Pharmacology, Biochemistry and Behavior 93 (2009) 177–182

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Dorsal hippocampal progesterone infusions enhance object recognition in young female mice

Patrick T. Orr [a], Michael C. Lewis [a], Karyn M. Frick [a,b,*]

[a] Department of Psychology, Yale University, New Haven, CT 06520, USA
[b] Interdepartmental Neuroscience Program, Yale University, New Haven, CT 06520, USA

ARTICLE INFO

Article history:
Received 30 June 2008
Received in revised form 12 May 2009
Accepted 17 May 2009
Available online 27 May 2009

Keywords:
Progestins
Object memory
Hippocampus
Mouse
Rodent

ABSTRACT

The effects of progesterone on memory are not nearly as well studied as the effects of estrogens. Although progesterone can reportedly enhance spatial and/or object recognition in female rodents when given immediately after training, previous studies have injected progesterone systemically, and therefore, the brain regions mediating this enhancement are not clear. As such, this study was designed to determine the role of the dorsal hippocampus in mediating the beneficial effect of progesterone on object recognition. Young ovariectomized C57BL/6 mice were trained in a hippocampal-dependent object recognition task utilizing two identical objects, and then immediately or 2 h afterwards, received bilateral dorsal hippocampal infusions of vehicle or 0.01, 0.1, or 1.0 μg/μl water-soluble progesterone. Forty-eight hours later, object recognition memory was tested using a previously explored object and a novel object. Relative to the vehicle group, memory for the familiar object was enhanced in all groups receiving immediate infusions of progesterone. Progesterone infusion delayed 2 h after training did not affect object recognition. These data suggest that the dorsal hippocampus may play a critical role in progesterone-induced enhancement of object recognition.

1. Introduction

Much recent interest has been focused on the ability of sex-steroid hormones, such as estrogens and progestins, to modulate cognitive function due, in part, to conflicting reports from studies using estrogen plus progestin therapy as a treatment for memory loss in menopausal women. For example, several studies in post-menopausal women have demonstrated beneficial effects of estrogen plus progestin treatment on working memory (Duff and Hampson, 2000), verbal memory (Grigorova and Sherwin, 2006; Maki et al., 2001), episodic memory, and verbal fluency (Yonker et al., 2006). An additional study reported a trend for improvement in figural memory, but also a detrimental effect on verbal memory (Resnick et al., 2006). Consistent with the latter effect, other reports find no benefit of estrogen plus progestin treatment on global cognitive function (Rapp et al., 2003), declarative memory, or working memory (Wolf et al., 2005). Together, these data suggest a potentially complex interaction between estrogens and progestins with regard to mnemonic function that may depend on various factors including timing of treatment, specific hormone formulation, and type of memory tested. One way to help tease apart the interactions between the two hormones may be to determine how each modulates memory, both independently and in combination, in animal models of memory formation.

The primary endogenous sources of progesterone are the adrenals and gonads (Nelson, 2000). Although both estrogens and progestins are produced in the ovaries in response to stimulation from the brain, the vast majority of basic research in this area has focused on effects of estrogens, such as 17β-estradiol, rather than progestins, such as progesterone. As a result, much less is known about the role progesterone may play in modulating memory, both alone and in combination with estradiol. However, there is abundant evidence to suggest that both estradiol and progesterone alter the physiology of regions of the brain that are critical for learning and memory, such as the hippocampus. For example, estradiol and progesterone have both been demonstrated to modulate CA1 dendritic spine density in rats (Woolley and McEwen, 1993). Whereas estradiol reverses the decrease in synaptic spine density of pyramidal CA1 cells following ovariectomy, the effects of progesterone are more complex, leading to an initial increase in spine density during the first 2–6 h after treatment, with a subsequent decrease over the next 18 h (Woolley and McEwen, 1993). Further, estradiol and progesterone in female monkeys have both been shown to increase hippocampal synaptic proteins; estradiol alone increased syntaxin, synaptophysin, and spinophilin, whereas progesterone alone increased synaptophysin (Choi et al., 2003). Interestingly, co-administration of both estradiol and progesterone reversed these increases (Choi et al., 2003). Additionally, estradiol and progesterone can modulate intracellular signaling. For example, both estradiol and progesterone alone

* Corresponding author. Department of Psychology, Yale University, P.O. Box 208205, New Haven, CT 06520, USA. Tel.: +1 203 432 4673; fax: +1 203 432 7172.
E-mail address: karyn.frick@yale.edu (K.M. Frick).

0091-3057/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2009.05.012

activate the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) pathway, and induce the nuclear translocation of phosphorylated ERK in hippocampal cultures (Nilsen and Brinton, 2002, 2003).

Although several previous studies have examined the ability of estradiol and progesterone to modulate hippocampal-dependent memory in young ovariectomized rats and mice, these findings are inconsistent. For example, estradiol plus progesterone treatment improved spatial working memory in the T-maze (Gibbs, 2000) and spatial reference memory in the Morris water maze (Markham et al., 2002), reversed scopolamine-induced impairments of spatial working and reference memory in a radial arm maze (Tanabe et al., 2004), protected against colchicine-induced impairments in water maze performance (Vongher and Frye, 1999), and improved object recognition (Walf et al., 2006). In contrast, estradiol plus progesterone treatment has also been reported to impair or not affect spatial memory in the water maze in rats (Bimonte-Nelson et al., 2006; Chesler and Juraska, 2000), to impair footshock learning in mice (Farr et al., 1995), and to block the neuroprotective effects of estradiol against kainite lesions in rats (Rosario et al., 2006). Inconsistencies among these studies may be due to a number of methodological factors, as many aspects of experimental design differed among them. However, these discrepant findings may also suggest that attempting to understand how progesterone modulates memory by administering it with estradiol is not the most effective method of examining the role of this hormone in memory formation. Rather, examining the effects of progesterone alone on memory may shed more light on this subject.

