



Published in final edited form as:

Horm Behav. 2009 September ; 56(3): 309–314. doi:10.1016/j.yhbeh.2009.06.008.

Chronic treatment with estrogen receptor agonists restores acquisition of a spatial learning task in young ovariectomized rats

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Abstract

Previous work has shown that continuous estradiol replacement in young ovariectomized rats enhances acquisition of a delayed matching-to-position (DMP) T-maze task over that of ovariectomized controls. The mechanism by which estradiol confers this benefit has not been fully elucidated. This study examined the role of selective estrogen receptor agonists of ER α , ER β , and GPR30 in the enhancement of spatial learning on a DMP task by comparing continuous estradiol replacement with continuous administration of PPT (an agonist of ER α), DPN (an agonist of ER β), or G-1 (an agonist of GPR30) relative to gonadally intact and ovariectomized vehicle-treated controls. It was found that ovariectomy impaired acquisition on this task, whereas all ER selective agonists restored the rate of acquisition to that of gonadally intact controls. These data suggest that estradiol can work through any of several estrogen receptors to enhance the rate of acquisition on this task.

Keywords

GPR30; estradiol; G-1; PPT; DPN; T-maze; working memory

Introduction

Animal studies have shown that estradiol can have beneficial effects on a variety of cognitive tasks and can even prevent age-related cognitive decline (Daniel, 2006; Dohanich, 2002; Gibbs and Gabor, 2003). Our own studies have shown repeatedly that estradiol has significant positive effects on acquisition of a delayed matching-to-position (DMP) T-maze task in young and middle-aged rats (Gibbs, 2000; Gibbs, 2007; Gibbs, Gabor, Cox, and Johnson, 2004; Gibbs, Mauk, Nelson, and Johnson, 2009). The DMP task is a spatial learning task in which rats are rewarded for returning to the same arm of the maze visited on the immediately preceding trial. The mechanisms that underlie the effect on the DMP task as well as other tasks remain unclear, although in some cases specific neural circuits have been implicated (Daniel, Hulst, and Lee, 2005; Fernandez, Lewis, Pechenino, Harburger, Orr, Gresack, Schafe, and Frick, 2008; Gibbs, 2007; Packard and Teather, 1997).

Estradiol exerts its effects by binding to specific receptors. Two nuclear receptors have been identified, ER α and ER β (Kuiper, Enmark, Peltö-Huikko, Nilsson, and Gustafsson, 1996;

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DISCLOSURE STATEMENT: The authors have nothing to disclose.

Toran-Allerand, 2004). These receptors are part of a large superfamily of nuclear receptors that regulate gene transcription, but which also can associate with specific membrane compartments and activate specific second messenger signaling pathways such as MAPK, CamKII, and CREB (Manavathi and Kumar, 2006; McEwen, 2002). Both receptors are expressed in the brain in a variety of isoforms, studied mainly at the mRNA level (Lewandowski, Kalita, and Kaczmarek, 2002; Pfeffer, 1996; Pfeffer, Fecarotta, Arena, Forlani, and Vidali, 1996). In the adult brain, ER α is highly expressed in areas of the hypothalamus that are responsible for reproduction (Ogawa, Eng, Taylor, Lubahn, Korach, and Pfaff, 1998), as well as in areas involved in learning, memory, emotionality, and attention such as the hippocampus and amygdala (Fugger, Foster, Gustafsson, and Rissman, 2000; Osterlund, Keller, and Hurd, 2000). ER β is more widely distributed throughout the adult brain and, in addition to the hypothalamus, hippocampus, and amygdala, is expressed in many regions of the neocortex (Li, Schwartz, and Rissman, 1997; Osterlund, Kuiper, Gustafsson, and Hurd, 1998b; Shughrue, Lane, and Merchenthaler, 1997).

It is assumed that binding to specific estrogen receptors results in the activation of neural circuits, which in turn enhance performance on specific cognitive tasks. Evidence that selective activation of ER α and/or ER β can affect specific tasks has been reported (Rissman, 2008). For example, Walf et al. (Walf, Rhodes, and Frye, 2006) reported that PPT (an ER α -selective agonist) as well as DPN (an ER β -selective agonist) were effective at enhancing object recognition memory in rats. Frye et al. (Frye, Duffy, and Walf, 2007), reported that PPT, but not DPN, enhances performance on a placement recognition task in rats. In contrast, Rhodes and Frye (Rhodes and Frye, 2006) reported that while DPN was effective at enhancing both inhibitory avoidance memory and place memory in rats, PPT was not. Several studies have shown that activity at ER β can reduce measures of anxiety (Bodo and Rissman, 2006; Day, Sung, Logue, Bowlby, and Arias, 2005; Walf and Frye, 2006), and some of the cognitive tasks that have been used (e.g., inhibitory avoidance, contextual fear conditioning, water maze tasks) include a significant acute anxiety or stress-related component, which may account for some of the estrogen-mediated effects described.

