Engram reactivation during memory retrieval predicts long-term memory performance in aged mice

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Abstract

Age-related cognitive decline preferentially targets long-lasting episodic memories that require intact hippocampal function. Memory traces (or engrams) are believed to be encoded within the neurons activated during learning (neuronal ensembles), and recalled by reactivation of the same population. However, whether engram reactivation dictates memory performance in late life is not known. Here, we labelled neuronal ensembles formed during object location recognition learning in the dentate gyrus, and analyzed the reactivation of this population by long-term memory recall in young adult, cognitively impaired- and unimpaired-aged mice. We found that reactivation of memory-encoding neuronal ensembles at long-term memory recall was disrupted in impaired- but not unimpaired-aged mice. Furthermore, we showed that the memory performance in the aged population correlated with the degree of engram reactivation at long-term memory recall. Overall, our data implicates recall-induced engram reactivation as a prediction factor of memory performance throughout aging. Moreover, our findings suggest impairments in neuronal ensemble stabilization and/or reactivation as an underlying mechanism in age-dependent cognitive decline.

Keywords: neuronal ensembles, aging, object location memory, dentate gyrus, hippocampus, memory trace

1. Introduction

Age-related cognitive decline refers to the gradual decrease in cognitive performance throughout the aging process, mostly affecting long-term storage of episodic and spatial memories that depend on hippocampal function (Burke and Barnes, 2006). The dentate gyrus (DG) is the hippocampal subregion most sensitive to the effects of advanced age (Small et al., 2004; Small et al., 2011). The DG undergoes anatomical and physiological changes as well as alterations in the transcriptomic profile that are thought to underlie the aging-associated dysfunction (Burke and Barnes, 2010; Ianov et al., 2017). It has recently been shown that subsets of DG neurons activated during learning (i.e., neuronal ensembles) belong to a memory engram, and that the reactivation of this neuronal population at memory recall is necessary and sufficient to evoke memory retrieval in young adult mice (Josselyn et al., 2015; Tonegawa et al., 2015). Moreover, dysfunctions in neuronal ensemble reactivation and memory retrieval mechanisms have been proposed as an underlying cause of dementia in pathological conditions, such as Alzheimer's disease (Perusini et al., 2017; Roy et al., 2016), in which aging presents a major risk factor. However, whether memory impairments observed in late life are associated with disruptions in neuronal ensemble reactivation is not known.

Here, we hypothesized that differences in DG neuronal ensemble dynamics underlie the inter-individual variability in long-term memory (LTM) performance in the aged population. We used tagging tools to label and characterize the formation and reactivation of neuronal ensembles associated with long-term spatial recognition memory, a form of memory particularly susceptible to decline with age. We showed that impairments in long-term spatial memory during aging are associated with impaired reactivation of memory-encoding neuronal ensembles at LTM recall. Moreover, we found that the degree of engram reactivation in aged mice correlated with long-term spatial memory performance. Overall, our findings identified novel cellular correlates of age-related memory decline.

2. Materials and Methods

2.1. Animals. 8-10 weeks or 18-20 months old male C57BL/6JRj mice (Janvier Labs, Germany) were individually housed on a 12 h light/dark cycle and *ad libitum* access to food and water (after stereotaxic surgery regular food was replaced by doxycycline-containing diet (40mg/kg, BioServ, Flemington, NJ, USA)). Experiments were carried out during the light phase. All of the animal procedures were approved and performed according to the European Community Council Directive 86/609/EEC.

2.2. Recombinant adeno associated viruses (rAAVs). RAAVs were produced and purified as described previously (Zhang et al., 2007). The RAM-HA-GFP viral construct was generated by insertion of a HA-tagged GFP expression cassette into the multiple cloning site (MCS) of rAAV-RAM (rAAV-RAM (rAAV-RAM-d2TTA::TRE-MCS-WPRE-pA) was a kind gift from Yingxi Lin (Addgene plasmid # 63931; http://n2t.net/addgene:6393; RRID:Addgene_63931)) (Sorensen et al., 2016).