The few studies that have examined the effects of progesterone alone on memory suggest an important effect of timing of administration. Chronic administration of progesterone prior to training (i.e., pre-training) impaired both spatial working memory and footshock avoidance learning in young ovariectomized rats and mice (Bimonte-Nelson et al., 2004; Farr et al., 1995). Acute pre-training progesterone injection had no effect on spatial memory in young ovariectomized rats (Chesler and Juraska, 2000; Sato et al., 2004). In contrast, single intraperitoneal injections of progesterone administered to young ovariectomized rats immediately after training (i.e., post-training) improved memory in Y-maze inhibitory avoidance and object recognition tasks (Frye and Lacey, 2000; Walf et al., 2006; Harburger et al., 2008). Systemic post-training progesterone injections also improved object recognition in ovariectomized middle-aged and aged mice (Lewis et al., 2008b). The discrepancy between the effects of pre- and post-training injections could stem from differences in task, dose, and/or side effects of progesterone treatment, given that this hormone has anxiolytic and analgesic effects (Bitran et al., 1991a,b; Frye and Duncan, 1994) which could influence non-mnemonic aspects of task performance (e.g., motor activity, motivation). As such, pre-training administration makes it more difficult to accurately interpret the effects of progesterone on memory, whereas administration of rapidly-metabolized forms of progesterone immediately after training allow for effects on memory consolidation to be observed on later testing in the absence of non-mnemonic confounds due to circulating progesterone.

The fact that the aforementioned studies administered progesterone systemically prevents specific localization of the modulatory effects of progesterone on the brain. The dorsal hippocampus is critical for several types of memory, including spatial and working memory, (Hock and Bunsey, 1998; Moser et al., 1993; Packard and McGaugh, 1996), and has been implicated more recently in object recognition memory (Baker and Kim, 2002; Broadbent et al., 2004). We have previously shown that estradiol infusion into the dorsal hippocampus enhances object recognition memory (Fernandez et al., 2008). Progesterone receptors are located in dorsal hippocampus (Guerra-Araiza et al., 2002, 2003; Kato et al., 1994), and progesterone can rapidly alter hippocampal morphology and cell signaling as described above (Choi et al., 2003; Nilsen and Brinton, 2002, 2003; Woolley and McEwen, 1993). Further, progesterone administration has been demonstrated to influence performance in memory tasks mediated by the dorsal hippocampus (e.g., Frye and Lacey, 2000; Gibbs, 2000; Harburger et al., 2008). As such, the dorsal hippocampus may play a critical role in the mnemonic effects of progesterone.

Therefore, the present study was designed to examine the effects of dorsal hippocampal progesterone infusions on object recognition memory. Young ovariectomized mice were implanted bilaterally with guide cannulae aimed at the dorsal hippocampus. Mice were then trained in an object recognition task, immediately infused with vehicle or one of three doses of rapidly metabolized water-soluble progesterone, and then tested 48 h later. This design was based on previous studies showing that systemic administration of water-soluble progesterone can enhance object recognition in young ovariectomized mice (Harburger et al., 2008). The version of the task used in this experiment is dependent on the hippocampus based on lesion (Clark et al., 2000) and pharmacological (Baker and Kim, 2002; Fernandez et al., 2008) studies. Based on the previous post-training studies discussed above, we hypothesized that intrahippocampal progesterone would enhance object recognition.

2. Methods

2.1. Subjects

Subjects were 46 female C57BL/6 mice received from Taconic (Germantown, NY) at 6 weeks of age. Mice were group-housed, five per shoebox cage in a room with a 12:12 light/dark cycle (lights on at 7:00). All behavioral testing was conducted during the light phase, and animals had ad libitum access to food and water. All procedures were approved by the Institutional Animal Care and Use Committee of Yale University, and conformed to the guidelines established by the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.2. Surgical procedures

All mice were ovariectomized prior to treatment in order to eliminate endogenous sources of ovarian hormones. Surgery was conducted at least one week prior to testing as previously described (Lewis et al., 2008a). Briefly, mice were anesthetized with isoflurane gas (2% isoflurane in 100% oxygen) and placed into a sterotaxic apparatus (Kopf Instruments, Tujunga, CA) in preparation for cannula implantation. The ovaries and tips of the uterine horns were isolated and removed through bilateral dorsal incisions made at the level of the pelvis. The muscle wall was sutured and the skin closed with wound clips.

Cannula implantation took place immediately following ovariectomy as previously described (Lewis et al., 2008a). The skull was exposed through an incision in the scalp. Bregma and Lambda were aligned in the same horizontal plane, and small bilateral holes (1 cm in diameter) were drilled for placement of stainless steel guide cannulae (C232GC, 22 gauge, Plastics One, Roanoke, VA) with dummy cannulae (C232 DC; Plastics One). Cannulae were directed toward the dorsal hippocampus (1.7 mm posterior to bregma, $+/-1.5$ mm lateral to midline, and 2.3 mm ventral to the surface of the skull), based on Paxinos and Franklin (2003). Cannulae were affixed to the skull with dental cement, which also closed the wound.

