# The mTOR and Canonical Wnt Signaling Pathways Mediate the Mnemonic Effects of Progesterone in the Dorsal Hippocampus

Ashley M. Fortress, John D. Heisler, and Karyn M. Frick*

**ABSTRACT: Although much is known about the neural mechanisms responsible for the mnemonic effects of 17β-estradiol ($E_2$), very little is understood about the mechanisms through which progesterone ($P_4$) regulates memory. We previously showed that intrahippocampal infusion of $P_4$ in ovariectomized female mice enhances object recognition (OR) memory consolidation in a manner dependent on activation of dorsal hippocampal ERK and mTOR signaling. However, the role of specific progesterone receptors (PRs) in mediating the effects of progesterone on memory consolidation and hippocampal cell signaling are unknown. Therefore, the goals of this study were to investigate the roles of membrane-associated and intracellular PRs in mediating hippocampal memory consolidation, and identify downstream cell signaling pathways activated by PRs. Membrane-associated PRs were targeted using bovine serum albumin-conjugated progesterone (BSA-P), and intracellular PRs (PR-A, PR-B) were targeted using the intracellular PR agonist R5020. Immediately after OR training, ovariectomized mice received bilateral dorsal hippocampal infusion of vehicle, $P_4$, BSA-P, or R5020. OR memory consolidation was enhanced by $P_4$, BSA-P, and R5020. However, only $P_4$ and BSA-P activated ERK and mTOR signaling. Furthermore, dorsal hippocampal infusion of the ERK inhibitor U0126 blocked the memory-enhancing effects of BSA-P, but not R5020. The intracellular PR antagonist RU486 blocked the memory-enhancing effects of R5020, but not BSA-P. Interestingly, $P_4$ robustly activated canonical Wnt signaling in the dorsal hippocampus, which is consistent with our recent findings that canonical Wnt signaling is necessary for OR memory consolidation. R5020, but not BSA-P, also elicited a modest increase in canonical Wnt signaling. Collectively, these data suggest that activation of ERK signaling is necessary for membrane-associated PRs to enhance OR, and indicate a role for canonical Wnt signaling in the memory-enhancing effects of intracellular PRs. This study provides the first evidence that membrane and intracellular PRs may employ different molecular mechanisms to enhance hippocampal memory.** © 2014 Wiley Periodicals, Inc.

**KEY WORDS: progesterone receptors; memory; ERK; mTOR; Wnt**

## INTRODUCTION

The role of sex steroid hormones in regulating hippocampal function has received much attention in recent years, in part, because steep drops in estrogen and progesterone levels during menopause are associated with memory impairments (Bove et al., 2014). Indeed, both 17β-estradiol ($E_2$) and progesterone ($P_4$) regulate hippocampal synaptogenesis, neurogenesis, and synaptic plasticity (Zhang et al., 2010; Foy, 2011; Kato et al., 2013). Although numerous studies have investigated the effects on hippocampal memory of $E_2$ alone or $E_2$ combined with $P_4$ (Luine et al., 2003; Walf et al., 2006; Frye et al., 2007; Harburger et al., 2009; Barron and Pike, 2012; Phan et al., 2012; Boulware et al., 2013; Fortress and Frick, 2014; Tuscher et al., in press), far fewer studies have investigated the effects of $P_4$ alone on hippocampal memory, perhaps because elevated levels of ovarian-derived $P_4$ are not typically observed in the absence of elevated $E_2$. However, $P_4$ itself is a potent neurosteroid, and $P_4$ alone is neuroprotective for hippocampal neurons in numerous animal models of brain injury, including stroke (Guennoun et al., in press; Yousuf et al., 2014). $P_4$ treatment has been tested in clinical trials for traumatic brain injury (Wright et al., 2007; Xiao et al., 2008), and so its therapeutic potential has already been recognized. Based on its effects on hippocampal neurons (e.g., Woolley and McEwen (1993)), $P_4$ is likely to affect memory function in patients receiving treatment, so it is important to understand the effects of this hormone on memory processes.