2.3. Stereotaxic surgery. 250 nl of rAAV-RAM viral solution was injected into the dentate gyrus (DG) of the hippocampus at the following coordinates relative to Bregma: − 2.0 mm anteroposterior, ± 1.3 mm medio-lateral, − 2.4 mm dorsoventral at the rate of 80-100 nl / min. The needle was left in place for 10 min before and after each injection to allow DG-specific diffusion of the fluid. Only the mice with viral spread throughout the DG of at least 60 µm range in the AP axis were considered for behavior and further analysis.

2.4. Behavior paradigms. Three weeks after stereotaxic surgeries, following 5 days of handling, the spatial object recognition (SOR) was performed. The open field test was performed in the first session of the SOR training as described previously (Gulmez Karaca et al., 2018; Brito et al., in press). The Smart Video Tracking Software (Panlab, Harvard Apparatus) was used to score the time spent in the central zone (32% of the arena), number of center entries and total distance travelled in the open field test. SOR memory performance (24 h post-training) was assessed by the formula ($T_{displaced}$ /($T_{nondisplaced}$ + $T_{displaced}$) × 100).

2.5. Immunohistochemistry. 2 h after the start of the SOR test, mice were perfused intracardially with 4% paraformaldehyde (PFA) (Sigma, Munich, Germany) and free-floating brain slices (at a thickness of 20 µm) were immunostained as previously described (Gulmez

Karaca et al., 2018; Oliveira et al., 2012). Primary antibodies were used at the following concentrations: anti-HA tag (Covance MMS-101R (1:1000)), anti-Fos (Cell Signaling #2250, 1:1000)).

2.6. Image acquisition and analysis. Z-stacks of DG images (3 frames with 2 µm interval) were acquired with Nikon A1R confocal microscopy (at Nikon Imaging Center, BioQuant, Heidelberg) at 20x magnification using NIS-Elements software. Maximum projection files of each stack were imported in Fiji (Schindelin et al., 2012), and Fos^+ or HA-GFP^+ neurons were manually marked after background subtraction and application of a signal threshold. Reactivation rate ((GFP^+Fos^+)/(GFP^+)×100), and similarity index ((GFP^+Fos^+)/((GFP^+)+(Fos^+)-(GFP^+Fos^+))×100 was calculated as previously described (Cowansage et al., 2014; Milczarek et al., 2018). Data was normalized to the mean of the aged impaired group to avoid artifacts that may be caused from different viral expressions among experimental batches.

2.7. Statistical analysis. Blinding to experimental conditions was applied to image and behavioral analysis. For normally distributed data (Shapiro-Wilk normality test; alpha=0.05), ordinary one-way ANOVA followed by Bonferroni's multiple comparisons test, whereas for non-normally distributed data, Kruskal-Wallis test followed by Dunn’s multiple comparisons test was used to compare three groups. For correlation analysis, Pearson correlation test was applied. Statistical analysis was performed using GraphPad prism for Mac OS X, version 7.

3. Results

3.1. Neuronal ensemble tagging in the DG of young, cognitively-impaired or -unimpaired aged mice. We aimed at characterizing DG neuronal ensembles that hold long-term representations of object location memory in order to identify possible correlates of cognitive dysfunction in the aged population. To label the neuronal ensemble activated during learning, we used the robust activity marking (RAM) system (Sorensen et al., 2016) that allowed learning-dependent expression of HA-tagged GFP (Sorensen et al., 2016) (Figure 1A). We

have previously confirmed that this tool reliably tags the DG neuronal ensembles associated with object location memory (KGK, AMMO, unpublished findings). First, we stereotaxically delivered recombinant adeno associated viruses (rAAVs) containing the RAM-HA-GFP viral construct into the DG of young-adult (2 months old) or aged (18-20 months old) mice. Through the removal of doxycycline diet, we tagged the neuronal population activated during object location training (Figure 1A). When tested 24 h after training all young adult mice displayed higher than chance (50%) preference for the displaced object (Figure 1B), indicating intact long-term object location memory. In contrast, the performance of the aged group was heterogenous; some mice performed similarly to young mice, whereas others showed preferences closer to chance level (Figure 1B). To be able to characterize the aged population according to individual differences in cognitive performance, we sorted the aged group into aged impaired (AI) and aged unimpaired (AU) based on the mice's long-term spatial memory scores (Figure 1B). Aged mice displaying preferences for the displaced object lower than one standard deviation from the mean of young mice (65%, represented with a dashed line in Figure 1B) were considered impaired. Importantly, we confirmed that all groups displayed comparable object exploration times during the training session (Figure 1C) and demonstrated similar locomotion and anxiety-like behavior in open-field test (Figure 1D-F). This indicates that poor LTM performance in AI mice was not due to lower exploratory interest or motor or anxiety impairments.