Mice were allowed to recover for one week after surgery and received 30 mg/kg ibuprofen in the drinking water as an analgesic during recovery. After surgery, mice were housed singly for the remainder of the experiment.

2.3. Hormone infusion

Mice were randomly divided into four groups receiving vehicle (2-hydroxypropyl-β-cyclodextrin (HBC) complex; Sigma, St. Louis, MO; $n=12$) or 0.01 ($n=11$), 0.1 ($n=14$), 1 ($n=9$) μg of water-soluble

progesterone (2-hydroxyproyl-β-cyclodextrin-encapsulated progesterone; Sigma) dissolved in physiological saline. Progesterone was purchased already encapsulated in HBC, and concentrations were based on the amount of progesterone, not the amount of HBC-progesterone, in solution. HBC enhances the water solubility of progesterone, and does not detrimentally affect the pharmacokinetic properties of steroid hormones (Brewster et al., 1995). Because this form of progesterone is metabolized within hours (Pitha et al., 1986), it is likely not in circulation during either phase of behavioral testing, thus, minimizing confounding effect of non-mnemonic performance factors (e.g., motivation, anxiety) on performance. Vehicle or progesterone was infused immediately following the sample phase of the object recognition task as described previously (Lewis et al., 2008a).

Briefly, dummy cannulae were replaced with injection cannulae (22 gauge; extending 0.8 mm beyond the tip of the guide cannula) that were attached to polyethylene tubing (PE50; Plastics One). This tubing was connected to a 10 μl Hamilton syringe controlled by a microinfusion pump (KDS 100, KD Scientific; New Hope, PA). After the injection cannula was inserted into the guide cannula, vehicle or one of three concentrations of progesterone (0.02, 0.2, or 2.0 μg/μl) was infused at a rate of 0.5 μl/min for 1 min at a volume of 0.5 μl/side of the hippocampus, resulting in doses of 0.01, 0.1, and 1.0 μg. Methylene blue infused into the entorhinal cortex using this protocol diffuses approximately 1 mm^3 (Lewis and Gould, 2007). Although the diffusion properties of HBC-progesterone in the hippocampus may differ from those of methylene blue, these data suggest that infusions were likely limited to the dorsal hippocampus.

To demonstrate that the effects of progesterone on object recognition were limited to the first 2 h after training, an additional set of mice was infused as described above with vehicle or 0.1 μg of progesterone 2 h after training.

2.4. Object recognition

Mice were tested in a hippocampal-dependent version of the novel object recognition task as previously described (Frick and Gresack, 2003) one week after surgery. The testing chamber was a white open field box (approximately 58 cm long by 58 cm wide by 46 cm high). A camera mounted on the ceiling above the chamber was connected to a monitor, VCR, and computer outside of the room that were used to collect data. Mice were first habituated to the testing chamber by placing them in the empty chamber and allowing them to explore freely for 5 min. To control for general levels of activity prior to hormone infusion, locomotor activity was measured by recording crossings of a 5×3 grid superimposed over the chamber on the computer monitor. Crossings in the inner grids (inner crossing), outer grids (outer crossings), and all grids (total crossings) were recorded.

Sample phase training occurred the following day. Each mouse was placed in the testing chamber with two identical objects that were positioned near the northeast and northwest corners of the chamber. Mice were allowed to freely explore the objects until the total amount of exploration time for both objects equaled 30 s. Maintaining a constant exploration time rather than a constant trial time in this fashion controlled for differences in propensity to explore between mice, and ensured that all mice experienced the objects for the same amount of time. This protocol has been shown to critically involve the dorsal hippocampus (Clark et al., 2000; Baker and Kim, 2002). Immediately after completion of the sample phase, mice were infused with vehicle or progesterone and then were returned to their home cages. The chamber and objects were cleaned with a 70% ethanol solution between mice.

Retention testing in the choice phase occurred 48 h later. Generally, untreated young ovariectomized mice do not demonstrate memory after 48 h (Lewis et al., 2008a). Thus, the 48 hour delay is appropriate

