CA4 [69-71]. This work strongly supports consideration of longitudinal intrahippocampal projections in playing a role in propagating aberrant hippocampal hyperactivity in psychiatric disorders to impair cognition.

The functional role of MC local and distant projections has primarily been studied in the context of seizure propagation. Since MCs target both granule cells and inhibitory interneurons, considerable work has addressed the question of whether the net effect of MC activation is to excite or inhibit DG granule cells [25,26,34,49,67,68]. Less attention has been devoted to the role of MCs, especially vMCs, in behavior relevant to neuropsychiatric disease including learning, memory, novelty, and anxiety, however there is growing research interest in these topics. Distinctions between vMCs and dMCs are supported by physiological [72-75], biochemical [29,30], and anatomical [75-77] evidence. It thus may not be sufficient to extrapolate findings from dMCs to all MCs across the HPC longitudinal axis. Recent studies have applied *in vivo* methods to correlate the activity primarily of dMCs with exploratory behavior in rodents, showing that dMCs are highly active compared to granule cells, have multiple place fields, and remap in response to contextual changes [31-33]. An elegant recent study using *in vivo* microscopy showed increased vMC activity in novel versus familiar environments that was not due to locomotion differences [75], consistent with our findings that vMC activity is increased specifically by rearing, a behavior related to novel contextual mapping.

Our finding that hyperactivation of dHPC-projecting vMCs impairs fine-grained spatial encoding, in conjunction with a recent report that *inhibition* of vMCs also impairs encoding in the same object location memory task [34], supports an overall model that bidirectional perturbation of vMC activity, either too much or too little, degrades spatial encoding (**Figure 5h**), without influencing exploratory or locomotor behavior itself. This effect is likely via projections to and effects on the dHPC, as previous work showed that optogenetic activation *or* inhibition of dDG but not vDG granule cells impaired contextual encoding [78] and that lesioning MCs across the longitudinal axis impairs dHPC-dependent contextual but not cued fear conditioning [49]. Two recent studies illustrate that the effect of vMCs on learning and memory is likely highly dependent on the specific task. Using Calb2-Cre mice to express hM3Dq in vMCs, Fredes *et al.* showed that vMC activation prior to shock-context pairing *enhanced* fear conditioning when the conditioning occurred in a familiar context [75], an important finding because contextual fear conditioning is markedly stronger in novel versus familiar contexts. Another study using Drd2-Cre mice to express hM3Dq in vMCs reported that vMC activation did not significantly impair encoding in an object location memory task when only a one hour delay was used between encoding and retrieval sessions [79], as opposed to the 24 hr delay used in the present study and that of Bui *et al.* [34].

The present results support several future investigations into basic vMC function, involvement of MCs in rodent models for schizophrenia, and therapeutic effects of MC manipulation in models for CIIS. Most important is to develop a mechanistic understanding of why vMC inhibition or excitation impairs spatial encoding. One plausible explanation based on the neuroanatomy of vMC projections to the dDG is through interactions with the medial perforant path (MPP) from the entorhinal cortex, which terminates in the middle molecular layer on granule cell dendrites just distal to vMC projection terminals. A functional interaction between the “associational” pathway (i.e. ipsilateral MC projections) and MPP has been shown in earlier, non-cell specific field stimulation studies [63,64], as well as recently using an optogenetic approach [76]. Hypo- or hyperactivation of vMC projections to dorsal granule cells may

impair the dDG's critical function of transforming highly active MPP inputs into sparse, orthogonal ensembles of activated granule cells for pattern separation [80-85]. Future studies using mouse models with genetic lesions conferring high risk for schizophrenia in humans that show vHPC hyperactivity and object location memory deficits, such as 22q11.2 deletion models [86,87], are well-suited to test whether modulating vMC longitudinal projections may rescue dHPC-dependent memory functions. Finally, chemogenetic manipulations in this study were acute, and it has been shown that learning and memory changes following viral MC ablations are surprisingly transient [49]. Given the increased recognition of schizophrenia as a condition of aberrant neurodevelopment, it will be important to test whether chronic chemogenetic vMC inhibition or excitation or MC ablation in the vHPC impairs spatial memory encoding in a similar manner to acute manipulations.

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Author contributions

J.B., S.L.R., M.E.J., W.K., J.Q, and C.Z. designed and performed research and analyzed data. P.J.C. designed research. A.S.L. designed and performed research, analyzed data, and wrote the paper.

