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Progesterone to ovariectomized mice enhances cognitive performance in the spontaneous alternation, object recognition, but not placement, water maze, and contextual and cued conditioned fear tasks

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Abstract

Research on how steroid hormones mediate mnemonic processes have focused on effects of 17 β -estradiol (E₂); yet, progesterone (P₄) co-varies with E₂ across endogenous hormonal milieu, and itself may influence cognitive processes. We investigated the hypothesis that acute P₄ treatment enhances cognitive performance compared to vehicle. Ovariectomized (OVX) c57/BL6J mice were randomly assigned to be subcutaneously injected with oil vehicle or P₄ (10 mg/kg). Mice were trained in the spontaneous alternation, object recognition, object placement, water maze, or fear conditioning tasks, and injected with vehicle or P₄ before training or immediately post-training, and then were tested 1, 4, or 24 h later. The data obtained from these experiments supported our hypothesis. P₄ increased the percentage of spontaneous alterations made in a T-maze more so than did vehicle. P₄, compared to vehicle, increased the percentage of time spent exploring the novel object in the object recognition task, but did not alter performance in the object placement task. P₄, compared to vehicle, decreased latencies to reach the location in the water maze where the platform had been during training in a probe trial, but did not alter performance in the control, cued trial. Compared to vehicle, P₄ treatment increased freezing in contextual and cued fear testing. Thus, acute P₄ treatment to OVX mice can improve cognitive performance across a variety of tasks.

Keywords

Allopregnanolone; Hippocampus; Cortex; Learning and memory; Neurosteroids

1. Introduction

Steroid hormones are important trophic factors that play a key role in the structure and functioning of the nervous system. Steroid hormones regulate essential neuronal and glial function (Schumacher et al., 2000), which contribute to behavioral outcomes. For example, many studies have demonstrated that 17 β -estradiol (E₂) has beneficial effects for learning and

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memory (Azcoitia, DonCarlos, & Garcia-Segura, 2003; Bisagno, Bowman, & Luine, 2003; Luine, Jacome, & Maclusky, 2004; Fillit & Luine, 1997; McEwen, 2001), neurogenesis within the hippocampus (Galea, in press; Tanapat, Hastings, & Gould, 2005), and may have neuroprotective effects (Brinton, 2004; Frye & Rhodes, 2005; Gibbs & Gabor, 2003). Progesterone (P_4), like E_2 , is a pleiotropic hormone in the nervous system that can exert cognitive and/or neuroprotective effects. Indeed, injections of E_2 and P_4 to young ovariectomized (OVX) rats enhances performance in several cognitive measures, including the object recognition, object placement, and water maze (Frye, Duffy, & Walf, 2007; Gibbs, 2003; Sandstrom & Williams, 2001; Walf, Rhodes, & Frye, 2006). However, E_2 and P_4 do not necessarily have the same effects on cognitive performance when rodents are treated with each steroid alone or when they are co-administered. Endogenous changes in E_2 over reproductive cycles of rats are accompanied by variations in progestogens, and each may influence cognitive performance differently. During the luteal phase P_4 levels positively correlate with motor coordination and better performance on perceptual, visual, and verbal memory tasks (Berman et al., 1997; Broverman et al., 1981; Hampson, 1990; Hampson & Kimura, 1988; Phillips & Sherwin, 1992a). Furthermore, performance on frontal lobe tasks, implicit memory, and attention is better when P_4 levels are high (Hollander, Hausmann, Hamm, & Corballis, 2005; Maki, Rich, & Rosenbaum, 2002; Solis-Ortiz, Guevara, & Corsi-Cabrera, 2004), whereas spatial performance is typically improved during menstruation, when E_2 and P_4 levels are lower (Hampson & Kimura, 1988; Hausmann, Slabbekoorn, Van Goozen, Cohen-Kettenis, & Gunturkun, 2000; McCormick & Teillon, 2001; Phillips & Silverman, 1997; Rosenberg & Park, 2002). In animal models, rats in proestrus (which have high E_2 and P_4) perform significantly better in the inhibitory avoidance, object recognition, and object placement tasks, and more readily acquire trace conditioning, than do diestrous rats with lower E_2 and P_4 levels (Frye et al., 2007; Walf et al., 2006; Wood, Beylin, & Shors, 2001). In contrast, rats in proestrus perform no differently or worse in the water maze (Frye, 1995; Stackman, Blasberg, Langan, & Clark, 1997). Thus, E_2 and P_4 may have different effects on cognitive performance, which likely depend upon many factors, such as the task utilized.

