Psychoneuroendocrinology (2013) 38, 1170—1177

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/psyneuen

Pubertal immune challenge blocks the ability of estradiol to enhance performance on cognitive tasks in adult female mice

Nafissa Ismail [a,b,*], Jeffrey D. Blaustein [a,b]

[a] Center for Neuroendocrine Studies, School of Psychology, University of Ottawa, 136 Jean-Jacques Lussier, Vanier Hall, Ottawa, ON, K1N 6N5 Canada
[b] Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01003, USA

Received 17 May 2012; received in revised form 3 October 2012; accepted 5 November 2012

KEYWORDS
Estradiol;
Hippocampus-dependent tasks;
Immune challenge;
Puberty

Summary Puberty is a period characterized by brain reorganization that contributes to the development of neural and behavioral responses to gonadal steroids. Previously, we have shown that a single injection of the bacterial endotoxin, lipopolysaccharide (LPS; 1.5 mg/kg IP), during the pubertal period (around 6 weeks old) in mice decreases sexual receptivity in response to estradiol and progesterone in adulthood. These findings suggest that pubertal immune challenge has an enduring effect of decreasing the behavioral responsiveness to gonadal steroid hormones. Since estradiol improves cognitive function in certain tasks in mice, we investigated the effect of pubertal immune challenge on the ability of estradiol to enhance cognitive function. We hypothesized that estradiol would be less effective at enhancing performance on particular cognitive tasks in female mice treated with LPS during puberty. Six-week old (pubertal) and 10-week old (adult) female CD1 mice were injected with either saline or LPS. Five weeks later, they were ovariectomized and implanted subcutaneously with either an estradiol- or oil-filled Silastic© capsule followed 1 week later with testing for cognitive function. The duration of juvenile investigation during social discrimination and recognition tests was used as a measure of social memory, and the duration of object investigation during object recognition and placement tests was used as a measure of object memory. Chronic estradiol treatment enhanced social and object memory in saline-treated females and in females treated with LPS in adulthood. In contrast, in females treated with LPS at 6 weeks old, estradiol failed to improve social and object memories. These results support the hypothesis that exposure to an immune challenge during puberty reduces at least some of the cognitive effects of estradiol. Moreover, these results support the idea that pubertal immune challenge compromises a wide variety of behavioral influences of ovarian hormones.
Published by Elsevier Ltd.

* Corresponding author at: School of Psychology, University of Ottawa, 136 Jean-Jacques Lussier, Vanier Hall, Ottawa, ON K1N 6N5, Canada.
E-mail address: nafissa.ismail@uottawa.ca (N. Ismail).

0306-4530/$ — see front matter. Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.psyneuen.2012.11.003

1. Introduction

Puberty is an important developmental stage during which sexual maturity is attained (Schulz and Sisk, 2006). This period is accompanied by numerous physiological (e.g., increases in hormone levels, development of secondary sexual characteristics), emotional, behavioral and social changes that then have an impact on behavior later in life (Marshall and Tanner, 1969; Forbes and Dahl, 2010). Puberty is also a period of significant brain reorganization (Levitt, 2003), as the brain undergoes further remodeling by sex steroid hormones (Schulz et al., 2009).

Exposure to stressors during the pubertal period can have lasting behavioral effects in adulthood. For example, exposure to heat, immobilization, or ether stress, around the time of puberty has long-term negative effects on the reproductive capacity of female mice (Paris et al., 1973). Moreover, exposing mice of both inbred and outbred strains to shipping stress or to the immune challenge, the bacterial endotoxin, lipopolysaccharide (LPS), during the pubertal period, decreases sexual receptivity in adulthood following treatment with estradiol and progesterone contrasted with mice stressed earlier or later in development (Laroche et al., 2009a,b; Ismail et al., 2011). This is not a response to all stressors; exposure to other stressors like restraint stress, food deprivation or a multiple stressor regimen during this period fails to reduce behavioral responsiveness to estradiol and progesterone in adulthood (Laroche et al., 2009a).