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Figure legends

Figure 1. Intersectional targeting of longitudinal ventral mossy cell projections. a Ventral mossy cell (vMC) to dorsal dentate gyrus (dDG) circuit of interest as illustrated by the Allen Mouse Brain Connectivity Atlas [88]. AAV expressing EGFP was infused into ventral hilus of wildtype C57BL/6J mouse (top). Serial two-photon tomography shows axonal projections throughout longitudinal extent of hippocampus, with strong projection to dDG inner molecular layer (https://connectivity.brain-map.org/projection/experiment/112672268). **b** To target vMCs, retrograde-AAV-pgk-Cre was infused into dDG inner molecular layer, and AAV-hSyn-Flex-GCaMP6f was infused into contralateral vDG hilus, enabling recombination only in vMCs that project to dDG. **c** Fluorescence microscopy from CD-1 mouse following targeting strategy shown in (b) reveals GCaMP6f is expressed in vMC somata with organized projections to contralateral vDG and bilateral dDG. **d** GCaMP6f from targeting strategy in (b) is highly colocalized with calretinin, a marker for ventral but not dorsal MCs. **e** Quantification of viral targeting strategy in (b) from n = 4 mice reveals most GCaMP6f+ hilar neurons are calretinin+, with limited off-target expression in nearby ventral granule cells and CA3 pyramidal neurons.

Figure 2. *In vivo* fiber photometry reveals ventral mossy cells are selectively activated by behavior related to contextual mapping. a Nissl stained coronal section from Allen Mouse Brain Atlas [89] depicting brain region targeted by photometry cannula (http://atlas.brain-map.org/atlas?atlas=1&plate=100960053#atlas=1&plate=100960053&resolution=10.87&x=6192.103794642857&y=4543.999055144074&zoom=-3). **b** Fluorescence microscopy of GCaMP6f expression in ventral mossy cells (vMCs) using the intersectional targeting strategy and fiber optic cannula placement. **c** $\Delta F/F_0$ Z-scores of rearing events aligned to time of rearing apogee in an individual mouse. Events are ordered by Z-score and consistently show increased photometry signal during rearing. **d** $\Delta F/F_0$ Z-scores for N = 5 mice were aligned to time of rearing apogee, averaged within mouse, then averaged across mice. Top, black line depicts mean $\Delta F/F_0$ Z-score, pink depicts s.e.m. Bottom, heatmap showing signal