Although the majority of studies investigating ovarian steroid regulation of cognitive behavior have investigated effects of E_2 , or its co-administration with P_4 , there is evidence that P_4 may have beneficial cognitive effects. Treatment with P_4 alone post-training to OVX rats enhances object recognition and object placement performance and increases progestogen levels in the cortex and hippocampus (Frye et al., 2007; Walf et al., 2006). However, there may be regimen-dependent effects of P_4 for cognitive performance that need to be addressed further. In studies of acute treatment of rats with dosages of P_4 , or its neuroactive metabolites (which would be expected to produce circulating levels of these progestogens higher than what is typically observed during proestrus), produce decrements in cognitive performance (Johansson, Birzniece, Lindblad, Olsson, & Backstrom, 2002; Zou, Yamada, Sasa, Nakata, & Nabeshima, 2000). We have demonstrated that chronic P_4 treatment to aged mice enhances cognitive function in the spontaneous alternation T-maze task and object placement task, and produces levels of progestogens in plasma and brain that are akin to those observed during proestrus (Frye & Walf, 2008). Together, these studies suggest that physiological dosing with P_4 may improve cognitive performance of female rats and mice. However, subtle differences, related to behavioral and/or hormonal responsiveness (Frick, Stillner, & Berger-Sweeney, 2000), in the nature of P_4 's effects for these processes likely exist. To begin to investigate this, the effects of acute treatment of physiological P_4 to young, adult OVX mice for cognitive performance needs to be determined.

Given the aging population, the potential for steroids to have beneficial effects on cognition (Brinton, 2004), and the potential relevance for human health, whether P_4 can exert beneficial cognitive effects across species is of interest. If P_4 has cognitive effects that are important and meaningful, one would expect conservation of these mnemonic effects across species. As

such, we tested the hypothesis that physiological dosing with P₄ to OVX mice would improve cognitive performance. OVX c57/BL6J mice were treated with P₄ pre-training, immediately post-training, and with a delay of 1.5 h post-training. Mice were tested 1, 4, and 24 h later when P₄ levels would expected to be physiological (1 h and 4 h post-injection) and at nadir (24 h post-injection).

2. Materials and methods

All methods utilized were pre-approved by the Institutional Animal Care and Use Committee at The University at Albany-SUNY. All experimentation was conducted in accordance with accepted standards of humane animal use, the US. Public Health Service “Policy on Humane Care and Use of Laboratory Animals” and the National Institutes of Health “Guide for the Care and Use of Laboratory Animals.”

2.1. Subjects and housing

Female C57/BL6J mice (Cohort 1, $n = 24$; Cohort 2, $n = 16$) were obtained from Jackson Laboratory (Bar Harbor, ME) and bred in our laboratory. Mice were housed in groups of 4–5, in cages that had woodchips for bedding and a Nestlet square. Mice were housed in the Laboratory Animal Care Facility at the University at Albany in the Life Sciences Research Building. The room had a 12/12 h reversed light/dark cycle, with the lights going off at 8:00 am. Mice had *ad libitum* access to Purina Rodent Chow and tap water in their home cages.

2.2. Ovariectomy

Under sodium pentobarbital anesthesia (80 mg/kg), 2-month-old mice were OVX via bilateral incisions on their flanks. Mice recovered from surgery for one week before habituation to handling/behavioral testing began.