Besides playing an important role in sexual behavior in many species, estradiol also plays a prominent role in many non-reproductive behaviors. Among other functions, estrogens have direct effects on the brain areas controlling mood and cognition (Spencer et al., 2008). Hormonal fluctuations across the menstrual cycle affect mood and cognitive function in women (Schmidt et al., 1998), and estradiol treatment in mice has similar effects (Ter Horst et al., 2012). In ovariectomized mice, estradiol treatment decreases anxiety-like and depression-like behavior; however, this effect of ovarian hormones is not seen in mice treated with LPS during pubertal development (Ismail et al., 2011; Olesen et al., 2011). In fact, pubertal immune challenge reverses the effects of estradiol treatment on two tests for depression-like behavior (Ismail et al., in press). These findings demonstrate that pubertal immune challenge alters the behavioral responsiveness of non-reproductive behaviors to estradiol.

The effects of estradiol on cognition depend on the cognitive task and the brain region(s) recruited during the particular task. For example, in female rats, estradiol impairs performance on striatum-dependent tasks (Korol, 2004; Davis et al., 2005), but it enhances performance on hippocampus-dependent tasks (Daniel et al., 1997; Luine et al., 1998; Sandstrom and Williams, 2004). Similarly, estradiol treatment to ovariectomized mice enhances performance on hippocampus-dependent tasks in mice (Li et al., 2004; Xu and Zhang, 2006).

The objective of this study was to determine if immune challenge during the pubertal period decreases behavioral responsiveness to estradiol on cognitive tasks in ovariectomized mice. We hypothesized that, as is the case with other ovarian hormone-influenced behaviors, pubertal LPS treatment will decrease the ability of estradiol to enhance performance on hippocampus-dependent tasks.

2. Materials and methods

2.1. Animals

All procedures were approved by the Institutional Animal Care and Use Committee of the University of Massachusetts Amherst. Sixty-four CD1 female mice ($n = 8$ per group) were purchased from Charles River Laboratories (Kingston, NY), shipped at 3 weeks of age and housed in an all-female colony room under controlled temperature ($24 \pm 2\ ^{\circ}C$) and reversed light:dark cycle (14:10; lights off at 1000 h). Mice were housed in groups of four in Polycarbonate cages with ad libitum access to food (Teklad 2014, Harlan Laboratories, Madison, WI, phytoestrogen-reduced diet) and water supplied in glass water bottles. Cages were lined with a combination of pine wood shavings and CareFRESH (International Absorbents, Inc., Ferndale, WA) bedding, and a fresh Nestlet (Ancare Corp., Baltimore, NY) was placed in each cage when they were changed. Testing and handling took place during the dark phase of the illumination cycle under dim red light.

2.2. Bacterial endotoxin, lipopolysaccharide (LPS)

Mice habituated to their housing environment until they were treated with saline or LPS. All mice were injected intraperitoneally (IP) at 6 (pubertal experimental group) or 10 (adult negative control group) weeks of age at the end of the light cycle with either sterile saline or 1.5 mg LPS/kg body weight (obtained from *Escherichia coli* serotype O26:B6; no. L3755; Sigma Chemical Co., St. Louis, MO) dissolved at a concentration of 0.1 mg/ml in sterile saline. Mice were returned to their home cage immediately after injection. Sickness behavior was scored as described below, and animals were weighed 24 and 48 h following treatment to assess the effect of the LPS treatment on body weight. The dose of LPS was chosen based on previous work showing long-term effects on hormonal response (Laroche et al., 2009a), as well as previous work in which it caused only mild sickness lasting less than 48 h under our housing conditions (Ismail et al., 2011).

2.3. Sickness behavior

Sickness behavior was scored 30 min, four, 24 and 48 h following treatment by two observers, who were blind to the treatment conditions. All mice were evaluated with respect to the number of symptoms displayed (one symptom = score of 1; two symptoms = score of 2, three symptoms = score of 3) (Gibb et al., 2008). The symptoms examined were lethargy (diminished locomotion), huddling (curled body posture), and ptosis (drooping eyelids). This rating procedure correlates with other methods of sickness scoring, such as scoring the severity of each symptom independently on a four-point scale (Gandhi et al., 2007).

2.4. Ovariectomy and capsule implantation

Five weeks after saline or LPS treatment, when all the mice were adult (either 11 or 15 weeks old), they were anesthetized with isoflurane (3% at one l min^{-1}, inhaled). Incisions were made to the ventral skin and the muscle layer. The