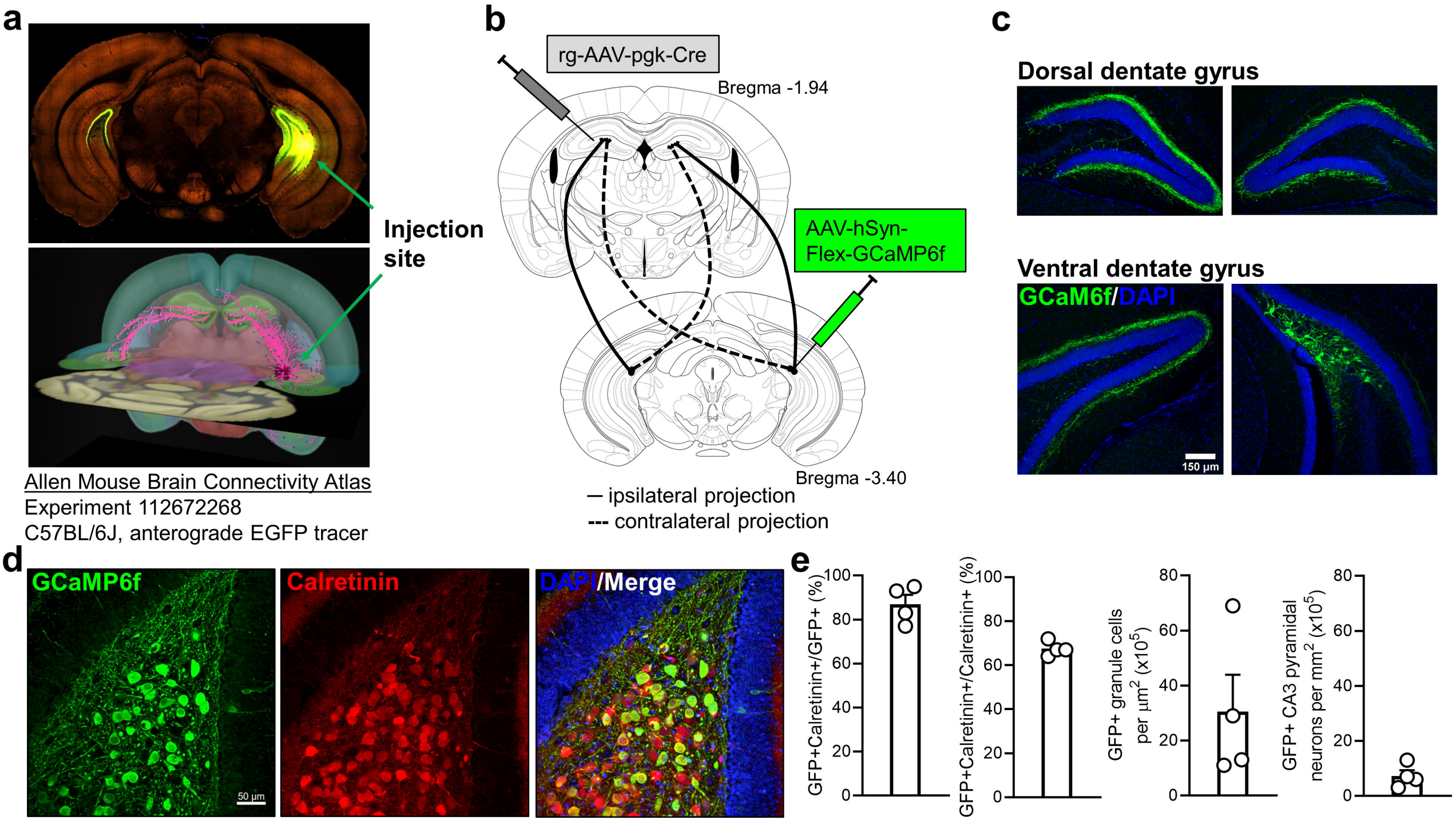
for individual mice. **e** As a control to test whether vMCs were activated by *any* motivated motor behavior, $\Delta F/F_0$ Z-scores for the same N = 5 mice from (d) during the same behavioral session were aligned to time of offensive attack on an interacting conspecific, averaged within mouse, then averaged across mice. Top, black line depicts mean $\Delta F/F_0$ Z-score, green depicts s.e.m. Bottom, heatmap showing signal for individual mice. **f** Area under the curve from -2 s to +2 s aligned to either rearing (pink) or attack (green) for mice in (d-e) shows significantly greater vMC activity associated with rearing than attack during the same behavioral social interaction session, suggesting vMCs are selectively activated by exploratory behavior related to contextual mapping but not activated by any motor output.

Figure 3. AAV1-mediated anterograde transsynaptic tracing reveals functional synapses between ventral mossy cells and dorsal granule cells. a AAV1-Cre was infused unilaterally into ventral dentate gyrus (DG) hilus, and Cre-dependent mCherry (AAV-DIO-mCherry) was infused either contralaterally (box 1) or ipsilaterally (box 2) to dorsal DG to detect transsynaptic trafficking. **b** AAV-DIO-mCherry does not express mCherry when infused alone (left), however additional infusion of AAV1-Cre in either contralateral (middle) or ipsilateral (right) DG results in dorsal DG granule cell expression of mCherry, supporting functional synaptic connectivity between the ventral hilus and dorsal DG most consistent with longitudinal mossy cell projections.

Figure 4. 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 (DG) and AAV-hSyn-DIO-hM3D(Gq)-mCherry or AAV-hSyn-DIO-mCherry was bilaterally infused into ventral DG hilus. **b** Fluorescence microscopy showing representative image of bilateral vMC targeting. **c** Whole cell recordings from vMCs expressing either mCherry (n = 3 cells|2 mice) or hM3Dq-mCherry (n = 4 cells|3 mice) revealed hM3Dq+ neurons showed increased mean inward current after bath application of 10 micromolar clozapine N-oxide (CNO),

whereas mCherry+ neurons did not respond to CNO. **p = 0.0068. **d** Mice with vMCs expressing mCherry (n = 3-4) or hM3Dq-mCherry (n = 4-5) were administered vehicle or 10 mg/kg CNO i.p., then perfused 90 mins later and immunostained for cFos. Only vMCs expressing hM3Dq-mCherry strongly expressed cFos, but also strongly expressed cFos in neighboring ventral DG granule cells. ****$p < 10^{-4}$. **e** Same method as in (d) but with reduced dose of CNO (1 mg/kg) showed increase in cFos in vMCs expressing hM3Dq, but no hyperactivation of neighboring granule cells. *p = 0.012. **f** 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. *p = 0.036.

