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Acute genistein treatment mimics the effects of estradiol by enhancing place learning and impairing response learning in young adult female rats

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Abstract

Endogenous estrogens have bidirectional effects on learning and memory, enhancing or impairing cognition depending on many variables, including the task and the memory systems that are engaged. Moderate increases in estradiol enhance hippocampus-sensitive place learning, yet impair response learning that taps dorsal striatum function. This memory modulation likely occurs via activation of estrogen receptors, resulting in altered neural function. Supplements containing estrogenic compounds from plants are widely consumed despite limited information about their effects on brain function, including learning and memory. Phytoestrogens can enter the brain and signal through estrogen receptors to affect cognition. Enhancements in spatial memory and impairments in executive function have been found following treatment with soy phytoestrogens, but no tests of actions on striatum-sensitive tasks have been made to date. The present study compared the effects of acute exposure to the isoflavone genistein with the effects of estradiol on performance in place and response learning tasks. Long-Evans rats were ovariectomized, treated with 17 β -estradiol benzoate, genistein-containing sucrose pellets, or vehicle (oil or plain sucrose pellets) for two days prior to behavioral training. Compared to vehicle controls, estradiol treatment enhanced place learning at a low (4.5 μ g/kg) but not high dose (45 μ g/kg), indicating an inverted

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pattern of spatial memory facilitation. Treatment with 4.4 mg of genistein over two days also significantly enhanced place learning over vehicle controls. For the response task, treatment with estradiol impaired learning at both the low and high doses; likewise, genistein treatment impaired response learning compared to rats receiving vehicle. Overall, genistein was found to mimic estradiol-induced shifts in place and response learning, facilitating hippocampus-sensitive learning and slowing striatum-sensitive learning. These results suggest signaling through estrogen receptor β and membrane-associated estrogen receptors in learning enhancements and impairments given the preferential binding of genistein to the ER β subtype and affinity for GPER.

Introduction

Estrogen status regulates learning and memory strategy in a region- and task-specific manner. Elevated levels of estrogens generally enhance performance of spatial memory tasks that tap hippocampal function (Daniel et al., 1997, Packard and Teather, 1997, Luine et al., 1998, Fader et al., 1999, Bimonte and Deneberg, 1999, Holmes et al. 2002, Korol and Kolo, 2002, Gibbs et al., 2004, Korol et al. 2004, Davis et al., 2005, Quinlan et al. 2008). However, the effects of estrogens on hippocampus-sensitive tasks are not unidirectional, as memory modulation varies with task demand (Galea et al., 2001, McLaughlin et al., 2008), estrogen dose (Packard and Teather, 1997, Holmes et al., 2002, McLaughlin et al., 2008, Barha et al., 2010, Inagaki et al., 2010), duration of hormone deprivation prior to treatment (Daniel et al., 2006, McLaughlin et al., 2008), stress (Bowman et al., 2009), and even daily handling (Bohacek and Daniel, 2007) and route of hormone administration (Garza-Meilandt et al., 2006).

In addition to these nuanced results, estrogens differentially impact cognition according to the memory system engaged. Elevated estrogen levels impair performance on memory tasks that engage the striatum (Galea et al., 2001, Korol and Kolo, 2002, Daniel and Lee, 2004, Korol et al., 2004, Davis et al., 2005, Zurkovsky et al., 2007, Quinlan et al., 2008) and prefrontal cortex (Wang et al., 2008, Wang et al., 2009). Unlike the well-studied behavioral effects of estrogens in the hippocampus, less is known regarding confounding factors that may influence memory modulation of striatum-sensitive tasks. Given these opposing effects, estrogens appear to shift the contributions of the hippocampus and striatum within a multiple memory systems framework whereby behaviors that rely on hippocampal processing are potentiated and those that rely on striatal contributions are diminished (Korol and Kolo, 2002, Korol 2004). These shifts in cognitive function are driven by the local actions of estradiol in the hippocampus and striatum (Zurkovsky et al., 2006, Zurkovsky et al., 2007).

Understanding how estrogens enhance or impair learning and memory is particularly important for women undergoing natural or surgical menopause, as declines in certain cognitive processes can be attributed to diminished estrogen levels. In some studies post-menopausal women demonstrate deficits in cognitive tasks that involve hippocampal function, such as declarative memory and verbal fluency (Maki and Hogervorst, 2003). Until recently, estrogen replacement, both with and without progesterone, was a popular therapy to preserve cognitive function and treat other symptoms of menopause such as hot flashes. However, the highly publicized findings from the Women's Health Initiative, such as increased risk of cardiovascular events and breast cancer, forced early termination of the study and prompted many women to discontinue HRT despite critical shortcomings in the WHI study design and interpretation (Gibbs and Gabor, 2003, Wise et al., 2005). Still searching for relief from menopausal complications, women began to turn to over-the-counter phytoestrogen-containing supplements as a "natural" alternative to HRT (Zhao and Brinton, 2007).

Phytoestrogens bind to estrogen receptors *in vivo* with mixed agonist/antagonist and tissue-specific actions (Brzezinski and Debi, 1999). Isoflavones, the class of phytoestrogens found in soy and a main component in many botanical supplements, are detected in many tissues following exposure, including the brain (Chang et al., 2000, Coldham and Sauer, 2000, Lund et al., 2001, Gu et al., 2005, Tsai 2005). A large proportion of women aged 40 to 60 consume soy foods or supplements (Mahady et al., 2003) despite a lack of research on the risks and benefits of isoflavone exposure, especially for brain health. Studies examining the effects of isoflavones on cognition are particularly sparse and document mixed results in humans (Lee et al., 2005, Zhao and Brinton, 2007). In experiments using laboratory rodents, isoflavones act like estradiol, enhancing performance for some memory tasks but impairing performance on others (Pan et al., 2000, Lund et al. 2001, Luine et al. 2006, Monteiro et al. 2008, Neese et al., 2010, Neese et al. 2012). Most research has focused on chronic isoflavone exposure, with few experiments examining the acute actions of isolated soy compounds commonly found in botanical supplements. Genistein, one of the most estrogenic isoflavones with twenty-fold selectivity for estrogen receptor (ER) β over ER α (Kuiper et al., 1998), is widely researched, yet only a handful of studies have investigated its mnemonic effects (Xu et al., 2007, Alonso et al., 2010, Huang and Zhang, 2010, Neese et al., 2010, Neese et al., 2012). Additionally, there have been no examinations of phytoestrogen actions on striatum-sensitive memory to date, which is surprising given that the striatum responds to agonists that target ER β (Morissette et al., 2008).

The current study compared the effects of acute estradiol and genistein treatment on place and response learning, tasks that rely on hippocampal and striatal function, respectively (Chang and Gold, 2003). Young adult Long-Evans rats were ovariectomized to remove endogenous estrogens and placed on a purified phytoestrogen-free diet following surgery to control dietary isoflavone intake. Three weeks after ovariectomy and forty-eight and twenty-four hours prior to behavioral training rats received acute doses of estradiol benzoate (single injections on each day), genistein (dosing throughout the day), or matching regimens of vehicle. Genistein treatment mimicked estradiol-induced enhancement of place learning and impairment of response learning. Our results indicated that genistein has robust estrogenic effects on learning and memory and point to activation of ER β and GPER possibly driving shifts in cognitive strategy.

