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Licorice root components mimic estrogens in an object location task but not an object recognition task

Payel Kundu[a,*], Donna L. Korol[b], Suren Bandara[a], Supida Monaikul[a], Caitlin E. Ondera[c], William G. Helferich[d], Ikhlas A. Khan[e], Daniel R. Doerge[f], Susan L. Schantz[a,c,g]

[a] University of Illinois at Urbana-Champaign, Neuroscience Program, 405 N Mathews Ave, Urbana, IL 61801, USA
[b] Syracuse University, Department of Biology, 107 College Place, Syracuse, NY 13244, USA
[c] University of Illinois at Urbana-Champaign, Department of Comparative Biosciences, College of Veterinary Medicine, 2001 S Lincoln Ave, Urbana, IL 61802, USA
[d] University of Illinois at Urbana-Champaign, Department of Food Science and Human Nutrition, 905 S. Goodwin, Urbana, IL 61801, USA
[e] The University of Mississippi, 1558 University Circle, P.O. Box 1848, University, MS 38677, USA
[f] National Center for Toxicological Research, U.S. Food & Drug Administration, 3900 NCTR Road, Jefferson, AR 72079, USA
[g] University of Illinois at Urbana-Champaign, Beckman Institute, 405 N Mathews Ave, Urbana, IL 61801, USA

A B S T R A C T

This study investigated the efficacy of components of licorice root to alter performance on two different recognition tasks, a hippocampus-sensitive metric change in object location (MCOL) task and a striatum-sensitive double object recognition (DOR) task. Isoliquiritigenin (ISL), licorice root extract (LRE), and whole licorice root powder (LRP) were assessed. Young adult female rats were ovariectomized (OVX) and exposed to ISL, LRE or LRP at 0.075%, 0.5% or 5% respectively in the diet. An estradiol group was included as a positive control based on our prior findings. Rats were allowed to explore two objects for three 5-min study trials (separated by 3-min intervals) before a fourth 5-min test trial where the objects were moved closer together (MCOL task) or replaced with two new objects (DOR task). Rats typically habituate to the objects across the three study trials. An increase in object exploration time in the test trial suggests the rat detected the change. Estradiol improved MCOL performance and impaired DOR performance, similar to previously shown effects of estradiol and other estrogens, which tend to improve learning and memory on hippocampus-sensitive tasks and impair striatum-sensitive cognition. LRP had no effect on recognition while exposure to ISL and LRE improved MCOL performance. Exposure to ISL, LRE and LRP failed to attenuate DOR, contrary to effects of estradiol shown here and to previous reports in young-adult OVX rats. These findings suggest components of licorice root may prove to be effective therapies targeting memory enhancement without unintended deleterious cognitive effects.

1. Introduction

Botanical estrogens are non-steroidal plant compounds that can mimic estrogens in the body (Glazier and Bowman, 2001). These compounds are widely sold as dietary supplements despite a dearth of research on their health effects. Many over the counter botanical supplements contain licorice root powder (LRP) or licorice root extracts (LREs), shown to exert estrogenic effects both in vitro in MCF7 breast cancer cells and in vivo in various tissues including the heart and the pituitary (Maggiolini et al., 2002; Tamir et al., 2001). Isoliquiritigenin (ISL) and liquiritigenin (LIQ) are two of the primary bioactive compounds in licorice root (Mersereau et al., 2008; Miksicek, 1993). ISL and LIQ convert readily back and forth in the body (Simmler et al., 2013). Both compounds are lipophilic and have relatively low molecular weight and thus likely cross into the brain through the blood brain barrier (Srihari et al., 2012).

Estrogens have broad ranging actions believed to modulate cognition (for reviews see Galea et al., 2017; Korol and Pisani, 2015; Korol and Wang, 2018; Luine and Frankfurt, 2015). However, the relationship between estrogens and cognition is a complicated one. The effects of estrogens on cognition vary widely depending on a variety of factors including the cognitive task and the brain areas engaged by the task. Much of the existing literature suggests that estrogen supplementation to ovariectomized (OVX) rodents improves performance on hippocampus-sensitive tasks, but impairs performance on striatum-sensitive tasks (e.g. Davis et al., 2005; Korol and Kolo, 2002; Pisani et al., 2012).

