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A role for hippocampal actin rearrangement in object placement memory in female rats

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ABSTRACT

Actin rearrangement, the polymerization of globular actin (G-actin) to filamentous actin, causes morphological changes in dendritic spines and is hypothesized to be a substrate of learning and memory. The ovarian hormone estradiol promotes hippocampal actin rearrangement and enhances performance on hippocampus-dependent tasks, including object placement memory. The goals of the current study were to determine a role for actin rearrangement and its regulatory pathway in object placement memory in female rats and to determine if estradiol impacts actin rearrangement in ovariectomized rats during the performance of the task. In an initial experiment, young adult Long-Evans rats were ovariectomized and implanted with capsules containing either cholesterol vehicle or estradiol. Bilateral intrahippocampal infusions of aCSF vehicle or the actin rearrangement inhibitor, latrunculin A, were administered 15 min prior to initiation of the object placement task. Latrunculin A dose-dependently impaired object placement memory. Estradiol had no impact on the ability of latrunculin A to affect performance. In a second experiment, rats were ovariectomized and received implants containing cholesterol or estradiol. Half of each hormone treatment group was exposed to the object placement memory task and half underwent control procedures. Immediately following completion of behavior, rats were euthanized and hippocampi removed. Western blotting was used to measure hippocampal levels of phosphorylated and total levels of a regulator of actin polymerization, the actin depolymerization factor cofilin. Exposure to the object placement memory task resulted in significant increases in phosphorylated levels of cofilin. Estradiol treatment had no impact on protein levels. These data support a role for hippocampal actin rearrangement and its regulatory proteins in object placement memory in female rats and suggest that chronic estradiol treatment does not impact hippocampal actin arrangement.

1. Introduction

Actin is the main structural component of dendritic spines and its rearrangement promotes morphological changes in synapses that are thought to be fundamental in the formation of memories (Holtmaat & Svoboda, 2009; Kasai et al., 2010; Lamprecht & LeDoux, 2004). Actin exists in a monomeric (globular actin, G-actin), or multimeric form (filamentous actin, F-actin) whereby G-actin polymerizes to F-actin, elongating the multimeric form (Lamprecht & LeDoux, 2004). The polymerization of G-actin to F-actin is linked to the formation or enlargement of dendritic spines and memory formation (Lamprecht & LeDoux, 2004). Actin dynamics are controlled by signaling pathways of Rho GTPases (Raftopoulou & Hall, 2004). Rho-associated kinase (ROCK) controls activation of LIM-kinase (LIMK; Maekawa et al., 1999). LIMK then controls activity of the actin severing protein cofilin, the ultimate regulator of actin polymerization, which in its active state depolymerizes F-actin. Phosphorylation of cofilin renders it inactive, thus allowing for actin polymerization and associated modifications in spine structure (Sumi, Matsumoto, Takai, & Nakamura, 1999; Yang et al., 1998). These changes in dendrite structure can be brought about independently of protein synthesis (Antonova et al., 2001) and thus, such rapid changes could be correlated to the beginning stages of learning.

Emerging experimental evidence supports a role for actin rearrangement in learning and memory. For example, LIMK knockout mice displayed decreases in actin filament accumulation as well as impairments in memory for a spatial location (Meng et al., 2002). Additionally, mice deficient in β-arrestin-2, a protein that mediates cofilin/LIMK scaffolding, had impaired spatial memory as assessed by a radial arm water maze (Pontrello et al., 2012). Furthermore, intrahippocampal infusions of latrunculin A, which forms a 1:1 complex with G-actin and

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prevents polymerization to F-actin (Spector, Shochet, Blasberger, & Kashman, 1989), blocked both acquisition and extinction of contextual fear conditioning in male mice (Fischer, Sananbenesi, Schrick, Spiess, & Radulovic, 2004) and attenuated conditioned place preference in a drug withdrawal paradigm in male rats (Hou et al., 2009). Likewise, infusion of latrunculin A into the insular cortex impaired acquisition of conditioned taste aversion in male rats (Bi et al., 2010). Intrahippocampal infusion of the actin rearrangement inhibitor, cytochalasin D, blocked acquisition of contextual fear in male rats (Motanis & Maround, 2011). Finally, pharmacologically disrupting the cofilin pathway via post-training intrahippocampal infusion of a ROCK inhibitor impaired long-term retention for a hidden escape platform in a water maze task in male rats (Dash, Orsi, Moody, & Moore, 2004). To date, there has been little investigation of the role of actin dynamics in learning using female subjects.