Materials and Methods

Subjects

Virgin Long-Evans female rats (75-90 days old) were obtained from Harlan (Barrier 202, Indianapolis, IN). Rats were individually housed in plastic cages on a 12:12 hour light:dark cycle, with *ad libitum* access to food and water prior to food restriction for behavioral training. At least two days after arrival and three weeks prior to training, rats underwent bilateral ovariectomies. From the day of surgery, rats were maintained on phytoestrogen-free AIN-93G chow (Research Diets, New Brunswick, NJ). Beginning ten days prior to training, rats were handled daily and food-restricted to 80-85% free feeding weight plus 5 g to allow for normal growth. To reduce neophobia on the day of testing, rats were given five to ten sucrose tablets (45 mg; TestDiet, Richmond, IN), the food reward used during behavioral training, each day of food restriction. Estradiol, genistein, or control treatments were initiated 48 h before training. Rats were decapitated immediately following training and tissue collected for later analysis.

All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee and were in compliance with the NIH Guide for the Care and Use of Laboratory Animals.

Ovariectomy

Rats were anesthetized with isoflurane and received injections of penicillin (100,000 units/kg, i.m.) and the analgesic carprofen (5 mg/kg, s.c.). Dorsolateral incisions were made through the skin, with fat and muscle layers bluntly dissected. The distal uterine horn was sutured at the oviduct and the ovary excised. Muscle and fat layers were individually sutured, and the incision closed with tissue adhesive and wound clips and treated with Bacitracin. Rats were given a post-operative injection of 10 mL physiological saline (s.c.) and a second carprofen injection 6–12 h after surgery for pain management.

Histological determination of hormone status

Vaginal smears were taken for five days before treatment to confirm ovariectomy and cessation of the estrous cycle. Small sterile calcium alginate-tipped swabs were moistened with sterile saline then gently inserted in the vagina to collect secretions. Cells were fixed to glass slides with ethanol, stained with hematoxylin and eosin, and coverslipped. Slides were examined on a light microscope and staged according to the methods of Long and Evans (1922).

Treatments

Estradiol—Rats were randomly assigned to receive either estradiol or vehicle treatments. Forty-eight and 24 hours prior to training, rats received a subcutaneous injection of either sesame oil (Sigma, St. Louis, MO; $n = 21$), 4.5 $\mu\text{g/kg}$ 17 β -estradiol benzoate (EB; Sigma) in oil (Lo-EB; $n = 19$), or 45 $\mu\text{g/kg}$ EB in oil (Hi-EB; $n = 17$); (Fig. 1A). The concentrations of EB solutions were 100 $\mu\text{g/mL}$ for the 45 $\mu\text{g/kg}$ dose and 10 $\mu\text{g/mL}$ for the 4.5 $\mu\text{g/kg}$ dose, such that injection volume corresponded to body weight for all treatments at 0.45 mL/kg.

Genistein—Rats were randomly assigned to either genistein-treated or sucrose control groups. Genistein treated rats ($n = 17$) were fed one 97 mg fruit punch-flavored sucrose tablet containing 485 μg genistein (TestDiet) every 4 hours during the light cycle beginning 48 h prior to training. This corresponds to 48 h, 44 h, 40 h, 28 h, 24 h, 20 h, 16 h, 4 h and 30 mins before training (Fig. 1B), for a total genistein exposure of 4.4 mg over two days. This dosing scheme was based on prior serum genistein dose-response data using these pellets (Neese et al., 2010) and was used to elevate serum genistein to levels physiologically relevant to humans consuming isoflavone supplements during the 48 h treatment period through behavioral training. Vehicle control rats ($n = 17$) received one 97 mg fruit punch sucrose pellet (TestDiet) at the same time points outlined above.

Genistein pharmacokinetics—A separate cohort of rats was ovariectomized, food-restricted, and assessed by vaginal smear as previously described ($n = 17$). Rats were placed on the genistein dosing regimen described above (Fig. 1B) and randomly split into three groups. For each group of rats, blood was only collected during a single day of treatment corresponding to one (t_2), two (t_1), or three days (t_0) of genistein exposure (Fig. 2E). Immediately prior to and 30 min following each genistein dose, blood was collected via tail puncture. The tail was warmed on a heating pad for approximately 30 s then punctured using a sterile 21 gauge needle. Blood droplets were gently milked into small glass test tubes to obtain a volume of 20–40 μL . The blood was allowed to clot then centrifuged at 2100g for 15 mins at RT, and the supernatant collected and frozen. Gentle pressure was applied to puncture sites with sterile gauze and topical Bacitracin applied. Whenever possible, the scab of a previous puncture was gently re-opened for the next collection point to avoid making a new wound. Following the last blood collection, rats were decapitated as described below.

Behavioral Training

General Training Protocol—Training procedures were adapted from Korol and Kolo (2002). Rats were trained in a single day approximately two to seven hours after lights on. Rats were allowed to acclimate to the training room in a clean holding cage for 10 mins prior to behavioral testing. The room was moderately lit by two floor lamps aimed at opposite ceiling corners and a standard house fan was used to mask extraneous noise. The training apparatus was a symmetrical plus-shaped maze made of black Plexiglas®, with arms measuring 105 cm long, 13 cm wide, and 7 cm high, and included a white center to emphasize the choice point. The maze was placed 70 cm off the floor on a small table in the center of the training room. Each arm contained a food reward boat under which inaccessible sucrose pellets were placed to reduce the use of odor cues during training. Each trial began by baiting the goal arm food boat with an accessible sucrose reward. The rat was then placed into the start arm and allowed to choose a single arm to enter. A choice was recorded when all four paws of the rat crossed into the arm; rats had a maximum of two mins to make a choice. Rats were allowed to remain in the arm ten seconds or until they turned to leave, and were then returned to the holding cage for an intertrial interval of thirty seconds. During the intertrial period, the maze was randomly rotated to minimize the use of intramaze cues. Rats were trained to one hundred trials. Criterion was set at 9 out of 10 correct choices, with at least six consecutive correct choices. Trials to criterion and percent correct choices over 10-trial blocks were used to assess learning.

Place task—Rats ($n = 50$) were trained to learn the spatial location of the goal arm containing the food reward using extra-maze cues. Various visual cues were located around the perimeter of the room, such as a door, desk, bookcase, shelves, high-contrast posters, etc. The location of the goal arm remained constant while the start location was randomized and counterbalanced between east and west arms (Fig. 1C).

Response task—Rats ($n = 41$) were trained to find the food reward by making a specific body movement to reach the goal arm, i.e. a 90° right or left turn. Rats were randomly assigned to be trained to turn right or left at the choice point. Beige curtains were placed around the outer walls and over the ceiling to obscure visual cues. The start location was randomly counterbalanced between east and west arms of the maze (Fig. 1D).

Tissue collection

Immediately following training, rats were overdosed with sodium pentobarbital (75 mg/kg) and decapitated. Trunk blood was collected, allowed to clot for 1 hr at RT, and centrifuged at 2100g for 15 mins. The serum supernatant was collected and frozen at -20°C until analysis. Uterine horns were harvested, trimmed of fat and vasculature, measured, and weighed.

Serum Analysis

Serum samples from rats that received injections were assessed for estradiol concentration using a commercially available RIA kit (DSL4800, Beckman Coulter, Brea, CA) with a minimum level of detection of 2.2 pg/mL. Serum genistein concentrations were determined for all rats that received sucrose or genistein treatments via liquid chromatography with electrospray mass spectrometry and tandem mass spectrometry (LC/MS/MS) detection as previously reported (Twaddle et al., 2002) with a detection threshold of 8-14 ng/mL for a 10 µL sample. For both serum estradiol and serum genistein measurements, samples that fell below the minimum level of detection were assigned a concentration of zero.