2.3. General procedure

Mice were randomly assigned to receive P₄ or vehicle throughout the experiment. A week post-surgery, mice experienced a 5-day habituation protocol prior to behavioral testing. After habituation, mice were tested in one task per day, every 4–5 days. The behavioral tasks utilized are described below. Mice were trained and tested in the dark cycle, between 8:00 am and 2:00 pm, so that they would be in the active phase of their circadian cycle.

2.4. Screening procedure

Approximately one week after OVX, each mouse was carefully evaluated to ascertain whether they exhibited normative responses (Moy et al., 2007). The general health, reflexes, motor behavior, and/or coordination of each mouse were examined. This was done to rule out any potential baseline differences in performance that may have influenced the cognitive endpoints under examination. The general appearance (fur, whiskers, posture, gait, muscle tone) and behavior (grooming, nest building, huddling, cage climbing, paw withdrawal) was examined for each mouse. No differences were observed and all mice were included in the study.

2.5. Handling procedure

The following procedure was utilized to habituate mice to handling and behavioral observation by the experimenter before behavioral testing began (Frye et al., 2006a). On day 1, mice were picked up from their home cage, handled for 15 s, and returned to their home cage. On day 2, mice were moved from their home cage to a transport cage (without food and water). On day 3, mice were weighed and then returned to their home cage. On day 4, mice in their home cages were transferred to another room on a cart and then placed in a transport cage for 5 min. On day 5, mice were transferred in their home cages on a cart, then were placed in transport cages,

and were subsequently placed in the open field utilized in the object recognition task described below) for 5 min.

2.6. Hormone treatment

Mice in Cohort 1 were assigned to receive P₄ (10 mg/kg, SC; Steraloids, Newport, RI) or vehicle (90% vegetable oil, 10% ethanol) immediately after training in each of the tasks described below, except the spontaneous alternation task. In the spontaneous alternation task, mice in Cohort 1 were injected with P₄ or vehicle 1 h before testing. Mice in Cohort 2 were injected with P₄ or vehicle 1.5 h post-training in the object recognition and conditioned fear tasks (and were not trained or tested in spontaneous alternation, water maze, or object placement tasks in this study). This regimen of P₄ produces plasma and hippocampus levels of progestogens that are typically observed during behavioral estrus of mice as measured by radioimmuno-assay in our laboratory (for P₄: ~34–38 ng/ml in plasma and ~18–21 ng/g in hippocampus, and for 3 α ,5 α -THP: ~19–22 ng/ml in plasma and ~19–21 ng/g in hippocampus —Frye, Sumida, Lydon, O'Malley, & Pfaff, 2006b).

2.7. Behavioral testing

Mice were trained and tested consecutively in the tasks in the order in which they are described below. Immediately before and after training and testing, mice were housed in transport cages. We investigated the effects of P₄ in a number of cognitive tasks. Furthermore, the tasks utilized have different inter-trial intervals (described below) and mice receive P₄ treatment pre-training (spontaneous alternation) or immediately post-training (object recognition, object placement, water maze, and fear conditioning). In the object recognition and fear conditioning tasks, performance of another cohort of mice (that had been similarly repeatedly injected with P₄ and behaviorally tested) that were treated with vehicle or P₄ 1.5 h after training, was also assessed. All mice were tested in the same order in all of the tasks. This was done so that potential carry-over effects could be accounted for across experimental conditions and behavioral tasks.

2.8. Spontaneous alternation

Performance in the spontaneous alternation task was assessed using a T-Maze as per previous methods (Frye & Walf, 2008; Hughes, 2004). The T-maze consisted of a clear plexiglas start box with a guillotine door, which is connected to one start (30.5 $\times$ 9 $\times$ 7 cm) and two goal (17.8 $\times$ 9 $\times$ 7 cm) arms made of black plexiglas. Briefly, mice were injected with P₄ or vehicle, and 1 h later, were placed in the start box and one forced arm choice was made with one goal arm blocked. After the forced trial, the blockade of the arm was removed. Mice then were tested for 13 trials (maximum latency to complete all 13 trials, 900 s) and the number of spontaneous alternations made (entering the right goal arm after the left goal arm was entered and vice versa), as a function of the total arm entries, during these trials was recorded. A higher percentage of alternations made in the free choice trials [(number of alternating entries made/ the total number of free choice entries made) $\times$ 100] was used as an index of enhanced performance. A chance level of responding of rodents in this task is about 50%, and we typically see that control mice make 40–60% alternations. Differences between mouse strains in this task are clear and may account for some of the variability in the task (Gerlai, 1998).

