Behavioural Brain Research 238 (2013) 211–226

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

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

Research report

Memory in aged mice is rescued by enhanced expression of the GluN2B subunit of the NMDA receptor

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HIGHLIGHTS

- Enhanced GluN2B expression improves memory in aged animals, similar to young.
- Enhanced GluN2B expression in the frontal cortex improves later long-term memory.
- Enhanced GluN2B expression in the hippocampus improves early long-term memory.
- Enhanced GluN2B expression increases NMDA receptor-mediated synaptic transmission.

ARTICLE INFO

Article history:
Received 26 July 2012
Received in revised form 9 October 2012
Accepted 13 October 2012
Available online 24 October 2012

Keywords:
Aging
Spatial memory
NMDA receptor
NR2B subunit
C57BL/6 mice
Adenoviral vector

ABSTRACT

The GluN2B subunit of the *N*-methyl-D-aspartate (NMDA) receptor shows age-related declines in expression across the frontal cortex and hippocampus. This decline is strongly correlated to age-related memory declines. This study was designed to determine if increasing GluN2B subunit expression in the frontal lobe or hippocampus would improve memory in aged mice. Mice were injected bilaterally with either the GluN2B vector, containing cDNA specific for the GluN2B subunit and enhanced green fluorescent protein (eGFP); a control vector or vehicle. Spatial memory, cognitive flexibility, and associative memory were assessed using the Morris water maze. Aged mice, with increased GluN2B subunit expression, exhibited improved long-term spatial memory, comparable to young mice. However, memory was rescued on different days in the Morris water maze; early for hippocampal GluN2B subunit enrichment and later for the frontal lobe. A higher concentration of the GluN2B antagonist, Ro 25-6981, was required to impair long-term spatial memory in aged mice with enhanced GluN2B expression, as compared to aged controls, suggesting there was an increase in the number of GluN2B-containing NMDA receptors. In addition, hippocampal slices from aged mice with increased GluN2B subunit expression exhibited enhanced NMDA receptor-mediated excitatory post-synaptic potentials (EPSP). Treatment with Ro 25-6981 showed that a greater proportion of the NMDA receptor-mediated EPSP was due to the GluN2B subunit in these animals, as compared to aged controls. These results suggest that increasing the production of the GluN2B subunit in aged animals enhances memory and synaptic transmission. Therapies that enhance GluN2B subunit expression within the aged brain may be useful for ameliorating age-related memory declines.

1. Introduction

Aging is associated with multiple functional declines, including declines in strength, balance, motor coordination, cognitive flexibility and memory [1,2]. One of the earliest cognitive functions to show declines with increasing age is memory; deterioration is evident by the fifth decade in humans and is associated with significant impairment in memory recall [3,4]. Spatial memory, which is responsible for the navigation of organisms within their

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http://dx.doi.org/10.1016/j.bbr.2012.10.026

environment, is particularly affected by the aging process [5–7]. In particular, spatial long-term and delayed short-term memory, as well as cognitive flexibility (*i.e.* the ability to switch a behavioral response according to the context of a situation), decline with age [8–13].

Specific brain regions are important for the acquisition, consolidation and retrieval of spatial memory, including the hippocampus and the prefrontal cortices within the frontal lobe [14–20]. Specifically, the medial and orbital prefrontal cortices are important for both spatial long-term and short-term memory in the radial arm and Morris water mazes [20,21]. The frontal lobe has also been shown to be necessary for the encoding of delayed short-term memories, as well as contributing to long-term memory by maintaining the learned information [22,23]. The medial prefrontal cortex has also been shown to be important for the retrieval of spatial information stored in the hippocampus [24]. The frontal lobe, including the orbital and medial prefrontal cortices, has also been shown to be important for cognitive flexibility [25–28]. In contrast, the hippocampus is responsible for the consolidation of less stable short-term memories into more stable long-term memories, which can be stored in the frontal lobe [19,29,30]. In particular, spatial long-term memory has been shown to require the hippocampus in a variety of studies using hippocampal lesions [14,31–33]. In particular, selective electrolytic lesions of the entorhinal projection to the CA1 disrupt the consolidation of long-term memory [15]. In contrast, lesions of the dentate gyrus (DG) and CA3, suggest that these regions of the hippocampus may be important for the acquisition of spatial memory [16,17]. The caudate nucleus is also believed to be involved in the acquisition and consolidation of spatial memory in the Morris water maze [34–36]. However, the striatum and medial temporal lobe are also believed to be involved in the formation of associative memory, which does not appear to be affected by aging [37,38].

