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Glutamate presynaptic vesicular transporter and postsynaptic receptor levels correlate with spatial memory status in aging rat models

Caroline Ménard [a,b,c,d], Rémi Quirion [a,b], Erika Vigneault [a], Sylvain Bouchard [e], Guylaine Ferland [f,g], Salah El Mestikawy [a,h], Pierrette Gaudreau [c,d,*]

[a] Douglas Mental Health University Institute, McGill University, Montreal, Quebec, Canada
[b] Department of Psychiatry, McGill University, Montreal, Quebec, Canada
[c] Laboratory of Neuroendocrinology of Aging, Centre Hospitalier de l'Université de Montréal Research Center, Montreal, Quebec, Canada
[d] Department of Medicine, University of Montreal, Montreal, Quebec, Canada
[e] Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada
[f] Institut Universitaire de Gériatrie de Montréal Research Center, University of Montreal, Montreal, Quebec, Canada
[g] Department of Nutrition, University of Montreal, Montreal, Quebec, Canada
[h] INSERM U952, CNRS UMR7224, Université Pierre et Marie Curie, Paris, France

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ABSTRACT

In humans, memory capacities are generally affected with aging, even without any reported neurologic disorders. The mechanisms behind cognitive decline are not well understood. We studied here whether postsynaptic glutamate receptor and presynaptic vesicular glutamate transporters (VGLUTs) levels may change in the course of aging and be related to cognitive abilities using various age-impaired (AI) or age-unimpaired rat strains. Twenty-four-month-old Long-Evans (LE) rats with intact spatial memory maintained postsynaptic ionotropic glutamate receptor levels in the hippocampal-adjacent cortex similar to those of young animals. In contrast, AI rats showed significantly reduced expression of ionotropic glutamate receptor GluR2, NR2A and NR2B subunits. In AI LE rats, VGLUT1 and VGLUT2 levels were increased and negatively correlated with receptor levels as shown by principal component analysis and correlation matrices. We also investigated whether glutamatergic receptors and VGLUT levels were altered in the obesity-resistant LOU/C/Jall (LOU) rat strain which is characterized by intact memory despite aging. No difference was observed between 24-month-old LOU rats and their young counterparts. Taken together, the unaltered spatial memory performance of 24-month-old age-unimpaired LE and LOU rats suggests that intact coordination of the presynaptic and postsynaptic hippocampal-adjacent cortex glutamatergic networks may be important for successful cognitive aging. Accordingly, altered expression of presynaptic and postsynaptic glutamatergic components, such as in AI LE rats, could be considered a marker of age-related cognitive deficits.

1. Introduction

In humans, normal aging is often characterized by a slow decline in cognitive abilities that can be exacerbated in neurologic disorders (Abbott, 2012). Remodeling of neuronal synapses is a central mechanism in memory formation. Activity-dependent plasticity can modulate either presynaptic or postsynaptic components through efficacy of neurotransmitter release or changes in the biophysical properties of receptors (Choquet and Triller, 2013). Thus, coordination of these processes is essential to strengthen specific neuronal networks and facilitate learning and memory mechanisms (Abraham, 2008; Bibb et al., 2010). To date, the impact of aging on the dynamic organization of presynaptic and postsynaptic glutamatergic components is still largely unknown.

Excitatory neurotransmission is involved in memory formation (Rebola et al., 2010; Shepherd, 2012). Before its regulated release, glutamate is concentrated in synaptic vesicles by vesicular glutamate transporters (VGLUTs, for review see El Mestikawy et al., 2011). VGLUT1 and VGLUT2 are expressed mainly by cortical and subcortical glutamatergic neurons, respectively (El Mestikawy et al., 2011). VGLUT1-heterozygous mice (characterized by 41% loss of VGLUT1 protein

* Corresponding author at: Department of Medicine, University of Montreal, Centre hospitalier de l'Université de Montréal Research Center, 900 St-Denis Street, Viger Tower, Rm R05.436B-02, Montreal, Quebec, Canada H2X 0A9. Tel.: (514) 890-8000 #23613; fax: (514) 412-7925.
E-mail address: pierrette.gaudreau@umontreal.ca (P. Gaudreau).

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level in the frontal cortex) show enhanced anxiety, depressive-like behaviors, and impaired recognition memory (Tordera et al., 2007). Conditional VGLUT2 knock-out (VGLUT2-KO) mice exhibit impaired spatial learning and memory associated with reduced neuronal plasticity, lower synaptic markers levels (including VGLUT1) and altered dendritic arborization in the hippocampus (He et al., 2012). VGLUTs are thus key anatomic and functional presynaptic markers of glutamatergic transmission.

Released glutamate binds to ionotropic glutamatergic receptor (IGLUR; α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid [AMPA] or N-methyl-D-aspartate [NMDA]) to trigger fast excitatory transmission. On the other hand, glutamate binding to metabotropic receptors (mGluR) provides slow modulatory (or even inhibitory) transmission (for reviews, see Huganir and Nicoll, 2013; Luscher and Huber, 2010). AMPA, NMDA, and group I mGluR are located on postsynaptic membranes, whereas group II and group III mGluR are involved in presynaptic inhibition of glutamate release (Sheng and Kim, 2002; Shigemoto et al., 1997). The activation of postsynaptic receptors triggers multiple signaling pathways, leading to short- or long-term strengthening or weakening of synaptic transmission and brain plasticity (Abraham, 2008; Huganir and Nicoll, 2013; Sheng and Kim, 2002). Interestingly, memory deficits in VGLUT2-KO adult mice can be partially reversed by increasing postsynaptic NMDA glutamate receptor transmission through treatment with D-serine and a D-amino acid oxidase inhibitor (He et al., 2012). These data suggest that both presynaptic VGLUTs and postsynaptic glutamate receptors contribute to glutamatergic synapses homeostasis.

