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Insulin-like growth factor 2 rescues aging-related memory loss in rats

Adam B. Steinmetz [1], Sarah A. Johnson [1], Dylan E. Iannitelli, Gabriella Pollonini, Cristina M. Alberini*

Center for Neural Science, New York University, New York, NY, USA

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ABSTRACT

Aging is accompanied by declines in memory performance, and particularly affects memories that rely on hippocampal-cortical systems, such as episodic and explicit. With aged populations significantly increasing, the need for preventing or rescuing memory deficits is pressing. However, effective treatments are lacking. Here, we show that the level of the mature form of insulin-like growth factor 2 (IGF-2), a peptide regulated in the hippocampus by learning, required for memory consolidation and a promoter of memory enhancement in young adult rodents, is significantly reduced in hippocampal synapses of aged rats. By contrast, the hippocampal level of the immature form proIGF-2 is increased, suggesting an aging-related deficit in IGF-2 processing. In agreement, aged compared to young adult rats are deficient in the activity of proprotein convertase 2, an enzyme that likely mediates IGF-2 post-translational processing. Hippocampal administration of the recombinant, mature form of IGF-2 rescues hippocampal-dependent memory deficits and working memory impairment in aged rats. Thus, IGF-2 may represent a novel therapeutic avenue for preventing or reversing aging-related cognitive impairments.

1. Introduction

Cognitive functions, and in particular long-term memory formation, storage, and retrieval, are impaired in aged populations (Balota et al., 2000; Cansino, 2009; Koen and Yonelinas, 2014). Not all types of memories are equally affected by the aging process; for example, implicit (procedural) learning, semantic memory, and verbal skills seem largely spared, whereas episodic and/or declarative memories, spatial memories, attention, and working memory are consistently impaired (Balota et al., 2000; Kausler, 1994). Thus, hippocampal- and cortical-dependent memories seem to be particularly vulnerable to impairments occurring with aging. This functional loss has been found in humans and in nonhuman animals (Gallagher and Pelleymounter, 1988; Rapp et al., 1997; Small et al., 2011). Moreover, in aged human subjects, memory performance remains unimpaired after brief, posttraining delays, but gradually decreases as time passes, in agreement with a compromised hippocampal-dependent consolidation process (Mitrushina et al., 1991; Pace-Schott and Spencer, 2011). Using contextual conditioning paradigms, a similar decay of memory over extended postlearning delays has been observed in aged relative to young adult rodents (Foster and Kumar, 2007; Gold et al., 1982; Winocur, 1988). Studies in aged animals show that memory deficits are associated with hippocampal synaptic alterations suggesting that mechanisms of hippocampal memory consolidation, the process by which memories become stabilized and long lasting over time (Alberini, 2009; Dudai, 2004; Squire and Alvarez, 1995), are compromised (Burke and Barnes, 2010; Driscoll et al., 2003; Foster, 2012; Morrison and Baxter, 2012; Yassa et al., 2010a, 2010b). During consolidation, memories are labile and their strength can be modulated (Alberini et al., 2012; Bambah-Mukku et al., 2014; Bekinschtein et al., 2007); therefore, targeting hippocampal mechanisms of consolidation might be an effective strategy for improving memory functions in aged individuals (Alberini and Chen, 2012; Stern and Alberini, 2013).

Using inhibitory avoidance (IA) in young adult rats, we have identified several components of gene expression cascades regulated in the dorsal hippocampus after learning and required for memory consolidation (Arguello et al., 2013; Chen et al., 2012; Garcia-Osta et al., 2006; Milekic and Alberini, 2002; Taubenfeld et al., 2001). Among these, the expression of insulin-like growth factor 2 (IGF-2 or IGF-II) increases during the first 24 hours after training, and this increase is essential for memory consolidation; in

* Corresponding author at: Center for Neural Science, New York University, 4 Washington Place, New York, NY 10003. Tel.: 212-998-7721; fax: 212-995-4011.
E-mail address: ca60@nyu.edu (C.M. Alberini).
[1] These authors contributed equally.

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fact, knockdown of its expression causes memory impairment (Chen et al., 2011). Furthermore, hippocampal or systemic administration of recombinant IGF-2, but not of another member of the IGF family, IGF-1, significantly enhances the retention and persistence of hippocampal-dependent memories in young adult rats and mice (Chen et al., 2011; Stern et al., 2014a, 2014b), as well as of hippocampal long-term potentiation (Chen et al., 2011). In contrast, IGF-1 may ameliorate memory deficits in aged animals (Deak and Sonntag, 2012; Markowska et al., 1998), suggesting distinct effects and/or mechanisms of IGFs on memory performance and deficit rescuing.

In the adult brain, IGF-2 is the most abundantly expressed among the insulin-like peptides, with the highest levels of mRNA expression found in myelin sheaths, leptomeninges, microvasculature, and the choroid plexus (Logan et al., 1994; Rotwein et al., 1988; Russo et al., 2005). In addition, relative to other brain regions, high levels of expression of IGF-2 protein and of its high affinity IGF-2 receptor (IGF-2R) are observed in the adult hippocampus and cortex (Couce et al., 1992; Fernandez and Torres-Alemán, 2012; Hawkes and Kar, 2004; Logan et al., 1994; Ye et al., 2015), brain regions essential for memory consolidation. Notably, both IGF-2 mRNA and mature protein levels are lower in the hippocampus of aged compared to young adult mice (Kitraki et al., 1993; Park and Buetow, 1991; Pascual-Lucas et al., 2014; Uddin and Singh, 2013). Because the effect of IGF-2 as a memory and synaptic plasticity enhancer is robust and very long-lasting, occurs via IGF-2R and not via IGF-1R, and targets multiple domains of hippocampal-dependent memory (Chen et al., 2011; Stern et al., 2014a), here we sought to determine the expression of IGF-2 and IGF-2 receptor (R) in aged rats and whether IGF-2 treatment is effective in preventing age-related memory deficits.