Fernandez et al. (Fernandez et al., 2008) recently reported that estradiol enhances object recognition memory in mice via a mechanism that requires activation of pERK in the dorsal hippocampus, and that the effect is mediated by membrane-bound receptors. Evidence that ER α and ER β can mediate rapid signaling at the cell membrane has been described (Kelly and Levin, 2001), and it has been suggested that the effects of estradiol involve both membrane and intracellular ER actions, which can potentiate each other (Vasudevan and Pfaff, 2007). Recently, a novel G-protein coupled receptor was described which is capable of mediating rapid signaling events in response to estradiol in a number of cell lines (Prossnitz, Arterburn, Smith, Oprea, Sklar, and Hathaway, 2008; Thomas, Pang, Filardo, and Dong, 2005). This receptor, referred to as GPR30, is a member of the seven transmembrane G-protein coupled receptor family, and is both genetically and structurally unrelated to ER α or ER β . In addition to mediating estrogen signaling in various cell lines, GPR30 is expressed in brain, including areas of the brain that play an important role in learning and memory processes such as the hippocampus and frontal cortex (Brailoiu, Dun, Brailoiu, Mizuo, Sklar, Oprea, Prossnitz, and Dun, 2007). Our recent analysis also suggests that GPR30 is expressed by the majority of cholinergic neurons in the medial septum and nucleus basalis magnocellularis (Hammond et al., unpublished observations). These neurons are the source of cholinergic inputs to the hippocampus and frontal cortex, and have been shown to play an important role in learning and memory processes (Baxter and Chiba, 1999; Everitt and Robbins, 1997). These neurons also play an important role in estrogen-mediated effects on DMP acquisition (Gibbs, 2002; Gibbs, 2007). Note that a subset of these cholinergic neurons also express ER α (Mufson, Cai, Jaffar, Chen, Stebbins, Sendera, and Kordower, 1999; Shughrue, Scrimo, and Merchenthaler, 2000), but do not appear to express ER β (Shughrue et al., 2000). A selective GPR30 agonist

(G-1) has been developed which binds with high affinity to GPR30, activates GPR30-mediated signaling in specific cell lines, and has no appreciable binding to ER α or ER β (Bologa, Revankar, Young, Edwards, Arterburn, Kiselyov, Parker, Tkachenko, Savchuck, Sklar, Oprea, and Prossnitz, 2006). Effects of this agonist on cognitive performance have not yet been reported. In the present study, our goal was to use the GPR30-selective agonist G-1, as well as agonists that are selective for ER α and ER β , to identify which estrogen receptors are involved in mediating estrogen effects on DMP acquisition.

Materials and Methods

Animals

A total of sixty-eight 3–4 month old female Sprague-Dawley rats (300–350 g) were purchased from Hilltop Laboratories. Rats were ovariectomized (with the exception of the gonadally intact group) prior to delivery and individually housed on a 12-hour day/night cycle with food and water available ad libitum. All procedures were carried out in accordance with PHS policies on the use of animals in research, and with the approval of the University of Pittsburgh's Institutional Animal Care and Use Committee.

Treatments

Two weeks prior to behavioral training, animals were handled daily, food restricted, and maintained at 85% of normal body weight during acquisition and testing. Rats were administered estradiol (Sigma Chemicals; St Louis, MO), G-1 (Calbiochem; La Jolla, CA), DPN (Tocris Cookson; Ellisville, MO), PPT (Tocris Cookson; Ellisville, MO), or vehicle by Alzet model 2006 mini-osmotic pumps implanted s.c. in the dorsal neck region. Gonadally intact rats received sham surgeries. The pumps delivered a volume of 0.15 μ l per hour over 42 days. G-1 has been shown to be an agonist specific to GPR30 – competitive binding studies showed that G-1 did not compete with estradiol binding in COS7 cells transfected with either ER α or ER β , whereas as it did compete with binding in COS7 cells transfected with GPR30 (Bologa et al., 2006). The ER α agonist used was propylpyrazole triol (PPT), a triarylpyrazole found to be approximately 410-fold more selective for ER α than ER β (Kraichely, Sun, Katzenellenbogen, and Katzenellenbogen, 2000; Stauffer, Coletta, Tedesco, Nishiguchi, Carlson, Sun, Katzenellenbogen, and Katzenellenbogen, 2000). The ER β agonist used was diarylpropionitrile (DPN), a compound with 70-fold higher relative binding affinity and 170-fold higher relative potency for ER β over ER α (Meyers, Sun, Carlson, Marriner, Katzenellenbogen, and Katzenellenbogen, 2001).

