Neurobiology of Aging 91 (2020) 1–4

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Brief communication

# Significance of inhibitory recruitment in aging with preserved cognition: limiting gamma-aminobutyric acid type A α5 function produces memory impairment

Ming Teng Koh, Audrey Branch, Rebecca Haberman*, Michela Gallagher

*Department of Psychological and Brain Sciences, Johns Hopkins University, Baltimore, MD, USA*

ARTICLE INFO

*Article history:*
Received 25 October 2019
Received in revised form 30 December 2019
Accepted 22 February 2020
Available online 5 March 2020

*Keywords:*
Aging
Inhibition
GABA α5 receptor
Spatial memory

ABSTRACT

Numerous aging studies have identified a shift in the excitatory/inhibitory (E/I) balance with heightened hippocampal neural activity associated with age-related memory impairment across species, including rats, monkeys, and humans. Neurobiological investigations directed at the hippocampal formation have demonstrated that unimpaired aged rats performing on par with young adult rats in a spatial memory task exhibit gene expression profiles, mechanisms for plasticity, and altered circuit/network function, which are distinct from younger rats. Particularly striking is a convergence of observational evidence that aged unimpaired rats augment recruitment of mechanisms associated with neural inhibition, a finding that may represent an adaptive homeostatic adjustment necessary to maintain neural plasticity and memory function in aging. In this study, we test the effect of limiting inhibition via administration of TB21007, a negative allosteric modulator of the alpha 5 subtype of gamma-aminobutyric acid type A α5 receptor, on a radial arm maze assessment of memory function. Impaired memory performance produced by this intervention in otherwise high-performing aged rats supports an adaptive role for gamma-aminobutyric acid in the functional maintenance of intact cognition in aging.



## 1. Introduction

Cognitive decline, although common in the elderly, is not inevitable. Many individuals maintain high cognitive performance well into the later decades of life, both relative to their own previous performance and compared with individuals at younger ages (Nyberg et al., 2012). Resilience in aging is evident even as the brain accumulates pathologic lesions characteristic of Alzheimer's disease. In asymptomatic Alzheimer's disease, some individuals have brain pathology at a level expected to cause clinical decline but who nonetheless maintain high levels of cognitive function, indicating that age- and pathology-related cognitive decline is not inevitable (Arnold et al., 2013; Driscoll and Troncoso, 2011). In the study of neurocognitive aging in humans, increasing interest has focused on factors that promote cognitive resilience (Gallagher et al., 2019). Numerous lines of evidence indicate that life-long experiences and exposures, differing greatly across individuals, such as education, occupational attainment, and lifestyle choices, may contribute to more optimal cognitive outcomes in late life.

Biological endowment may also contribute to late life outcomes. Aged outbred Long-Evans rats show variability in age-related cognitive performance, with some animals displaying impaired memory function (Aged Impaired, AI) and others demonstrating preserved function on par with young adult performance (Aged Unimpaired, AU) (Gallagher et al., 1993). In this well-characterized study population for individual differences in neurocognitive aging, rats are maintained under uniform conditions throughout life, allowing a more constrained life history for examination of the biological basis of individual differences in age-related cognitive decline. Studies of AU rats suggest that adaptations in brain function beyond mature adulthood contribute to resilience at older ages. Although considerable data indicate that AU rats do not differ significantly from young adults in numerous structural and functional changes that characterize AI rats (Smith et al., 2000; Spiegel et al., 2013; Stranahan et al., 2011), there is an increasing body of evidence that neural mechanisms recruited by AU animals differ from those of young adults (Branch et al., 2019; Haberman, 2019; Menard and Quirion, 2012; Tran et al., 2018, 2019). Of particular note, data from AU animals consistently point to adaptive recruitment of inhibitory mechanisms to balance excitation and inhibition in the medial temporal lobe, including the hippocampal formation (McQuail et al., 2015). This potentially adaptive function in AU rats

* Corresponding author at: Department of Psychological and Brain Sciences, The Johns Hopkins University, 3400 North Charles Street, 116 Dunning Hall, Baltimore, MD 21218, USA. Tel.: 410-516-5914; fax: 410-516-0494.
*E-mail address:* rahabs@jhu.edu (R. Haberman).