2. Materials and methods

2.1. Animals

Young adult (4 months) and aged (26 months) male Fischer 344 × Brown Norway (FBN) F1 hybrid rats were obtained from the National Institute on Aging's colony at Harlan (Indianapolis, IN, USA) and Charles River (Frederick, MD, USA). This hybrid rat strain is widely used for aging studies as they offer several advantages as an aging model (Lipman et al., 1996). Animals were singly housed, permitted ad libitum access to food and water, and maintained on a standard 12-hour light-dark cycle. All rats used in the experiments were monitored daily for health parameters by the experimenters and the animal care staff and were weighed at least 3 times per week to confirm health status and appropriate levels of food and water intake. Experiments were performed during the light phase of the cycle. Rats were allowed to acclimate to the experimental facility for a minimum of 7 days, and were then handled for 3 minutes (min) per day for 5 days before beginning experimental manipulations. All protocols were approved by the Institutional Animal Care and Use Committee at New York University and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Surgery and intrahippocampal injections

Cannulation and injections were carried out as described previously (Chen et al., 2011; Stern et al., 2014b). Rats were anesthetized with ketamine (65 mg/kg)-xylazine (7.5 mg/kg) intraperitoneal and stainless-steel guide cannulae (22-gauge; Plastics One) were stereotactically implanted to bilaterally target the hippocampus (relative to Bregma anterior/posterior: −4.0 mm, medial/lateral: +2.6 mm, dorsal/ventral: −2.0 mm in young adult or −2.3 mm in aged to account for increased skull thickness). Cannulae were secured in place with anchor screws and dental cement, and dummy stylets were inserted to maintain patency. Rats were returned to their home cages and allowed to recover from surgery for a minimum of 7 days. After behavioral training, rats received bilateral hippocampal injections using 28-gauge needles extending 1.5 mm beyond the tips of the guide cannulae, connected to a 10-μL Hamilton syringe by polyethylene tubing. Injections were delivered at a rate of 0.33 μL/min using an infusion pump (Harvard Instruments), and needles were left in place for 2 minutes to allow dispersion of the solution. Rats received 250 ng of recombinant mouse IGF-2 (catalog number 792-MG, R&D Systems) in a volume of 1 μL per hemisphere, or an equal volume of vehicle solution (sterile phosphate buffered saline with 0.1% bovine serum albumin). To establish the correct cannula placements, rats were euthanized and their brains were postfixed with 10% buffered formalin. Coronal sections (40 μm) were cut through the hippocampus and examined under a light microscope. Rats with incorrect placements were excluded from subsequent analyses.

2.3. Inhibitory avoidance

Inhibitory avoidance training and testing were carried out as described previously (Chen et al., 2011; Stern et al., 2014b). The IA chamber (Med Associates. Inc) consisted of a rectangular Perspex box divided into a safe compartment and a shock compartment. The safe compartment was white and illuminated with a house lamp and the shock compartment was black and remained dark. Footshocks were delivered via the grid floor of the shock chamber with a constant current scrambler circuit. The apparatus was located in a sound-attenuated, dimly lit room. During training sessions, each rat was placed in the safe compartment facing the lit wall of the chamber, away from the door. After 10 seconds, the guillotine door separating the light and dark compartments opened, allowing the rat access to the shock compartment. The door closed within 2 seconds of the rat entering the shock compartment, and a 2 second footshock of either 0.6 or 0.9 mA, as specified, was administered. Rats remained in the shock compartment for 10 seconds. The animals were transported to a different room and received a bilateral intrahippocampal injection of IGF-2 or vehicle and then returned to their home cage. Rats were tested for memory retention at the designated time point(s). Tests for IA memory consisted of placing the rat back in the safe compartment and recording its latency to cross to the shock compartment. Footshock was not administered during retention tests, and each test session was terminated after 900 seconds. Testing was carried out blind to treatments.

2.4. Object location test

Rats were trained and tested in a square open field arena with clear Plexiglas walls and floor (42 cm × 42 cm × 30 cm) located in a dim room. Visual cues were provided within the box and on the walls of the room. The walls of the box were covered with white and black paper. One black and one white wall also contained symbols (circle and square) to create 4 unique walls. Behavior was recorded with a video camera positioned approximately 1.5 m above the arena. Rats were first habituated to the arena for 5 minutes each day for 2 consecutive days. The next day, training consisted of exposing the rats to 2 identical objects constructed from Mega Bloks secured to the floor of the arena. Rats were initially placed facing a wall, away from the objects, and were allowed to explore the arena and objects for 5 minutes. Rats then received a bilateral intrahippocampal injection of IGF-2 or vehicle as described for IA experiments, and were returned to their home