Statistical Analysis

Trials to criterion data were compared within place and response tasks using a one-way analysis of variance (ANOVA) with estradiol treatment as the independent variable. *A priori* planned paired comparisons were also conducted across groups receiving injections to assess dose-response differences versus control rats. The effects of genistein treatment one trials to criterion were assessed via unpaired t-tests. Learning curves were generated by graphing percent correct choices over blocks of ten trials. Repeated-measures ANOVAs were used to evaluate treatment differences in task acquisition, with treatment as the between subjects variable, trial block as the within subjects variable, and percent correct choices within each block as the dependent measure. Differences in uterine horn weights, serum estradiol levels, and serum genistein levels across repeated days of dosing were tested using a one-way ANOVA to evaluate the measures according to treatment, followed by planned *post hoc* t-tests. Unpaired t-tests were used to compare serum genistein levels. All analyses were performed using $\alpha = 0.05$.

Results

Serum estradiol levels and bioactivity

All rats demonstrated diestrous vaginal smears prior to estradiol treatment indicating a successful ovariectomy and cessation of the estrous cycle. Uterine horn wet weights increased according to treatment (mean uterine horn wet weight, mg/cm: oil = 14.1, Lo-EB = 32.6, Hi-EB = 40.9; $F[2,53] = 119.71$; $p < 0.0001$, Fig. 2A). Uterine weight differed significantly between each treatment group (all paired comparisons $p < 0.0001$) and increased in a dose-dependent manner, with greater uterine weights seen in Hi-EB rats than in Lo-EB rats. ANOVA revealed a significant treatment effect on serum estradiol levels (mean serum estradiol, pg/mL: oil = 2.2, Lo-EB = 11.12, Hi-EB = 120.86; $F[2,53] = 127.86$; $p < 0.0001$, Fig. 2B). Serum estradiol concentrations differed significantly between each treatment group ($p < 0.0001$).

Serum genistein levels and bioactivity

All rats treated with sucrose or genistein demonstrated diestrous smears prior to and throughout treatment. There was no effect of genistein treatment on uterine horn weight (mean uterine horn wet weight, mg/cm: sucrose = 12.5, genistein = 11.7; $F[1,32] = 1.08$; $p > 0.30$, Fig. 2C). Genistein-treated rats had significantly elevated serum levels of total genistein, approximately 2% of which is aglycone (Holder et al., 1999; mean serum genistein, in ng/mL: sucrose = 6.75, genistein = 337; $F[1,32] = 35.53$; $p < 0.0001$; Fig. 2D). As expected, no detectable levels of the isoflavone daidzein or its metabolite equol were present in the serum of sucrose- or genistein-treated animals (data not shown).

The serum concentration-time profiles for genistein dosing revealed spikes in serum levels 30 min after each treatment, followed by a gradual decline over 4 h (Fig. 2E). Serum genistein concentrations across days of dosing were statistically compared for the blood draw 30 min following the second genistein dose on each day of treatment. There was a significant effect of treatment on circulating genistein concentrations for each day of dosing (mean serum genistein, ng/mL: 4.5 h = 404, 24.5 h = 482, 48.5 h = 1167; $F[2,14] = 16.27$; $p < 0.0002$; Fig. 2E). Post-hoc tests showed that serum genistein levels were significantly elevated on the third day of treatment as compared to one and two days of dosing ($p < 0.0003$).

Behavioral testing: place and response learning tasks

Place learning—Two days of estradiol treatment enhanced place learning at the Lo-EB dose, but not the Hi-EB dose, suggesting an inverted U-shaped dose-response function. Rats that received Lo-EB injections demonstrated a significant decrease in the number of trials to reach criterion (mean trials to criterion oil = 59.4, Lo-EB = 43.4; $p < 0.02$; Fig. 3A), indicating an improvement in place learning relative to oil controls. An improvement in place learning was not observed in rats treated with Hi-EB injections (mean trials to criterion oil = 59.4, Hi-EB = 58.6; $p > 0.9$). Due to the inverted dose-response function, there was no main effect of treatment ($F[2,30] = 2.56$; $p > 0.09$).

Genistein treatment enhanced place learning compared to sucrose controls. Rats treated with genistein reached criterion in significantly fewer trials than did control rats (mean trials to criterion: sucrose = 68.8, genistein = 48.0; $F[1,14] = 6.71$; $p < 0.03$; Fig. 3B). The trials to criterion data for the different treatment regimens (injection vs. oral, Fig. 3A, 3B) revealed a similar magnitude of learning enhancement (approximately 30%) in rats treated with Lo-EB or genistein over the performance of the corresponding oil- or sucrose-treated controls.

All oil- and estradiol-treated rats demonstrated learning, seen as significant changes in percent correct across trial blocks ($F[9,270] = 104.68$; $p < 0.0001$, Fig. 3C). In contrast to trials to criterion data, there was no significant interaction between treatment and percent correct ($F[9,270] = 0.80$; $p > 0.6$) and no main effect of treatment ($F[2,270] = 2.06$; $p > 0.1$).

Analysis of the learning curves for sucrose- or genistein-treated rats indicated that the genistein group learned the place task more quickly than did sucrose controls. All rats learned over trial blocks ($F[9, 126] = 44.02$; $p < 0.0001$, Fig. 3D). There was a significant interaction between treatment and percent correct across trial blocks ($F[9,126] = 1.98$; $p < 0.05$) but a non-significant main treatment trend ($F[1,126] = 3.79$; $p > 0.07$).

Response learning—In contrast to effects on place learning, estradiol treatment impaired response learning. Compared to oil controls, the groups treated with Lo-EB and Hi-EB both required significantly more trials to reach criterion (mean trials to criterion oil = 29.8, Lo-EB = 67.9, Hi-EB = 54. 7; $F[2,19] = 28.14$; $p < 0.0001$; Fig. 4A). Performance between the Lo-EB group and the Hi-EB group did not differ significantly ($p > 0.088$).

Response learning speed was also significantly reduced by genistein exposure. Genistein-treated rats reached learning criterion at significantly later trials than did sucrose controls (mean trials to criterion sucrose = 29.1, genistein = 53.9; $F[1,16] = 11.17$; $p < 0.004$; Fig. 4B). Genistein also mimicked estradiol impairment of response learning, as qualitative examination of the trials to criterion data shows similar performance in control (oil vs. sucrose) and treatment groups (Lo-EB and Hi-EB vs. genistein, Fig. 4A, 4B).

For the learning curves, all injected rats showed significant learning as percent correct increased across trial blocks ($F[9,171] = 95.85$; $p < 0.0001$, Fig. 4C). In contrast to place learning, the results of hormone treatment on response learning across trial blocks were quite robust. There was a main effect of treatment ($F[2,171] = 14.25$; $p < 0.0003$) and a significant interaction between treatment and percent correct ($F[9,171] = 6.91$; $p < 0.0001$), as both Lo-EB and Hi-EB treated rats acquired the response task more slowly than did oil controls.