The effects of $P_4$ alone on hippocampal memory depend on timing, dose, duration of treatment, and type of memory tested (Farr et al., 1995; Chesler and Juraska, 2000; Bimonte-Nelson et al., 2004; He et al., 2011). However, post-training administration of $P_4$ consistently enhances the consolidation of hippocampal-dependent object recognition (Walf et al., 2006; Frye and Walf, 2008; Harburger et al., 2008; Orr et al., 2009; Orr et al., 2012) and object placement (Frye et al., 2007; Frye et al., 2013) memories in ovariectomized rats and mice. The memory-enhancing effects of $P_4$ have been observed after either systemic injection or dorsal hippocampal infusion, and are restricted to a 1 to 2 h window after treatment, suggesting that $P_4$ specifically enhances memory consolidation (Walf et al., 2006; Frye et al., 2007; Orr et al., 2009). Our laboratory has found that post-training infusions of $P_4$ enhance object recognition memory consolidation in a manner dependent on rapid activation of the extracellular signal regulated kinase (ERK) and mammalian target

**Department of Psychology, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin**
Grant sponsors: University of Wisconsin-Milwaukee and an Ellison Medical Foundation/AFAR Postdoctoral Fellowship in Aging (to A.M.F.).
*Correspondence to: Karyn M. Frick, Department of Psychology, University of Wisconsin-Milwaukee, 2441 E. Hartford Ave., Milwaukee, WI 53211, USA. E-mail: frickk@uwm.edu
Accepted for publication 3 December 2014.
DOI 10.1002/hipo.22398
Published online 00 Month 2014 in Wiley Online Library (wileyonlinelibrary.com).

of rapamycin (mTOR) signaling cascades in the dorsal hippocampus (Orr et al., 2009; Orr et al., 2012). However, it is unknown which progesterone receptors (PRs) trigger the rapid ERK and mTOR cell signaling activation necessary for $P_4$ to enhance object recognition memory consolidation.

$P_4$ binds to PRs located both at the membrane and within the cytoplasm. Because cell-signaling cascades are typically triggered near the plasma membrane, membrane-associated PRs are natural candidates to mediate the effects of $P_4$ on hippocampal cell signaling and memory consolidation. Two types of membrane-associated PRs have been identified: mPRs and PGRMCs (Singh et al., 2013). Post-training systemic injection of $P_4$ enhances object recognition, spatial memory, and contextual fear conditioning in mice lacking intracellular PRs (Frye and Walf, 2010), suggesting a role for membrane-associated PRs in the memory-enhancing effects of $P_4$. However, the molecular mechanisms through which membrane-associated PRs may contribute to hippocampal memory are not known. Phosphorylation of ERK downstream from PGRMC1/2 is necessary for $P_4$ to promote adult hippocampal neurogenesis in rat neural precursor cells (Liu et al., 2009). Because ERK activation is critical for long-term memory consolidation in the hippocampus (Sweatt, 2004), this finding suggests a potential role for membrane-associated PRs in the $P_4$-induced facilitation of hippocampal ERK and mTOR activation, as well as object recognition memory consolidation (Orr et al., 2012). ERK phosphorylation activates the mTOR cell-signaling pathway, which then triggers local protein synthesis in hippocampal dendrites (Tsokas et al., 2007). Activation of both pathways is critical for hippocampal spine remodeling and memory formation (Sweatt, 2004; Hoeffer and Klann, 2010). Within 5 min of dorsal hippocampal infusion, $P_4$ increases phosphorylation of p42 ERK and the downstream mTOR pathway kinase p70S6K (Orr et al., 2012). Given the important role of ERK activation in hippocampal neurogenesis induced by PGRMC1/2 (Liu et al., 2009), we hypothesized that membrane-associated PRs would facilitate object recognition memory consolidation by activating ERK-mediated mTOR signaling in the dorsal hippocampus.