Estradiol, DPN, and PPT treatments were administered at a rate of 5 μ g/day at concentrations of 1.39 mg/ml in vehicles consisting of 13.9% DMSO + 20% hydroxypropyl- β -cyclodextrin (HP β CD). G-1 treatments were also administered at a rate of 5 μ g/day at a concentration of 1.39 mg/ml in a vehicle 33.7% DMSO+ 13.3% HP β CD. Previous literature was used to select doses of estradiol, PPT, and DPN (Gibbs et al., 2004; Walf, Rhodes, and Frye, 2004), but because no previous research has been published using G-1, the dose was chosen to be similar based on molecular weight. Vehicle controls contained either one of the vehicles listed above minus any drug treatment.

Behavior

The DMP task is a spatial learning and memory T-maze task. The T-maze consists of an approach alley (4 in. wide \times 14 in. long) and two goal arms (4 in. wide \times 12 in. long). The walls of the maze are 5 in. high, and the doors are constructed of clear plexiglass, thus allowing animals to view the surrounding room. Sliding doors are positioned 8 in. down the approach alley and at the entrance to each goal arm.

Behavior training was performed as previously described (Gibbs, 1999). Animals were first adapted to the maze by placing them in the maze with food (formula 5TUM 45 mg pellets from Test Diets, Inc.) for 5 days. Starting on day 6 through 9 animals were trained to run to the ends of the goal arms by using a series of forced choices and rewarding with four pellets. Right and left arms were alternated to avoid introducing a side bias. Next, animals began DMP testing, which was performed as 8 trial pairs per day. The first trial of each pair consisted of a forced choice in which one arm was blocked, forcing the animal to enter the unblocked arm to receive the food reward. The animal was then returned to the approach alley for the second trial in which both goal arms were open. A choice was defined as the animal placing both front legs and part of both rear legs into a goal arm. Returning to the same arm as the forced choice trial resulted in a food reward, while entering the incorrect arm resulted in no food reward and confinement for 10 seconds. Forced choices were randomized and balanced to avoid introducing a side bias. Animals were run in squads of 4 to 6. After each trial pair, the animal was returned to its home cage for 5–10 min while other animals were tested. Animals received 8 trial pairs per day until they reached the criterion of at least 15/16 correct choices over two days.

After reaching criterion, animals received a probe trial during which the T-maze was rotated 180° (relative to extramaze cues) between the forced and open trial. This was done to assess whether rats were using a place strategy (relying on extramaze cues) or a response strategy (independent of extramaze cues) to perform the task. Rats using a place strategy would be impaired by maze rotation, whereas rats using a response strategy would be unaffected. For analysis purposes, rats were given a score of 1 if they entered the same goal arm during the open choice and a score of 0 if they entered the opposite arm. After the probe trial, animals received 8 trial pairs per day for 4 days with increased intertrial delays (10, 30, 60, 90 seconds on each of the 4 consecutive days).

Following training, animals were given an overdose of ketamine (40 mg/kg) and xylazine (28 mg/kg) injected i.p. and euthanized by decapitation. Trunk blood was collected for the determination of serum estradiol and luteinizing hormone (LH) levels. For estradiol analysis, samples were extracted with ether and resuspended in buffer. Samples were then analyzed in duplicate by RIA using a kit from Diagnostic Systems Laboratories, Inc. (Webster, TX). All samples were analyzed in one assay. The estradiol assay was performed by the assay core of the Center for Reproductive Physiology at the University of Pittsburgh. The minimum detectable dose was 1.2 pg/ml, and the intra-assay CV was 5.8%. Samples also were analyzed for circulating levels of LH. Samples were assayed in singlet using a sensitive sandwich immunoradiometric assay by the University of Virginia Center for Research in Reproduction Ligand Assay and Analysis Core. This assay had a reportable range of 0.07 – 37.4 ng/ml with a sensitivity of 0.07 ng/ml. The inter- and intra-assay CVs were 10% and 5%, respectively.

Data Analysis

Rate of acquisition was defined as the number of days to reach criterion (DTC). DTC was analyzed for each treatment group by one-way ANOVA. Learning curves were constructed by plotting the mean performance (percent correct) for each group across Blocks of training. Each Block represented average performance across three consecutive days of training. Upon reaching criterion, a value of 93.8% correct, reflecting the criterion of 15/16 correct choices over 2 days, was used in calculating group performance on subsequent days. The learning curves were compared using a two factor (Treatment×Block) ANOVA with repeated measures on Block. Performance on the probe trial was analyzed by contingency table and Chi-square. Performance during the increased intertrial delays was analyzed by ANOVA with repeated measures on Delay. Post-hoc comparisons were made using a Tukey test. Significance was set at $p \leq 0.05$. All statistical analysis was performed using JMP software for Macintosh.

Results

Serum estradiol levels

The mean levels of estradiol in the estradiol treatment group were 116.0 ± 39.6 pg/ml, with a range of 65.0–166.1 pg/ml. Mean estradiol levels in the gonadally intact vehicle group were 15.0 ± 7.0 pg/ml, with a range of 4.7–23.1 pg/ml. Levels of estradiol in non-estradiol treatment groups were undetectable.