0197-4580/$ – see front matter © 2020 Elsevier Inc. All rights reserved.
https://doi.org/10.1016/j.neurobiolaging.2020.02.019

contrasts with a condition of neural overactivity affecting the same medial temporal lobe circuits in AI rats, the magnitude of which is correlated with poorer memory performance.

In the current model for individual differences in neurocognitive aging, AU rats display increased inhibitory postsynaptic currents in recordings from dentate gyrus granule cells and a larger tonic inhibitory current in recordings from pyramidal neurons (Tran et al., 2018), while AI rats show diminished recruitment of synaptic inhibition following lateral entorhinal cortex stimulation (Tran et al., 2019). Behavioral studies have reported that AU animals recruit gene expression related to inhibitory function in the hippocampus in response to behavioral tasks with a memory component (Haberman et al., 2013) and in a task designed to maximally engage hippocampal encoding of new information (Branch et al., 2019). In striking contrast, young rats show no such change, even trending toward a reduction in inhibitory gene expression in these same tasks (Branch et al., 2019). Given that hippocampal neural overactivity is observed in the brains of AI rats as well as in functional magnetic resonance imaging studies of human aging and amnestic mild cognitive impairment (Bakker et al., 2015; Putcha et al., 2011; Yassa et al., 2010, 2011), recruitment of inhibitory regulation by AU animals may provide an adaptive and functionally significant homeostatic adjustment in the face of a propensity for heightened hippocampal neural activity in aging.

Here we tested the functional significance of augmented inhibition in AU rats, to examine a potential dissociation between the neurobiological mechanisms that preserve cognitive function in the aged brain and those operating in the young adult brain. Prior research into preservation of cognitive performance in aging focused on targeting the α5 subtype of gamma-aminobutyric acid type A ($GABA_A$) α5 receptors. $GABA_A$ α5 receptors display highest expression localized to the hippocampal formation (Sur et al., 1998), mediating tonic inhibition of principle hippocampal neurons via extrasynaptic receptors (Glykys and Mody, 2006) as well as synaptic inhibitory control (Jacob, 2019). Hypothesizing that blockade of hippocampal inhibition might improve hippocampal-dependent cognitive performance, efforts to enhance cognitive performance in the past focused on selective α5 inverse agonists which function as negative allosteric modulators (NAMs) (Atack et al., 2006; Ballard et al., 2009; Chambers et al., 2003; Dawson et al., 2006; Etherington et al., 2019). Somewhat surprisingly, although $GABA_A$ α5 NAMs effectively enhanced cognitive performance in young adult rodents, such treatment failed in development as a therapeutic for cognitive decline in aging (Atack et al., 2006). Given evidence for neural overactivity in neurocognitive aging, a prior study compared both negative and positive $GABA_A$ α5 modulators in young adults and AI rats. While young adult rats treated with a $GABA_A$ α5 receptor NAM improved hippocampal-dependent memory performance, age-related impairment was ameliorated by $GABA_A$ α5 receptor positive allosteric modulator treatment without benefit from $GABA_A$ α5 NAM administration (Koh et al., 2013), consistent with previous studies suggesting augmented inhibition improves cognitive function in aging (Koh et al., 2010). If a naturally occurring increase in inhibitory function observed in aged rats with preserved memory plays a significant functional role in maintaining cognition in aging, the use of a $GABA_A$ α5 receptor NAM might have a uniquely detrimental effect in AU rats that is not observed in young adults. To evaluate this possibility, we challenged aged rats with intact memory using a $GABA_A$ α5 receptor NAM, using an identical protocol to that previously tested in young animals (Koh et al., 2013).

## 2. Materials and methods

All procedures were approved by the Institutional Animal Care and Use Committees in accordance with the National Institutes of Health directive. Pathogen-free, aged, male Long-Evans rats were obtained at 8–9 months of age from Charles River Laboratories (Raleigh, NC) and housed in a vivarium at Johns Hopkins University until 24–26 months of age. Aged rats were screened in a standardized water maze assessment of hippocampal-dependent spatial cognition before behavioral studies with experimental treatments, with young rats (6 months) included in each test run (for details, refer to the study by Gallagher et al., 1993). The proximity of the rat's position to the escape platform location on probe tests interpolated over the course of training was used to calculate a learning index score for each rat, providing a composite graded measure of spatial learning capacity (Gallagher et al., 1993). Approximately 40%–50% of the aged rats (AU) have index scores that are distributed within the range of young performance, and the remaining aged rats (AI) from the same cohort have index scores that fall outside the entire range of young performance. Cue training (visible escape platform; 6 trials) occurred on the last day of training to test for sensorimotor and motivational factors independent of spatial learning.