Figure 5. Ventral mossy cell hyperactivation impairs object location memory encoding. a Schematic of the object location memory task. Mice are handled for three days, followed by six days of habituation to an arena with spatial cues on the wall. The following day they undergo a 10 min encoding session during which they are exposed to two identical objects. 24 hrs later they are returned to the arena for a 5 min retrieval 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 encoding session only. **b** CNO administration during encoding reduced object location retrieval in mice with vMC hM3Dq expression (n = 10 mCherry, n = 9 hM3Dq). **c** Total time spent investigating both objects did not significantly differ between the mCherry or hM3Dq-expressing mice during the encoding (left) or retrieval session (right). **d** 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). 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**). **h** Schematic suggesting that an optimal setpoint of vMC activity exists to promote optimal encoding performance, which is supported by previous work showing that inhibition of vMCs impairs spatial encoding [34] and the current study suggesting that hyperactivation of vMCs also impairs spatial encoding.


a
Injection site
Allen Mouse Brain Connectivity Atlas
Experiment 112672268
C57BL/6J, anterograde EGFP tracer
b
rg-AAV-pgk-Cre
Bregma -1.94
AAV-hSyn-Flex-GCaMP6f
Bregma -3.40
— ipsilateral projection
--- contralateral projection
c
Dorsal dentate gyrus
Ventral dentate gyrus
GCaM6f/DAPI
150 µm
d
GCaMP6f
Calretinin
DAPI/Merge
50 µm
e
GFP+Calretinin+/GFP+ (%)
GFP+Calretinin+/Calretinin+ (%)
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GFP+ CA3 pyramidal neurons per mm2 (x10^5)
0
20
40
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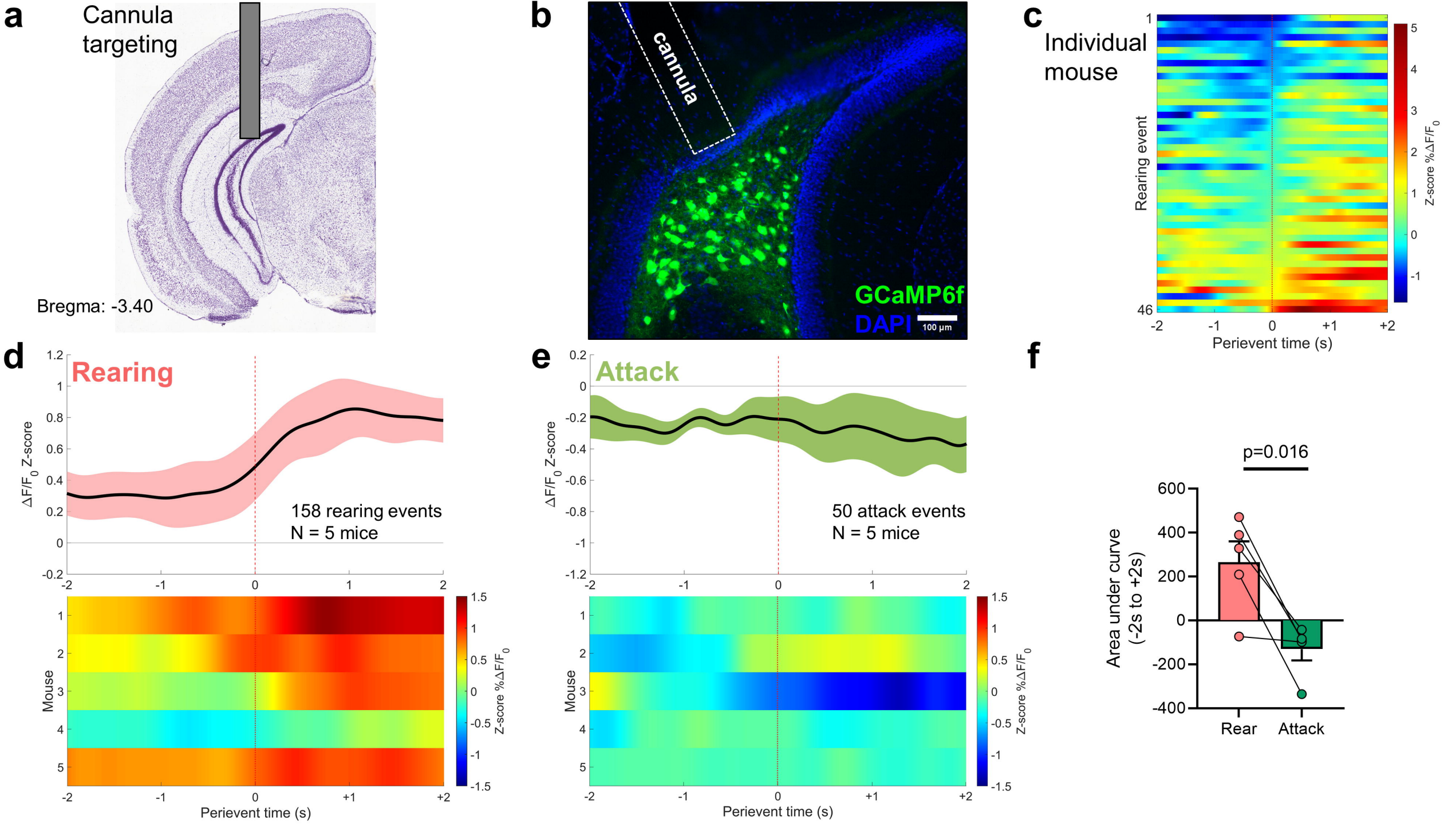
a
Cannula targeting
Bregma: -3.40
