Estradiol Alters Fos-Immunoreactivity in the Hippocampus and Dorsal Striatum during Place and Response Learning in Middle-Aged But Not Young Adult Female Rats

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Evidence from lesion and inactivation studies suggests that the hippocampus (HPC) and dorsal striatum compete for control over navigation behavior, and there is some evidence in males that the structure with greater relative activation controls behavior. Estradiol has been shown to enhance HPC-dependent place learning and impair dorsal striatum-dependent response learning in female rats, possibly by increasing hippocampal activation and/or decreasing striatal activation. We used Fos-immunoreactivity (Fos-IR) to examine the activation of several subregions of the HPC and striatum in ovariectomized female rats with or without estradiol replacement 30 min after place or response learning. In 4-month-old rats, neither task nor estradiol increased Fos-IR above explore control levels in any subregion analyzed, even though estradiol impaired response learning. In 12-month-old rats, estradiol increased Fos-IR in the dentate gyrus, dorsal medial striatum, and dorsal lateral striatum in place task learners, while the absence of estradiol increased Fos-IR in these regions in response task learners. However, learning rate was not affected by estradiol in either task. We also included a group of long-term ovariectomized 12-month-old rats that displayed impaired place learning and altered Fos-IR in CA1 of the HPC. These results suggest that task-specific effects of estradiol on hippocampal and striatal activation emerge across age but that relative hippocampal and striatal activation are not related to learning rate during spatial navigation learning. (Endocrinology 152: 946–956, 2011)

Lesions or inactivation of the dorsal hippocampus (HPC) impair place learning and in some cases enhance response learning, while lesions or inactivation of the dorsal striatum (DS) impair response learning but sometimes enhance place learning (1–4). These findings suggest that the HPC and DS may compete for behavioral control and the neural system with greater relative output guides spatial navigation (5). Estradiol has been shown to bias rats to use a place strategy on navigation tasks that can be solved using either strategy, and it can enhance explicit place learning and impair explicit response learning (e.g., Ref. 6). Evidence that these effects of estradiol occur locally within the HPC and DS (7, 8) suggests that estradiol may modulate navigation learning by altering the engagement of hippocampal and/or dorsal striatal ensembles at the onset or during navigation learning, however this hypothesis has never been assessed.

The immediate-early gene c-fos has been used to measure neural activation during other hormonally-modulated behaviors, including aggression, sex, and maternal behaviors (9–13), revealing that estradiol may promote behavioral responses by enhancing activation in relevant neural regions (13). c-fos has also been used to examine the relative activation of the HPC and DS during place and response learning in males (14–16). However, while one study found that activation in the DS but not HPC distin-
guished response from place learners (14), two other studies found that activation in hippocampal but not dorsal striatal subregions was greater in rats that used a place strategy to solve a navigation task than those that used a response strategy (15, 16). Despite the varied results, these data suggest that differential activation within subregions of the HPC and DS occurs during place and response navigation.

While estradiol appears to be a strong modulator of navigation behavior in the young adult female, its effects during middle age, as cycling ovarian hormones decline, remain unclear (17, 18). While some studies have shown that estradiol improves place navigation in middle-aged females (19–24), others have shown estradiol to be ineffective at this age (25, 26). The DS may also lose responsiveness to estradiol as rats age (e.g., Ref. 27). In addition, long-term ovarian hormone deprivation has been shown to be detrimental to general cognition and hippocampal function in particular (28, 29; see also Ref. 30). Thus, the effects of estradiol and long-term hormone deprivation on the function of the HPC and DS in middle-aged females during spatial navigation need further examination.

The current study had several goals: 1) to determine whether the HPC and DS show different activation patterns when female rats are engaged in place vs. response learning, 2) to examine whether estradiol’s effects on place and response learning are reflected in changes in hippocampal and striatal activation, and 3) to determine whether aging and long-term estradiol deprivation influence hippocampal and striatal activation during navigation learning.

