Available online at www.sciencedirect.com
ScienceDirect
Neurobiology of Learning and Memory 88 (2007) 208–216
Neurobiology of Learning and Memory
www.elsevier.com/locate/ynlme

Estrogens and progestins enhance spatial learning of intact and ovariectomized rats in the object placement task

Cheryl A. Frye [a,b,c,d,*], Caryn K. Duffy [a,b], Alicia A. Walf [a]

[a] Department of Psychology, The University at Albany – State University of New York, United States
[b] Department of Biology, The University at Albany – State University of New York, United States
[c] Center for Life Science, The University at Albany – State University of New York, United States
[d] Center for Neuroscience Research, The University at Albany – State University of New York, United States

Received 22 December 2006; revised 5 April 2007; accepted 6 April 2007
Available online 15 May 2007

Abstract

Steroid modulation of cognitive function has focused on estrogen (E_2), but progestins naturally co-vary with E_2 and may also influence cognitive performance. Spatial performance in the object placement task over endogenous hormonal states in which E_2 and progestins vary, and when E_2 and/or progestins were administered, was examined. *Experiment 1*: Rats in proestrus or estrus had significantly better performance in the object placement task than did diestrus rats. *Experiment 2*: Rats in the third trimester, post-partum, or lactation exhibited significantly better performance in the object placement task than did rats in the first trimester. *Experiment 3*: Ovariectomized (ovx) rats administered 17β-estradiol (0.9 mg/kg), subcutaneously (sc), progesterone (P; 4 mg/kg, sc), or E_2 and P, immediately after training in the object placement task, performed significantly better when tested 4 h later, than did control rats administered vehicle (sesame oil 0.2 cc). *Experiment 4*: ovx rats administered E_2 or P with a 1.5 h delay after training in the object placement task, did not perform differently than vehicle-administered controls. *Experiment 5*: ovx rats administered post-training E_2, which has a high affinity for both E_2 receptor (ER)α and β isoforms, or propyl pyrazole triol (PPT; 0.9 mg/kg, sc), which is more selective for ERα than ERβ, had significantly better performance in the object placement task than did rats administered vehicle or diarylpropionitrile (DPN; 0.9 mg/kg, sc), an ERβ selective ligand. *Experiment 6*: ovx rats administered P, or its metabolite, 5α-pregnan-3α-ol-20-one (3α,5α-THP; 4 mg/kg, sc), immediately post-training performed significantly better in the object placement task than did vehicle control rats. Thus, performance in the object placement task is better when E_2 and/or P are naturally elevated or when E_2, the ERα selective ER modulator PPT, P, or its metabolite, 3α,5α-THP, are administered post-training.

Keywords: Estrous cycle; Allopregnanolone; Neurosteroids; SERMs; Estrogen receptor; Learning and memory

1. Introduction

Ovarian steroids, such as estradiol (E_2), can influence cognitive processes of female rodents. Performance in the eye-blink conditioning, passive avoidance, and object recognition tasks, which involve the hippocampus and other regions of the brain (i.e. amygdala, cortex), is better during behavioral estrus when E_2 levels are acutely elevated than during low E_2 phases of the estrous cycle (Frye & Bayon, 1999; Rhodes & Frye, 2004; Shors, Lewczyk, Pacynski, Mathew, & Pickett, 1998; Walf, Rhodes, & Frye, 2006; Wood, Beylin, & Shors, 2001). When E_2 is administered to ovariectomized (ovx) rats, such that physiological E_2 concentrations are achieved during training and consolidation, performance in hippocampally mediated tasks, such as the water maze, radial arm maze, 4-arm plus maze, and passive avoidance are improved over that produced by vehicle-administration (Bimonte & Denenberg, 1999; Daniel, Fader, Spencer, & Dohanich, 1997; Davis, Jacobson,

* Corresponding author. Address: Department of Psychology, The University at Albany – State University of New York, Life Science Building, Room 01058, NY 12222, United States. Fax: +1 518 591 8848.
E-mail address: cafrye@albany.edu (C.A. Frye).

1074-7427/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.nlm.2007.04.003

Aliakbari, & Mizumori, 2005; Diaz-Veliz, Urresta, Dussaubat, & Mora, 1991; Fader, Hendricson, & Dohanich, 1998; Frye & Rhodes, 2002; Gibbs, 1999, 2000; as reviewed in Korol, 2004; Korol & Kolo, 2002; Luine, Richards, Wu, & Beck, 1998; Marriott & Korol, 2003; O'Neal, Means, Poole, & Hamm, 1996; Packard, 1998; Sandstrom & Williams, 2001, 2004). These data suggest a role of the hippocampus as a target for steroid hormones' effects for cognitive processes and that the timing of hormone exposure and testing is critical. Indeed, when rats are trained and tested in different phases of the estrous cycle, little evidence for cognitive-enhancing effects of endogenous ovarian steroids in the water maze are observed (Frye, 1995). We have utilized the object recognition task, which has a 4-h intertrial interval, to assess steroids' effects on object memory in a task that relies on functioning of the prefrontal cortex and hippocampus. Given the 4-h intertrial interval, we were able to assess these effects over the estrous cycle when rats are trained and tested in the same estrous cycle state (Walf et al., 2006). As such, using a hippocampally mediated task with a short interval between training and testing, such as the object placement task, to investigate effects of endogenous changes in ovarian hormones is essential to assess the role of the hippocampus for these effects.