Similar outcomes were observed with genistein treatment, as accuracy across training trials was diminished. All sucrose- and genistein-treated rats learned over trial blocks ($F[9,135] = 52.69$; $p < 0.0001$, Fig. 4D). Repeated-measures ANOVA of the learning curves revealed a

main effect of genistein treatment ($F[1,135] = 8.37$; $p < 0.02$) and a significant interaction of treatment and percent correct ($F[9,135] = 6.59$; $p < 0.0001$).

Discussion

The current study examined the effects of 48 hours of treatment with low estradiol, high estradiol, or the soy isoflavone genistein on the hippocampus-sensitive place task and the striatum-sensitive response task. Our results are consistent with previous experiments reporting estradiol-induced enhancement of place learning and impairment of response learning, but here demonstrated a distinct dose-response relationship in rats free of dietary phytoestrogens. Moreover, acute treatment with a physiologically-relevant dose of genistein facilitated performance in the place task but slowed learning in the response task. Genistein therefore mimics the opposing actions of estradiol-induced shifts in learning strategy, augmenting hippocampal-sensitive cognition while diminishing striatum-sensitive cognition. These results point to task- and brain region-specific actions of genistein: like estradiol, isoflavones may facilitate or impair performance depending on cognitive demands and neural systems engaged.

Estradiol and genistein bioactivity

Estradiol treatments were physiologically active, as indicated by increases in uterine horn wet weights and serum estradiol levels. Despite being quite uterotrophic, the Lo-EB dose only moderately elevated serum estradiol levels. The seemingly low level of estradiol detected in serum from these rats could largely be due to the method of analysis, the direct RIA. The commercial estradiol RIA kit used here does not involve a steroid extraction step and has been optimized for use with human serum, opening the possibility for reduced accuracy due to the presence of serum binding proteins or inter-species matrix differences as discussed by Klee (2004) and Ström et al. (2008). Additionally, the kit in question (DSL4800, Beckman Coulter) has previously been reported to yield serum estradiol values lower than other RIA kits or alternative methods of analysis (unpublished data; Stanczyk et al., 2003, Ström et al., 2008). Preliminary findings from pilot LC/MS/MS detection of estradiol in serum samples from treated animals detected 2-3.5 times greater concentrations than values obtained via RIA (data not shown).

Genistein treatment failed to increase uterine horn wet weight, indicating a lack of appreciable ER α activation in the periphery. This was expected given the preferential binding affinity of genistein for the ER β subtype and is consistent with reports indicating no uterine proliferation with low-to-moderate genistein exposures (Mäkelä et al., 1999, Whitten and Patisaul, 2001, Owens et al., 2003). Serum genistein concentrations paralleled those found in human adults consuming high-soy diets or isoflavone-containing supplements (Adlercreutz 1998, Doerge et al., 2000, Gardner et al., 2009). Thus, the effective doses of genistein for modulation of learning and memory are physiologically relevant to human isoflavone exposures.

Place learning was facilitated both by low estradiol and genistein treatment

Rats treated with a low dose of estradiol benzoate (4.5 $\mu\text{g/kg}$) demonstrated enhanced learning in the place task, while treatment with a high dose of estradiol benzoate (45 $\mu\text{g/kg}$) had no effect on place learning. These findings contrast previous results from our laboratory reporting facilitated performance on the place task using the same treatment schedule and a similarly high dose of estradiol (10 μg ; Korol and Kolo, 2002, Zurkovsky et al. 2006). An important distinction is that the current study used Long-Evans rats maintained on phytoestrogen-free chow while prior experiments used Sprague-Dawley rats fed standard chow with variable isoflavone content, where the presence of dietary phytoestrogens that

have mixed agonist/antagonist properties (Brzezinski and Debi, 1999) may have modulated estrogen signaling. Additionally, strain differences in estrogen sensitivity and metabolism are well described (Kacew and Festing, 1996, Diel et al. 2004, Chapin et al. 2008) and may contribute to differences in observed dose-response functions. However, no direct comparisons of the mnemonic actions of estrogens across rat strains have been published to date.

Our results indicate that the enhancing effect of estradiol on hippocampal-sensitive place learning does not follow a monotonic dose-response function, but rather demonstrates an inverted U-shaped pattern. Inverted-U dose effects, whereby an optimal dose of estradiol enhances memory while lower and higher doses are ineffective or impairing, have been reported for a number of tasks that tap hippocampal function, including a spatial version of the swim task (Packard and Teather, 1997, McLaughlin et al., 2008), radial arm maze (Holmes et al. 2002), contextual fear conditioning (Barha et al. 2010), and object placement (Inagaki et al. 2010). The inverted U dose-response is indeed a well-documented pattern of neuromodulation, appearing across many treatments and memory tasks (see Baldi and Bucherelli, 2005, Gold and Korol, 2010, for review). Packard (1998) proposes that the effective dose of estrogen results in optimal receptor activation required for hippocampal-dependent memory enhancements, with lower doses insufficiently activating receptors and hyper-activation at higher doses, leading to “neural noise” that interferes with information processing. Our data point to such an optimal dose for estrogenic modulation of place learning.

Forty-eight hours of genistein treatment enhanced place learning with a similar magnitude of improvement over controls as that obtained with estradiol treatment. While several published reports have examined the effects of chronic mixed isoflavone exposure on hippocampal-sensitive memory, few studies documenting the mnemonic actions of isolated genistein exist; to our knowledge, our findings represent only the second report of acute genistein effects on hippocampal-sensitive learning and memory. A single high dose of genistein (40 mg/kg) enhances memory in the swim task (Alonso et al., 2010), as does chronic genistein treatment (Xu et al., 2007, Huang and Zhang, 2010). The mnemonic effects of extended exposure to mixed soy isoflavones have been more frequently described, with enhancements reported in the radial arm maze (Pan et al., 2000, Lund et al., 2001), object placement (Luine et al., 2006), and swim task (Monteiro et al., 2008, Lee et al., 2009, Pan et al., 2010). While the current study used only a single genistein dosage, other findings (Huang and Zhang, 2010) suggest that genistein may demonstrate a similar inverted-U pattern of spatial memory modulation, as rats chronically treated with low (15 mg/kg) but not high (30 mg/kg) doses of genistein showed memory enhancements in the swim task. Potential nonlinear effects of genistein on learning and memory are not surprising, as isoflavones demonstrate biphasic responses across a host of physiological systems (Calabrese 2001, Patisaul and Adewale, 2009). Non-monotonic dose-response functions of these compounds may be particularly important given the widespread use of soy-based products and supplements: estrogenic botanical compounds may be harmful, beneficial, or ineffective depending on the doses and formulations in which they are used.

Estradiol or genistein treatment impaired response learning

Treatment with both a low (4.5 µg/kg) and high (45 µg/kg) dose of estradiol benzoate impaired response learning. These results are consistent with findings from previous studies demonstrating that absence or low levels of estrogens bias rats towards response strategies, while elevated hormone levels shift rats toward hippocampal-based strategies and thus impair performance of striatum-sensitive tasks. Elevated estradiol impairs acquisition of the cued win-stay task (Galea et al., 2001) and response maze learning (Korol and Kolo, 2002, Davis et al., 2005), biases rats against the use of a stimulus-response strategy in a water

maze (Daniel and Lee, 2004), biases rats away from response strategies in the dual-solution T-maze (Korol et al. 2004, Quinlan et al. 2008), and impairs performance of operant conditioning tasks that engage prefrontal cortex and cortico-striatal function (Wang et al., 2008, Wang et al., 2009).

