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Erasure of striatal chondroitin sulfate proteoglycan–associated extracellular matrix rescues aging-dependent decline of motor learning

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ABSTRACT

Cognitive decline is a feature of aging. Accumulating evidence suggests that the brain extracellular matrix (ECM) is involved in the process of aging-dependent cognitive impairment and neurodegeneration by regulating synaptic neurotransmission and affecting neuroplasticity. Age-related changes in brain structure and cognition are not uniform across the whole brain. Being one of the most vulnerable brain regions to aging-dependent alterations, striatum is integral to several central nervous system functions, such as motor, cognition, and affective control. However, the striatal ECM is largely understudied. We first describe 2 major types of chondroitin sulfate proteoglycan (CSPG)–associated ECM in striatum: perineuronal nets and diffusive ECM. Both types of ECM accumulate in an aging-dependent manner. The accumulation of CSPG-associated ECM correlates with aging-dependent decline in striatum-related cognitive functions, including motor learning and working memory. Enzymatic depletion of CSPG-associated ECM in aged mice via chondroitinase ABC significantly improves motor learning, suggesting that changes in neural ECM CSPGs regulate striatal plasticity. Our study provides a greater understanding of the role of neural ECM underlying striatal plasticity, which is an important precursor to design appropriate therapeutic strategies for normal and pathologic aging.

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1. Introduction

The global population of people aged 60 years and older is predicted to increase from 688 million in 2010 to 1.96 billion in 2050 and is expected to yield a significant health care burden worldwide (Lunenfeld, 2008). Cognitive impairment has emerged as one of the greatest health threats to the aged population and is the most common shared symptom in multiple aging-related neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, frontotemporal dementia, Huntington's disease, and others. Developing therapeutic interventions for such conditions demands a greater understanding of the substrates at the molecular and circuitry levels, which underlie both normal and pathological brain aging.

Rapidly accumulating evidence suggests that the neural extracellular matrix (ECM), a complex molecular network surrounding all neural cells, is involved in the enigmatic process of aging-dependent cognitive impairment and neurodegeneration (Bonneh-Barkay and Wiley, 2009; Soleman et al., 2013; Yang et al., 2014). The neural ECM plays a key role in brain development, aging, and adult neural functions by regulating synaptic neurotransmission (Senkov et al., 2014), which has led the evolution of the conceptual synapse from the traditional 2 components into a tetrapartite system (a "synaptic quadriga") which includes a presynaptic and postsynaptic element, astroglia, and synaptic/perisynaptic (or diffusive) ECM (Faissner et al., 2010). Genetic and enzymatic targeting of neural ECM has profound effects on modulation of neuroplasticity, including acquisition of memories (Carulli et al., 2010; Romberg et al., 2013), cognitive flexibility (Happel et al., 2014), fear memory (Gogolla et al., 2009), and drug/reward memories (Slaker et al., 2015, 2016) in a bidirectional manner.

The neural ECM is associated with critical periods of neuroplasticity, which are periods during development in which intrinsic and extrinsic experiences shape immature neuronal circuits into mature, adult-like circuitry (Hensch and Bilimoria, 2012). During these critical periods, the neural ECM plays a role in synaptogenesis and synaptic maturation, and therefore the establishment of functional and anatomic neuroarchitecture (Busch and Silver, 2007; Hensch, 2005). An overall reduction in neuroplasticity occurs

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during the closure of critical periods and coincides with the accumulation of a unique form of ECM that is characterized by lattice-like ECM structures termed perineuronal nets (PNNs) (Frischknecht and Gundelfinger, 2012). The major constituents of the PNNs are chondroitin sulfate proteoglycans (CSPGs), hyaluronan and its synthesizing enzymes hyaluronan synthases, tenascin-R, and proteoglycan link proteins. Because of the relationship between the neural ECM and plasticity, it is imperative to determine if a life-long accumulation of neural ECM contributes to aging-related alteration of the brain structure and function. A recent report showed that hippocampal ECM molecules were significantly accumulated in aged mice and associated with aging-related decline of spatial memory (Vegh et al., 2014). Aging-related accumulation of neural ECM in other brain regions has yet to be reported.