3.2. Cognitively impaired aged mice exhibit impaired recall-induced neuronal ensemble reactivation in the upper blade of the DG. Next, we investigated the reactivation of spatial memory-associated neuronal ensembles in response to LTM recall. To this end, the expression of HA-GFP and Fos in the DG, that represent active neurons during learning or LTM recall respectively, was analyzed 2 h after object location test. The neuronal population coexpressing HA-GFP$^+$ and Fos$^+$ represented the population active in both episodes, i.e., reactivated neuronal ensemble. Given the previously described differences in responsiveness of the upper and lower blades of the DG to environmental exposure (Chawla et al., 2013; Marrone et al., 2012; Ramirez-Amaya et al., 2013), we analyzed the two

subregions separately. The percentage of DG neurons activated after object location memory recall (Figure 2B) or after learning (Figure 2C) was not statistically different between the three groups. Interestingly, we found that AI mice exhibited reduced recall-driven reactivation of the neuronal population activated by learning in the DG compared to young or AU mice (Figure 2D). The reactivation rates observed in the DG of young and AU mice were similar (Figure 2D). Notably, the impaired reactivation rate was specific for the neuronal ensembles located in the upper, but not the lower, blade of the DG in AI mice (Figure 2D). Furthermore, to evaluate whether the learning-activated neuronal ensemble pattern was reinstated equally in all of the three groups at memory recall, we applied a second formula, the similarity index. We observed the same selective impairment in AI mice (Figure 2E). Altogether, this set of experiments revealed that despite being able to activate a similar proportion of neurons upon learning or memory recall, cognitively impaired mice exhibit disrupted neuronal ensemble reactivation at LTM recall in the upper DG blade.

3.3. LTM performance of aged mice correlates with the reactivation rate in the upper blade of the DG. Finally, we tested whether the neuronal ensemble reactivation rate in the upper DG blade correlates with memory performance in aged mice. For this, we performed Pearson correlation analysis between LTM performance and reactivation rate in the aged population (i.e., pooled data of AI and AU mice). Remarkably, we found a significant positive correlation between the rate of neuronal ensemble reactivation in the upper blade of the DG and the memory score of the mice (Figure 2F). Such a correlation was not present for the lower blade of the DG (Figure 2G), nor for the total DG (Figure 2H). Overall, these results demonstrated that cognitive performance during aging positively correlates with neuronal ensemble reactivation during the recall of LTM.

4. Discussion

In this study, we tagged the neuronal ensemble formed during a spatial recognition task in the DG of young, aged-impaired and aged-unimpaired mice and identified a cellular correlate of cognitive performance in aged mice. We showed that cognitively impaired aged mice