A hippocampal-dependent radial arm maze task was used to assess the effect of drug treatment as described in detail in the study by Koh et al. (2010). The protocol allowed repeated within-subject assessment using systemic administration of drugs at different doses. Prior research using this model has shown that reliable individual differences in hippocampal-dependent memory among aged rats translate across the water maze used for initial characterization and the radial maze task (Koh et al., 2010).

Pretraining consisted of habituation, standard win-shift training, and win-shift training with delays interposed between information and memory test phases on the eight-arm maze. Drug treatments began a day after the completion of pretraining. Three arms were blocked at the beginning of each trial (information phase). The identity and configuration of the blocked arms were varied across trials. Food-deprived rats were allowed to retrieve food reward (Kellogg's Froot Loops cereal) from the five unblocked arms. The rat was then removed from the maze for 5 hours, during which time the barriers on the blocked arms were removed allowing access to all eight arms. Rats were then placed back onto the center platform and allowed to retrieve the remaining food rewards (memory test phase). An error consisted of returning to an arm (all four paws on the arm) from which food had already been obtained. The number of errors made in the retention phase was used to assess memory performance. Rats were tested with a series of drug doses in ascending/descending order; each dose, including vehicle alone, was thus tested twice. Repeated measures analyses of variance and t-tests were used to analyze the results.

TB21007, a $GABA_A$ α5 receptor NAM (Tocris Bioscience, Ellisville, MO; Chambers et al., 2003), was dissolved in dimethyl sulfoxide (5% of final volume) and then with polyethylene glycol 300 (20%) and distilled water (75%). The drug and vehicle were injected intraperitoneally at 1 mL/kg approximately 30 minutes before each training session.

## 3. Results

Rats were tested for cognitive status before the drug treatment using a standardized water maze protocol developed in this study population to assess hippocampal-dependent function. Only AU rats were selected for the present drug experiment ($n = 8$) and had learning index scores ranging from 182 to 222, which are in the normative range for this study population and on par with that in young rats (Gallagher et al., 1993).

Fig. 1 shows the performance of aged rats with intact memory treated with increasing doses of TB21007, a selective $GABA_A$ α5 receptor NAM (Chambers et al., 2003), as indexed by the number of

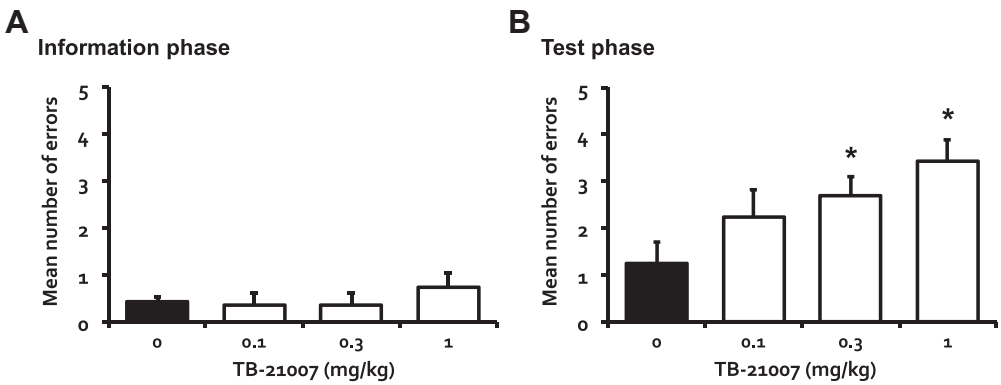


**Fig. 1.** Aged rats with a background of unimpaired memory function (n = 8) were treated with varying doses of TB21007, a $GABA_A$ α5 receptor NAM, in a radial arm maze task. (A) The rats showed no differences in performance in response to the drug during the information phase. (B) The drug, however, dose-dependently disrupted memory performance of the rats after delay during the test phase. Doses at 0.3 and 1 mg/kg significantly increased memory errors relative to vehicle (0 mg/kg). *$p < 0.05$. Abbreviation: $GABA_A$, gamma-aminobutyric acid type A; NAM, negative allosteric modulator.