2.9. Object recognition

Object recognition was implemented as previously described (Ennaceur & Delacour, 1988; Frye & Walf, 2008; Luine, Jacome, & Maclusky, 2003; Walf et al., 2006). During training, mice were placed in a corner (NE) of a white open field (46 $\times$ 57 cm with 35 cm high walls), which did not contain shavings, in the brightly-lit testing room. Training in this task utilized two identical objects (plastic toy mouse or bottle) that were placed in the southwest and southeast quadrants of the open field. Mice were allowed to explore these objects for 3 min.

The duration of time spent within 5 cm of the object, directly in contact, investigating and/or orienting towards the objects, was automatically recording using a tracking program (Any-Maze; Stoelting Co., Wood Lawn, IL). Immediately, or 1.5 h, after training, mice were injected SC with P₄ or vehicle, and were returned to their transport cages. Four hours later, mice were returned to the open field for 3 min. One of the objects used during training was replaced by a colored spherical plastic toy (“orange” or “lemon”). Whether the novel object was on the southeast or southwest side of the open field was alternated to reduce potential confounds of a side preference influencing results. The time mice spent exploring the identical and novel objects was recorded. More time spent exploring the novel object during testing (duration spent with novel object/(duration spent with novel object + duration spent with familiar object) × 100) is considered an index of cognitive performance in this task.

2.10. Object placement

Object placement testing was done in accordance with previous methods employed in our laboratory (Frye & Walf, 2008; Frye et al., 2007). The training methods utilized were the same as described above for object recognition, except that differently shaped plastic objects were utilized. Mice were trained for 3 min and then 4 h later were tested. During testing, one object was displaced from its original location during training to the opposite corner of the open field. Displacement of the novel object to the southeast or southwest side of the open field was alternated to reduce potential confounds of a side preference influencing results. The duration spent by mice exploring the identical and novel objects was recorded. An increase in time spent exploring the displaced object during testing (duration spent with displaced object/(duration spent with displaced object + duration spent with stationary object) × 100) is considered an index of cognitive performance in this task.

2.11. Water maze

The Morris Water Maze (San Diego Instruments, San Diego, CA, USA; 97 cm in diameter) was used according to previously described methods (Bennett, McRae, Levy, & Frick, 2006; Frye, Rhodes, & Dudek, 2005). Temperature of the water in the maze was 22–25 °C. The water was made opaque with non-toxic, white, tempera paint. Mice were habituated to the maze by allowing them to swim in the water maze with the hidden platform (8 × 8 cm) in it. After 1 min, mice were placed on the hidden platform for 10 s. Following habituation, mice were trained in 12, 1-min trials, which were organized into three blocks of 4 trials. In each block of trials, mice were placed in one of four start positions in each of the quadrants of the maze. The inter-trial interval within each block of trials was 10 s. Each block of trials was separated by 30 min. In each trial, the maximum latency for mice to reach the platform was 60 s. Immediately after training, mice were injected with P₄ or vehicle SC. Mice were tested in a cued trial of the water maze to assess their ability to swim to a platform in the maze. In this, the latency of mice to swim to a platform that is made visible and cued is determined to rule out potential differences due to treatment for swimming ability or the ability to perform the task. Twenty-four hours later, the latency of mice to swim to the precise location in the maze where the platform had been during training, and the duration spent in the quadrant where the platform was located during training (probe trial), were recorded. These measures can be utilized as indicators of performance in this task.