The striatum is the major input nucleus of the basal ganglia and contributes to voluntary movement, procedural learning (Graybiel and Grafton, 2015; Yin and Knowlton, 2006), motor learning (Yin et al., 2009), and working memory (Lewis et al., 2004). The striatum is significantly impacted in aging-related disorders of the basal ganglia, including Parkinson's and Huntington's diseases. The striatal ECM has been reported to accumulate during the motor critical period, postnatal day 8 (P8) to P16, during which ECM CSPG expression shifts from striosome to matrix compartment with concomitant appearance of PNNs (Lee et al., 2008). Elimination of striatal CSPG-associated ECM has been linked to changes in gait and acquisition learning in the Morris water maze (Lee et al., 2012), suggesting a functional role of ECM CSPGs in striatal plasticity. We hypothesize that the striatal CSPG-associated ECM inversely regulates neuroplasticity and contributes to aging-related cognitive decline. We first sought to address a possible aging-related accumulation of the striatal CSPG-associated ECM throughout the lifespan of mice. Second, we determined if striatum-associated cognitive functions decline in very old mice. Finally, by erasure of CSPG-associated ECM, we explored the relationship between the striatal CSPG-associated ECM and cognitive function in aged mice.

2. Materials and methods

2.1. Animals

All procedures were performed in accordance with the Institutional Animal Care and Use Committee at LSU Health Sciences Center-Shreveport. Mice were raised on a standard 12-hour light/dark cycle; behavioral experiments were performed between 9 AM and 5 PM during the light cycle. All mice used in this study were in a mixed C57Bl/6J background. Both male and female mice were used for behavioral and immunohistological experiments; animal genders are noted where appropriate. For the young adult (2–3 months) mice, animals from 4 litters were used for behavior and 1 litter for immunohistochemical staining. For the aged (18–22 months) mice, animals from 6 litters were used for behavior and 1 litter for immunohistochemical staining. A subgroup of mice from the aged cohort that previously underwent behavioral testing were chosen for the chondroitinase ABC (ChABC) experiments. Immunohistochemistry experiments were performed in young and aged mice naïve to behavioral testing.

2.2. Accelerating rotarod

The accelerating rotarod is often used to measure motor coordination and motor learning in mice (Buitrago et al., 2004; Hirata et al., 2016; Shiotsuki et al., 2010; Yin et al., 2009). To assess potential aging-related changes in motor learning, mice were trained and tested in the accelerating rotarod using the following procedure.

2.2.1. Habituation

Before training on the first day, mice were handled briefly and placed on the rotarod platform for 15 minutes to reduce novelty of the rotarod apparatus.

2.2.2. Training

Mice were trained to stay on the rotarod at a constant speed of 4 rpm for three 3-minute trials. The procedure was repeated for 3 days for a total of 9 trials per mouse. The experimenter placed the mouse on the rod and assisted the animals to stay on the rod during each trial until no further assistance was necessary, usually by the second or third training day.

2.2.3. Testing

After the final training day, mice underwent 3 test trials per day with the rotarod set at an acceleration rate of 4–40 rpm/10 minutes. Latency to fall from the accelerating rotarod was recorded for each trial. Animals were allowed a minimum time of 20 seconds to be counted as a successful trial and 600 seconds as the maximum trial time. The procedure was repeated for 3 days for a total of 9 trials per mouse. Latency to fall data were normalized to the average latency from trial 1 (T1) within each cohort to analyze learning curves. The normalized data were analyzed using repeated-measures analysis of variance (RM-ANOVA). For rotarod comparison between young and aged mice, age was used as the between-subjects factor and training (improvement over time in the rotarod) as the within-subjects factor. For rotarod comparison between ChABC and saline-treated mice, treatment was used as the between-subjects factor and training as the within-subjects factor.

2.3. Open field

To assess potential age-related differences in spontaneous locomotion, mice were tested in an open field chamber (AccuScan Instruments) equipped with vertical and horizontal activity sensors (Liu et al., 2011; Prut and Belzung, 2003; Shoji et al., 2016). Mice were allowed to habituate to the testing room for 1 hour before open field testing. After habituation, mice were placed into the testing chambers, and activity parameters including total distance traveled, time spent moving, horizontal movement, vertical movement, and rearing time were measured for a total of 30 minutes per animal. Data were analyzed using Versamax and transformed to excel format using Versadat software. Data from each open field parameter were compared between either age groups or treatment groups using independent-samples *t*-test.

2.4. T-maze

To assess aging-related differences in working memory, mice underwent T-maze testing for spontaneous alternation (Deacon and Rawlins, 2006). Mice were allowed 30 minutes to habituate to the testing room before the procedure. Mice were placed into the starting arm of the maze facing away from goal arms with a central partition to facilitate arm choice and allowed up to 2 minutes to enter the left or right goal arm. Following each goal arm choice, a sliding guillotine door was used to block the animal in the chosen arm for an intertrial interval of 30 seconds. After the intertrial interval, mice were placed into the starting arm and allowed to make a secondary choice with the omission of the central partition. Alternate choices in each test were counted as successful trials. Each animal underwent 3 trials per day for 9 total trials. The total number of correct alternations over the 9 trials per mouse was expressed as a percentage. Percentage of correct alternations was compared between age or treatment groups using independent-samples *t*-test.