Experiments from our lab show that estrogens act in a site-specific manner to modulate memory: infusion of estradiol into the hippocampus enhanced place learning, while infusion into the striatum had no effect. Conversely, estradiol infusion into the dorsal striatum but not the hippocampus impaired response learning (Zurkovsky et al. 2007). Taking the local actions of estrogens into account, it follows that dose-response effects on learning and memory will vary according to the estrogen receptor milieu of the brain structure critical for the cognitive task. Compared to the hippocampus, the striatum has a far lower density of ERs (Shughrue et al., 1997) and thus may be less sensitive to processing disruptions that occur with exposure to high doses of estradiol. In contrast to studies of hippocampus-sensitive tasks, there is a paucity of research examining dose-response patterns for estrogen impairment of striatum-sensitive learning and memory. Our results across place and response learning support the contributions of site-specific sensitivity to the diverse effects of estrogen on various cognitive tasks.

Rats that received genistein treatment also showed impaired performance on the response learning task. To our knowledge, this is the first report of the mnemonic effects of phytoestrogen treatment on a striatum-sensitive task. Our previous results indicate that genistein treatment impairs performance on operant conditioning measures of executive function in a delayed spatial alternation task that relies primarily on the prefrontal cortex but also engages cortico-striatal function (Neese et al., 2010, Neese et al., 2012). Importantly, these findings of impaired learning indicate that isoflavone treatment does not produce global cognitive enhancements. The benefits of soy isoflavones and other phytoestrogens on brain health are oft-reported, with such treatments frequently proposed as alternatives to estrogen replacement therapy to protect against cognitive decline (Lee et al., 2005, Zhao and Brinton, 2007). However, like endogenous estrogens, our results demonstrate that the effects of genistein on learning and memory depend on the cognitive demands of the task and the brain structures that are engaged during training. Thus, like estradiol, phytoestrogens may in fact impair certain types of cognition.

Genistein may modulate memory via activation of ER β and GPER

Genistein treatment enhanced place learning and impaired response learning, mimicking the direction and magnitude of estrogenic effects on learning in these tasks. Unlike 17 β -estradiol, which binds to both classical estrogen receptors ER α and ER β with approximately equal high affinity, genistein binds to ER β with a twenty-fold selectivity over ER α (Kuiper et al., 1998). The absence of a uterotrophic response following genistein treatment suggests that insufficient tissue concentrations were achieved to activate ER α . In the hippocampus, ER α and ER β are expressed in a sub-region specific fashion, with both receptors detected at nuclear sites and in abundance at extranuclear sites, including dendritic spines and axon terminals (Shughrue et al., 1997, Milner et al., 2001, Milner et al., 2005, Milner et al., 2008). Low-to-moderate expression of both ER α and ER β in the striatum has been detected via sensitive methodology, with ER β predominantly expressed in striatal afferents; subcellular localization of the receptors has not been conclusive (Küppers and Beyer, 1999, Shughrue and Merchenthaler, 2001, Creutz and Kritzer, 2002, Mitra et al., 2003). However, results from our lab suggest extranuclear ER signaling in the striatum contributes to modulation of response learning as impairments were observed following only two hours of exposure to intra-striatal estradiol (Zurkovsky et al., 2011).

Non-classical receptors may also contribute to mnemonic effects, as genistein binds to the G-protein coupled estrogen receptor (GPER) with relatively high affinity, approximately 7-fold less than that of estradiol and intermediate between genistein's affinity for ER α and ER β (Maggiolini et al., 2004, Kuiper et al., 1998). Importantly, genistein potently activates downstream molecules believed to be involved in neural plasticity associated with learning and memory, such as cFos and ERK, likely through GPER signaling. (Maggiolini et al., 2004, Thomas and Dong, 2006). GPER has been detected in all major cell layers of the hippocampal formation (Brailoiu et al., 2007, Matsuda et al., 2008). In contrast to the relatively low levels of ER α and ER β in the striatum, GPER is highly expressed (Brailoiu et al., 2007, Hammond et al., 2011). Our behavioral results coupled with the distribution of ER β and GPER in the hippocampus and striatum suggest that activation of these ER subtypes underlie the mnemonic effects of genistein.

Conclusion

In sum, the results of the current study indicate that genistein mimics estradiol-induced shifts in hippocampal and striatal learning, enhancing performance in the place task and impairing performance in the response task. The pharmacology of genistein points to ER β and GPER as possible receptor subtypes through which estrogens may exert their mnemonic effects on the tasks used here. Our data demonstrate that like estradiol, the cognitive effects of genistein can be dissociated by task attributes and neural systems engaged. Thus, phytoestrogen enhancements of certain types of memory do not generalize to global improvements in cognitive processes. The use of genistein-containing supplements should be approached with caution as this compound demonstrates robust estrogenic modulation of learning and memory that include impairments in some categories of cognition.

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References

- Adlercreutz H. Epidemiology of phytoestrogens. *Baillières Clin. Endocrinol. Metab.* 1998; 12:605–623.
- Alonso A, González-Pardo H, Garrido P, Conejo NM, Llaneza P, Díaz F, del Rey CG, González C. Acute effects of 17 β -estradiol and genistein on insulin sensitivity and spatial memory in aged ovariectomized female rats. *Age (Dordr.)*. 2010; 32:421–434. [PubMed: 20467821]
- Baldi E, Bucherelli C. The inverted “u-shaped” dose-effect relationships in learning and memory: modulation of arousal and consolidation. *Nonlinearity Biol. Toxicol. Med.* 2005; 3:9–21. [PubMed: 19330154]
- Barha CK, Dalton GL, Galea LA. Low doses of 17alpha-estradiol and 17beta-estradiol facilitate, whereas higher doses of estrone and 17alpha- and 17beta-estradiol impair, contextual fear conditioning in adult female rats. *Neuropsychopharmacology*. 2010; 35:547–559. [PubMed: 19847162]
- Bimonte HA, Denenberg VH. Estradiol facilitates performance as working memory load increases. *Psychoneuroendocrinology*. 1999; 24:161–173. [PubMed: 10101725]
- Bohacek J, Daniel JM. Increased daily handling of ovariectomized rats enhances performance on a radial-maze task and obscures effects of estradiol replacement. *Horm. Behav.* 2007; 52:237–243. [PubMed: 17524404]