errors made during the information phase (Fig. 1A) and memory test phase (Fig. 1B) in the radial arm maze task. TB21007 did not have any significant effect on performance during the information phase at any dose, $F(3, 21) = 0.81$, $p = 0.501$. Differences in memory performance were however evident under drug treatment as a function of dose during the memory test phase, $F(3, 21) = 4.58$, $p = 0.013$. The doses at 0.3 and 1 mg/kg produced significantly more memory errors relative to vehicle, $t(7) = 2.96$, $p = 0.021$ and $t(7) = 3.97$, $p = 0.005$, respectively. These results show that $GABA_A$ α5 receptor NAM treatment interfered with memory performance in aged rats with a background of unimpaired memory function.

## 4. Discussion

The study of aged individuals who retain high levels of cognitive performance has the potential to reveal important insights into the underlying neurobiology to prevent age-related cognitive decline, involving processes which may not be necessary or beneficial in the brains of young adults. Indeed, drugs developed to improve cognitive function in young adults via negative allosteric modulation of $GABA_A$ α5 receptors failed in development as a therapeutic for cognitive decline in aging (Atack, 2010). Preclinical data from young adult animals showed treatment with a $GABA_A$ α5 receptor NAM improved hippocampal-dependent memory performance (Atack et al., 2006; Chambers et al., 2003; Dawson et al., 2006; Etherington et al., 2019) but did not improve performance in neurocognitive aging with memory impairment (Koh et al., 2013), indicating that in the aging brain, limiting $GABA_A$ α5 function is not an effective strategy for improving cognition.

Multiple studies have shown that AU animals retain structural and functional signatures similar to young ones but do so alongside an upregulation of inhibitory mechanisms not seen in young or AI animals. This intriguing finding strongly suggests that upregulation of inhibition is a functional contributor to their cognitive success. Here, we treated AU animals with a negative allosteric modulator of the $GABA_A$ α5 receptor to directly test the functional significance of inhibitory upregulation in these animals. This compound has high efficacy against $GABA_A$ α5 receptors (Chambers et al., 2003) and has been demonstrated to reduce amplitude and decay rate of inhibitory postsynaptic currents of hippocampal and cortical neurons (Chen et al., 2017). Use of this compound in young animals was previously shown to decrease errors in a hippocampal-dependent radial arm task in a dose-dependent fashion (Koh et al., 2013), building on previous data that had shown spatial memory improvement in rats with use of this compound (Chambers et al., 2003) and similar effects with other $GABA_A$ α5 negative modulators (Atack et al., 2006; Dawson et al., 2006; Etherington et al., 2019). Here we show that application of an identical dose response with an identical protocol produced an opposite effect in AU animals. While the TB21007-dependent reduction in memory performance could be due to nonhippocampal effects, there was no indication of such responses in any study in young adult subjects (Chambers et al., 2003; Koh et al., 2013). Coupled with gene expression data within this model, these results strongly suggest that inhibitory mechanisms play a profoundly different role in the operation of the hippocampal system in the young and aged brain and present further mechanistic insight into the therapeutic role for this class of drugs in selectively targeting age-related cognitive performance.

## Disclosure statement

Dr. Gallagher is the founder of AgeneBio Incorporated, a biotechnology company that is dedicated to discovery and development of therapies to treat cognitive impairment. She has a financial interest in the company. Dr. Koh, Dr. Haberman, and Dr. Gallagher are inventors on Johns Hopkins University's intellectual property that is licensed to AgeneBio. Otherwise, Dr. Gallagher has had no consulting relationships with other public or private entities in the past 3 years and has no other financial holdings that could be perceived as constituting a potential conflict of interest. All conflicts of interest are managed by Johns Hopkins University. Dr. Branch has no conflicts of interests to declare.

## CRediT authorship contribution statement

**Ming Teng Koh:** Methodology, Investigation, Formal analysis, Visualization, Writing - review & editing. **Audrey Branch:** Conceptualization, Writing - original draft. **Rebecca Haberman:** Conceptualization, Writing - review & editing. **Michela Gallagher:** Conceptualization, Funding acquisition, Writing - review & editing.

## Acknowledgements

Supported by the NIA, United States grant P01AG009973-21A1 to Dr. Gallagher.