Estradiol binds with a high affinity to both E_2 receptor (ER) isoforms, ERα, and ERβ. Although there is differential distribution of ERα and ERβ throughout the central nervous system, both ERα and ERβ are expressed in the hippocampus and cortex (Shughrue & Merchenthaler, 2001; Shughrue, Lane, & Merchenthaler, 1997; Shughrue, Scrimo, & Merchenthaler, 1998) and may influence cognitive processes that rely on hippocampal and cortical function. Indeed, ERα and ERβ selective ER modulators (SERMS) that have high affinity for ERα, enhance exploration and performance in tasks that involve exploration, hippocampal, and cortical function, such as the object recognition task (Luine, Jacome, & Maclusky, 2003; Morgan, Schulkin, & Pfaff, 2004; Walf et al., 2006). SERMS or dietary phytoestrogens, which have selective actions at ERβ, enhance performance in hippocampal tasks, such as the water and radial arm mazes and passive avoidance (Lephart et al., 2002; Rhodes & Frye, 2006). Thus, actions at ERα and/or ERβ may underlie E_2-enhanced performance in hippocampally mediated tasks.

Progesterone and its metabolites, dihydroprogesterone (DHP), and 5α-pregnan-3α-ol-20-one (3α,5α-THP), covary with E_2 over reproductive cycles and may influence cognitive performance, yet few studies have addressed the role and/or mechanisms of progestins for spatial, hippocampus-dependent learning. Although pregnancy is characterized by more marked elevations in progestin than E_2 levels, one of the few animal studies investigating spatial learning over pregnancy attributed differences to E_2 concentrations (Galea et al., 2000). Notably, E_2 enhances P's metabolism to DHP and 3α,5α-THP (Cheng & Karavolas, 1973; Vongher & Frye, 1999) and P or its metabolites can enhance performance in various cognitive tasks. Administration of P, DHP, or 3α,5α-THP to ovx rats enhances performance in the object recognition and Y-maze tasks, both of which involve prefrontal and hippocampal processes, as well as conditioned and passive avoidance, tasks (Diaz-Veliz, Urresta, Dussubat, & Mora, 1994; Ebner, Richardson, & Riccio, 1981; Frye & Lacey, 2000; van Wimersma Greidanus, 1977; Walf et al., 2006). Indeed, regression analyses revealed significant positive correlations between E_2 and 3α,5α-THP levels in the hippocampus and 3α,5α-THP levels in the prefrontal cortex for performance in the object recognition task (Walf et al., 2006). Thus, it is necessary to ascertain the nature and extent to which E_2 and progestins can have integrated and/or independent effects on hippocampus-dependent spatial performance.

To further address the respective roles and/or mechanisms by which E_2 and/or progestins may influence cognitive performance in a hippocampally mediated task, the effects of endogenous fluctuations in ovarian steroids, removal of the ovaries, and selective replacement of estrogens and progestins on performance in the object placement task were examined. We hypothesized that endogenous fluctuations and exogenous administration of E_2 and/or progestins would alter object placement performance. To test this hypothesis, we examined performance of rats across the estrous cycle (Experiment 1), various stages of pregnancy (Experiment 2), and following ovx and administration of E_2 and/or P (Experiments 3 and 4), SERMs (Experiment 5), or progestins (Experiment 6).

2. Methods

The Institutional Animal Care and Use Committee pre-approved these methods.

2.1. Animals and housing

Subjects were 2–4 month old, female Long-Evans rats ($N = 161$), bred at SUNY-Albany from stock originally purchased from Taconic Farms, Germantown, NY. Rats were housed 4–5 per cage (45 cm × 24 cm × 21 cm) with *ad libitum* access to Purina Rat Chow and tap water, in a temperature-controlled room (21 ± 1 °C), that had a 12/12 h reversed-light cycle (lights off at 0800) in The Laboratory Animal Care Facility in The Life Sciences Research Building. After testing in this experiment, rats became breeder females or were tested in other ongoing experiments in the laboratory.

2.2. Determination of estrous cycle/pregnancy

For some rats, phase of estrous cycle was determined by daily examination of vaginal cytology between 08:00 and 09:00 (Frye & Bayon, 1999) and were trained immediately after status was determined (and tested 4 h after training in the same cycle phase). Vaginal lavages of rats in proestrus, estrus, and diestrus are differentiated by the preponderance of nucleated, cornified epithelial, and heterogeneous cell types, respectively. Some rats in behavioral estrus were not tested that day, but were mated and were utilized in Experiment 1 as the pregnant, post-partum, and/or lactating rats. Only rats that showed typical cycling for at least 3 cycles were utilized in this study.

2.3. Extirpation and steroid replacement

Some rats had the primary endogenous sources of E_2 and progestins, the ovaries, removed under Rompum (12 mg/kg; Bayer Corp., Shawnee Mission, KS) and Ketaset (80 mg/kg; Fort Dodge Animal Health, Fort