**Materials and Methods**

**Subjects**

Subjects were 4- and 12-month-old female Sprague Dawley CD strain rats purchased from Charles Rivers Laboratories (Kingston, NY) at 3 months of age (n = 90). They were pair-housed in individually-ventilated cages and given ad libitum access to water and a standard diet (Rodent Diet 5001, PMI Nutrition International, Inc., Brentwood, MO). The temperature-controlled colony room was maintained on a 12:12 h light:dark cycle with lights on at 0700 daily, and all behavioral training took place during the lights-on phase of the day. Rats were handled daily for 10 d before ovariectomy and then an additional 7 d before behavioral testing. All procedures were approved by and conducted in accordance with the Institutional Animal Care and Use Committee of Duke University.

**Experimental design**

The current study used rats in three hormonal conditions, as shown in Fig. 1: A) Rats ovariectomized (OVX) at 3 mo and behaviorally trained at 4 mo (n = 46); B) Rats OVX at 11 mo and behaviorally trained at 12 mo (n = 26); and C) Rats OVX at 3 mo and behaviorally trained at 12 mo (n = 18). Short-term OVX rats (groups A and B) received either two sc injections of 10 μg 17β-estradiol (Sigma-Aldrich, St. Louis, MO) dissolved in 0.1 ml sesame oil (Sigma-Aldrich, St. Louis, MO) or the oil vehicle alone 48 and 24 h before behavioral training, as illustrated in Fig. 1, A and B. This hormone replacement regimen has been used previously to demonstrate estradiol’s modulation of place and response learning (6) and has been shown to increase a number of mechanisms of hippocampal plasticity (31, 32). All long-term OVX rats received oil injections (Fig. 1C). Thirty minutes after place or response training or control trials, rats were perfused transcardially and their brains harvested for quantification of Fos-immunoreactivity (Fos-IR) in several hippocampal and dor-

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**FIG. 1.** Experimental timeline of hormonal manipulations and behavioral training for rats trained at 4 months of age (A) and 12 months of age (B and C), and timeline of behavioral training, probe testing, and sacrifice (D) and illustration of samples taken from left hemisphere of DLS and DMS (E, **left**) and DG, hilus, CA1, and CA3 of the HPC (E, **right**).
sal striatal subregions, as delineated in Fig. 1. This sacrifice time point was chosen because Fos expression peaks 90–120 min after induction (24, 33–35) and all rats completed training within 60–100 min of the start of training. This time point has previously been used to reveal differences in Fos-IR between male place and response task learners (14).

Ovariectomies
To remove the source of circulating gonadal hormones, rats were anesthetized with a combination of 80 mg/kg ketamine plus 10 mg/kg xylazine and OVX via bilateral incisions through the abdomen. The ovary and ovarian fat on each side of the body were exposed, tied off, and surgically removed. The site of removal was cauterized and placed back into the abdominal cavity, and the muscle wall and skin were sutured. Antibiotic cream and buprenorphine (0.5 mg/kg, ip) were administered at the end of all surgeries and again 12 h later to prevent infection and pain, respectively. To confirm that rats were no longer cycling, vaginal samples were collected from each rat daily for 1 week before estradiol or oil injections. Cells were collected on a moistened cotton swab, rolled onto a glass slide, and examined at ×10 magnification to determine estrous cycle status based on the proportion of leukocytes, epithelial, cornified, and nucleated cells (36).

Apparatus and behavioral training
All behavioral training and probe testing took place within a single session in a black plastic pool with a diameter of 1.8 m filled with approximately 30 cm of water mixed with black water-based paint maintained at 24–25°C. The maze was located in a 6.5 m × 3.8 m rectangular room that was rich with cues including a curtain, cart with cages, metal counter, shelves, and posters with high-contrast patterns.