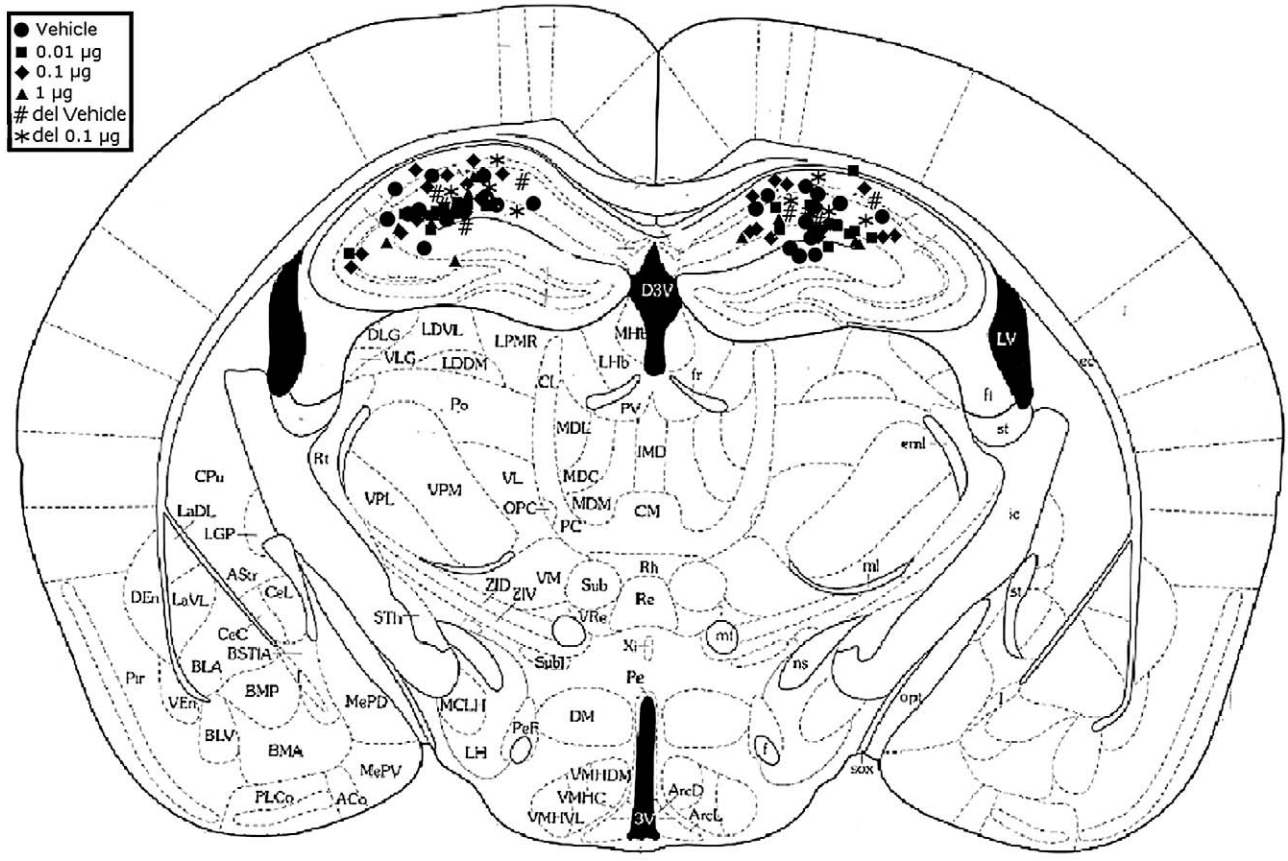

Fig. 1. Cannula placements for each group in both experiments. Each point represents the tip of the guide cannulae; infusion cannulae extended 0.8 mm beyond the tip of the guide cannulae. All injection sites were within the dorsal hippocampus. del = delayed. Figure adapted from Paxinos and Franklin (2003).

for examining hormone-mediated enhancements in memory. Each mouse was placed back into the testing chamber along with one object previously seen in the sample phase (familiar) and one novel object. The position (northeast or northwest corner) and identity of the novel object were counterbalanced across mice. Mice were again allowed to explore until they had accrued 30 s of object exploration. Time spent with the objects was recorded. Because mice innately tend to prefer novelty, a mouse that remembers the familiar object from the sample phase should spend significantly more time than chance (15 s) exploring the novel object and less time than chance exploring the familiar object. Such a result would suggest a preference for the novel object. In addition, elapsed time to accumulate 30 s of object exploration was recorded to control for effects of progesterone infusion on non-mnemonic aspects of performance. Again, 70% ethanol was used to clean the chamber and objects between mice.

2.5. Data analysis

One-way ANOVAs were conducted on inner, outer and total grid crossings collected during habituation to examine pre-existing differences in locomotor activity.

To assess memory retention, one-way analyses of variance (ANOVAs) were conducted on time spent exploring the novel object during retention testing. Separate analyses were conducted on the data from the four dose–response groups and the data from the two groups testing effects of a two-hour delay (see below). A one-way ANOVA was also conducted on elapsed time during retention testing to measure effects of progesterone infusion on non-mnemonic aspects of task performance.

For all ANOVAs, significant main effects of Treatment were followed up with Fisher's LSD post-hoc tests. All analyses were conducted using SPSS (SPSS Inc., Chicago, IL).

2.6. Histology

Cannula placements were examined after behavioral testing to ensure correct placement. Mice were cervically decapitated and brains were removed and stored in 10% formalin solution (Fisher Scientific, Pittsburgh, PA). A freezing microtome was used to cut coronal sections (60 μm thick) proximal to cannula tracts and mounted on PLUS slides (Fisher Scientific) and dried overnight. Cannula placements were verified under a light microscope. Because injection sites were within dorsal hippocampus for all mice (Fig. 1), no mice were excluded from the data analyses.

3. Results

Grid crossings during habituation are presented in Table 1. The main effect of Treatment was not significant for the inner, outer, or total grid crossings during the habituation phase of testing (inner, $F(3, 37) = 1.038$, $p > 0.05$; outer, $F(3, 37) = 2.194$, $p > 0.05$; total, $F(3, 37) = 1.828$, $p > 0.05$), indicating that the groups exhibited similar levels of general locomotor activity prior to progesterone infusion.

The main effect of Treatment was significant for time spent with the novel object during retention testing ($F(3, 42) = 4.26$, $p = 0.01$; Fig. 2), indicating a significant effect of progesterone on object recognition. Post-hoc tests revealed that each progesterone-treated group explored the novel object significantly more than the vehicle group (0.01 μg, $p = 0.006$; 0.1 μg, $p = 0.015$; 1.0 μg, $p = 0.004$; Fig. 2). These data suggest that bilateral dorsal hippocampal progesterone treatment enhances object recognition in young ovariectomized mice.

