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Specific disruption of contextual memory recall by sparse additional activity in the dentate gyrus

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ABSTRACT

The dentate gyrus (DG) of the hippocampus is essential for contextual and spatial memory processing. While lesion or silencing of the DG impairs contextual memory encoding and recall, overly activated DG also prevents proper memory retrieval. Abnormally elevated activity in the DG is repeatedly reported in amnesic mild cognitive impairment (aMCI) patients or aged adults. Although the correlation between memory failure and abnormally active hippocampus is clear, their causal relationship or the underlying nature of such interfering activity is not well understood. Using optogenetics aided by a carefully controlled adeno-associated virus infection system, we were able to examine the differential effects of abnormally activated hippocampus on mice motor behavior and memory function, depending on the extent of the stimulation. Optogenetic stimulation of massive proportion of dorsal DG cells resulted in memory retrieval impairment, but also induced increase in general locomotion. Random additional activity in a sparse population of dorsal DG neurons, however, interfered with contextual memory recall without inducing hyperactivity. Our findings thus establish the causal role of elevated DG activity on memory recall failure, suggesting such aberrant DG activity may contribute to amnesic symptoms in aMCI patients and aged adults.

1. Introduction

The dentate gyrus of the hippocampus is well known for its contribution to processing of contextual memories and to discrimination between different contexts (O'Reilly and McClelland, 1994; Gilbert, Kesner, & Lee, 2001; Leutgeb, Leutgeb, Moser, & Moser, 2007; Hernández-Rabaza et al., 2008; Hunsaker & Kesner, 2008). Neurons in the DG are activated in response to contextual learning, reactivation of which is sufficient for retrieval of that memory (Liu et al., 2012). Highlighting its role in learning, lesion or inactivation of the DG impairs spatial memory acquisition and recall (Morris, Churchwell, Kesner, & Gilbert, 2012; Xavier, Oliveira-Filho, & Santos, 1999; Conejo, Cimadevilla, González-Pardo, Méndez-Couz, & Arias, 2013; Denny et al., 2014; Bernier et al., 2017).

Although DG activity is necessary for remembering contextual information, excess activity can also cause problems. Abnormally elevated activity in the DG is observed in aMCI, disorder associated with risk for Alzheimer's disease (Bakker et al., 2012). Increased blood-oxygenation level-dependent (BOLD) signal was observed from functional magnetic resonance imaging (fMRI) in the DG of older adults, which also correlated with aging-related memory deficits (Yassa et al., 2011). Contextual memory recall failures have been reported in rodent models as well when overall DG activity was artificially increased (Collier, Miller, Travis, & Routtenberg, 1982; Kheirbek et al., 2013). Opposed to the view that greater activity in the brain region involved in encoding of a memory might have a beneficial impact on memory function, these reports propose otherwise. As such, substantial number of studies shows that elevated activity in the DG occurs with disrupted memory recall, but this correlation is not sufficient to indicate causality. Previous research has reported that artificial stimulation of the DG neurons disrupts recall of contextual fear (Kheirbek et al., 2013). However, the study also reported that elevated DG activity induced hyperactive behavior. To clarify the consequence of randomly increased activity in the DG on memory retrieval, experimental conditions of this study was designed to distinguish between the effects on locomotion and on memory function.

In the present study we sought to investigate how memory recall is influenced by artificially stimulating a spectrum of (from small to large) population of the dorsal DG, taking advantage of the optogenetics technique aided by the adeno-associated virus (AAV) viral expression

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system. We first tested contextual memory recall while activating a mass of dorsal DG cell layer of both hemispheres and observed that massive activation of the dorsal DG interferes with contextual fear memory recall and induces hyperactive behavior, consistent with previous reports. Then we increasingly restricted expression of the excitatory opsin channelrhodopsin-2 (ChR2) to only a sparse number of neurons in the unilateral DG. As a result, we demonstrate that sparse additional activation of dorsal DG population as small as approximately 1% specifically blocks contextual memory retrieval without effects on locomotor behavior, which lead us to better understand memory recall deficits in aMCI patients and aged adults with elevated hippocampal activity.

2. Materials and methods

2.1. Mice

Adult (2–3 months old) 129/C57Bl/6 hybrid mice were used for all experiments. Mice were group housed (3–5 mice per cage) and the vivarium was maintained in 12 h light/dark cycle at a constant temperature of 22 ± 1 °C with 40–60% humidity. Food and water were available *ad libitum*. All procedures were approved by the Animal Ethics Committee at the Korea Advanced Institute of Science and Technology.

2.2. Virus production

All AAV virus used in this study was prepared as described previously (Kwon et al., 2014). Briefly, AAV expression vector and viral vector (pAdΔF6 and $AAV_{2/1}$) were co-transfected into HEK293T cells by calcium phosphate precipitation. After 72 h, cells containing AAV virus were harvested, and AAV virus was purified on iodixanol gradient using an ultracentrifuge. Five AAV viral constructs were used in this study, and titers were calculated by quantitative PCR: AAV-CaMKIIα-ChR2-Venus (3.7×10^{11} vg ml^{-1}), AAV-CaMKIIα-eGFP (5.2×10^{11} vg ml^{-1}), AAV-CaMKIIα-mCherry-IRES-Cre (1.1×10^{11} vg ml^{-1}), AAV-EF1α-DIO-hChR2(H134R)-eYFP (7.0×10^{11} vg ml^{-1}) and AAV-EF1α-DIO-eYFP (1.2×10^{12} vg ml^{-1}).