However, intracellular PRs may also mediate the rapid effects of $P_4$ on hippocampal cell signaling and memory consolidation. The two ligand-sensitive cytoplasmic PR isoforms, PR-A and PR-B, are expressed throughout hippocampal neurons in rats (Kato et al., 1994; Camacho-Arroyo et al., 1998; Guerra-Araiza et al., 2002, 2003; Mitterling et al., 2010). A role for PR-A and PR-B in memory is supported by rodent data showing that the ability of $P_4$ to enhance spatial memory in the Morris water maze is blocked by the intracellular PR antagonist RU486 (Zhang et al., 2010). Traditionally, PR-A and PR-B are thought to bind to $P_4$ in the cytoplasm and form homo- or heterodimers which either translocate to the nucleus to bind to progesterone response elements in promoter regions of target genes (Moore et al., 1997) or indirectly alter transcription of target genes by interacting with transcriptional machinery (Proietti et al., 2005). Although the nuclear actions of intracellular PRs do not lend themselves to rapid regulation of hippocampal cell signaling, PR-A and PR-B regulate uterine and mammary gland development by activating the canonical Wnt/β-catenin signaling pathway (Robinson et al., 2000; Satterfield et al., 2008; Hagan et al., 2012; Boras-Granic and Hamel, 2013; Cooke et al., 2013), thus, raising the possibility that these intracellular receptors may regulate some types of cell signaling in the hippocampus. Canonical Wnt/β-catenin signaling regulates hippocampal plasticity, is activated in the hippocampus by object recognition and spatial learning, and is necessary for object recognition memory consolidation (Chen et al., 2006; Tabatadze et al., 2012; Fortress et al., 2013a; Vargas et al., 2014). Furthermore, canonical Wnt/β-catenin signaling is regulated by endogenous $E_2$ levels in the hippocampus (Zhang et al., 2008; Varea et al., 2009; Scott and Brann, 2013), suggesting that this pathway could mediate effects of $P_4$ in the hippocampus. Canonical Wnt signaling is initiated by the binding of a Wnt ligand (e.g., Wnt7a) to a frizzled receptor, thereby inactivating GSK3β and increasing levels of the transcription regulator β-catenin, which then translocates to the nucleus to upregulate downstream target genes such as *Cyclin D1* (*CCND1*) and *c-myc* (*myc*) (Zhang et al., 2011). *Cyclin D1* and *c-myc* are both regulated by $P_4$ in non-neural tissues (Moore et al., 1997; Gregory et al., 2001), supporting the notion that $P_4$ may regulate canonical Wnt signaling in the hippocampus through PR-A and PR-B. Given that canonical Wnt signaling is necessary for memory consolidation (Fortress et al., 2013a) and can be regulated by $E_2$ in the hippocampus and by $P_4$ in non-neural tissues, we hypothesized that activation of PR-A and PR-B may facilitate object recognition memory consolidation by activating canonical Wnt signaling in the dorsal hippocampus.

The goals of the this study were to determine the extent to which intracellular and membrane-associated PRs mediate the effects of $P_4$ on object recognition memory consolidation, and to identify downstream cell signaling pathways activated by these PRs. We found that activation of either intracellular or membrane-associated PRs facilitated object recognition memory consolidation, however, each type of receptor did so via a different cell signaling mechanism in the dorsal hippocampus. Specifically, membrane-associated PRs required dorsal hippocampal ERK activation to enhance memory consolidation and activate mTOR cell signaling, whereas intracellular PRs signaled independent of ERK and activated canonical Wnt signaling. Our findings are the first to demonstrate an independent role of membrane-associated and intracellular PRs in regulating $P_4$-induced memory enhancement, and to identify specific cell signaling mechanisms in the dorsal hippocampus through which each class of receptors may do so.

## METHODS

### Subjects

Female C57BL/6 mice were obtained from Taconic (Cambridge City, Indiana) at 8 to 10 weeks of age. Mice were

housed four to five per cage before surgery and then singly-housed thereafter. All mice were provided standard rodent chow and water ad libitum, and were housed with a standard 12:12 light/dark cycle (lights on at 7:00 a.m.). All procedures followed the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee at the University of Wisconsin-Milwaukee.

## Surgery

Mice were ovariectomized and implanted with guide cannulae in the same surgical session as described previously (Fernandez et al., 2008; Zhao et al., 2012; Fortress et al., 2013b). Using a stereotaxic apparatus (Kopf Instruments, Tujunga, CA), mice were implanted with either bilateral or triple stainless steel guide cannulae (C232GC, 22 gauge; Plastics One, Roanoke, VA). Double guide cannulae aimed bilaterally at the center of the dorsal hippocampus (−1.7 mm AP, ±1.5 mm ML, −2.3 mm DV [injection site]) were used in the experiments depicted in Figures 1A,B. Triple guide cannulae aimed bilaterally at the dorsal hippocampus and the dorsal third ventricle (intracerebroventricular (ICV); −0.9 mm AP, ±0.0 mm ML, −2.8 mm DV) were used in all other studies. All guide cannulae were fitted with a dummy cannula (C232DC) and dental cement was used to affix the guide cannulae to the skull and close the wound. All surgical procedures were performed under isofluorane gas anesthesia (5% for induction and 2% for maintenance). Mice recovered 7 days before the start of behavioral testing. Ibuprofen (20 mg/ml) in the drinking water provided postoperative analgesia.