- Bowman RE, Micik R, Gautreaux C, Fernandez L, Luine VN. Sex-dependent changes in anxiety, memory, and monoamines following one week of stress. *Physiol. Behav.* 2009; 97:21–29. [PubMed: 19419681]
- Brailoiu E, Dun SL, Brailoiu GC, Mizuo K, Sklar LA, Oprea TI, Prossnitz ER, Dun NJ. Distribution and characterization of estrogen receptor G protein-coupled receptor 30 in the rat central nervous system. *J. Endocrinol.* 2007; 193:311–321. [PubMed: 17470522]
- Brzezinski A, Debi A. Phytoestrogens: the “natural” selective estrogen receptor modulators? *Eur. J. Obstet. Gynecol. Reprod. Biol.* 1999; 85:47–51. [PubMed: 10428321]
- Calabrese EJ. Estrogen and related compounds: biphasic dose responses. *Crit. Rev. Toxicol.* 2001; 31:503–515. [PubMed: 11504177]
- Chang HC, Churchwell MI, Delclos KB, Newbold RR, Doerge DR. Mass spectrometric determination of Genistein tissue distribution in diet-exposed Sprague-Dawley rats. *J. Nutr.* 2000; 130:1963–1970. [PubMed: 10917909]
- Chang Q, Gold PE. Intra-hippocampal lidocaine injections impair acquisition of a place task and facilitate acquisition of a response task in rats. *Behav. Brain Res.* 2003; 144:19–24. [PubMed: 12946591]
- Chapin RE, Adams J, Boekelheide K, Gray LE, Hayward SW, Lees PS, McIntyre BS, Portier KM, Schnorr TM, Selevan SG, Vandenberg JG, Woskie SR. NTP-CERHR expert panel report on the reproductive and developmental toxicity of bisphenol A. *Birth Defects Res. B. Dev. Reprod. Toxicol.* 2008; 83:157–395. [PubMed: 18613034]
- Coldham NG, Sauer MJ. Pharmacokinetics of [(14)C]Genistein in the rat: gender-related differences, potential mechanisms of biological action, and implications for human health. *Toxicol. Appl. Pharmacol.* 2000; 164:206–215. [PubMed: 10764634]
- Creutz LM, Kritzer MF. Estrogen receptor-beta immunoreactivity in the midbrain of adult rats: regional, subregional, and cellular localization in the A10, A9, and A8 dopamine cell groups. *J. Comp. Neurol.* 2002; 446:288–300. [PubMed: 11932944]
- Daniel JM, Fader AJ, Spencer AL, Dohanich GP. Estrogen enhances performance of female rats during acquisition of a radial arm maze. *Horm. Behav.* 1997; 32:217–225. [PubMed: 9454673]
- Daniel JM, Hulst JL, Berbling JL. Estradiol replacement enhances working memory in middle-aged rats when initiated immediately after ovariectomy but not after long-term period of ovarian hormone deprivation. *Endocrinology.* 2006; 147:607–614. [PubMed: 16239296]
- Daniel JM, Lee CD. Estrogen replacement in ovariectomized rats affects strategy selection in the Morris water maze. *Neurobiol. Learn. Mem.* 2004; 82:142–149. [PubMed: 15341799]
- Davis DM, Jacobson TK, Aliakbari S, Mizumori SJ. Differential effects of estrogen on hippocampal- and striatal-dependent learning. *Neurobiol. Learn. Mem.* 2005; 84:132–137. [PubMed: 16054404]
- Diel P, Schmidt S, Vollmer G, Janning P, Upmeyer A, Michna H, Bolt HM, Degen GH. Comparative responses of three rat strains (DA/Han, Sprague-Dawley and Wistar) to treatment with environmental estrogens. *Arch. Toxicol.* 2004; 78:183–193. [PubMed: 14689164]
- Doerge DR, Chang HC, Churchwell MI, Holder CL. Analysis of soy isoflavone conjugation in vitro and in human blood using liquid chromatography-mass spectrometry. *Drug Metab. Dispos.* 2000; 28:298–207. [PubMed: 10681374]
- Fader AJ, Johnson PE, Dohanich GP. Estrogen improves working but not reference memory and prevents amnesic effects of scopolamine of a radial-arm maze. *Pharmacol. Biochem. Behav.* 1999; 62:711–717. [PubMed: 10208377]
- Galea LA, Wide JK, Paine TP, Holmes MM, Ormerod BK, Floresco SB. High levels of estradiol disrupt conditioned place preference learning, stimulus response learning and reference memory but have limited effects on working memory. *Behav. Brain Res.* 2001; 126:115–126. [PubMed: 11704257]
- Gardner CD, Chatterjee LM, Franke AA. Effects of isoflavone supplements vs. soy foods on blood concentrations of genistein and daidzein in adults. *J. Nutr. Biochem.* 2009; 20:227–234. [PubMed: 18602820]
- Garza-Meilandt A, Cantu RE, Claiborne BJ. Estradiol's effects on learning and neuronal morphology vary with route of administration. *Behav. Neurosci.* 2006; 120:905–916. [PubMed: 16893296]

- Gibbs RB, Gabor R. Estrogen and cognition: applying preclinical findings to clinical perspectives. *J. Neurosci. Res.* 2003; 74:637–643. [PubMed: 14635215]
- Gibbs RB, Gabor R, Cox T, Johnson DA. Effects of ralozifene and estradiol on hippocampal acetylcholine release and spatial learning in the rat. *Psychoneuroendocrinology.* 2004; 29:741–748. [PubMed: 15110923]
- Gold PE. Coordination of multiple memory systems. *Neurobiol. Learn. Mem.* 2004; 82:230–242. [PubMed: 15464406]
- Gold, PE.; Korol, DL. Hormones and Memory.. In: Koob, GF.; Le Moal, M.; Thompson, RF., editors. *Encyclopedia of Behavioral Neuroscience.* Vol. 2. Academic Press; Oxford: 2010. p. 57-64.
- Gu L, Laly M, Chang HC, Prior RL, Fang N, Ronis MJ, Badger TM. Isoflavone conjugates are underestimated in tissues using enzymatic hydrolysis. *J. Agric. Food Chem.* 2005; 53:6858–6863. [PubMed: 16104811]
- Hammond R, Nelson D, Gibbs RB. GPR30 co-localizes with cholinergic neurons in the basal forebrain and enhances potassium-stimulated acetylcholine release in the hippocampus. *Psychoneuroendocrinology.* 2011; 36:182–192. [PubMed: 20696528]
- Holmes MM, Wide JK, Galea LA. Low levels of estradiol facilitate, whereas high levels of estradiol impair, working memory performance on the radial arm maze. *Behav. Neurosci.* 2002; 116:928–934. [PubMed: 12369813]
- Huang YH, Zhang QH. Genistein reduced the neural apoptosis in the brain of ovariectomized rats by modulating mitochondrial oxidative stress. *Br. J. Nutr.* 2010; 104:1297–1303. [PubMed: 20579403]
- Inagaki T, Gautreaux C, Luine V. Acute estrogen treatment facilitates recognition memory consolidation and alters monoamine levels in memory-related brain areas. *Horm. Behav.* 2010; 58:415–426. [PubMed: 20553724]
- Kacew S, Festing MF. Role of rat strain in the differential sensitivity to pharmaceutical agents and naturally occurring substances. *J. Toxicol. Environ. Health.* 1996; 47:1–30. [PubMed: 8568909]
- Klee GG. Interferences in hormone immunoassays. *Clin. Lab Med.* 2004; 24:1–18. [PubMed: 15157554]
- Korol DL. Role of estrogen in balancing contributions from multiple memory systems. *Neurobiol. Learn. Mem.* 2004; 82:309–323. [PubMed: 15464412]
- Korol DL, Kolo LL. Estrogen-induced changes in place and response learning in young adult female rats. *Behav. Neurosci.* 2002; 116:411–420. [PubMed: 12049322]
- Korol DL, Malin EL, Borden KA, Busby RA, Couper-Leo J. Shifts in preferred learning strategy across the estrous cycle in female rats. *Horm. Behav.* 2004; 45:330–338. [PubMed: 15109907]
- Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson JA. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology.* 1998; 139:4252–4263. [PubMed: 9751507]
- Küppers E, Beyer C. Expression of estrogen receptor- α and β mRNA in the developing and adult mouse striatum. *Neurosci Lett.* 1999; 276:95–98. [PubMed: 10624800]
- Lee YB, Lee HJ, Sohn HS. Soy isoflavones and cognitive function. *J. Nutr. Biochem.* 2005; 16:641–649. [PubMed: 16169201]
- Lee YB, Lee KH, Sohn HS, Lee SJ, Cho KH, Kang IJ, Kim DW, Shin YK, Pai T, Hwang IK, Won MH. Effects of soy phytoestrogens on reference memory and neuronal cholinergic enzymes in ovariectomized rats. *J Med Food.* 2009; 12:64–70. [PubMed: 19298197]
- Long, JA.; Evans, HM. *Memoir of the University of California.* Vol. 6. University of California Press; Berkeley: 1922. The oestrous cycle in the rat and its associated phenomena.; p. 1-48.
- Lund TD, West TW, Tian LY, Bu LH, Simmons DL, Setchell KD, Adlercreutz H, Lephart ED. Visual spatial memory is enhanced in female rats (but inhibited in males) by dietary soy phytoestrogens. *BCM Neurosci.* 2001; 2:20.
- Luine V, Attalla S, Mohan G, Costa A, Frankfurt M. Dietary phytoestrogens enhance spatial memory and spine density in the hippocampus and prefrontal cortex of ovariectomized rats. *Brain Res.* 2006; 1126:183–187. [PubMed: 16945354]