Table 1
Grid crossings during habituation.

Group	Inner	Outer	Total
Vehicle	27.45 ± 3.96	130.55 ± 11.12	158.00 ± 12.93
0.01 μg	23.10 ± 3.09	172.60 ± 17.35	195.70 ± 19.27
0.1 μg	23.91 ± 3.38	144.27 ± 17.20	168.18 ± 19.20
1.0 μg	17.67 ± 3.08	116.50 ± 9.49	134.17 ± 12.16

Values represent mean ± SEM.

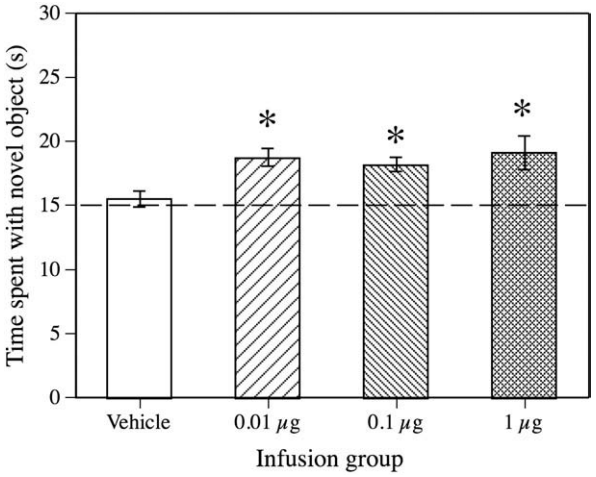

Fig. 2. Time spent with novel object during retention testing. Each bar represents the group mean ± the standard error of the mean (SEM). The dotted line indicates chance performance (15 s). Asterisks indicate a significant ($p < 0.05$) difference from the vehicle group. All mice receiving progesterone spent significantly more time exploring the novel object than mice receiving vehicle, thereby demonstrating that progesterone enhanced memory for the familiar object 48 h after infusion.

The main effect of Treatment was not significant for elapsed time to accumulate 30 s of exploration during retention testing ($F(3, 42) = 1.082$, $p > .05$), demonstrating that hormone treatment did not affect motivation to explore the objects. Elapsed time (± standard error of the mean) for each group was as follows: Vehicle: 522.1 ± 92.8; 0.01 μg: 395.3 ± 86.3; 0.1 μg: 567.3 ± 150.6; 1.0 μg: 738.0 ± 149.8.

To demonstrate that the effects of progesterone were limited to the period immediately following training, another set of mice received bilateral dorsal hippocampal infusions of vehicle or a moderate dose of progesterone (0.1 μg) 2 h after training. There was no difference in novel object exploration during testing between mice receiving delayed infusion of vehicle or of 0.1 μg progesterone ($t(6) = 0.711$, $p > 0.05$, Fig. 3). These data suggest that the effects of the immediate progesterone treatments observed above occur within the first 2 h after training.

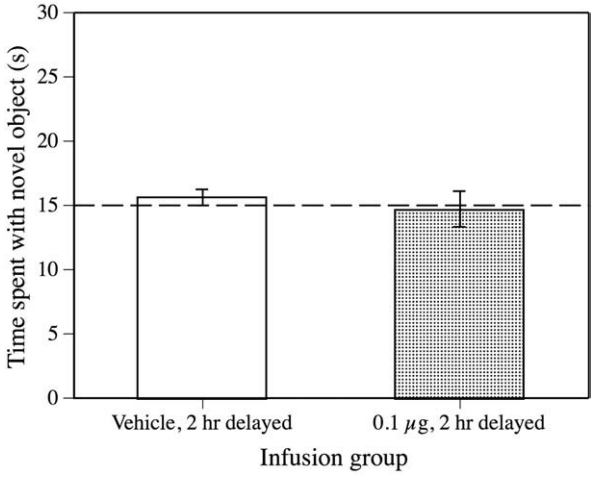

Fig. 3. Time spent with the novel object during the retention test after delayed infusions. Each bar represents the group mean ± the standard error of the mean (SEM). The dotted line indicates chance performance (15 s). The delayed vehicle and delayed 0.1 μg progesterone groups did not differ in the time spent exploring the novel object.

4. Discussion

The present data demonstrate that immediate post-training infusion of progesterone into the dorsal hippocampus enhances object recognition in young ovariectomized female mice, and that this effect occurs within 2 h of infusion. Although peripheral post-training injections of progesterone have previously been shown to enhance object recognition throughout the lifespan in female mice using this object recognition paradigm (Harburger et al., 2008; Lewis et al., 2008b), the present data suggest that the dorsal hippocampus may be critical for the beneficial effects of progesterone on object recognition. Thus, this finding is an important step towards elucidating the neural mechanisms by which progesterone modulates memory.