2.12. Conditioned contextual/cued fear

Conditioned Fear was modified from previously described methods (Frye, Edinger, & Sumida, 2008). Mice were habituated to the conditioning chamber for 4 min. During training, a tone (10 s) is sounded, followed by an electric foot-shock (2 s, 0.35 mA). Mice receive the tone-shock pairing over 3 training trials. When mice were shocked, their response to this shock was rated (0-no response, 1-twitch, 2-jump, 3-squeak, 4-jump and squeak) and recorded by

investigators. Immediately after training, mice were injected with P₄ or vehicle SC. Mice were tested 24 h later. During testing, freezing behavior is observed for eight 1-min intervals in the same chamber for contextual fear (mediated by the hippocampus), and in a novel environment with the same tone for cued fear (mediated by the amygdala; Phillips & LeDoux, 1992). More time spent freezing during contextual or cued fear testing trials is utilized as an indication of improved performance in this task.

2.13. Statistical analyses

One-way analyses of variance were utilized to determine effects of hormone condition (vehicle vs. P₄) on performance in tasks. A *P*-value of ≤ 0.05 was considered significant.

3. Results

3.1. Spontaneous alternation

P₄ enhanced performance in the spontaneous alternation T-maze $F(1, 22 = 3.99; P \leq 0.05)$. Mice treated with P₄ made significantly more spontaneous alternations than did mice injected with vehicle (Fig. 1).

3.2. Object placement

During training, mice spent similar durations investigating each object located on the left (vehicle: 7.3 ± 1.4 s; P₄: 8.2 ± 2.6 s) or right (vehicle: 11.0 ± 2.4 s; P₄: 16.2 ± 5.5 s) side of the chamber. There were no significant differences on percent of time investigating the displaced object in the object placement task of mice that received injections of vehicle ($52.1 \pm 10.4\%$ sem) or P₄ ($52.7 \pm 8.1\%$ sem).

3.3. Object recognition

During training, mice spent similar durations investigating each object located on the left (vehicle: 11.7 ± 3.2 s; P₄: 9.5 ± 1.6 s) or right (vehicle: 10.5 ± 3.1 s; P₄: 11.5 ± 3.9 s) side of the chamber. P₄ enhanced performance in the object recognition task $F(1, 22 = 4.69; P < 0.04)$. Mice treated with P₄, compared to vehicle, immediately post-training spent significantly more time exploring the novel object during testing in the object recognition task (Fig. 2, left). If mice were injected with P₄ 1.5 h after training, performance in this task was similar to that observed in vehicle controls (Fig. 2, right).

3.4. Water maze

Differences were not observed during training in the water maze. During training, there were no differences in mean latencies, averaged across 12 training trials, for mice injected with vehicle (33.8 ± 6.1 sem) or P₄ (43.2 ± 6.4 sem) and see Fig. 3. During testing, P₄ enhanced performance in the probe trial of the water maze task. In the probe trial, mice treated with P₄ had significantly shorter latencies to the location where the hidden platform had been located during training $F(1, 22 = 5.21; P \leq 0.03)$, but were not different in the mean time spent in the quadrant where the platform had been during training (25.8 ± 4.0 sem), than mice treated with vehicle (23.7 ± 4.4 sem). When the platform was made visible and a cue was used to highlight its location, there were no differences in the latencies to reach the platform of P₄ and/or vehicle-treated mice (Fig. 3).

3.5. Conditioned contextual/cued fear

During training, there were no differences in flinch-jump scores of mice in the vehicle (3.8 ± 0.1 sem) or P₄ (3.9 ± 0.1 sem) treatment groups. P₄ enhanced performance in the context $F(1, 22 = 5.49; P \leq 0.03)$ and the cued $F(1, 22 = 4.17; P < 0.05)$ components of the conditioned fear task. Mice injected with P₄ spent significantly more time freezing in the same context as they

were shocked in. Moreover, when tested in a novel context, but with the same auditory cue that had been associated with shock, mice treated with P₄ spent more time freezing than did vehicle-treated mice (Fig. 4, left). If mice were injected with P₄ 1.5 h after training, performance in the contextual or cued tasks was similar to that observed in mice injected with vehicle (Fig. 4, right).