## References

Arnold, S.E., Louneva, N., Cao, K., Wang, L.S., Han, L.Y., Wolk, D.A., Negash, S., Leurgans, S.E., Schneider, J.A., Buchman, A.S., Wilson, R.S., Bennett, D.A., 2013.

Cellular, synaptic, and biochemical features of resilient cognition in Alzheimer's disease. Neurobiol. Aging 34, 157e168.
Atack, J.R., 2010. Preclinical and clinical pharmacology of the GABAA receptor alpha5 subtype-selective inverse agonist alpha5IA. Pharmacol. Ther. 125, 11–26.
Atack, J.R., Bayley, P.J., Seabrook, G.R., Wafford, K.A., McKernan, R.M., Dawson, G.R., 2006. L-655,708 enhances cognition in rats but is not proconvulsant at a dose selective for alpha5-containing GABAA receptors. Neuropharmacology 51, 1023–1029.
Bakker, A., Albert, M.S., Krauss, G., Speck, C.L., Gallagher, M., 2015. Response of the medial temporal lobe network in amnestic mild cognitive impairment to therapeutic intervention assessed by fMRI and memory task performance. Neuroimage Clin. 7, 688–698.
Ballard, T.M., Knoflach, F., Prinssen, E., Borroni, E., Vivian, J.A., Basile, J., Gasser, R., Moreau, J.L., Wettstein, J.G., Buettelmann, B., Knust, H., Thomas, A.W., Trube, G., Hernandez, M.C., 2009. RO4938581, a novel cognitive enhancer acting at GABAA alpha5 subunit-containing receptors. Psychopharmacology (Berl) 202, 207–223.
Branch, A., Monasterio, A., Blair, G., Knierim, J.J., Gallagher, M.G., Haberman, R.P., 2019. Aged rats with preserved memory dynamically recruit hippocampal inhibition in a local/global cue mismatch environment. Neurobiol. Aging 76, 151–161.
Chambers, M.S., Atack, J.R., Broughton, H.B., Collinson, N., Cook, S., Dawson, G.R., Hobbs, S.C., Marshall, G., Maubach, K.A., Pillai, G.V., Reeve, A.J., MacLeod, A.M., 2003. Identification of a novel, selective GABA(A) alpha5 receptor inverse agonist which enhances cognition. J. Med. Chem. 46, 2227–2240.
Chen, X., Keramidas, A., Lynch, J.W., 2017. Physiological and pharmacological properties of inhibitory postsynaptic currents mediated by alpha5beta-a1gamma2, alpha5beta2gamma2 and alpha5beta3gamma2 GABAA receptors. Neuropharmacology 125, 243–253.
Dawson, G.R., Maubach, K.A., Collinson, N., Cobain, M., Everitt, B.J., MacLeod, A.M., Choudhury, H.I., McDonald, L.M., Pillai, G., Rycroft, W., Smith, A.J., Sternfeld, F., Tattersall, F.D., Wafford, K.A., Reynolds, D.S., Seabrook, G.R., Atack, J.R., 2006. An inverse agonist selective for alpha5 subunit-containing GABAA receptors enhances cognition. J. Pharmacol. Exp. Ther. 316, 1335–1345.
Driscoll, I., Troncoso, J., 2011. Asymptomatic Alzheimer's disease: a prodrome or a state of resilience? Curr. Alzheimer Res. 8, 330e335.
Etherington, L.A., Mihalik, B., Pálvölgyi, A., Ling, I., Pallagi, K., Kertész, S., Varga, P., Gunn, B.G., Brown, A.R., Livesey, M.R., Monteiro, O., Belelli, D., Barkóczy, J., Spedding, M., Gacsályi, I., Antoni, F.A., Lambert, J.J., 2017. Selective inhibition of extra-synaptic α5-GABAA receptors by S44819, a new therapeutic agent. Neuropharmacology 125, 353–364.
Gallagher, M., Burwell, R., Burchinal, M., 1993. Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris water maze. Behav. Neurosci. 107, 618–626.
Gallagher, M., Okonkwo, O.C., Resnick, S.M., Jagust, W.J., Benzinger, T.L.S., Rapp, P.R., 2019. What are the threats to successful brain and cognitive aging? Neurobiol. Aging.
Glykys, J., Mody, I., 2006. Hippocampal network hyperactivity after selective reduction of tonic inhibition in GABA A receptor alpha5 subunit-deficient mice. J. Neurophysiol. 95, 2796–2807.