- Luine VN, Richards ST, Wu VY, Beck KD. Estradiol enhances learning and memory in a spatial memory task and effects levels of monoaminergic neurotransmitters. *Horm. Behav.* 1998; 34:149–162. [PubMed: 9799625]
- Maggiolini M, Vivacqua A, Fasanella G, Recchia AG, Sisci D, Pezzi V, Montanaro D, Musti AM, Picard D, Andò S. The G protein-coupled receptor GPR30 mediates c-fos up-regulation by 17beta-estradiol and phytoestrogens in breast cancer cells. *J. Biol. Chem.* 2004; 279:27008–27016. [PubMed: 15090535]
- Mahady GB, Parrot J, Lee C, Yun GS, Dan A. Botanical dietary supplement use in peri- and postmenopausal women. *Menopause.* 2003; 10:65–72. [PubMed: 12544679]
- Mäkelä S, Savolainen H, Aavik E, Myllärniemi M, Strauss L, Taskinen E, Gustafsson JA, Häyry P. Differentiation between vasculoprotective and uterotrophic effects of ligands with different binding affinities to estrogen receptors alpha and beta. *Proc. Natl. Acad. Sci. USA.* 1999; 96:7077–7082. [PubMed: 10359841]
- Maki P, Hogervorst E. The menopause and HRT; HRT and cognitive decline. *Best Pract. Res. Clin. Endocrinol. Metab.* 2003; 17:105–122. [PubMed: 12763515]
- Matsuda K, Sakamoto H, Mori H, Hosokawa K, Kawamura A, Itose M, Nishi M, Prossnitz ER, Kawata M. Expression and intracellular distribution of the G protein-coupled receptor 30 in rat hippocampal formation. *Neurosci. Lett.* 2008; 441:94–99. [PubMed: 18586395]
- McLaughlin KJ, Bimonte-Nelson H, Neisewander JL, Conrad CD. Assessment of estradiol influence on spatial tasks and hippocampal CA1 spines: evidence that the duration of hormone deprivation after ovariectomy compromises 17beta-estradiol effectiveness in altering CA1 spines. *Horm. Behav.* 2008; 54:386–395. [PubMed: 18579142]
- Mermelstein PG, Becker JB, Surmeier DJ. Estradiol reduces calcium currents in rat neostriatal neurons via a membrane receptor. *J. Neurosci.* 1996; 16:595–604. [PubMed: 8551343]
- Milner TA, Ayoola K, Drake CT, Herrick SP, Tabori NE, McEwen BS, Warrier S, Alves SE. Ultrastructural localization of estrogen receptor beta immunoreactivity in the rat hippocampal formation. *J. Comp. Neurol.* 2005; 491:81–95. [PubMed: 16127691]
- Milner TA, Lubbers LS, Alves SE, McEwen BS. Nuclear and extranuclear estrogen binding sites in the rat forebrain and autonomic medullary areas. *Endocrinology.* 2008; 149:3306–3312. [PubMed: 18356271]
- Milner TA, McEwen BS, Hayashi S, Li CJ, Reagan LP, Alves SE. Ultrastructural evidence that hippocampal alpha estrogen receptors are located at extranuclear sites. *J. Comp. Neurol.* 2001; 429:355–371. [PubMed: 11116225]
- Mitra SW, Hoskin E, Yudkovitz J, Pear L, Wilkinson HA, Hayashi S, Pfaff DW, Ogawa S, Rohrer SP, Schaeffer JM, McEwen BS, Alves SE. Immunolocalization of estrogen receptor beta in the mouse brain: comparison with estrogen receptor alpha. *Endocrinology.* 2003; 144:2055–2067. [PubMed: 12697714]
- Monteiro SC, de Mattos CB, Ben J, Netto CA, Wyse ATS. Ovariectomy impairs spatial memory: prevention and reversal by a soy isoflavone diet. *Metab Brain Dis.* 2008; 23:243–253. [PubMed: 18648916]
- Morissette M, Le Saux M, D'Astous M, Jourdain S, Al Sweidi S, Morin N, Estrada-Camarena E, Mendez P, Garcia-Segura LM, Di Paolo T. Contribution of estrogen receptors alpha and beta to the effects of estradiol in the brain. *J. Steroid Biochem. Mol. Biol.* 2008; 108:327–338. [PubMed: 17936613]
- Neese SL, Wang VC, Doerge DR, Woodling KA, Andrade JE, Helferich WG, Korol DL, Schantz SL. Impact of dietary genistein and aging on executive function in rats. *Neurotoxicol. Teratol.* 2010; 32:200–211. [PubMed: 19945528]
- Neese SL, Bandara SB, Doerge DR, Helferich WG, Korol DL, Schantz SL. Effects of multiple daily genistein treatments on executive function in middle-aged rats. *Neurotoxicol. Teratol.* 2012; 34:187–195. [PubMed: 21945133]
- Owens W, Ashby J, Odum J, Onyon L. The OECD program to validate the rat uterotrophic bioassay. Phase 2: dietary phytoestrogens analyses. *Environ. Health Perspect.* 2003; 111:1559–1567. [PubMed: 12948898]