4. Discussion

The hypothesis that acute physiological dosing with P₄ would enhance cognitive performance of OVX mice across several tasks was supported. In the spontaneous alternation task, P₄ to mice prior to testing increased the number of spontaneous alternations made compared to that observed in OVX mice treated with vehicle. In the object placement task, no differences due to P₄ treatment were observed. In the object recognition task, mice injected with P₄ immediately post-training (but not 1.5 h post-training) performed better than did vehicle controls. In the water maze probe trial, mice that were injected with P₄ immediately after training had shorter latencies to swim to the location in the water maze where the hidden platform had been during training, compared to vehicle controls. There were no differences based upon condition for performance in the control, cued trial or, in the probe trial, for duration spent in the quadrant where the platform had been during training. In the conditioned fear task, P₄, compared to vehicle, immediately after training (but not 1.5 h later) increased the duration of time spent freezing in response to re-exposure to the training context or when re-exposed to the tone cue that had been paired with footshock during training. Thus, P₄ treatment to young, OVX mice improves cognitive performance.

The present data begin to address the nature of P₄'s effects on cognitive processes. In the present experiment, beneficial effects of P₄ were demonstrated when P₄ was injected before training in the spontaneous alternation, as well as when P₄ was injected immediately post-training in the object recognition, water maze, and conditioned fear task. That post-training treatment of P₄ was sufficient to enhance cognitive performance implies P₄ may have effects on consolidation. Utilizing a 1 h or 1.5 h delay in post-training treatment with P₄, such that P₄ does not have effects during consolidation, does not improve object recognition and object placement performance of rats (Frye et al., 2007; Walf et al., 2006). We found a similar pattern of results in the present study. When mice were treated with P₄ 1.5 h post-training in the object recognition and conditioned fear tasks, no improvements were observed in these tasks when mice were tested 4 h or 24 h post-training. Moreover, beneficial effects of P₄ were observed when P₄ was circulating (i.e. 1 h after injections in the spontaneous alternation task, 4 h after priming in the object recognition task) and when at nadir (i.e. 24 h following injections in the water maze and conditioned fear tasks). We have previously found that this regimen of P₄ in mice produces progesterone levels similar to those observed during behavioral estrus within 1 h that are sustained for 4–8 h, and reach nadir 24 h after treatment (Frye et al., 2006a, 2006b). Together, these data suggest that the beneficial effects of P₄ are not limited to situations in which there are increases in circulating concentrations of P₄, and instead suggest that they may be attributable to P₄'s modulation of memory consolidation.

The present findings extend the existing literature on the potential for progestogens to have mnemonic effects to demonstrate that acute, physiological P₄ priming has mnemonic effects in mice. For instance, in a study using adult, intact cycling mice, performance in a spatial reference memory task was better during estrus, than metestrus, when endogenous steroid hormone levels are greater (Frick & Berger-Sweeney, 2001). A recent study from our laboratory demonstrated that chronic P₄ treatment improves cognitive performance of mid-aged OVX mice. Mice were OVX at 6 months of age and then implanted with SC pellets of P₄ or placebo between 6 and 12 months of age. Chronic P₄ in these mice improved performance in the spontaneous alternation and object recognition tasks, than placebo implants (Frye &