Rats in each hormonal condition were randomly assigned to receive training on either a place or response version of the water maze or to serve as explore controls. Cage mates were transported to the test room in individual holding cages on a cart that remained in a stable location throughout training. To habituate rats to the maze, each rat was placed in the pool and allowed to swim for 30 s. During training, rats were placed in the water at one of four locations (N, S, E, and W) at the beginning of each trial in a pseudo-random order. Each trial ended when the rat climbed onto a black escape platform with diameter 10.2 cm. If one of four locations (N, S, E, and W) at the beginning of each trial in a pseudo-random order. Each trial ended when the rat climbed onto a black escape platform with diameter 10.2 cm. If the rat failed to find the platform at the end of 60 s, the experimenter guided it to the platform where it remained for 10 s before being returned to its holding cage. Cage mates alternated trials in this fashion until each had reached the learning criterion (see below) or 40 trials were completed. Training trials were immediately followed by one probe trial in which the platform was removed and the rat was allowed to swim for 60 s. During all trials, the path of the animal was recorded in real time using HVS Image (Buckingham, WH, UK). Some rats were randomly assigned to serve as controls for motor activity and latent learning associated with exploration of the environment while swimming in the pool. Each explore control was yoked to a behaviorally-trained rat such that it swam for the same amount of time and the same number of trials.

Place task
Rats were required to find an escape platform below the surface of the water that was in the center of one quadrant of the maze throughout training by using extramaze cues (e.g., salient landmarks and room geometry). Platform locations were counterbalanced within and across experimental groups. Criterion for learning was achieved when the rat performed four consecutive trials from random start locations with latencies under 10 s. Then the rat was given one probe trial as described above.

Response task
Rats were required to find a visible escape platform when extramaze cues were hidden by a curtain drawn around the pool. The platform was always located in the quadrant either to the left or right of the random start position of the rat, so that it was always in the same relative position to the rat’s start location. Thus, rats could navigate to the platform by using a visual strategy with the platform as a beacon and/or acquiring a response strategy (e.g., go to the left). To encourage rats to acquire a response strategy, criterion for learning was reached when the rat completed four consecutive trials in which the latencies were within 10 s of each other and the rat made a similar path or turn sequence to reach the platform. A probe trial with no platform was conducted on the following trial.

Perfusion, immunohistochemistry, and quantification of Fos-IR
Rats were anesthetized with an ip injection of 80 mg/kg ketamine and 10 mg/kg xylazine and then perfused transcardially with 0.9% saline followed by 4% paraformaldehyde. Brains were removed and postfixed in 4% paraformaldehyde for 4 d and then stored in sodium azide at 4°C until sectioned. Every fifth 50-μm section of HPC and DS was collected and used for immunohistochemistry, and subjects from all experimental groups were included in each immunohistochemistry run to ensure identical labeling conditions for all groups. Tissue was washed in 30 mM PBS, incubated in 1% hydrogen peroxide to remove endogenous peroxidase, and washed in PBS. Tissue was blocked in 0.1% BSA and 0.2% TX-100 and incubated in the primary antibody solution containing 1:5000 c-Fos polyclonal rabbit IgG (Sigma, St. Louis, MO) overnight. Tissue was washed in PBS and incubated in a secondary solution of 0.1% BSA and 1:500 concentration of biotinylated antirabbit made in goat. Tissue was washed in PBS, incubated in an avidin biotin complex (Vector Laboratories, Burlingame, CA) solution with 0.1% BSA, and washed in PBS. Tissue was incubated in a 3,30-diaminobenzidine solution (Sigma) until brown, indicating that Fos-IR had occurred. Sections were washed in PBS, mounted onto glass slides, dehydrated, and coverslipped with Permount mounting medium (Fisher Scientific, Fair Lawn, NJ).

The optical fractionator method was used to estimate the number of Fos-IR cells in subregions of the HPC and DS (StereoInvestigator, MicroBrightField, Colchester, VT). Cells were only counted as Fos-IR if they were in focus in the inner 20-μm block of tissue and the solid reaction product covered at least half of the nucleus. In the dorsal lateral striatum (DLS) and dorsal medial striatum (DMS), a 150 μm × 150 μm sample was counted within a 187.5 μm × 187.5 μm grid throughout a uniform-sized square sample outlined by the experimenter, illustrated in Fig. 1E (left). In CA1, CA3, dentate gyrus (DG), and hilus of the HPC, a 120 μm × 120 μm sample was counted within a 150 μm × 150 μm grid throughout the extent of each subregion within a contour drawn by the experimenter to include principle cell layers and adjacent cell layers, illustrated in Fig. 1E (right). Three left-hemisphere sections were analyzed for each subregion of each rat at an interval of five sections, between +1.2
mm anterior to bregma and −0.2 mm posterior to bregma for the DS and between −2.8 and −4.0 mm posterior to bregma for the HPC according to Paxinos & Watson’s rat brain atlas (37). Estimated counts based on section thickness were divided by estimated volume to attain a density measure of Fos-IR for each brain subregion of each rat.