exhibit impairments in recruiting the original memory engram at long-term spatial memory recall compared to young or cognitively unimpaired aged mice. We further showed that the degree of engram reactivation correlates with the strength of LTM in the aged population. We focused on long-term object location memory which has previously been shown to be impaired by aging (Oliveira et al., 2012; Wimmer et al., 2012). Increasing evidence suggests that each memory is encoded by a subset of neurons (neuronal ensembles) that are synchronously activated upon learning and reactivated by the retrieval of the memory (Josselyn et al., 2015; Tonegawa et al., 2015). A previous study by Penner and colleagues showed that neuronal reactivation upon short-term re-exposure to a previously visited context is reduced in the DG, but not CA1, of aged compared to young adult mice, suggesting that impairments in neuronal reactivation may be associated with cognitive deficits in aged individuals (Penner et al., 2011). Here, we characterized DG neuronal ensemble during the formation and retrieval of long-term recognition memory and correlated with the performance in the same task. We found that aging-related impairments in long-term object location memory are not associated with the size of the DG neuronal population activated by learning or recall, but rather with the fidelity of reactivation of the encoding population during memory retrieval. Aged impaired and unimpaired mice showed similar number of neurons activated by learning or by recall. In contrast, cognitively impaired mice had significantly disrupted reactivation rates compared to unimpaired aged, or young adult mice. Intriguingly, the differences were found in the neuronal population located in the upper, but not lower, blade of the DG. This is in line with previous studies showing that behaviorally-induced expression of activity regulated genes occurs primarily in the upper blade of the DG (Chawla et al., 2005; Chawla et al., 2013; Marrone et al., 2012; Ramirez-Amaya et al., 2013). These reports indicate that the sparse population of DG upper blade neurons form the spatial memory neuronal ensemble. Therefore, it may be expected that alterations in ensemble dynamics are specific to this subregion. Finally, our findings showed a positive correlation between the neuronal ensemble reactivation rate in the upper blade of the DG and LTM

performance of aged mice. These findings suggest a novel mechanism that may underlie long-term spatial memory integrity in the aged population.

The underlying molecular and physiological causes of disrupted neuronal ensemble reactivation in aged cognitively-impaired aged mice are not understood. It is well established that aging is accompanied by anatomical and physiological changes in the DG. Namely, fewer synaptic contacts and impaired synaptic plasticity in aged versus young adults have been reported (Burke and Barnes, 2010). Interestingly, age-related impairments in LTP maintenance correlated with memory performance (Rosenzweig and Barnes, 2003). Thus, deficits in the reactivation of the original encoding neuronal population may result from impaired neuronal ensemble stabilization during memory consolidation, which could emerge from the inability to strengthen the connectivity between ensemble neurons.

Recently, two studies showed that LTM impairments in a mouse model of Alzheimer's disease (AD) are linked to deficits in neuronal ensemble reactivation (Perusini et al., 2017; Roy et al., 2016). Our findings now show that alterations in neuronal ensemble properties are a common mechanism in aging and aging-associated pathological conditions. This underscores the need for therapeutic approaches targeted at facilitating engram reactivation to restore age-associated memory impairments.

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Disclosure statement

The authors declare no competing financial interests.

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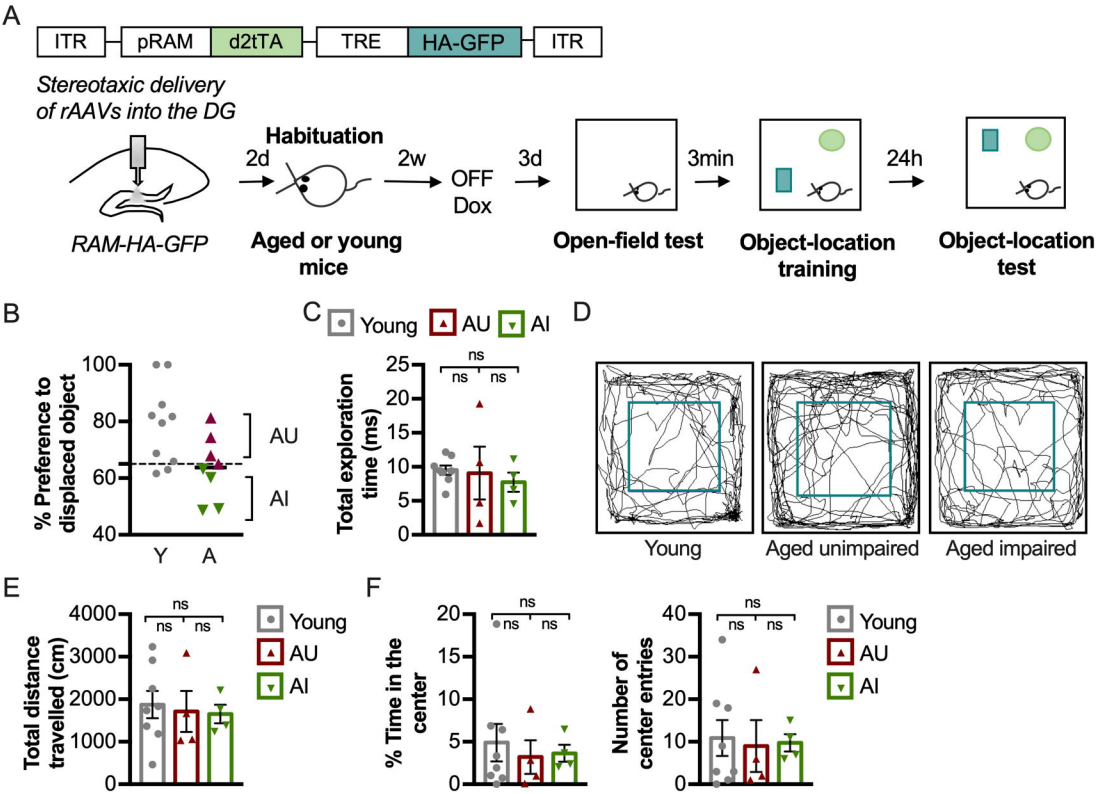
Figure legends