Walf, 2008). Similar to some of the present results, effects of P₄ in the object placement task were less clear and no effects of P₄ were observed in the water maze in this study. Another consideration to make in interpreting the results of the present study is whether P₄ is altering the strategies utilized by mice in these cognitive tasks. Although this was not systematically investigated or manipulated in the present study, shorter latencies to the location of the hidden platform during the probe trial in mice injected with P₄, and no differences between groups for time spent in the quadrant, suggest that P₄-treated mice may have oriented towards the platform location faster or more reliably. Future studies could assess this directly. Other factors to consider is how the observed behavioral effects may be related to its capacity to protect the brain from damage (Gonzalez Deniselle et al., 2002; Jiang, Chopp, Stein, & Feit, 1996; Stein, 2008; Thomas, Nockels, Pan, Shaffrey, & Chopp, 1999; Yu, 1989), or promote neurological and functional recovery after damage (Frye, Rhodes, Walf, & Harney, 2002; Rhodes, McCormick, & Frye, 2004; Stein, 2008; Thomas et al., 1999; Yu, 1989). Indeed, the beneficial effects of chronic P₄ are observed in a murine model of early-onset Alzheimer's Disease, in which there is overexpression of mutations of amyloid precursor protein and presenilin (Frye & Walf, 2008). Together, these data, in addition to those from the present study, suggest that mice can be responsive to the cognitive-enhancing effects of physiological dosing with acute pre- or post-training P₄ and/or chronic P₄. However, it must be noted that mice were repeatedly exposed to P₄ (or vehicle) for five weeks. Although this type of P₄ exposure may approximate what is observed for mice across endogenous estrous cycles, the cumulative effects of P₄ on cell morphology or other structural changes in the brain need to be addressed in the future.

Although these data suggest that physiological levels during consolidation, rather than testing, may be critical for enhancing performance in some cognitive tasks, other factors in interpreting these results need to be considered. The beneficial effects of chronic P₄ were not observed across all tasks utilized, suggesting that there may be some site specificity of P₄'s effects. Although some lesion studies suggest that brain areas, such as the hippocampus, cortex, and/or amygdala, may contribute to performance in specific tasks utilized (Ennaceur, Neave, & Aggleton, 1997; Gerlai, 1998; Lee, Jerman, & Kesner, 2005; Morris, Garrud, Rawlins, & O'Keefe, 1982; Phillips & LeDoux, 1992; but also see Gerlai, McNamara, Williams, & Phillips, 2002; Mumby, 2001), whether there is site specificity of P₄'s actions in these regions needs to be addressed directly in future studies. Additionally, the present data that P₄ improved performance in both the contextual and cued test trials of the conditioned fear task suggest that P₄ has actions in the hippocampus and amygdala for cognitive performance. Progestogens have actions through the amygdala and hippocampus to attenuate fear and anxiety (Akwa, Purdy, Koob, & Britton, 1999; Bitran, Foley, Audette, Leslie, & Frye, 2000; Frye & Walf, 2004). This provokes the question about whether the cognitive-enhancing effects of P₄ are indirect and associated with changes in arousal (Pfaff, Martin, & Ribeiro, 2007). Evidence from the present study suggests that all effects observed were not due to changes in general arousal, altering emotional learning, and/or affect. First, we found that P₄ improved performance in the water maze and conditioned fear, 24 h after P₄ was injected, which is a time when P₄ and other progestogen levels should be at nadir. We have previously found that cognitive performance is enhanced, and affective behavior is not changed, 24 h after OVX rats are injected with P₄ SC (Frye & Lacey, 2000). As such, the cognitive-enhancing effects of progestogens can occur independent of demonstrable changes in affective behavior. Second, delay in P₄ treatment following training did not enhance performance of mice when they were tested 4 h post-training when P₄ would be in circulation. Third, in relation to how repeated exposure of mice to P₄ may have produce cumulative effects that may be related to changes in affect, we did not see differences in flinch-jump ratings (i.e. their responsiveness to footshock) of mice during training in the conditioned fear task. Thus, these data suggest that P₄ may have mnemonic effects.