Haberman, R.P., Colantuoni, C., Koh, M.T., Gallagher, M., 2013. Behaviorally activated mRNA expression profiles produce signatures of learning and enhanced inhibition in aged rats with preserved memory. PLoS One 8, e83674.
Haberman, R.P., Monasterio, A., Branch, A., Gallagher, M., 2019. Aged rats with intact memory show distinctive recruitment in cortical regions relative to young adults in a cue mismatch task. Behav. Neurosci. 133, 537–544.
Jacob, T.C., 2019. Neurobiology and therapeutic potential of alpha5-GABA Type A receptors. Front. Mol. Neurosci. 12, 179.
Koh, M.T., Haberman, R.P., Foti, S., McCown, T.J., Gallagher, M., 2010. Treatment strategies targeting excess hippocampal activity benefit aged rats with cognitive impairment. Neuropsychopharmacology 35, 1016–1025.
Koh, M.T., Rosenzweig-Lipson, S., Gallagher, M., 2013. Selective GABA(A) alpha5 positive allosteric modulators improve cognitive function in aged rats with memory impairment. Neuropharmacology 64, 145–152.
McQuail, J.A., Frazier, C.J., L Bizon, J., 2015. (2015) Molecular aspects of age-related cognitive decline: the role of GABA signaling. Trends Mol. Med. 21, 450–460.
Ménard, C., Quirion, R., 2012. (2012). Successful cognitive aging in rats: a role for mGluR5 glutamate receptors, homer 1 proteins and downstream signaling pathways. PLoS One 7, e28666.
Nyberg, L., Lovden, M., Riklund, K., Lindenberger, U., Backman, L., 2012. Memory aging and brain maintenance. Trends Cogn. Sci. 16, 292–305.
Putcha, D., Brickhouse, M., O'Keefe, K., Sullivan, C., Rentz, D., Marshall, G., Dickerson, B., Sperling, R., 2011. Hippocampal hyperactivation associated with cortical thinning in Alzheimer's disease signature regions in non-demented elderly adults. J. Neurosci. 31, 17680–17688.
Smith, T.D., Adams, M.M., Gallagher, M., Morrison, J.H., Rapp, P.R., 2000. Circuit-specific alterations in hippocampal synaptophysin immunoreactivity predict spatial learning impairment in aged rats. J. Neurosci. 20, 6587–6593.
Spiegel, A.M., Koh, M.T., Vogt, N.M., Rapp, P.R., Gallagher, M., 2013. Hilar interneuron vulnerability distinguishes aged rats with memory impairment. J. Comp. Neurol. 521, 3508–3523.
Stranahan, A.M., Haberman, R.P., Gallagher, M., 2011. Cognitive decline is associated with reduced reelin expression in the entorhinal cortex of aged rats. Cereb. Cortex 21, 392–400.
Sur, C., Quirk, K., Dewar, D., Atack, J., McKernan, R., 1998. Rat and human hippocampal alpha5 subunit-containing gamma-aminobutyric AcidA receptors have alpha5 beta3 gamma2 pharmacological characteristics. Mol. Pharmacol. 54, 928–933.
Tran, T., Bridi, M., Koh, M.T., Gallagher, M., Kirkwood, A., 2019. Reduced cognitive performance in aged rats correlates with increased excitation/inhibition ratio in the dentate gyrus in response to lateral entorhinal input. Neurobiol. Aging 82, 120–127.
Tran, T.T., Gallagher, M., Kirkwood, A., 2018. Enhanced postsynaptic inhibitory strength in hippocampal principle cells in high-performing aged rats. Neurobiol. Aging 70, 10.
Yassa, M.A., Lacy, J.W., Stark, S.M., Albert, M.S., Gallagher, M., Stark, C.E., 2011. Pattern separation deficits associated with increased hippocampal CA3 and dentate gyrus activity in nondemented older adults. Hippocampus 21, 968–979.
Yassa, M.A., Stark, S.M., Bakker, A., Albert, M.S., Gallagher, M., Stark, C.E., 2010. High-resolution structural and functional MRI of hippocampal CA3 and dentate gyrus in patients with amnestic Mild Cognitive Impairment. Neuroimage 51, 1242–1252.