**Statistical analysis**

Similar but separate analyses were calculated for 4- and 12-month-old rats treated with oil and estradiol, as well as for short-term and long-term OVX 12-month-olds. To examine the effects of task and estradiol replacement on learning place and response tasks, the number of trials to reach behavioral criterion was examined using ANOVAs with estradiol status (oil, estradiol) and task (place, response, explore control) as independent variables. Because behavioral criteria were based on latencies, mean swim speed across the first 10 trials (before any rats reach criterion) was compared between oil- and estradiol-treated rats within each task and revealed no differences (P > 0.10), suggesting that differences in latency reported in the Results section were not attributable to differences in swim speed. t tests were also used to compare oil- and estradiol-treated rats in the number of platform crossings and percent time spent in each maze quadrant during the place task probe to determine the accuracy and precision of the platform location learned by rats trained on the place task. In the response task, rats that made a similar turn sequence on the probe trial as the last four training trials were categorized as having acquired a response strategy, while those that searched randomly for the platform during the probe were categorized as having used a visual strategy.

To examine effects of estradiol and task on hippocampal and striatal Fos-IR, we used ANOVAs with the number of Fos-IR cells per mm³ as the dependent variable and task (place, response, explore control) and estradiol treatment (oil, estradiol) as the independent variables for each subregion quantified (DG, hilus, CA1, CA3, DMS, and DLS). t tests were calculated to determine whether strategy used on the response probe affected Fos-IR, but only hippocampal analyses in 4-month-olds were significant, so they are the only ones reported in the results (all others, P > 0.15).

To compare the relative activation of striatal and hippocampal subregions between oil- and estradiol-treated rats during place and response learning, ratios between Fos-IR in different subregions of HPC and DS (DG:DMS, DG:DLS, CA1:DMS, CA1:DLS, and DMS:DLS) were compared. We also calculated correlations between subregions (DMS vs. DLS, DG vs. CA1, DG vs. DMS, DG vs. DLS, CA1 vs. DMS, and CA1 vs. DLS) to determine whether there was a linear relationship between Fos-IR in different subregions, and we examined the relationship between Fos-IR in each subregion and the number of trials to reach behavioral criterion to determine whether any effects in activation might be related to the amount of training or motor behavior rather than only task or estradiol replacement, but these correlations were not significant and so are not reported below (P > 0.05). Because explore controls used in the place and response tasks did not differ in any measure analyzed, they were combined for data analysis. All analyses described above were also used to determine whether duration of OVX in 12-month-old rats influenced these measures.

**Results**

**Effects of estradiol treatment on learning and Fos-IR in 4-month-old rats**

**Learning**

Estradiol replacement did not affect the number of training trials to reach behavioral criterion on the place task but estradiol-treated rats were impaired in the rate of response learning, revealed by a main effect of task [F (1, 14) = 6.29, P = 0.025] and an estradiol status × task interaction [F (1, 14) = 4.71, P = 0.048], but no main effect of estradiol status (P > 0.40; Fig. 2A). Direct comparisons confirmed that estradiol-treated rats required more trials to reach criterion than oil-treated rats in the response task [t (19) = 4.02, P = 0.001] but not the place task (P > 0.15). However, oil-treated rats learned the place and response tasks at the same rate (P > 0.55).