b
cannula
GCaMP6f
DAPI
100 µm
c
Individual mouse
Rearing event
Perievent time (s)
Z-score %ΔF/F0
d
Rearing
ΔF/F0 Z-score
158 rearing events
N = 5 mice
Mouse
Perievent time (s)
Z-score %ΔF/F0
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ΔF/F0 Z-score
50 attack events
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Area under curve (-2s to +2s)
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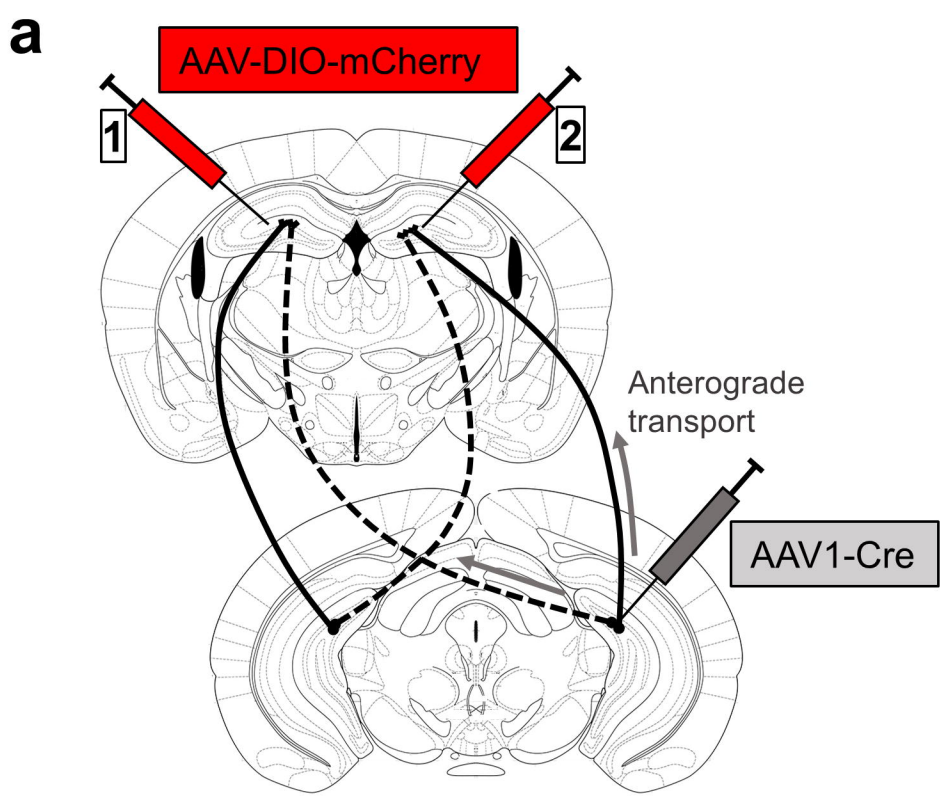
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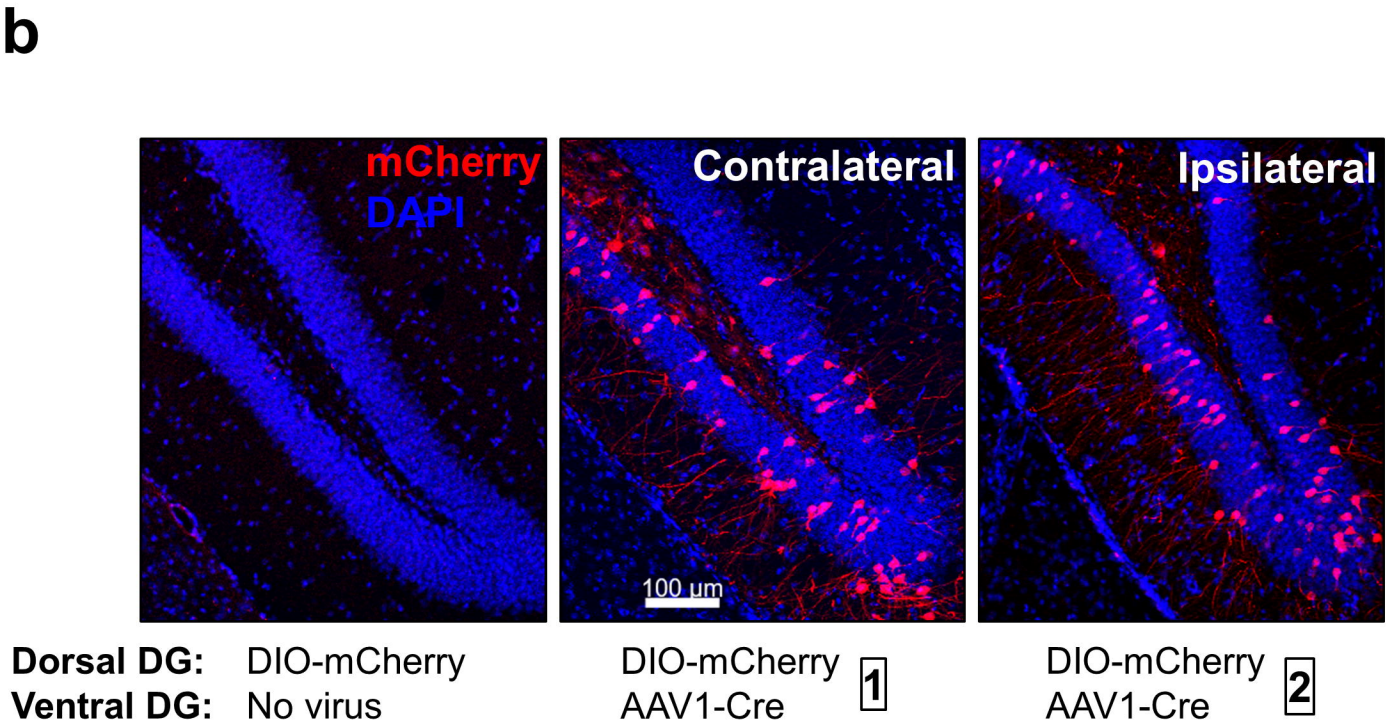
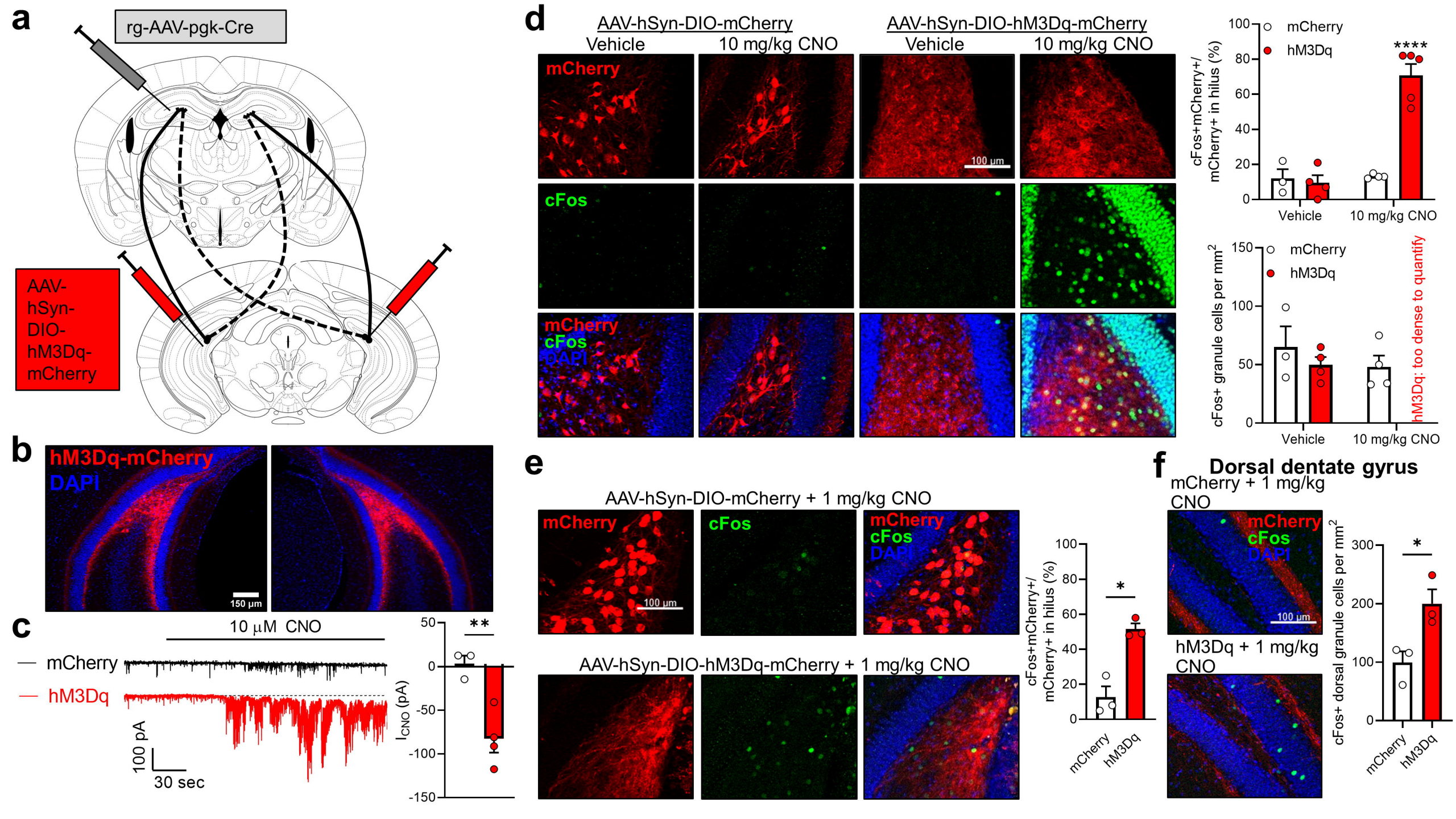
1
2
Anterograde
transport
AAV1-Cre