The results of the present study are consistent with previous findings that post-training progesterone treatment enhances object recognition using a different testing protocol (Frye and Lacey, 2000; Walf et al., 2006), and fits particularly well with data from our laboratory using the same testing protocol showing that progesterone treatment immediately post-training enhances object recognition in young (Harburger et al., 2008), middle-aged, and aged (Lewis et al., 2008b) female mice. It is also consistent with beneficial effects of post-training progesterone on spatial memory consolidation in the Morris water maze in aged female mice (Lewis et al., 2008b). However, the beneficial effects of post-training hormone administration contrast with previous reports of detrimental effects (Bimonte-Nelson et al., 2004; Farr et al., 1995) or no effects (Chesler and Juraska, 2000; Sato et al., 2004) of pre-training progesterone administration in various learning and memory tasks including the Morris water maze, radial arm maze, and inhibitory avoidance. Given that all post-training studies to date have shown a beneficial effect of progesterone, whereas pre-training studies have not, it is likely that timing of administration is critical to the effect of this hormone. Thus, anxiolytic and analgesic effects (Bitran et al., 1991a,b; Frye and Duncan, 1994) of circulating progesterone and progestin metabolites may interfere with task performance if treatment is administered prior to training. However, such effects may still influence behavior during retention testing if they lead to long-term genomic alterations, a possibility that has yet to be fully addressed. The timing of progesterone treatment may also be important with regard to its effects on the brain. The present data, and previous reports, demonstrate that object recognition memory, in particular, is sensitive to modulation of the dorsal hippocampus (Clark et al., 2000; Baker and Kim, 2002; Fernandez et al., 2008). In the dorsal hippocampus, progesterone has a biphasic effect on CA1 synaptic spine density (Woolley and McEwen, 1993), such that progesterone increases spine density in the first 2–6 h following treatment, and subsequently decreases spine density below baseline over the next 18 h. Such biphasic morphological changes in the hippocampus could also account for the apparently conflicting reports of the effects of progesterone administration on hippocampal memory and underscore the sensitivity of such effects to timing of treatment.

Although the present data indicate that the progesterone-induced enhancement of object recognition is associated with the dorsal hippocampus, the specific cellular mechanisms underlying this enhancement remain unclear. As previously described, progesterone reportedly increases specific synaptic proteins (Choi et al., 2003), and exerts a biphasic modulatory effect on CA1 dendritic spines (Woolley and McEwen, 1993). Additionally, progesterone, like estradiol, has been shown to modulate intracellular signaling in hippocampal cultures (Nilsen and Brinton, 2002, 2003), specifically the ERK/MAPK pathway, leading to an increase in nuclear translocation of phosphorylated ERK. Interestingly, the effects of progesterone on the ERK phosphorylation can be blocked by both progesterone and estrogen receptor antagonists (Migliaccio et al., 1998). Progesterone has also been shown to influence the cAMP/PKA (Collado et al., 1985) and PI-3K (Singh, 2001) pathways. Given the importance of these signaling pathways to hippocampal memory (Adams and Sweatt, 2002), activation of cell signaling in the dorsal hippocampus may be critical to the mnemonic effects of progesterone.

The receptor mechanisms through which progesterone affects memory are unclear, although there are a number of possibilities (Singh, 2005). Among these possibilities are nuclear progesterone receptors (PRA and PRB), which have been demonstrated to inhibit estradiol-induced transcription (Chalbos and Galtier, 1994), or to more recently described membrane-bound progesterone receptors, which have been demonstrated to alter both cAMP and MAPK activity *in vitro* (Zhu et al., 2003). Progesterone can modulate $GABA_A$ receptors (Reddy et al., 2005), which are common in the hippocampus and have been demonstrated to affect hippocampal memory tasks (Car et al., 1996; Mohler et al., 2008), although it is unclear if effects on behavior are due to specifically to a disruption of memory or of non-mnemonic aspects of task performance such as anxiety. Additionally, the effects of progesterone on memory could be the indirect result of one of the many progesterone metabolites (e.g. androgens, estrogens, allopregnanolone), which can independently influence memory (Melchior and Ritzmann, 1996; Silvers et al., 2003). As such, understanding how this complex hormone influences memory will prove challenging, given the many possible routes through which progesterone can modulate hippocampal physiology. Nevertheless, such an understanding should be possible to achieve given the consistency of post-training progesterone's effects on memory.

In conclusion, the present data suggest that the dorsal hippocampus plays a critical role in progesterone-based enhancement of object recognition, which extends the previous literature on systemic post-training progesterone treatment. Identifying a specific brain region, such as the hippocampus, as critical for the beneficial mnemonic effects of progesterone is a necessary first step in understanding how this hormone modulates memory alone and in combination with estradiol. Future studies will undoubtedly reveal how progestins and estrogens interact in the hippocampus and related brain regions to modulate memory processes, allowing for more effective treatment of age-related cognitive decline, particularly in post-menopausal women.

Acknowledgements

This work was supported by Yale University and NIH grant AG022525 to K.M.F.