Serum LH levels

The mean serum LH levels were 0.96 ± 0.85 , 0.25 ± 1.02 , 8.45 ± 0.88 , 10.16 ± 0.99 , 9.08 ± 1.04 , and 10.22 ± 0.95 ng/ml for the intact, estradiol, vehicle, G-1, DPN, and PPT treatment groups, respectively. ANOVA revealed a significant effect of Treatment ($F(5,61)=14.9$, $p < 0.01$), and post-hoc analysis revealed vehicle, G-1, DPN, and PPT treatment groups differed significantly from intact and estradiol groups.

DMP acquisition

Results show that ovariectomized (OVX) animals took more days to reach criterion than gonadally intact animals, and that estradiol and all three selective estrogen receptor agonists were able to restore the rate of acquisition to that of gonadally intact controls (Fig. 1). On average, OVX controls took 13 ± 0.59 days to learn the task. All other groups took an average of approximately 9 to 10 days to reach criterion on the task. ANOVA revealed a significant effect of Treatment ($F(5,62)=6.60$, $p < 0.01$). Post-hoc analysis revealed that estradiol treatment and all selective estrogen receptor agonist treatments differed significantly from OVX controls. Estradiol and selective estrogen receptor agonist treatments did not significantly differ from gonadally intact controls. There was no significant correlation between DTC and circulating estradiol levels in the E-treated or intact groups. Likewise there was no significant correlation between DTC and serum LH levels in any of the treatment groups.

Examination of the learning curves revealed that all groups performed similarly below chance at the start of training (no effect of treatment on Block 1), and that rats receiving estradiol or agonist treatments acquired the task at a faster rate than the vehicle group (Fig. 2). ANOVA revealed a significant effect of Treatment ($F(5,62)=5.74$, $p < 0.01$), a significant effect of Block ($F(3,60)=94.4$, $p < 0.01$), and a significant Treatment \times Block interaction ($F(15,166)=1.88$, $p < 0.028$). Post-hoc analysis of the main effects of treatments collapsed across Blocks 2–5 revealed significant differences between OVX controls and intact ($p < 0.01$), estradiol ($p < 0.0041$), G-1 ($p < 0.01$), PPT ($p < 0.0025$), and DPN ($p < 0.01$) treatments. Significance was adjusted to $p \leq 0.01$ to account for multiple comparisons. One way ANOVA for each block followed by a post-hoc Tukey test showed that the intact group was significantly different from OVX controls at Blocks 2–3, and that the G-1 treatment group was significantly different from OVX controls at Blocks 2–4.

Previous studies show that some rats will adopt a persistent turn early on during testing and that this can affect DTC (Gibbs, 2007; Gibbs and Johnson, 2007). To quantify this, we examined whether rats adopted a persistent turn, defined as entering the same arm at least 15/16 times during the choice trial over two days. Results show that a minority (26%) of rats adopted a persistent turn (consistently entering either arm of the maze). Treatments had no effect on the percentage of rats that adopted a persistent turn ($X^2(5)=2.9$, $p=0.71$). Among rats that did adopt a persistent turn, treatments did not affect the number of days that this pattern persisted ($F(5,17)=1.3$, $p=0.32$).

Post-criterion Testing

After reaching criterion, rotating the maze 180° between the forced and open choices significantly disrupted performance within most groups. Performance dropped from 93.8% (criterion level) to 60%, 44.4%, 72.7%, 72.7%, 83.3% and 53.3% for intact, estradiol, G-1, DPN, PPT, and vehicle-treated groups respectively as a result of rotating the maze 180° between the forced and open trials. Analysis showed that none of these values differed significantly from chance with the exception of the PPT-treated group, which scored significantly above chance ($X^2=9.7$, $p<0.02$). Nevertheless, no significant overall effect of Treatment on performance during the probe trial was detected ($X^2=5.9$, $p > 0.4$).

Once rats had reached criterion, increasing the intertrial delay impaired performance for all treatment groups (Fig. 3). ANOVA revealed a significant effect of Delay ($F(3,60)=8.9$, $p < 0.01$), no significant effect of Treatment ($F(5,62)=0.7$, $p > 0.6$), and no significant interaction between Treatment and Delay ($F(15,166)=1.15$, $p > 0.3$).