## Drugs and Infusions

Infusions were conducted as described previously (Fernandez et al., 2008; Zhao et al., 2012; Boulware et al., 2013). The fact that $P_4$ enhances memory when given immediately after training suggests that the anxiolytic and analgesic effects of $P_4$ (Bitran et al., 1991a,1991b) given before training may interfere with performance during training or testing. This potential confound is minimized by the use of post-training treatments and a form of $P_4$ (cyclodextrin-encapsulated and water-soluble) that is rapidly metabolized within hours (Pitha et al., 1986).

For all infusions, mice were gently restrained and dummy cannulae were replaced with infusion cannulae (313I, intrahippocampal: 28 gauge, extending 0.8 mm beyond the 1.5 mm guide cannulae; ICV: 28 gauge, extending 1.0 mm beyond the 1.8 mm guide cannulae; Plastics One). Infusions were conducted with a microinfusion pump (KD Scientific, Holliston, MA) using 10 μl Hamilton syringes attached to PE50 tubing. Infusions were delivered at a rate of 0.5 μl/min for 1 min to yield a final total volume of 0.5 μl per hemisphere for dorsal hippocampal infusions and 1.0 μl for ICV infusions. Infusion cannulae were left in place for an additional 1 min to prevent drug diffusion up the cannula track. Dorsal hippocampal infusions were always delivered immediately before ICV infusions.

Cyclodextrin-encapsulated $P_4$ (Sigma, St. Louis, MO) was dissolved in physiological saline to a concentration of 0.2 μg/μl, resulting in doses of 0.1 μg/side for the dorsal hippocampus and 0.2 μg for the dorsal third ventricle. The 0.1 μg/side dose of $P_4$ enhances hippocampal-dependent object recognition memory in young ovariectomized mice (Orr et al., 2009, 2012). This form of $P_4$ is used because it is metabolized within hours and, therefore, is not present in the hippocampus during object recognition testing. The vehicle for $P_4$, 2-hydroxypropyl-β-cyclodextrin (HBC; Sigma), was dissolved in physiological saline to contain the same concentration of cyclodextrin present in the cyclodextrin encapsulated-$P_4$ solution.