References

Adams JP, Sweatt JD. Molecular psychology: roles for the ERK/MAP kinase cascade in memory. Annu Rev Pharmacol Toxicol 2002;42:135–63.
Baker KB, Kim JJ. Effects of stress and hippocampal NMDA receptor antagonism on recognition memory in rats. Learn Memory 2002;9:58–65.
Bimonte-Nelson HA, Singleton RS, Williams BJ, Granholm AC. Ovarian hormones and cognition in the aged female rat: II. Progesterone supplementation reverses the cognitive enhancing effects of ovariectomy. Behav Neurosci 2004;118:707–14.
Bimonte-Nelson HA, Francis KR, Umphlet CD, Granholm AC. Progesterone reverses the spatial memory enhancements initiated by tonic and cyclic oestrogen therapy in middle-aged ovariectomized female rats. Eur J Neurosci 2006;24:229–42.
Bitran D, Hilvers RJ, Kellogg CK. Anxiolytic effects of 3 alpha-hydroxy-5 alpha[beta]-pregnan-20-one: endogenous metabolites of progesterone that are active at the GABAA receptor. Brain Res 1991a;561:157–61.
Bitran D, Hilvers RJ, Kellogg CK. Ovarian endocrine status modulates the anxiolytic potency of diazepam and the efficacy of gamma-aminobutyric acid-benzodiazepine receptor-mediated chloride ion transport. Behav Neurosci 1991b;105:653–62.
Brewster ME, Anderson WR, Loftsson T, Huang MJ, Bodor N, Pop E. Preparation, characterization, and anesthetic properties of 2-hydroxypropyl-beta-cyclodextrin complexes of pregnanolone and pregnenolone in rat and mouse. J Pharm Sci 1995;84: 1154–9.
Broadbent NJ, Squire LR, Clark RE. Spatial memory, recognition memory, and the hippocampus. Proc Natl Acad Sci USA 2004;101:14515–20.
Car H, Kuziemka-Leska M, Wisniewski K. The NMDA and GABA-A receptors in behavioral activity of rats. Acta Physiol Hung 1996;84:269–70.
Chalbos D, Galtier F. Differential effect of forms A and B of human progesterone receptor on estradiol-dependent transcription. J Biol Chem 1994;269:23007–12.
Chesler EJ, Juraska JM. Acute administration of estrogen and progesterone impairs the acquisition of the spatial Morris water maze in ovariectomized rats. Horm Behav 2000;38:234–42.
Choi JM, Romeo RD, Brake WG, Bethea CL, Rosenwaks Z, McEwen BS. Estradiol increases pre- and post-synaptic proteins in the CA1 region of the hippocampus in female rhesus macaques (*Macaca mulatta*). Endocrinology 2003;144:4734–8.
Clark RE, Zola SM, Squire LR. Impaired recognition memory in rats after damage to the hippocampus. J Neurosci 2000;20:8853–60.