Discussion

Effects of Estradiol and Estrogen Receptor Agonists on DMP Acquisition

Our results confirm that ovariectomy slowed the rate of DMP acquisition in young adult rats. PPT, DPN, G-1, and estradiol each were able to restore the rate of DMP acquisition to that of gonadally intact controls. This is consistent with previous studies showing that estradiol enhances DMP acquisition in young and middle-aged rats (Gibbs, 1999; Gibbs, 2007; Gibbs et al., 2004; Gibbs et al., 2009), and suggests that activity at any of the three established estrogen receptors might underlie this effect. Moreover, similar performance was observed in intact cycling rats and OVX rats with sustained estradiol replacement. Although we did not monitor cycles, we assume that the intact rats were undergoing estrous cycles. The fact that estradiol levels were low in some of these rats suggests that they were killed on diestrus, whereas others were killed at other phases of the cycle. This suggests that sustained elevated estradiol levels are not required to enhance acquisition of the DMP task. Controversy exists in regards to whether cyclical or sustained estradiol treatment is more effective in enhancing cognitive performance (Gresack and Frick, 2006; Iivonen, Heikkinen, Puolivali, Helisalml, Hiltunen, Soininen, and Tanila, 2006; Rapp, Morrison, and Roberts, 2003). We also note that the effect of estradiol on the learning curve, though significant, was not as robust as in some previous reports, possibly due to the relatively high levels of estradiol achieved in the estradiol treatment group. Some studies have reported that high doses of estradiol can impair spatial working memory in rats (Wide, Hanratty, Ting, and Galea, 2004).

Recent studies suggest that elevated levels of LH that occur in response to ovariectomy may contribute to the cognitive impairment associated with ovariectomy, aging, and neurodegenerative diseases (Casadesus, Milliken, Webber, Bowen, Lei, Rao, Perry, Keri, and Smith, 2007; Webber, Stocco, Casadesus, Bowen, Atwood, Previll, Harris, Zhu, Perry, and Smith, 2006). It has been shown that intraperitoneal or intracerebroventricular administration of human chorionic gonadotropin produces deficits on T-maze tasks in rats (Lukacs, Hiatt, Lei, and Rao, 1995), and that increased LH levels in mice are associated with declines in cognitive performance on a Y-maze task (Casadesus et al., 2007). In the present study, we detected no correlation between LH levels and the number of days required to reach criterion on the task in any of the treatment groups. In addition, G-1, DPN, and PPT all significantly enhanced the rate of DMP acquisition despite the fact that LH levels were very high relative to intact and E-treated groups. This suggests that the effects of ovariectomy and estrogen treatments on the rate of DMP acquisition are not due to effects of LH.

Treatment Effects on Learning Strategy and Working Memory

Rats can use various strategies to solve T-maze tasks, such as place strategies, which use extramaze visual cues, and response strategies, which use body positioning and kinetic cues (Dudchenko, 2001; Korol, Malin, Borden, Busby, and Couper-Leo, 2004). Rats also have been shown to change strategy with repeated testing or in response to estradiol treatment (Korol and Kolo, 2002; Packard and McGaugh, 1996). Evidence suggests place learning is mediated by hippocampal circuits (Zurkovsky, Brown, and Korol, 2006) while response learning is mediated by extrapyramidal circuits, such as through the caudate (Packard and McGaugh, 1996; Squire, 1998). Moreover, place learning is acquired faster than response learning (Chang and Gold, 2003; Packard and McGaugh, 1996). Results of the probe trial suggest that upon reaching criterion most treatment groups used extramaze cues to a significant degree while performing the DMP task. PPT-treated rats were slightly less affected by rotating the maze than the other groups, which may indicate a slightly greater predisposition to use a response vs. a place-based strategy; however, this difference was not statistically significant. In addition, treatments had no effect on the predisposition to adopt a persistent turn. This suggests that the effects of the selective estrogen receptor agonists on DMP acquisition were not due to differential effects on strategy. The hippocampus also plays an important role in spatial working memory. Results of the delay trials indicate that the effects of the selective estrogen receptor agonists on DMP acquisition cannot be accounted for by effects on spatial working memory. These findings are consistent with a recent analysis of the effects of estradiol on DMP acquisition in young rats (Gibbs, 2007).

Comparison with Previous Studies

The present study showed that chronic estradiol treatment throughout training and testing enhanced acquisition of the DMP task. Others have used a post-training regimen and have shown that estradiol can be administered in the period immediately after training to enhance performance. For example, ovariectomized rats that were trained in a hidden platform water maze and given estradiol injections less than two hours post training performed better than vehicle controls when returned to the maze 24 hours later for a retention test. This enhancement in retention did not occur when estradiol was given later than two hours post training (Packard, 1998). In another case, ovariectomized rats were trained in an inhibitory avoidance task and tested following a 24 hour delay, and it was shown that estradiol administration immediately, but not 1, 2, or 3 hours post training, increased crossover latencies compared to vehicle (Rhodes and Frye, 2004). Taken together, these suggest that estradiol may affect both acquisition and memory consolidation.