* Corresponding author.
E-mail addresses: kundupayel1@gmail.com (P. Kundu), dlkorol@syr.edu (D.L. Korol), conder2@illinois.edu (C.E. Ondera), helferic@illinois.edu (W.G. Helferich), khan@olemiss.edu (I.A. Khan), daniel.doerge@fda.hhs.gov (D.R. Doerge), schantz@illinois.edu (S.L. Schantz).

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Studies using the same basic training paradigm to assess different cognitive attributes are especially useful in comparing the effects of estrogens on different brain systems, given that several factors like the dose of estradiol used, the pattern of administration as well as aspects of the task environment are held constant. For example, compared to OVX vehicle-treated controls, administration of estradiol to OVX young adult female rats improves performance on an allocentric, place learning version of a 4-arm radial maze task, known to rely on intact functioning of the hippocampus (Chang and Gold, 2003), but impairs performance on an egocentric, response learning version of the same maze task that engages the striatum during learning (Davis et al., 2005; Korol and Kolo, 2002; Korol and Pisani, 2015; Zurkovsky et al., 2006). Moreover, two days of oral dosing with the estrogen receptor (ER) ERβ-selective botanical estrogen genistein produced a similar shift in learning strategy by enhancing place learning while impairing response learning. These bidirectional effects are not limited to ERβ activation, as several estrogen receptor agonists have also been investigated using this place and response learning paradigm (Korol and Pisani, 2015). The ERα-selective compound propyl pyrazole triol (PPT) and the ERβ-selective compounds diarylpropionitrile (DPN) and Br-ERb-041 were all found to improve place learning and impair response learning, albeit at different doses (Pisani et al., 2016). These findings suggest that estrogen supplementation to OVX young adult rodents facilitates learning and memory that depends on the hippocampus and impairs cognition that depends on the striatum. It should be noted that these bidirectional effects depend on dosing, timing of exposure, and other aspects of the animal history and training environment such as stress history, parity, and age (Korol and Pisani, 2015).

Estrogens have also been shown to affect performance on object recognition and placement memory in paradigms developed to match task attributes much like the place and response learning paradigm described above. Administration of estrogens to OVX adult rodents enhance performance on hippocampus-sensitive object location tasks (Luine et al., 2003; Tunur et al., 2012). The effects of estrogens on object recognition are mixed with some evidence that estrogens enhance performance on these tasks (Gresack and Frick, 2006) and some evidence that they impair performance (Tunur et al., 2015).

Diet has also been shown to affect cognition, with several studies showing that a high fat diet can impair various aspects of cognition including spatial learning, working memory, object recognition, and fear conditioning (for a review see Freeman et al., 2014). Impairments have been found on a hippocampus-sensitive radial arm maze task (Granholm et al., 2008; Winocur and Greenwood, 2005), a prefrontal cortex-dependent operant delayed spatial alternation task (Winocur and Greenwood, 2005), an operant-based delayed matching to position task (McNeilly et al., 2011), and an object recognition task (Camer et al., 2015; Carey et al., 2014; Jurdak and Kanarek, 2009; Kaczmarczyk et al., 2013). However, it is worth noting that nearly all of the studies investigating the effects of a high fat diet on cognition in a rodent model, including all of the studies mentioned above, used only male rodents. In the few studies investigating both males and females, some have found sex differences in response to a high fat diet in peripheral metabolism, performance on a contextual fear conditioning task, as well as the magnitude of long-term potentiation (Hwang et al., 2010; Underwood and Thompson, 2016), highlighting the need to investigate the effects of a high fat diet in females as well as males.