b
mCherry
DAPI
Contralateral
Ipsilateral
100 µm
Dorsal DG: DIO-mCherry
Ventral DG: No virus
DIO-mCherry
AAV1-Cre
1
DIO-mCherry
AAV1-Cre
2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DAPI
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c
10 µM CNO
mCherry
hM3Dq
100 pA
30 sec
I_{CNO} (pA)
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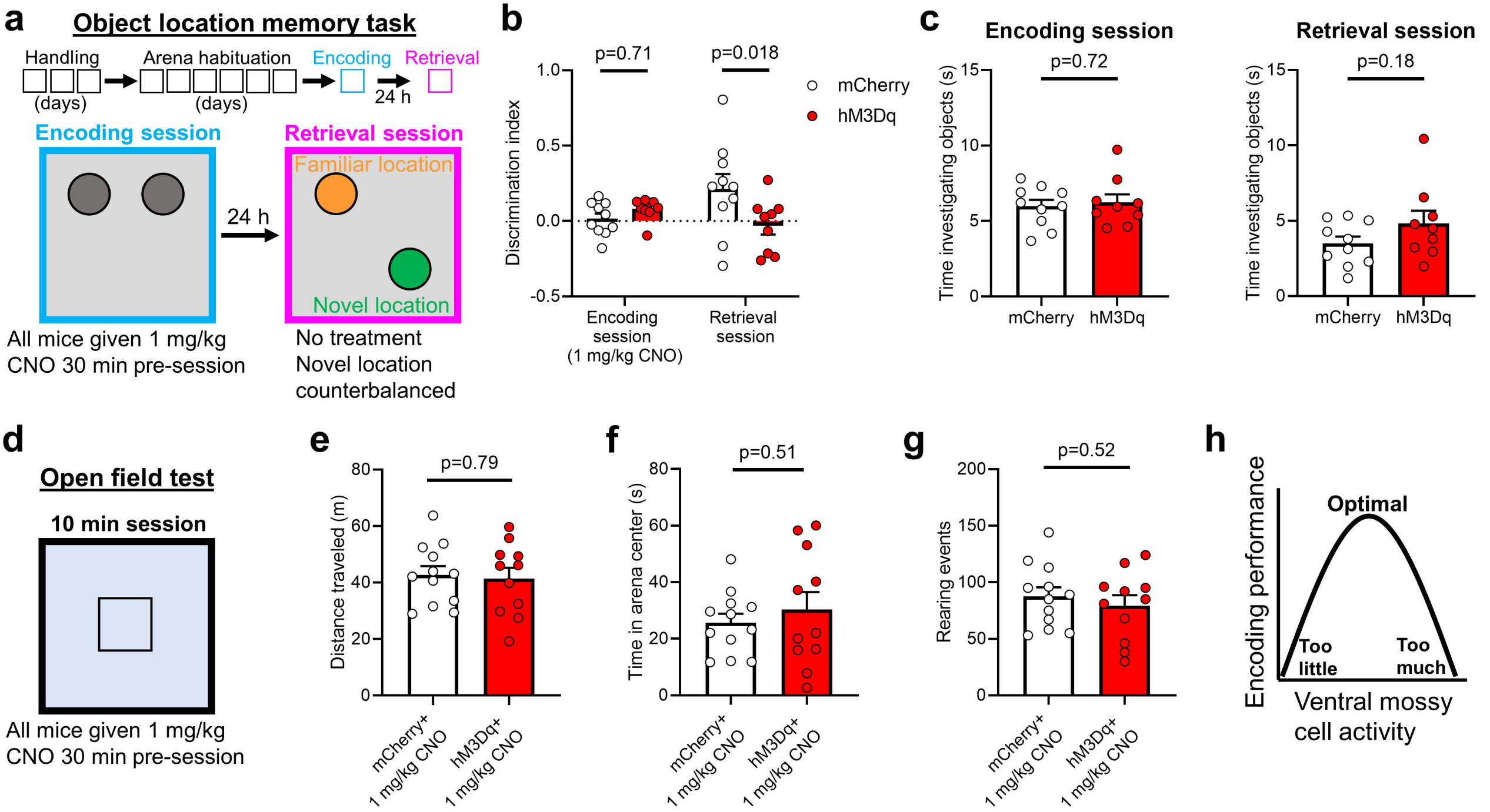
d
AAV-hSyn-DIO-mCherry
AAV-hSyn-DIO-hM3Dq-mCherry
Vehicle
10 mg/kg CNO
mCherry
cFos
DAPI
100 µm
cFos+mCherry+/ mCherry+ in hilus (%)
****
cFos+ granule cells per mm^2
hM3Dq; too dense to quantify
e
AAV-hSyn-DIO-mCherry + 1 mg/kg CNO
AAV-hSyn-DIO-hM3Dq-mCherry + 1 mg/kg CNO
*
f
Dorsal dentate gyrus
mCherry + 1 mg/kg CNO
hM3Dq + 1 mg/kg CNO
cFos+ dorsal granule cells per mm^2


a
Object location memory task
Handling
(days)
Arena habituation
(days)
Encoding
Retrieval
24 h
Encoding session
Retrieval session
Familiar location
Novel location
24 h
All mice given 1 mg/kg
CNO 30 min pre-session
No treatment
Novel location
counterbalanced
b
p=0.71
p=0.018
Discrimination index
1.0
0.5
0.0
-0.5
mCherry
hM3Dq
Encoding
session
(1 mg/kg CNO)
Retrieval
session
c
Encoding session
Retrieval session
p=0.72
p=0.18
Time investigating objects (s)
15
10
5
0
mCherry
hM3Dq
d
Open field test
10 min session
All mice given 1 mg/kg
CNO 30 min pre-session
e
p=0.79
Distance traveled (m)
80
60
40
20
0
mCherry+
1 mg/kg CNO
hM3Dq+
1 mg/kg CNO
f
p=0.51
Time in arena center (s)
80
60
40
20
0
mCherry+
1 mg/kg CNO
hM3Dq+
1 mg/kg CNO
g
p=0.52
Rearing events
200
150
100
50
0
mCherry+
1 mg/kg CNO
hM3Dq+
1 mg/kg CNO
h
Encoding performance
Optimal
Too
little
Too
much
Ventral mossy
cell activity