Analyses of the 60-s place task probe trial revealed that both oil- and estradiol-treated rats learned the platform location with a high level of accuracy and precision (target quadrant: oil: 54 ± 3%, estradiol: 52 ± 5%; all other quadrants: oil: <18 ± 4%, estradiol: <18 ± 4%; P > 0.80). In the response task, analysis of the path used on the probe trial revealed that a majority of oil-treated rats (90%) and estradiol-treated rats (67%)
used a response strategy to locate the target in the absence of a visible platform during the probe trial. That is, they traveled the same path on the probe trial as they did on the last four criterion trials, even though there was no visible platform. The remainder of the rats swam randomly throughout the maze on the probe trial, indicating that they had probably learned to find the platform by using it as a visual beacon, but they learned the response task at a similar rate ($P > 0.10$).

**Fos-immunoreactivity**

**Hippocampus**

Neither task nor estradiol replacement increased Fos-IR in any hippocampal subregions analyzed, as shown in Fig. 2B and Table 1 ($P > 0.20$), nor were there any interactions between the two. However, response task learners that used a response strategy on the probe trial had more Fos-IR in the DG and hilus than rats that used a visual strategy [DG: $t (17) = 2.84, P = 0.011$; hilus: $t (17) = 2.45, P = 0.025$]. In contrast, no differences in Fos-IR between these two groups were detected in CA1 or CA3 ($P > 0.10$).

**Dorsal striatum**

Overall, task did not affect Fos-IR in the DS, but estradiol-treated rats had increased Fos-IR in the DMS [F (1, 40) = 4.68, $P = 0.037$] and DLS [F (1, 40) = 10.23, $P = 0.003$]. However, there were no interactions between task and estradiol status ($P > 0.20$), suggesting that estradiol effects on Fos-IR were slight.

**Ratios and correlations between Fos-IR in hippocampal and striatal subregions**

We found a main effect of estradiol status in several of the ratios between hippocampal and striatal subregions [DG:DLS (F [1, 40] = 8.32, $P = 0.006$), CA1:DLS (F [1, 40] = 9.51, $P = 0.003$), CA1:DMS (F [1, 40] = 4.17, $P = 0.048$), and DMS:DLS (F [1, 40] = 4.61, $P = 0.038$)], but no other effects or interactions ($P > 0.10$; data not shown), suggesting that estradiol altered the relative engagement of hippocampal and striatal subregions during navigation learning. However, these differences were driven by the fact that estradiol-treated rats had increased striatal but not hippocampal activation. Fos-IR in the DMS and DLS were positively correlated for response task learners ($r = 0.791, P < 0.0001$), place task learners ($r = 0.699, P = 0.003$), and all 4-month-old rats combined ($r = 0.760, P < 0.0001$), suggesting direct communication between these two subregions. No other correlations were significant ($P > 0.15$).

**Effects of estradiol treatment on learning and Fos-immunoreactivity in 12-month-old rats**

**Learning**

Similar to 4-month-olds, estradiol replacement did not affect the number of training trials needed to reach behavioral criterion in the place task, but unlike 4-month-olds, estradiol did not impair response learning rate ($P > 0.60$). However, response task learners took significantly more trials than place task learners to reach criterion [F (1, 18) = 19.06, $P = 0.0004$; t (9) = 2.83, $P = 0.020$], as shown in Fig. 3A.

Analyses of the percent time spent in each quadrant of the maze and the number of platform location crossings during the place task probe trial revealed that both oil- and estradiol-treated rats learned the platform location with a high level of accuracy and precision (target quadrant: oil: $49 \pm 7\%$, estradiol: $47 \pm 5\%$; all other quadrants: oil: $<23 \pm 10\%$, estradiol: $<23 \pm 8\%; P > 0.30$). On the response probe trial, while a majority of oil-treated rats (67%) used a response strategy to locate the target in the absence of a visible platform, only 17% of estradiol-treated rats used a response strategy, but they learned at a similar rate ($P > 0.10$), suggesting that estradiol impaired response learning to the point where rats were unable to acquire a response strategy.