In addition to spatial learning and memory, others have examined the effects of DPN and PPT on stress and anxiety. Daily treatment with DPN has been shown to decrease anxiety-related behaviors on tasks such as the elevated plus maze or open field, suggesting that ER β mediates select anxiolytic effects of estradiol (Lund, Rovis, Chung, and Handa, 2005; Walf and Frye, 2005). In contrast, daily injections of PPT have been reported to increase anxiogenic behaviors such as the number of fecal boli and time spent grooming (Lund et al., 2005). Thus, it is important when analyzing effects of PPT and DPN on learning tasks to consider the degree to which stress and anxiety play a role in each task. Though we did not measure corticosterone levels, to our knowledge stress and anxiety are not major components of the DMP task, analogous to other food-motivated land-based navigational tasks. While rats do experience the stress of food deprivation, the task does not include an acute stressor such as foot shock or swimming, which are major components of standard inhibitory avoidance and water maze tasks.

The parallel effect of the three agonists in facilitating acquisition raises the possibility that estradiol and the three agonists may affect non-mnemonic processes such as sensory-motor

functions, motivational factors, or attentional mechanisms. Previous work from our lab has shown that ovariectomy and estradiol treatments that affect DMP acquisition have no effect on a configural association operant conditioning task (Gibbs and Gabor, 2003). This task requires rats to distinguish between visual and auditory stimuli and is motivated by the same food reward associated with the DMP task. The fact that ovariectomy and estradiol treatments have no effect on this task reduces the likelihood that effects on motivation and sensory perception underlie the effects on DMP acquisition.

What circuits underlie the effects of the selective estrogen receptor agonists on DMP acquisition is unknown. As mentioned earlier, ER α immunoreactivity and mRNA have been found in the hippocampus, and ER β immunoreactivity and mRNA have been found in the hippocampus and neocortex (Mitra, Hoskin, Yudkovitz, Pear, Wilkinson, Hayashi, Pfaff, Ogawa, Rohrer, Schaeffer, McEwen, and Alves, 2003; Osterlund, Kuiper, Gustafsson, and Hurd, 1998a). This raises the possibility that DPN and PPT may act directly at one of these two regions to affect DMP acquisition.

To our knowledge, this is the first study to show an effect of G-1 administration on a cognitive task. Our results show that G-1 had as much or more of a beneficial effect on DMP acquisition as any of the other agonists, suggesting that activation of GPR30 is a viable strategy for enhancing performance within specific cognitive domains. G-1 is still in the early stages of development; however, like other selective estrogen receptor agonists, it may offer numerous advantages over standard estrogen therapy, most notably with respect to cancer risks and stroke. Also, unlike ER α and ER β , GPR30 is a membrane-bound receptor which can mediate rapid estrogen signaling via activation of adenylate cyclase and the liberation of membrane-bound EGF (Filardo, Quinn, Frackelton, and Bland, 2002). Where in the brain G-1 acts to affect DMP acquisition is not known. Further studies that evaluate the functional consequences of GPR30 expression and activation within specific regions of the brain are needed to fully elucidate the potential of GPR30 agonists to enhance neuronal function and cognitive performance.

Acknowledgments

This work was supported by NIH grant R01 AG021471. Work by The University of Virginia Center for Research in Reproduction Ligand Assay and Analysis Core is supported by the Eunice Kennedy Shriver NICHD/NIH (SCCRIR) Grant U54-HD28934.

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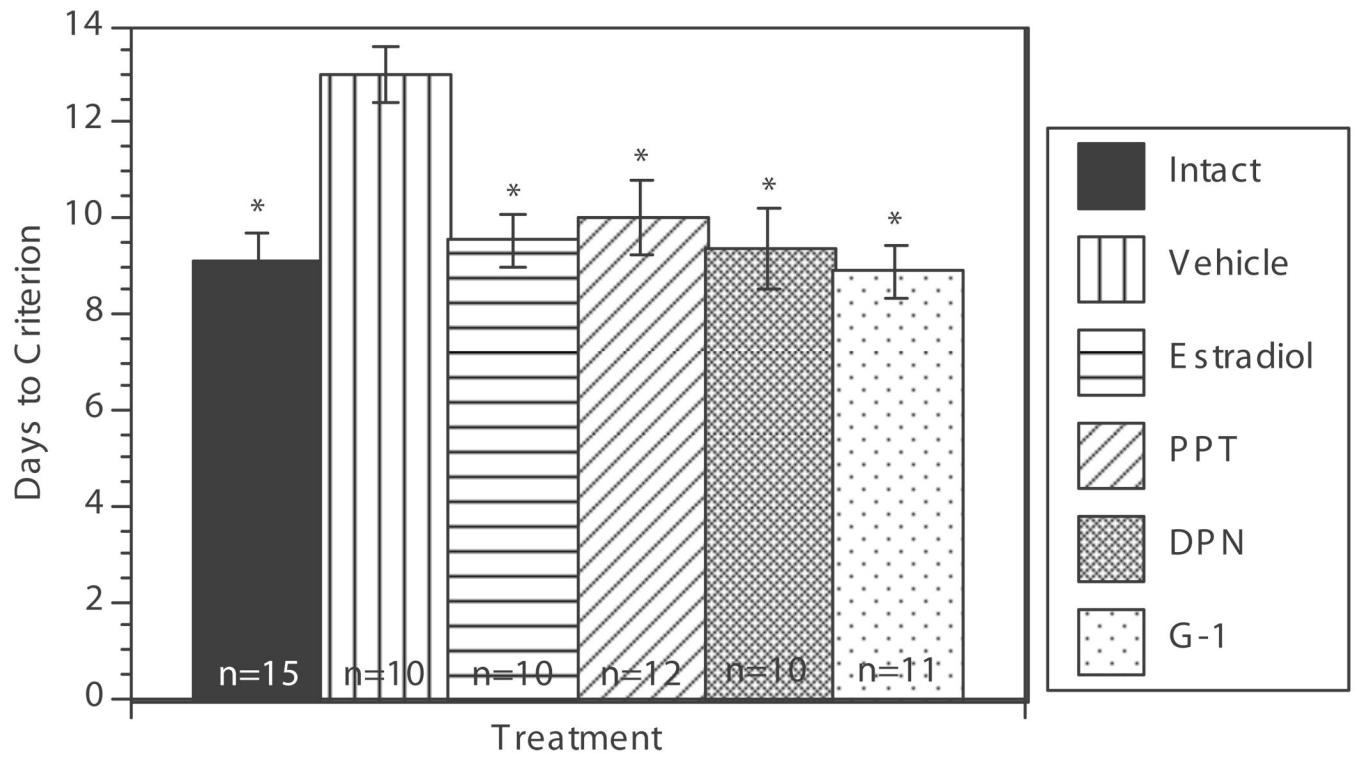