Collado ML, Rodriguez-Manzo G, Cruz ML. Effect of progesterone upon adenylate cyclase activity and cAMP levels on brain areas. Pharmacol Biochem Behav 1985;23: 501–4.
Duff SJ, Hampson E. A beneficial effect of estrogen on working memory in postmenopausal women taking hormone replacement therapy. Horm Behav 2000;38: 262–76.
Farr SA, Flood JF, Scherrer JF, Kaiser FE, Taylor GT, Morley JE. Effect of ovarian steroids on footshock avoidance learning and retention in female mice. Physiol Behav 1995;58:715–23.
Fernandez SM, Lewis MC, Pechenino AS, Harburger LL, Orr PT, Gresack JE, et al. Estradiol induced enhancement of object memory consolidation involves hippocampal ERK activation and membrane-based estrogen receptors. J Neurosci 2008;28:8660–7.
Frick KM, Gresack JE. Sex differences in the behavioral response to spatial and object novelty in adult C57BL/6 mice. Behav Neurosci 2003;117:1283–91.
Frye CA, Duncan JE. Progesterone metabolites, effective at the GABAA receptor complex, attenuate pain sensitivity in rats. Brain Res 1994;643:194–203.
Frye CA, Lacey EH. Progestins influence performance on cognitive tasks independent of changes in affective behavior. Psychobiology 2000;28:500–63.
Gibbs RB. Long-term treatment with estrogen and progesterone enhances acquisition of a spatial memory task by ovariectomized aged rats. Neurobiol Aging 2000;21:107–16.
Grigorova M, Sherwin BB. No differences in performance on test of working memory an executive functioning between healthy elderly postmenopausal women using or not using hormone therapy. Climacteric 2006;9:181–94.
Guerra-Araiza C, Coyoy-Salgado A, Camacho-Arroyo I. Sex differences in the regulation of progesterone receptor isoforms expression in the rat brain. Brain Res Bull 2002;59:105–9.
Guerra-Araiza C, Villamar-Cruz O, Gonzalez-Arenas A, Chavira R, Camacho-Arroyo I. Changes in progesterone receptor isoforms content in the rat brain during the oestrus cycle and after oestradiol and progesterone treatments. J Neuroendocrinol 2003;15:984–90.
Harburger LL, Pechenino AS, Saadi A, Frick KM. Post-training progesterone dose-dependently enhances object, but not spatial, memory consolidation. Behavioural Brain Research 2008;194:174–80.
Hock BJ, Bunsey MD. Differential effects of dorsal and ventral hippocampal lesions. J Neurosci 1998;18:7027–32.
Kato J, Hirata S, Nozawa A, Yamada-Mouri N. Gene expression of progesterone receptor isoforms in the rat brain. Horm Behav 1994;28:454–63.
Lewis MC, Gould JT. Reversible inactivation of the entorhinal cortex disrupts the establishment and expression of latent inhibition of cued fear conditioning in C57BL/6 mice. Hippocampus 2007;17:462–70.
Lewis MC, Kerr KM, Orr PT, Frick KM. Estradiol-induced enhancement of object memory consolidation involves NMDA receptors and protein kinase A in the dorsal hippocampus of female C57BL/6 mice. Behav Neurosci 2008a;122:716–21.
Lewis MC, Orr PT, Frick KM. Acute progesterone enhances spatial and object memory in aged female C57BL/6 mice. Horm Behav 2008b;54:455–62.
Maki PM, Zonderman AB, Resnick SM. Enhanced verbal memory in nondemented elderly women receiving hormone-replacement therapy. Am J Psychiatry 2001;158: 227–33.
Markham JA, Pych JC, Juraska JM. Ovarian hormone replacement to aged ovariectomized female rats benefits acquisition of the Morris Water Maze. Horm Behav 2002;42:284–93.
Melchior CL, Ritzmann RF. Neurosteroids block the memory-impairing effects of ethanol in mice. Pharmacol Biochem Behav 1996;53:51–6.
Migliaccio A, Piccolo D, Castoria G, Di Domenico M, Bilancio A, Lombardi M, et al. Activation of the Src/p21^{ras}/Erk pathway by progesterone receptor via cross-talk with estrogen receptor. EMBO J 1998;17:2008–18.
Mohler H, Rudolph U, Boison D, Singer P, Feldon J, Yee BK. Regulation of cognition and symptoms of psychosis: focus on GABA(A) receptors and glycine transporter 1. Pharmacol Biochem Behav 2008;90:58–64.
Moser E, Moser MB, Andersen P. Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions. J Neurosci 1993;13:3916–25.
Nelson RJ. An Introduction to Behavioral Endocrinology. Sunderland, MA: Sinauer Associates, Inc.; 2000.
Nilsen J, Brinton RD. Impact of progestins on estrogen-induced neuroprotection: synergy by progesterone and 19-norprogesterone and antagonism by medroxyprogesterone acetate. Endocrinology 2002;143:205–12.
Nilsen J, Brinton RD. Divergent impact of progesterone and medroxyprogesterone acetate (Provera) on nuclear mitogen-activated protein kinase signaling. Proc Natl Acad Sci USA 2003;100:10506–11.
Packard MG, McGaugh JL. Inactivation of hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. Neurobiol Learn Mem 1996;65:65–72.
Paxinos G, Franklin KBJ. The Mouse Brain in Stereotaxic Coordinates. 2nd ed. San Diego, CA: Academic Press; 2003.
Pitha J, Harman SM, Michel ME. Hydrophillic cyclodextrin derivatives enable effective oral administration of steroidal hormones. J Pharm Sci 1986;75:165–7.
Rapp SR, Espeland MA, Shumaker SA, Henderson VW, Brunner RL, Manson JE, et al. Effect of estrogen plus progestin on global cognitive function in postmenopausal women. J Am Med Assoc 2003;289:2663–72.
Reddy DS, O'Malley BW, Rogawski MA. Anxiolytic activity of progesterone in progesterone receptor knockout mice. Neuropharmacology 2005;48:14–24.
Resnick SM, Maki PM, Rapp SR, Espeland MA, Brunner R, Coker LH, et al. Effects of combination estrogen plus progestin hormone treatment on cognition and affect. J Clin Endocrinol Metab 2006;91:1802–10.
Rosario ER, Ramsden M, Pike CJ. Progestins inhibit the neuroprotective effects of estrogen in rat hippocampus. Brain Res 2006;1099:206–10.
Sato T, Tanaka K, Ohnishi Y, Teramoto T, Irifune M, Nishikawa T. Effects of estradiol and progesterone on radial maze performance in middle-aged female rats fed a low-calcium diet. Behav Brain Res 2004;150:33–42.
Silvers JM, Tokunaga S, Berry RB, White AM, Matthews DB. Impairments in spatial learning and memory: ethanol, allopregnanolone, and the hippocampus. Brain Res Rev 2003;43:275–84.
Singh M. Ovarian hormones elicit phosphorylation of Akt and extracellular-signal regulated kinase in explants of the cerebral cortex. Endocrine 2001;14:407–15.
Singh M. Mechanisms of progesterone-induced neuroprotection. Ann NY Acad Sci 2005;1052:145–51.
Tanabe F, Miyasaka N, Kubota T, Aso T. Estrogen and progesterone improve scopolamine-induced impairment of spatial memory. J Med Dent Sci 2004;51:89–98.
Vongher JM, Frye CA. Progesterone in conjunction with estradiol has neuroprotective effects in an animal model of neurodegeneration. Pharmacol Biochem Behav 1999;64:777–85.
Walf AA, Rhodes ME, Frye CA. Ovarian steroids enhance object recognition in naturally cycling and ovariectomized, hormone-primed rats. Neurobiol Learn Mem 2006;86: 35–64.
Wolf OT, Heinrich AB, Hanstein B, Kirschbaum C. Estradiol or estradiol/progesterone treatment in older women: no strong effects on cognition. Neurobiol Aging 2005;26: 1029–33.
Woolley CS, McEwen BS. Roles of estradiol and progesterone in regulation of hippocampal dendritic spine density during the estrous cycle in the rat. J Comp Neurol 1993;336:293–306.
Yonker JE, Adolfsson R, Eriksson E, Hellstrand M, Nilsson L, Herlitz A. Verified hormone therapy improves episodic memory in healthy postmenopausal women. Aging Neuropsychol Cogn 2006;13:291–307.
Zhu Y, Rice CD, Pang Y, Pace M, Thomas P. Cloning, expression, and characterization of a membrane progestin receptor and evidence it is an intermediary in meiotic maturation of fish oocytes. Proc Natl Acad Sci USA 2003;100:2231–6.