**Fos-immunoreactivity**

**Hippocampus**

Neither task nor estradiol alone affected Fos-IR in any hippocampal subregion examined ($P > 0.25$), similar to 4-month-olds. However, task and estradiol interacted to modulate Fos-IR in the DG [F (2, 20) = 6.36, $P = 0.007$] but not in other hippocampal subregions ($P > 0.15$), as shown in Fig. 3B and Table 2 and illustrated in Fig. 4. Direct comparisons revealed that estradiol-treated rats had increased Fos-IR in the DG dur-
Effects of long-term ovariectomy on learning and Fos-IR in 12-month-old rats

Learning

An ANOVA for the number of training trials to reach behavioral criterion revealed a main effect of task \([F (1, 19) = 8.33, P = 0.009]\) and a main effect of duration of OVX \([F (1, 19) = 5.93, P = 0.025]\) but no interaction \((P > 0.45)\). However, direct comparisons showed that compared with short-term OVX rats, long-term OVX rats (9 mo) were impaired in learning rate on the place task \([t (8) = 2.33, P = 0.048]\) but not the response task \((P > 0.25)\), as shown in Fig. 5A.

For the place task, analysis of the percent time spent in each quadrant of the maze and the number of platform location crossings during probe trial revealed that both short-term and long-term OVX rats learned the platform location with a high level of accuracy and precision (long-term OVX target quadrant: 43 ± 7%; all other quadrants: <24 ± 9%; \(P > 0.55\)). Path analysis from the response probe trial revealed that a majority of long-term OVX rats (67%) and short-term OVX rats (67%) used a response strategy to locate the target in the absence of a visible platform. These results suggest that long-term OVX impaired place learning rate but not the degree to which rats

Dorsal striatum

Estradiol replacement alone did not affect Fos-IR in either subregion of the DS \((P > 0.85)\). However, task affected Fos-IR in the DMS \([F (2, 20) = 4.15, P = 0.031]\), with response task learners having greater Fos-IR than explore controls \([t (6) = 3.55, P = 0.012]\) and place task learners \([t (9) = 2.38, P = 0.041]\), as illustrated in Figs. 3C and 4. However, task did not affect Fos-IR in the DLS \((P > 0.20)\). Similar to effects on Fos-IR in the DG, estradiol status and task interacted to modulate Fos-IR in the DMS \([F (2, 20) = 9.26, P = 0.001]\) and DLS \([F (2, 20) = 5.23, P = 0.015]\), as shown in Fig. 3, C and D. Estradiol-treated rats had greater striatal Fos-IR than oil-treated rats in the place task \(DMS: t (8) = 2.85, P = 0.021; DLS: t (8) = 2.43, P = 0.041\) but did not display the increase in Fos-IR observed in oil-treated rats in the response task \(DMS: t (10) = 2.95, P = 0.015; DLS: t (10) = 2.04, P = 0.069\); Fig. 3, C and D). Thus, the patterns of Fos-IR in the DMS and DLS were similar to the pattern observed in the DG but not other hippocampal subregions.

Ratios and correlations between Fos-IR in hippocampal and striatal subregions

Neither task nor estradiol replacement affected the ratios of Fos-IR between any hippocampal and striatal subregions \((P > 0.15)\). However, Fos-IR in the DG, DMS, and DLS were positively correlated with one another for all rats, as shown in Table 3, but CA1 Fos-IR was not significantly correlated with Fos-IR in any of these subregions \((P > 0.20)\). When place and response task learners were analyzed separately, similar correlations were significant for place task learners \(DMS vs. DLS, DG vs. DMS, DG vs. DLS; all others, \(P > 0.10)\), as shown in Table 3. There were fewer significant correlations for response task learners \(DMS vs. DLS, DG vs. DMS; all others, \(P > 0.10)\), but similar patterns of and correlations between Fos-IR across tasks suggest that activation of these three subregions are related during navigation, regardless of the strategy used.
learned the platform location, and it did not affect any aspect of response learning.