FIG 1. Bar graph summarizing Days to Criterion (DTC) for each treatment group. Bars indicate mean number of days \pm s.e.m. * $p < 0.05$ relative to OVX vehicle.

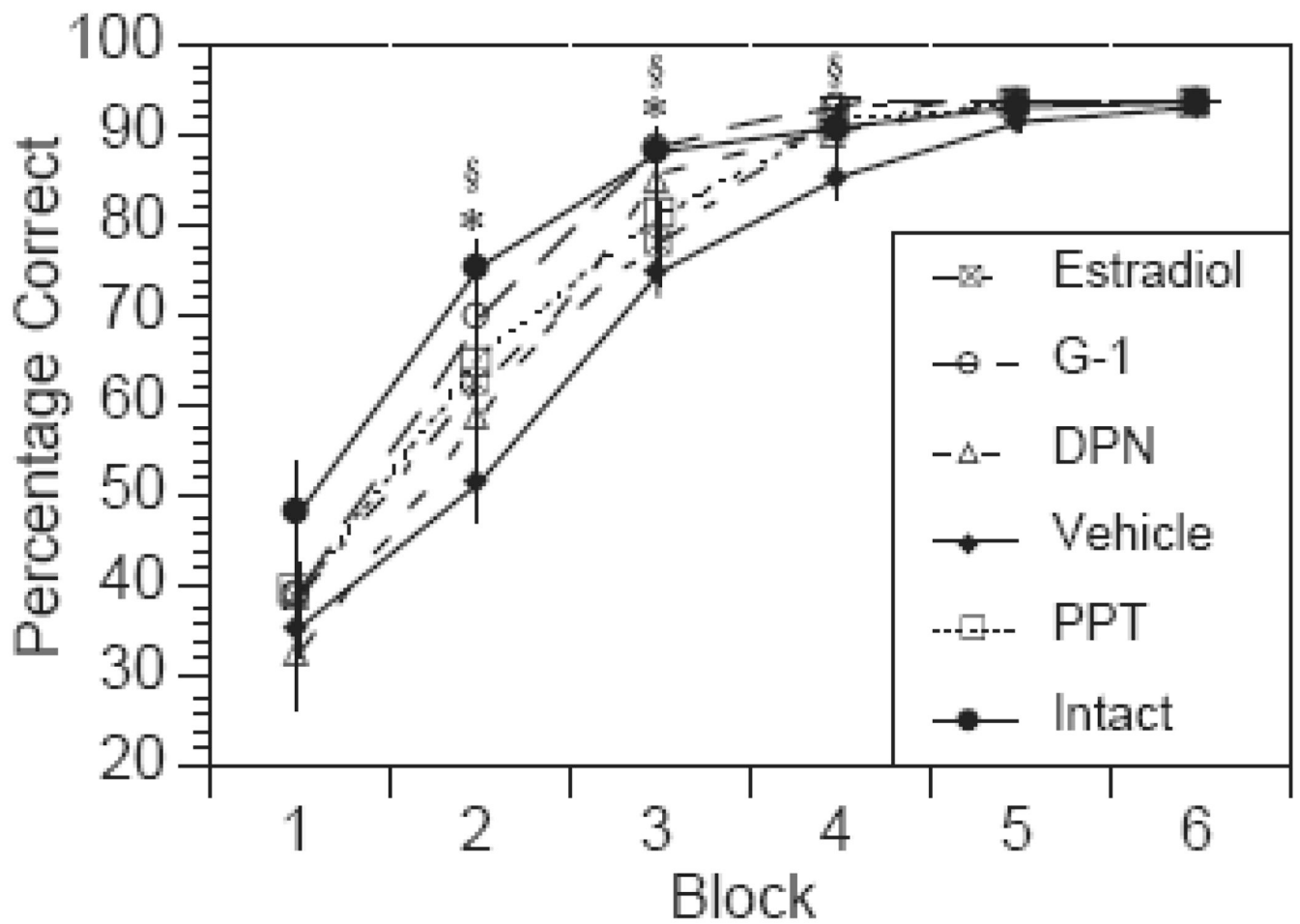


FIG 2. Learning curves showing acquisition of the DMP task over time. Values represent the mean percent correct \pm s.e.m. within a 3-day block of training for