One subtype of glutamate receptor, the *N*-methyl-D-aspartate (NMDA) receptor, is highly expressed throughout the frontal lobe, caudate nucleus and hippocampus [39,40] and has been shown to be important for learning and memory [41,42]. Specifically, NMDA receptors are especially important for spatial memory [43] and are also thought to be involved in cognitive flexibility [44]. Antagonists of the receptor block initiation of long-term potentiation (LTP), a cellular mechanism believed to underlie learning and memory, in both the hippocampus and regions of the frontal lobe [45–51]. Memory and learning, including spatial memory, are also impaired by use of NMDA receptor specific antagonists such as AP5, MK801, ketamine and Ro 25-6981 [43,52–60]. In addition, correlations have been seen between NMDA-displaceable [^{3}H]glutamate binding and NMDA subunit expression and spatial memory performance in the Morris water maze [61,62,9,63–67].

Aging animals exhibit declines in NMDA receptor binding densities. A number of binding studies, employing [^{3}H]glutamate or glutamate analogs, have shown that the NMDA receptors are more susceptible to the effects of aging than any other type of glutamate receptor in the ventrolateral frontal cortex, including orbital and insular cortices, and in the hippocampus of the mouse brain [68–71]. Similar results were also observed in other species, including rats [11,44,72], canids [65], non-human primates [66] and humans [73]. Several studies have used spatial memory tasks to characterize the relationship between age-related declines in memory and NMDA receptor expression [8–12,61,62,67,74–78]. Specifically, the age-related decline in densities of NMDA receptor binding and expression of its subunits in regions of the frontal lobe and hippocampus of the rodent brain have been shown to be associated with declines in spatial memory, including spatial long-term and delayed short-term memory, during aging [8–12,44,61,62,67,78].

NMDA receptors are heteromeric tetramers composed of combinations of subunits from different families of proteins, now termed GluN1 (NR1), GluN2 (NR2) and GluN3 (NR3) subunit families [79,80]. Of the NMDA receptor subunits, the GluN2B subunit is most affected by the aging process. The GluN2B subunit mRNA has been shown to be especially vulnerable to the effects of aging in the cerebral cortex and dentate gyrus of the hippocampus [63,65]. Significant declines in GluN2B mRNA expression have also been observed in the prefrontal cortex and caudate nucleus of aged macaques and in the hippocampus of aged rats [81,82]. In the frontal lobes of C57BL/6 mice, it appears that the decline in mRNA during adult aging may be a continuation of the developmental decline and, therefore, may be programmed [64]. Protein expression of the GluN2B subunit also declines with age across regions of the frontal lobe and hippocampus [9,81,83]. However, the protein levels of the GluN2B subunit show a greater decline with age within the synaptic membrane of the frontal lobe than in the tissue as a whole [12]. Within the hippocampus, there is a significant decline in GluN2B subunit expression in the synapse with age, similar to the tissue changes [12]. Functional studies of NMDA receptors also suggest that aging results in a decrease in or loss of functional GluN2B-containing receptors. Specifically, NMDA receptors from aged animals appear to be less sensitive to ifenprodil, a GluN2B specific antagonist, and have an increased rate of deactivation (faster channel closing) [84,85].

The GluN2B subunit has also been shown to be important for spatial memory. Its decrease, *via* experimental manipulation, has been shown to be sufficient to account for the degree of spatial long-term memory impairment seen in aged rodents [86]. Moreover, aging studies have shown that there is a significant correlation between decreased GluN2B subunit expression and impaired spatial long-term memory in aged animals [9]. Specifically, age-related decreases in the protein expression of the GluN2B subunit within crude synaptosomes of the frontal cortex of C57BL/6 mice show a relationship to the declines in performance in the long-term spatial memory task across age groups. However, those expressing the highest levels of the GluN2B subunit within the synaptic membrane of the hippocampus, among aged mice, are the poorest performers in the same task [12]. Previous research has shown that increasing GluN2B subunit expression throughout multiple brain regions from birth is beneficial to memory, including spatial long-term and delayed short-term memory, and remains beneficial even into middle-age (18 months) [87,88]. These data suggest that maintaining higher levels of the GluN2B subunit during aging, than are seen in normal aged mice, could be beneficial for memory.

The present paper explored the effects of increasing the expression of the GluN2B subunit within the frontal lobe or the hippocampus of aged mice using a replication deficient adenoviral vector to deliver cDNA specific to the GluN2B subunit. The orbital cortex was targeted because the ventrolateral frontal cortex, which includes the orbital and insular cortices, exhibits consistent declines in GluN2B subunit mRNA during aging in C57BL/6 mice [65] and shows significant relationships between age-related declines in NMDA receptor binding and spatial memory [61]. In addition, the orbital cortex is centrally located within the frontal lobe, which provided the best target for enhancement throughout the lobe.

Adenoviral vectors have previously been used to effectively deliver genes to the central nervous system (CNS) [89]. An adenoviral vector was chosen because of its size (packaging capacity) [90]. Specifically, the GluN2B vector expresses two transgenes, GluN2B and eGFP, from two independent promoters. This cannot be accomplished in an adeno-associated viral vector because the packaging capacity is too small [91]. A lentivector, such as feline immunodeficiency virus (FIV), can be engineered to express two transgenes