Figure 1. Tagging neuronal ensembles formed during spatial object-location learning in the DG of young, cognitively-impaired or -unimpaired aged mice. A) Schematic representation of the RAM-based viral construct used for neuronal ensemble tagging and of the experimental design. B) Percentage of preference for displaced object during the object location test session. Dashed line (65%) represents the threshold applied to identify AI and AU mice. C) Total object exploration time during the training session of object-location test. D) Representative trajectories during open-field test. E) Total distance travelled in open-field test. F) Percentage of time in the center and number of center entries in open-field test. In all graphs: Y (n=8), AI (n=4), AU (n=4), ns: not significant. ITR: inverted terminal repeat, DG: Dentate gyrus of the hippocampus, Dox: doxycycline, AU: aged-unimpaired, AI: aged-impaired, rAAV: recombinant adeno-associated viruses, TRE: tetracycline responsive element, pRAM: robust activity marking promoter. Error bars represent s.e.m.

Figure 2. Age-related cognitive decline is associated with disrupted DG neuronal ensemble reactivation at LTM recall. A) Schematic representation of the experimental design. B) Percentage of Fos^+ neurons. C) Percentage of HA-GFP^+ neurons. D) Normalized reactivation rates of learning-activated neuronal ensembles at memory recall. E) Normalized similarity indices of neuronal ensemble populations activated at learning or at LTM recall. F-H) Pearson correlation between the normalized neuronal ensemble reactivation in the upper (F), lower (G) DG blades or in total DG (H) and memory performance of aged mice at long-term object-location test. In all graphs: Y (n=8), AI (n=4), AU (n=4), *$p<0.05$; ns: not significant. DG: Dentate gyrus of the hippocampus, Dox: doxycycline, AU: aged-unimpaired, AI: aged-impaired, rAAV: recombinant adeno-associated viruses. Error bars represent s.e.m.