2.3. Surgery

Mice were pretreated with atropine (12 μg, regardless of body weight), and then anesthetized with pentobarbital (83 mg per kg of body weight) by intraperitoneal injection. To infect massive population of the dorsal DG, AAV-CaMKIIα-ChR2-Venus or AAV-CaMKIIα-eGFP was loaded into a glass micropipette and stereotaxically located in the bilateral DG (AP −2.0 mm, ML ±1.3 mm, DV −2.0 mm). Virus solution was injected into the DG at a rate of 0.1 μl min^{-1} for 8 min (total 0.8 μl). After additional 10 min to allow for diffusion of the virus, micropipette was withdrawn from the brain very slowly. To achieve sparse infection of the DG, cocktail of AAV-CaMKIIα-mCherry-IRES-Cre and AAV-EF1α-DIO-hChR2(H134R)-eYFP or AAV-EF1α-DIO-eYFP were used (Nomura et al., 2015). AAV-CaMKIIα-mCherry-IRES-Cre was diluted in phosphate buffered saline (PBS) in various dilution factors, then added to AAV-EF1α-DIO-hChR2(H134R)-eYFP or AAV-EF1α-DIO-eYFP in 1:1 ratio. Total of 1.0 μl of cocktail virus was injected into the unilateral DG (AP −2.0 mm, ML −1.3 mm, DV −2.0 mm). For all optogenetic experiments, optic ferrule implantation surgery was conducted 2 weeks after the virus injection. Optic ferrules were located slightly above the virus injection site (AP −2.0 mm, ML ±1.3 mm, DV −1.6 mm for bilateral implantation, AP −2.0 mm, ML −1.3 mm, DV −1.6 mm for unilateral implantation) and fixed with dental cement. Mice were single housed for an additional week for recovery and sufficient expression of transgene before starting behavioral experiments.

2.4. Immunohistochemistry (IHC)

To test whether our light stimulation setup induces neuronal activation in the dorsal DG, c-Fos immunohistochemistry was conducted. ChR2-expressing mice received light stimulation (20 Hz with 10 msec pulse) for 3 min in home cage while control mice received the same handling procedure without light stimulation. After 90 min, mice were perfused transcardially with 100 ml of PBS followed by same volume of 4% paraformaldehyde (PFA). Brains were extracted and stored in 4% PFA overnight. Coronal brain sections (40 μm) were acquired using vibratome (VT-1200S, Leica). In the diaminobenzidine (DAB, Sigma) staining, c-Fos was detected by anti-c-Fos primary rabbit polyclonal antibody (1:2000, Santa Cruz) followed by biotinylated goat-anti-rabbit secondary antibody (1:2000, Vector Laboratories). Staining was visualized using avidin–biotin based peroxidase system (Vectastain Elite ABC kit, Vector Laboratories) coupled with DAB as a chromogen. Brain sections assigned to the virus injection site by reference to the Mouse Brain Atlas (Franklin 2008) were imaged using ECLIPSE 80i microscope (Nikon). In the cell counting experiments, AlexaFluor594 (1:2000, Molecular Probes) or AlexaFluor647 AffiniPure goat-anti-rabbit antibody (1:2000, Jackson Laboratory) was used as secondary antibody.

2.5. Cell counting analysis

The total number of DAPI, ChR2-eYFP positive (ChR2+) and c-Fos positive (c-Fos+) cells in the dorsal DG were counted from three coronal sections per mouse. The section assigned to the virus injection site by reference to the Mouse Brain Atlas (Franklin & Paxinos, 2008) was centered and two additional sections 120 μm anterior and posterior from the center section were selected to be averaged (Liu et al., 2012). The DG in selected three sections was tile-scanned by a ZEISS LSM780 upright confocal laser-scanning microscope with 40× water immersion objective lens using Zen 2012 software. For quantification of virus infection and c-Fos induction in a granule cell layer under massive infection condition, DAPI+, ChR2+, c-Fos+ cells were manually counted in the region of interest (ROI, 0.4 × 0.3 mm; Stefanelli, Bertollini, Lüscher, Muller, & Mendez, 2016).

To analyze co-localization between ChR2+ and c-Fos+ cells in sparse infection condition (Fig. 2D and E), total ten optical sections (1 μm each) were obtained using a z-stack analysis (Liu et al., 2012). To obtain percentage ChR2+ cells according to various virus dilution factors, total number of granule cells in the dorsal DG was automatically counted from DAPI signal in the granule cell layer of DG using spot detection algorithm in IMARIS software (BITPLANE). Finally, ChR2+ cells were manually counted and divided by the total DAPI+ DG granule cells to obtain the percentages.

Same cell counting method as above was used to measure c-Fos induction after context fear memory recall (Fig. 4C and E), but c-Fos+ and ChR2+ cells within a defined field were manually counted using ImageJ software. At the constant AP sites across all the brain sections analyzed, the ROI was selected to include the granule cell layer of the DG.

2.6. Fear conditioning

Fear conditioning was conducted 1 week after ferrule implantation (Day 1). For contextual fear conditioning, mice were placed in a metal grid-floored, square-shaped conditioning chamber (Coulbourn Instruments) with 70% alcohol scent. Mice were allowed to freely explore the chamber for 2 min. Unconditioned stimulus (US) of electrical foot shock (0.5 mA, 2 s) was delivered via metal grid three times with 1 min inter-stimulus-interval (ISI). For auditory fear conditioning, a conditioned stimulus (CS; 2,800 Hz, 85 dB tone lasting 30 s) was presented and co-terminated with an US (0.5 mA, 2 s) three times with random ISI of an average of 150 sec. After the last foot shock, mice were kept in the chamber for additional 30 sec to monitor post shock