In the present studies, we investigated the effects of ISL, LRE and LRP on a hippocampus-sensitive metric change in object location (MCOL) task and a striatum-sensitive double object recognition (DOR) task (Goodrich-Hunsaker et al., 2008; Korol and Pisani, 2015). Importantly, the tasks differ only in the final trial and are otherwise identical. Thus, the roles of ISL, LRE and LRP on two distinct cognitive tasks engaging distinct brain areas/memory systems can be compared using these tasks. We chose to investigate LRP and LRE because those are the forms of licorice root typically found in dietary supplements. We included the pure compound, ISL because it is a component of licorice root that has demonstrated estrogenic properties both in vitro and in vivo (Maggiolini et al., 2002; Miksicek, 1993; Tamir et al., 2001).

We used an OVX rat model to investigate the ability of ISL, LRE and LRP to impact cognition in the absence of endogenous estrogens that would compete for ERs. Additionally, we wished to address the issue that the standard rodent diet is much lower in fat than the typical western diet, and previous rodent studies have reported cognitive deficits in rats fed high fat diets relative to those fed standard laboratory chow. Thus, the inclusion of high fat diet groups allowed us to evaluate a diet that more closely models the typical western diet consumed by humans and to investigate whether these botanicals interact with this high fat diet to produce a pattern of effects different from that seen in rats consuming a low fat diet.

2. Materials and methods

2.1. Animals and treatment

Due to the large number of rats required in these studies, they were each conducted in a series of cohorts or replicates, as described below. The effects of ISL, LRE and LRP on the MCOL task were investigated in three separate studies consisting of three cohorts each. The effects of ISL, LRE and LRP on the DOR task were investigated in one study consisting of three cohorts. Estradiol groups were also included in all of the studies because previous work has shown that estradiol improves performance on the MCOL task and impairs performance on the DOR task in OVX young adult rats (Korol and Pisani, 2015; Tunur et al., 2012; Tunur et al., 2015). For each MCOL study, 72 young adult virgin female Long-Evans rats (53 days old) were obtained from Harlan (Indianapolis, IN) in 3 cohorts of 24 rats each, spaced 1 week apart. In each of the three cohorts 4 rats were assigned to each of 6 treatment groups (high fat control, low fat control, high fat estradiol, low fat estradiol, high fat botanical, low fat botanical). With all cohorts included, there was a total of 12 rats per treatment group in each of the three studies (Table 1A). For the DOR study, a total of 90 female Long-Evans rats was obtained from Harlan (Indianapolis, IN) in cohorts of 30 rats each, spaced 1 week apart. In each cohort 4 rats were assigned to each of five treatment groups (control, estradiol, ISL, LRE and LRP). With all cohorts included, there was a total of 12 rats per treatment group (Table 1B).

Rats were housed in a temperature and humidity controlled room (22 °C, 40–55% humidity) on a 12-h light–dark cycle (lights on at 7:00 am). Rats were pair-housed in standard plastic cages (17.7 × 9.4 × 7.9 in.) with Beta Chip® bedding, and food and water were available ad libitum. The housing facility was fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Illinois at Urbana-Champaign and were in accordance with the guidelines of the Public Health Service Policy on Humane Care and Use of Laboratory Animals and the Guidelines for the Care and Use of

Table 1A
MCOL was assessed in three separate studies that tested the effects of ISL, LRE or LRP, respectively. Each study consisted of three cohorts, each with the study design shown above.

	Control	Estradiol	Botanical (ISL, LRE or LRP)
High fat	Cohort 1: n = 4	Cohort 1: n = 4	Cohort 1: n = 4
	Cohort 2: n = 4	Cohort 2: n = 4	Cohort 2: n = 4
	Cohort 3: n = 4	Cohort 3: n = 4	Cohort 3: n = 4
	Total: n = 12	Total: n = 12	Total: n = 12
Low fat	Cohort 1: n = 4	Cohort 1: n = 4	Cohort 1: n = 4
	Cohort 2: n = 4	Cohort 2: n = 4	Cohort 2: n = 4
	Cohort 3: n = 4	Cohort 3: n = 4	Cohort 3: n = 4
	Total: n = 12	Total: n = 12	Total: n = 12