The present findings that P₄ can have mnemonic effects in mice are intriguing in light of potential implications for hormone-replacement therapies. Hormone-replacement therapies are used by some women to promote successful aging. E₂-based hormone-replacement therapies may protect against age-related cognitive decline and/or decrease the risk, or delay the onset of, neurodegenerative disorders (Asthana et al., 1999, 2001; Henderson et al., 2000; Paganini-Hill & Henderson, 1994, 1996; Phillips & Sherwin, 1992b). Recently, re-analyses of data from the Women's Health Initiative (WHI) trials demonstrated that beneficial effects of E₂ may be limited to the perimenopausal period and that older women, who are long past menopause, can experience detrimental effects of E₂-based hormone-replacement therapies (Sherwin, 2007). Notably, it is a standard practice for progestogens to be given to menopausal women with E₂-based hormone-replacement therapies to reduce the risk of cancers of reproductive tissue (Sitruk-Ware, 2002, 2007). As such, it is important to understand the capacity for progestogens, apart from effects of E₂ or effects of these steroids in conjunction, to influence cognitive processes and/or have neurotrophic effects. Although it is likely that E₂ and P₄ may produce dissimilar cognitive effects depending upon the task utilized, in addition to other factors, we have found that physiological dosing with E₂ and/or P₄ to OVX rats produces similar enhancements in the object recognition task (Walf et al., 2006). In our laboratory, we are interested in determining whether formation, and subsequent actions, of the P₄ metabolite and neurosteroid, allopregnanolone, may underlie these similar beneficial effects of E₂ and P₄ treatment. Indeed, P₄ is a precursor of allopregnanolone and E₂ can increase allopregnanolone by enhancing activity of rate-limiting enzymes required for allopregnanolone formation (Cheng & Karavolas, 1973; Frye & Rhodes, 2005). This possibility is currently being investigated in our laboratory.

In summary, the present results demonstrate that pre-training P₄ treatment increased spontaneous alternations. Post-training P₄ injections improved probe trial performance in the water maze when mice were tested 24 h after treatment. Mice injected with P₄ immediately post-training had improved performance in the object recognition task and the cued and contextual conditioned fear tasks. These performance improvements were not observed if there was a delay in post-training P₄ treatment. These data suggest that physiological P₄ during consolidation enhances cognitive performance of young, OVX mice. The present data that P₄ has mnemonic effects in an *in vivo* mouse model enables us to begin to further investigate the nature and mechanisms of P₄'s effects for learning and memory. This basic research is sorely needed for its clinical relevance in the etiology and/or therapeutic treatment of age-related cognitive sequelae.

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Fig. 1.

The mean ($\pm$ sem) percentage of spontaneous alternations made in the spontaneous alternation task of ovariectomized mice injected with vehicle (veh; open bar; $n = 11$) or progesterone (P₄; closed bar; $n = 13$) 1 h before testing. * vs. vehicle condition, $P < 0.05$.

Fig. 2.

The mean ($\pm$ sem) percentage of time spent exploring a novel object in the object placement task of ovariectomized mice injected with vehicle (veh; open bar; $n = 11$) or progesterone (P_4 ; closed bar; $n = 13$) immediately post-training (left panel) or 1.5 h post-training (right panel; $n = 8$ for each condition). condition, * vs. vehicle $P < 0.05$.

Fig. 3.

(Top panel) The mean (s + sem) latencies to reach the hidden platform during the training trials of the water maze. (Bottom panel) On the left, the mean (s + sem) latency to reach the location that the hidden platform had been during training in the water maze probe trial of ovariectomized mice injected with vehicle (veh; open bar; $n = 11$) or progesterone (P_4 ; closed bar; $n = 13$) immediately post-training is depicted. On the right, the latency to reach the visible platform during the control, cued trials of these mice is depicted. * vs. vehicle condition, $P < 0.05$.

Fig. 4.

The mean (s + sem) duration spent freezing in the contextual (top) and cued (bottom) testing trials of the conditioned fear task of ovariectomized mice injected with vehicle (veh; open bar; $n = 11$) or progesterone (P_4 ; closed bar; $n = 13$) immediately post-training (left panel) or 1.5 h post-training (right panel; $n = 8$ for each condition). * vs. vehicle condition, $P < 0.05$.