- Packard MG. Posttraining estrogen and memory modulation. *Horm. Behav.* 1998; 34:126–139. [PubMed: 9799623]
- Packard MG, Teather LA. Posttraining estradiol injections enhance memory in ovariectomized rats: cholinergic blockade and synergism. *Neurobiol. Learn. Mem.* 1997; 68:172–188. [PubMed: 9322259]
- Pan X, Li Z, Yeung V, Xu R. Dietary supplementation of soy germ phytoestrogens or estradiol improves spatial memory performance and increases gene expression of BDNF, TrkB receptor and synaptic factors in ovariectomized rats. *Nutr Metab (Lond)*. 2010; 7:75. [PubMed: 20843342]
- Pan Y, Anthony M, Watson S, Clarkson TB. Soy phytoestrogens improve radial arm maze performance in ovariectomized retired breeder rats and do not attenuate benefits of 17beta-estradiol treatment. *Menopause*. 2000; 7:230–235. [PubMed: 10914615]
- Patisaul HB, Adewale HB. Long-term effects of environmental endocrine disruptors on reproductive physiology and behavior. *Front. Behav. Neurosci.* 2009; 3:10. [PubMed: 19587848]
- Quinlan MG, Hussain D, Brake WG. Use of cognitive strategies in rats: the role of estradiol and its interaction with dopamine. *Horm. Behav.* 2008; 53:185–191. [PubMed: 17976601]
- Roy EJ, Buyer DR, Licari VA. Estradiol in the striatum: effects on behavior and dopamine receptors but no evidence for membrane steroid receptors. *Brain Res. Bull.* 1990; 25:221–227. [PubMed: 2224537]
- Shughrue PJ, Lane MV, Merchenthaler I. Comparative distribution of estrogen receptor-alpha and -beta mRNA in the rat central nervous system. *J. Comp. Neurol.* 1997; 388:507–525. [PubMed: 9388012]
- Shughrue PJ, Merchenthaler I. Distribution of estrogen receptor beta immunoreactivity in the rat central nervous system. *J. Comp. Neurol.* 2001; 436:64–81. [PubMed: 11413547]
- Stanczyk FZ, Cho MM, Endres DB, Morrison JL, Patel S, Paulson RJ. Limitations of direct estradiol and testosterone immunoassay kits. *Steroids*. 2003; 68:1173–1178. [PubMed: 14643879]
- Ström JO, Theodorsson A, Theodorsson O. Substantial discrepancies in 17beta-oestradiol concentrations obtained with three different commercial direct radioimmunoassay kits in rat sera. *Scand. J. Clin. Lab Invest.* 2008; 68:806–813. [PubMed: 18622802]
- Thomas P, Dong J. Binding and activation of the seven-transmembrane estrogen receptor GPR30 by environmental estrogens: a potential novel mechanism of endocrine disruption. *J. Steroid Biochem. Mol. Biochem.* 2006; 102:175–179.
- Tsai TH. Concurrent measurement of unbound genistein in the blood, brain and bile of anesthetized rats using microdialysis and its pharmacokinetic application. *J. Chromatogr. A*. 2005; 1073:317–322. [PubMed: 15909536]
- Twaddle NC, Churchwell MI, Doerge DR. High throughput quantification of soy isoflavones in human and mouse plasma using liquid chromatography with electrospray mass spectrometry and tandem mass spectrometry detection. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2002; 777:139–145.
- Wang VC, Neese SL, Korol DL, Schantz SL. Chronic estradiol replacement impairs performance on an operant delayed spatial alternation task in young, middle-aged, and old rats. *Horm. Behav.* 2009; 56:382–390. [PubMed: 19631212]
- Wang VC, Sable HJ, Ju YH, Allred CD, Helferich WG, Korol DL, Schantz SL. Effects of chronic estradiol treatment on delayed spatial alternation and differential reinforcement of low rates of responding. *Behav. Neurosci.* 2008; 122:794–804. [PubMed: 18729633]
- Whitten PL, Patisaul HB. Cross-species and interassay comparisons of phytoestrogen action. *Environ. Health Perspect.* 2001; 109(Suppl 1):5–20. [PubMed: 11250801]
- Wise PM, Dubal DB, Rau SW, Brown CM, Suzuki S. Are estrogens protective or risk factors in brain injury and neurodegeneration? Reevaluation after the Women's health initiative. *Endocr. Rev.* 2005; 26:308–312. [PubMed: 15851820]
- Xu J, Zhu J, Shi C, Guo K, Yew DT. Effects of genistein on hippocampal neurodegeneration of ovariectomized rats. *J Mol Neurosci.* 2007; 31:101–112. [PubMed: 17478884]
- Zhao L, Brinton RD. WHI and WHIMS follow-up and human studies of soy isoflavones on cognition. *Expert Rev Neurother.* 2007; 7:1549–1564. [PubMed: 17997703]

- Zurkovsky L, Brown SL, Boyd SE, Fell JA, Korol DL. Estrogen modulates learning in female rats by acting directly at distinct memory systems. *Neuroscience*. 2007; 144:26–37. [PubMed: 17052857]
- Zurkovsky L, Brown SL, Korol DL. Estrogen modulates place learning through estrogen receptors in the hippocampus. *Neurobiol. Learn. Mem.* 2006; 86:336–343. [PubMed: 16979357]
- Zurkovsky L, Serio SJ, Korol DL. Intra-striatal estradiol in female rats impairs response learning within two hours of treatment. *Horm. Behav.* 2011; 60:470–477. [PubMed: 21820439]

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Research Highlights

- Tested acute genistein effects on place and response learning in female rats
- Estradiol produced an inverted dose-response for enhancement of place learning.
- Genistein enhanced place learning but impaired response learning.
- Genistein treatment mimics estradiol-induced shifts in place and response learning.

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Fig. 1.

A) Treatment schedule for subcutaneous estradiol benzoate injections. B) Treatment schedule for oral genistein dosing. Graphic representations of the C) place and D) response learning tasks. In the place task, rats must use extra-maze cues to learn to reach the static goal arm. For the response task, extra-maze visual cues are absent and the rat must learn to make an egocentric body turn to reach the food reward.

Fig. 2.

A) Uterine horn wet weights (mg/cm) following 17β-estradiol benzoate treatment. Uterine horns proliferated in a dose-dependent manner. B) Serum estradiol levels (pg/mL) were determined via RIA. Treatment significantly elevated serum estradiol concentrations. C) Uterine horn wet weight in genistein- and sucrose-treated rats. Genistein had no uterotrophic effect. D) Serum genistein concentrations (ng/mL) as determined by LC/MS. Genistein treatment greatly elevated serum levels. E) Serum genistein levels in response to multiple dosings. Light/dark cycle is indicated by the open/shaded bars below the time axis. Blood samples were drawn just prior to and 30 mins following the dosing schedule outlined in Fig. 1B. There was a significant effect of treatment on serum genistein concentrations. Different lower case letters represent significant differences between groups 30 min following the second genistein dose of each day, $p < 0.0003$.

Fig. 3.

Place learning results. A) Trials to criterion data (9 out of 10, at least 6 consecutively correct) for oil ($n = 11$), Lo-EB ($n = 12$) and Hi-EB ($n = 11$) treated rats. Trials to criterion were lower following treatment with $4.5 \mu\text{g/kg}$ but not $45 \mu\text{g/kg}$ EB producing an inverted pattern of neuromodulation. B) Trials to criterion for sucrose ($n = 8$) and genistein ($n = 8$) treated rats. Genistein treatment facilitated place learning, similar to the effect of low EB in Fig. 3A. C). Acquisition curves for oil- and EB-treated rats. D). Acquisition curves for sucrose- and genistein-treated rats. There was a significant interaction between genistein treatment and task acquisition. Different lower case letters represent significant differences between groups, $p < 0.02$.

Fig. 4.

Response learning results. A) Trials to criterion for oil ($n = 10$), Lo-EB ($n = 7$) and Hi-EB ($n = 6$) treated rats. Treatment with both $4.5 \mu\text{g/kg}$ and $45 \mu\text{g/kg}$ EB impaired response learning as rats required more trials to reach criterion. B) Trials to criterion data for rats treated with sucrose ($n = 9$) and genistein ($n = 9$). Genistein treatment impaired response learning similar to effects observed with EB treatment. C) Acquisition curves for oil- and EB-treated rats. Rats treated with EB acquired the task more slowly than did oil controls. D) Acquisition curves for sucrose- and genistein-treated rats. Genistein treatment impaired performance on the response task, as rats took longer to reach high levels of accuracy. Different lower case letters represent significant differences between groups, $p < 0.004$.