Figure 1


A
ITR
pRAM
d2tTA
TRE
HA-GFP
ITR
Stereotaxic delivery of rAAVs into the DG
RAM-HA-GFP
Habituation
2d
2w
OFF Dox
3d
3min
24h
Aged or young mice
Open-field test
Object-location training
Object-location test
B
% Preference to displaced object
100
80
60
40
AU
AI
Y
A
C
Young
AU
AI
Total exploration time (ms)
25
20
15
10
5
0
ns
ns
ns
D
Young
Aged unimpaired
Aged impaired
E
Total distance travelled (cm)
4000
3000
2000
1000
0
ns
ns
ns
Young
AU
AI
F
% Time in the center
20
15
10
5
0
ns
ns
ns
Number of center entries
40
30
20
10
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ns
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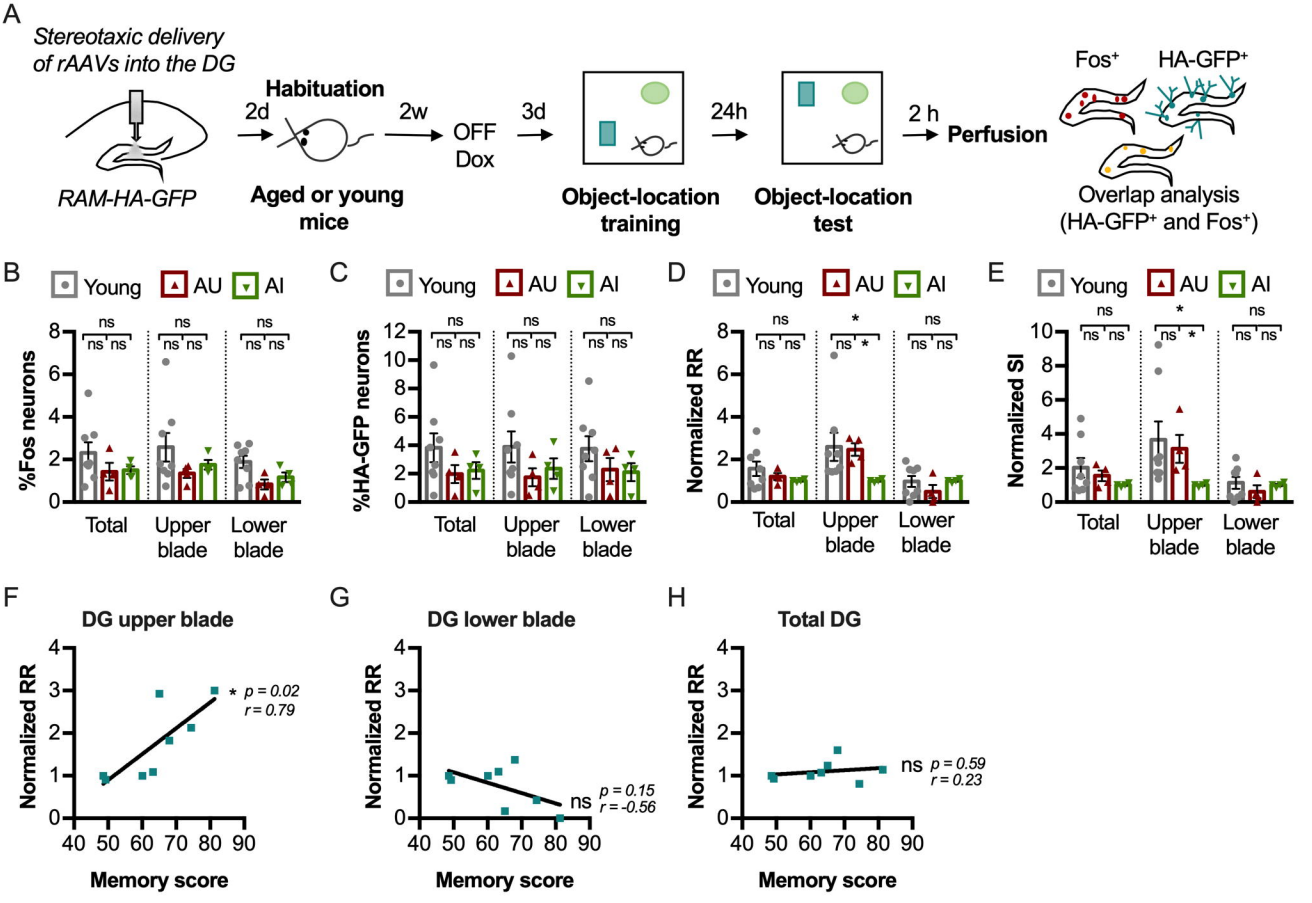
ns
Young
AU
AI

Figure 2


A
Stereotaxic delivery of rAAVs into the DG
RAM-HA-GFP
Habituation
2d
2w
OFF Dox
3d
24h
2 h
Perfusion
Aged or young mice
Object-location training
Object-location test
Fos+
HA-GFP+
Overlap analysis (HA-GFP+ and Fos+)
B
C
D
E
Young
AU
AI
%Fos neurons
%HA-GFP neurons
Normalized RR
Normalized SI
Total
Upper blade
Lower blade
ns
*
F
G
H
DG upper blade
DG lower blade
Total DG
Memory score
* p = 0.02
r = 0.79
ns p = 0.15
r = -0.56
ns p = 0.59
r = 0.23