**Fos-immunoreactivity**

**Hippocampus**

Long-term OVX explore controls had significantly less Fos-IR than short-term OVX rats in CA1 ($P < 0.05$) but not other hippocampal subregions ($P > 0.45$), as shown in Fig. 5B. Long-term OVX response task learners had less DG Fos-IR than short-term OVX response task learners [$F (2, 25) = 3.43, P = 0.048$; $t (10) = 3.07, P = 0.012$], but short-term OVX and long-term OVX rats had similar Fos-IR in the place task and in the control condition ($P > 0.70$). In CA1, there was a main effect of duration of OVX [$F (1, 25) = 6.69, P = 0.016$] and a trend of task ($P = 0.075$), but no interaction ($P > 0.15$; Fig. 5B). While response and control long-term OVX rats had less Fos-IR than short-term OVX rats [$t (10) = 3.70, P = 0.004$; $t (6) = 2.39, P = 0.05$, respectively], place task learners did not ($P > 0.95$), as shown in Fig. 1. These results suggest that long-term OVX rats that learned the place task increased CA1 activation up to short-term OVX levels to learn the place task. All other ANOVAs revealed no effects or interactions ($P > 0.10$).

**Dorsal striatum**

There were no effects of task or duration of OVX on DMS Fos-IR ($P > 0.05$). In the DLS, there was a main effect of task [$F (2, 25) = 4.20, P = 0.027$] and OVX duration [$F (1, 25) = 5.54, P = 0.027$] but no interaction ($P > 0.80$). However, no direct comparisons were significant ($P > 0.05$).

**Ratios and correlations between Fos-IR in hippocampal and striatal subregions**

Neither task nor long-term OVX affected the relative amount of activation in the hippocampal and striatal subregions examined. While several ANOVAs revealed main effects of either task or duration of OVX (DG:DLS, DMS:DLS, CA1:DLS), there were no significant interactions in any analyses ($P > 0.05$), and no direct comparisons revealed significant differences ($P > 0.15$). Similar to other groups, Fos-IR in the DG, DMS, and DLS were correlated with one another. In addition, Fos-IR in the DG and CA1 were also positively correlated (data not shown). However, CA1 was not correlated with either the DMS or DLS.

**TABLE 3. Correlations between hippocampal and striatal Fos-IR in 12-month-old short-term OVX rats**

<table>
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<th>Place</th>
<th>Response</th>
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<tr>
<td>DMS vs. DLS</td>
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<tr>
<td>CA1 vs. DLS</td>
<td>0.255</td>
<td>0.299</td>
<td>0.135</td>
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*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$.

**FIG. 4.** Examples of Fos-IR in the DMS of short-term OVX 12-month-old rats (top six panels) administered oil (left column) or estradiol (right column) that learned place (first row) or response (second row) tasks or served as explore controls (third row). Bottom two panels are representative photomicrographs from the DG.
When place and response task learners were analyzed separately, similar correlations were significant or nearly significant for response task learners (DMS vs. DLS, DG vs. DMS, DG vs. DLS, DG vs. CA1; all others, \( P > 0.10 \)). There were few significant correlations for place task learners (DG vs. CA1, DG vs. DMS; all others, \( P > 0.25 \)). These results suggest that as in other groups, activation in the DG, DMS, and DLS were related, but additionally, CA1 activation corresponded with these subregions.

**Discussion**

The current results suggest a complex interaction between hippocampal and striatal subregions activated during place and response learning that is influenced by aging and is highly modulated by estradiol. However, our data do not support the hypothesis that the HPC and DS compete for control over navigation behavior via selective activation in females as has been suggested for males (5), nor that activation in the HPC and DS are directly related to ease of learning a response task. In the HPC, estradiol administration increased DG Fos-IR in 12-month-old place task learners but did not alter learning. While these results suggest that learning rate is not related to hippocampal activation, there was some evidence that successful place learning required CA1 activation to be at threshold. Long-term OVX rats had less CA1 Fos-IR than short-term OVX rats in all groups but place task learners, suggesting that long-term OVX rats had to increase CA1 activation to reach the threshold of activation required to learn the place task.

**Estradiol affects learning and Fos-IR differently in 4- and 12-month-olds**

Estradiol has been shown to increase baseline levels of neural activation in the HPC and DS (e.g., Refs. 39, 40), but in this study estradiol did not increase neural activation in explore controls that simply swam in the maze. In behaviorally-trained rats, estradiol replacement and task interacted to modulate activation in the DG, DLS, and DMS beyond explore control levels in 12-month but not 4-month-olds. One possible reason for this age-related difference is that the level of neural activation that occurred during swimming/exploring alone may have already been

![FIG. 5. The mean number of trials to reach behavioral criterion in the place and response tasks (A) and Fos-IR in CA1 (B) of short-term and long-term OVX 12-month-olds. *, \( P < 0.05 \)].