each treatment group. * $p < 0.05$ for intact group relative to OVX vehicle. § $p < 0.05$ for G-1 treatment relative to OVX vehicle.

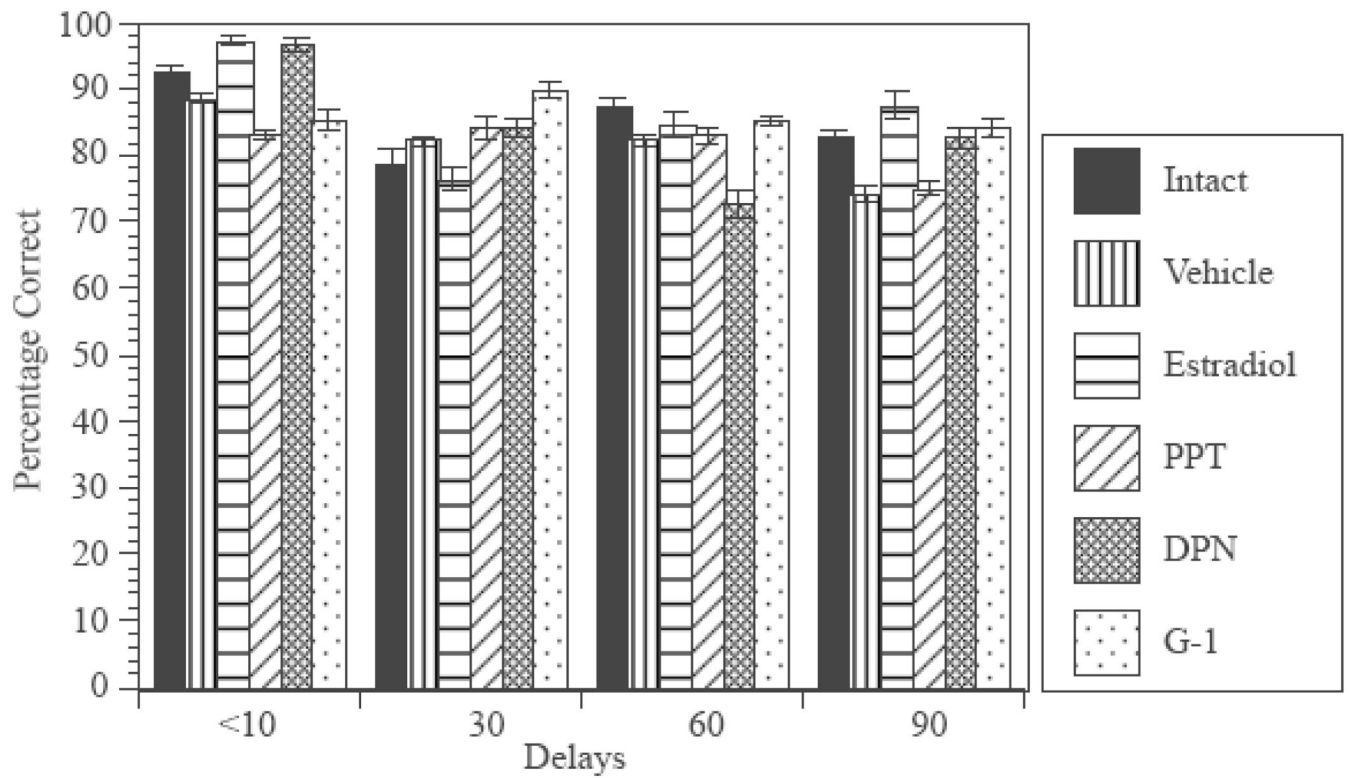


FIG 3. Effects of increasing the delay between the forced and open trial to 30, 60, and 90 seconds on performance. Bars represent the percentage of correct choices \pm s.e.m. for each group.