The importance of investigation of the role of actin dynamics in learning and memory in females is supported by the multiple effects exerted by ovarian hormones on the structure and function of hippocampal neurons. For example, synapse structure in the hippocampus is responsive to the presence of gonadal hormones and fluctuates across the estrous cycle in rats (Woolley, Gould, Frankfurt, & McEwen, 1990). Additionally, treatment with 17β-estradiol (estradiol), the primary estrogen produced by the ovaries, increased dendritic spine density in the CA1 region of the hippocampus of ovariectomized rats (Gould, Woolley, Frankfurt, & McEwen, 1990; MacLusky, Luine, Hajszan, & Leranth, 2005; Woolley & McEwen, 1992). Hippocampal slices treated with estradiol showed increased dendritic spine density that was blocked by addition of latrunculin A, suggesting that estradiol effects change in dendrite morphology through actin polymerization (Kramar et al., 2009). Both *in vivo* and *in vitro* molecular evidence demonstrate that estradiol increases phosphorylation of cofilin in the hippocampus, thereby promoting actin polymerization (Kramar et al., 2009; Yildirim et al., 2008; Yuen, McEwen, & Akama, 2011). In addition to its impacts on the hippocampus, estradiol enhances hippocampus dependent memory across a variety of tasks (Daniel, Fader, Spencer, & Dohanich, 1997; Daniel, Roberts, & Dohanich, 1999; Korol & Kolo, 2002; Luine, Richards, Wu, & Beck, 1998; Sandstrom, 2004) including the object placement task used in the current study (Frye, Duffy, & Walf, 2007; Luine, Jacome, & Maclusky, 2003). Although molecular data exist to support a relationship between estradiol and actin rearrangement, there has been no *in vivo* behavioral evidence linking the beneficial cognitive effects of estradiol treatment to actin dynamics.

The goals of the present study were to test the hypotheses that (1) actin rearrangement in the hippocampus mediates object placement memory in female rats, and (2) chronic estradiol treatment promotes actin rearrangement during performance of an object placement task. Two experiments were conducted. In the first, we assessed the impact of intrahippocampal administration of latrunculin A on object placement memory in ovariectomized rats that were and were not receiving chronic estradiol treatment. We predicted that latrunculin A would dose-dependently block object placement memory. We also predicted that because estradiol would promote actin polymerization, estradiol-treated rats would be less sensitive to the impairing effects of latrunculin A on performance. In a second experiment, we used western blotting to determine the impact of performance on an object placement task on total and phosphorylated levels of cofilin in the hippocampus of ovariectomized rats that were and were not receiving chronic estradiol treatment. We predicted that performance of the object placement task would increase phosphorylated levels of cofilin and that these levels would be enhanced by estradiol treatment.

2. Material and methods

2.1. Experiment 1

2.1.1. Subjects and treatments

Twenty female Long-Evans hooded rats, approximately 2 months of age, were purchased from Harlan Sprague–Dawley (Indianapolis, IN). Animal care was in accordance with guidelines set by the *National Institutes of Health Guide for the Care and Use of Laboratory Animals* and all procedures were approved by the Institutional Animal Care and Use Committee of Tulane University. Rats were housed individually in a temperature controlled vivarium under a 12-h light/dark cycle (lights on at 7:00 am). One week after arrival, all rats were ovariectomized while under anesthesia induced by injections of ketamine (100 mg/kg i.p., Bristol Laboratories, Syracuse, NY) and xylazine (7 mg/kg i.p., Miles Laboratories, Shawnee, KS). At the time of surgery, animals were implanted with 5 mm SILASTIC brand capsules (0.058-in i.d. and 0.077-in. o.d., Dow Corning, Midland, MI) containing either 25% 17β-estradiol (Sigma Chemical, St. Louis, MO) diluted with cholesterol or 100% cholesterol vehicle. We have reported previously that implants of these dimensions maintain blood plasma estradiol levels of 26–47 pg/ml, which fall within the physiological range of cycling female rats (Bohacek & Daniel, 2007, 2010).

2.1.2. Cannulation

Two weeks after ovariectomy, all rats underwent stereotaxic surgery while deeply anesthetized via injections of ketamine and xylazine. At the time of the surgery, animals were implanted with bilateral guide cannulae purchased from Plastics One (Roanoke, VA) that were anchored to the skull with dental acrylic. Guide cannulae (28 gauge) were aimed at the dorsal hippocampus as shown

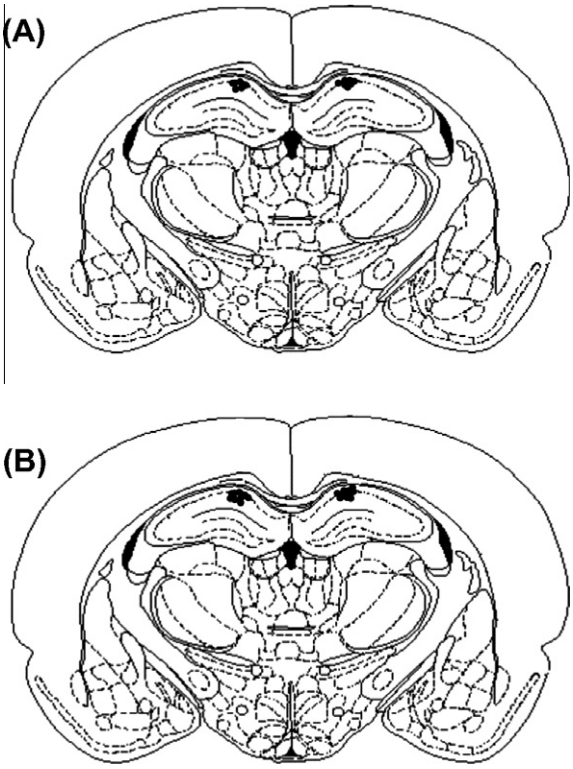

Fig. 1. Drawing of a coronal section of the rat brain at 3.3 mm posterior to bregma (Paxinos & Watson, 1998) illustrating cannulae placement for hippocampal infusions in Experiment 1. (A) Cannulae placement as indicated by black circles is shown for cholesterol-treated rats. (B) Cannulae placement is shown for estradiol-treated rats.