Estradiol’s effects on Fos-IR required rats to be engaged in navigation task, because estradiol did not alter activation in any hippocampal or striatal subregions in rats that simply explored the pool. In contrast to some previous published findings (e.g., Refs. 6, 19, 22), estradiol replacement did not improve place learning at either age, however mild stress from exposure to the water may have promoted rapid learning and masked an effect of estradiol. Consistent with previous work (e.g., 6), estradiol impaired response learning in young adult females. Further, we showed for the first time that aging impairs response learning in females and that estradiol exacerbates this impairment in middle age. These results suggest that under some conditions, estradiol modulates both learning and neural activation in females during spatial navigation, but these are not directly related.
sufficient to support learning in 4-month-olds but not in 12-month-olds. Thus, estradiol modulation of neural activation during training may have functioned to enhance exploration-induced activation to ensure quick learning in 12-month-olds that may have found the task more difficult than 4-month-olds. This proposed role of estradiol is similar to its known function in mediating lordosis in the female rodent, in which strong tactile stimulation can elicit lordosis, but estradiol (and progesterone) sensitizes the neural circuit so that gentle flank stimulation is sufficient to elicit lordosis (41). Alternatively, while our sacrifice time point was chosen to capture peak Fos-IR induced by the learning phase of the task, we may have actually observed the neural correlate for later memory. Estradiol administration aids in the consolidation of spatial memory in an age-dependent manner (42–45), so it is possible that the Fos-IR pattern we observed would have been more reflective of later success for memory of the platform location.

Functions of and coordination between hippocampal and striatal subregions during navigation learning

Together, our results suggest somewhat different functions of hippocampal and striatal subregions during navigation in females than what has previously been reported in males. While we found that DLS activation is higher in response task learners than other groups (like 14), we found a more robust effect in the DMS than the DLS, possibly because our training required subjects to form a representation of the task that included the outcome (46, 47), and we attempted to capture peak Fos-IR during the learning phase of the task (as in 14). However, this pattern of activation in the DS was also observed in the DG, suggesting that Fos-IR measured here may reflect the similar sensory inputs received by the DG and DS from the entorhinal cortex (48–50) rather than similar functions of all three subregions. Therefore, these results do not suggest that the HPC and DS compete for control during navigation learning via selective activation (15, 16). These differences highlight the importance of task and sacrifice time point on activation of the HPC and DS, and they suggest that there may be marked sex differences in the roles of hippocampal and striatal subregions during navigation learning, with females highly tuned to the effects of estradiol for these functions.

Measuring activation vs. plasticity

Results from the current and previous studies indicate that both the HPC and DS are engaged during navigation tasks, but our results suggest that there may be a different relationship between engaged brain regions, estradiol, and behavioral control during spatial navigation than simple neural activation. Evidence that estradiol increases basal forebrain cholinergic input to the HPC (e.g., 51) and that cholinergic transmission in the HPC and DS is correlated with strategy use (e.g., 52, 53) suggests that estradiol may influence the relative engagement of the HPC and DS via differential cholinergic activity in these regions. Alternatively, control over navigation behavior may better indexed by measures of plasticity. For example, the same estradiol administration paradigm used in this and other studies (e.g., 6) has also been shown to increase a number of NMDA receptor-dependent mechanisms of plasticity in the HPC (31, 32, 54–58) that have been correlated with performance on hippocampal-dependent tasks (59, 60). Further, estradiol decreases striatal NMDA receptor binding (61) and enhances the impairment in response learning caused by blocking dopamine receptors (7), which are required for several forms of striatal plasticity (62, 63). Thus, estradiol’s modulation of hippocampal and striatal plasticity may be a potential mechanism by which it influences navigation strategy use.

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