Several postsynaptic effectors are known to regulate synaptic efficiency underlying cognition (Huganir and Nicoll, 2013; Malenka and Nicoll, 1999; Sheng and Kim, 2002). Altered IGLUR function has been linked to age-related memory impairments in several animal models (Burke and Barnes, 2006, 2010). For example, recognition memory is impaired in 24-month-old SD rats, and this deficit is associated with reduced NMDA-mediated plasticity (Kollen et al., 2010). Group I mGluR signaling and function have also recently been correlated with intact spatial memory in aged rats (Menard and Quirion, 2012b; Yang et al., 2013) and mice (Menard et al., 2013).

In the present study, spatial learning, as well as VGLUT and glutamatergic receptor (AMPAR, NMDAR, and group I mGluR) expression, was investigated in the hippocampal formation of 2 aging rat models, the Long-Evans (LE) and Lou/C/Jall (LOU) strains. The LE rat has been extensively used for aging studies. Interestingly, a subgroup of older LE animals maintains high cognitive abilities despite aging, whereas another one shows impairments which is similar to individual differences observed in humans (Menard and Quirion, 2012a). The LOU rat strain is considered a model of successful aging, as it is characterized by increased lifespan, maintenance of a low and stable adipose tissue mass throughout life, and low incidence of age-related diseases (Alliot et al., 2002). Intact spatial memory in 24-month-old animals was correlated with VGLUT and postsynaptic receptor expression comparable with those of young rats for both LE and LOU strains. By contrast, age-related memory impairment was linked to higher VGLUT protein expression and reduced postsynaptic AMPAR, NMDAR, and group I mGluR levels in LE rats. These results are supported by principal component analysis (PCA) and correlation matrix analyses, suggesting that the expression of presynaptic and postsynaptic glutamatergic components is associated and can be altered in the hippocampus and adjacent cortex of older rats, leading to memory deficits.

2. Methods

2.1. Animals

Animal care, handling, and experimental procedures were approved by the McGill University Animal Care Committee for the LE rats and by the CHUM Research Center and University of Montreal Animal Care Committees for the LOU rats, in compliance with Canadian Council for Animal Care guidelines.

2.1.1. LE rats

For the aged groups, male LE rats were purchased from Charles River Laboratories (St. Constant, Quebec, Canada) at the age of 12 months and housed at the Douglas Mental Health University Institute (DMHUI) animal facility until the age of 24 months. For the young group, male LE rats were purchased at 3 months of age and kept at the DMHUI animal facility until they reached 6 months. A large colony of 6- and 24-month-old LE rats (N = 108) was previously tested in the reference memory version of the Morris water maze (MWM) task, as reported earlier (Menard and Quirion, 2012b). Some of these rats were used for the immunoblot analyses reported in the present study (N = 5–7/group). LE rats were kept on a 12:12-hour light-dark cycle; lights on at 07:00 hours with *ad libitum* access to water and food (Purina Lab Chow; Mondou, Montreal, Quebec, Canada). Animals were housed 2 or 3 per cage, depending on body weight.

2.1.2. LOU rats

Six-, 12-, and 24-month-old male and female LOU rats were obtained from the Quebec Network for Research on Aging's rat colonies. A large group of aging LOU rats (N = 52) were previously tested in the reference memory version of the MWM task (Menard et al., 2014b). The performances of rats used for immunoblotting are shown in the present study (N = 6/group, 3 males, 3 females). Rats were housed 3 per cage in temperature-, humidity-, and lighting-controlled rooms on a 12:12-hour light-dark cycles; lights on at 07:00 hours like for the LE rats. They were fed chow A03 SAFE growing diet for 3 weeks after weaning and the maintenance A04 SAFE diet thereafter (Perotech, Toronto, CA) (Menard et al., 2014b; Veyrat-Durebex et al., 2005).

On completion of behavioral training (2–4 hours following the last probe test), LE and LOU non-fasted non-anesthetized rats were quickly sacrificed by rapid decapitation for ex vivo biochemical analyses (Menard and Quirion, 2012b; Menard et al., 2014b; Whittington et al., 2013).

2.2. Morris water maze

The long-term reference memory version of the MWM task (Morris, 1984) was performed as described previously to discriminate between aged rat populations according to memory status (Brouillette and Quirion, 2008; Farso et al., 2013; Gallagher et al., 2003; Lee et al., 2005; Menard and Quirion, 2012b; Rowe et al., 1998).

2.2.1. Learning acquisition and retention

Briefly, in the learning acquisition phase, rats were pseudorandomly started from a different position on each trial (3 trials per day for 4–5 consecutive days) and had to find a submerged platform (15 cm diameter) in a pool (1.5 m diameter), located 2 cm below the surface of the water (24 °C) rendered opaque by nonallergic white gouache paint (Menard and Quirion, 2012b; Menard et al., 2014b). Animals used distal visual-spatial cues (posters on the walls of the room) to find the hidden escape platform located in the center of the target quadrant. If it was not reached within 90 seconds, the animal was gently guided to the platform. Before removal, all the rats remained on the platform for 15 seconds to let them time to orientate themselves in space. Sixty minutes after the last trial of the last day of acquisition phase, rats were given 1 probe trial of 90 seconds (learning probe) for which the platform was removed from the pool (Menard and Quirion, 2012b; Menard et al.,