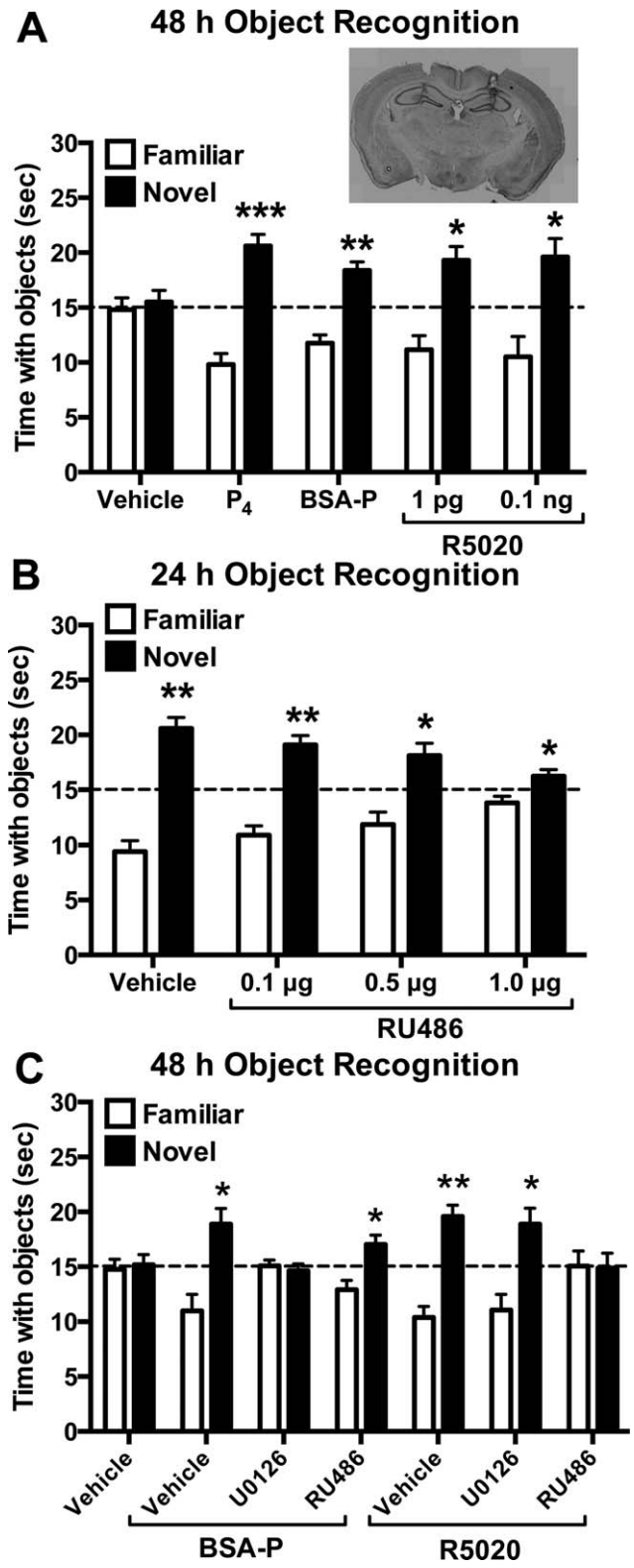
To determine the extent to which membrane-associated PRs are responsible for the mnemonic effects of $P_4$, we used bovine serum albumin-conjugated progesterone (BSA-P, Sigma) (Karteris et al., 2006; Josefsberg Ben-Yehoshua et al., 2007). BSA-P was reconstituted as a 100× stock solution in physiological saline. To eliminate any free $P_4$ in solution, a centrifugal filter unit (Ultracel 10K Membrane, Millipore) was used to concentrate BSA-P to 24× (according to manufacturer's protocol) before being diluted to a 1× stock solution that was further diluted to 73.4 pg/μl. The final concentration of 36.7 pg/0.5 μl dissolved in physiological saline was used for all infusions, and thus, physiological saline was used as the vehicle control.

To examine the role of intracellular PRs in mediating the mnemonic effects of $P_4$ in the dorsal hippocampus, we used the intracellular PR agonist R5020 (Promegestone, Perkin Elmer). R5020 was reconstituted to a 5 mg/ml stock solution in 100% ethanol (EtOH) and diluted 1:25 to obtain 0.2 ng/μl in 50% EtOH, which was then diluted 1:10 to obtain 0.002 ng/μl (2.0 pg/μl) in 50% EtOH. The final concentration of 1.0 pg/0.5 μl in 50% EtOH was used for all studies. The vehicle control was 50% EtOH in physiological saline.

We have previously used the MEK/ERK inhibitor 1,4-diamino-2,3-dicyano-1,4-bis(o-aminophenylmercapto)butadiene (U0126, Promega, Madison, WI) to show that p42 ERK phosphorylation is necessary for $P_4$ to facilitate hippocampal-dependent object recognition memory (Orr et al., 2012). Therefore, U0126 was infused into the dorsal hippocampus to determine if the effects of BSA-P and R5020 were dependent on ERK activation. U0126 was infused bilaterally at a concentration of 1.0 μg/μl in 50% dimethylsulfoxide (DMSO), which resulted in a dose of 0.5 μg/0.5 μl. We have previously shown in ovariectomized mice that this dose of U0126 does not impair object recognition memory on its own (Fernandez et al., 2008; Boulware et al., 2013; Fortress et al., 2013b). The vehicle control was 50% DMSO in physiological saline.

To demonstrate the specificity of R5020 for intracellular PRs, we infused the PR antagonist RU486 (Mifepristone, Sigma) bilaterally into the dorsal hippocampus immediately before ICV infusion of R5020 or BSA-P. We first determined a dose of RU486 that did not impair memory on its own so that it could be later used in conjunction with R5020 and BSA-P. RU486 was reconstituted to a stock concentration of 10 μg/μl in 20% DMSO in physiological saline and then further diluted to concentrations of 2.0 μg/μl, 1.0 μg/μl, and 0.2

μg/μl (Dong et al., 2006). The final concentration of 1.0 μg/0.5 μl in 20% DMSO in physiological saline was used for all inhibitor studies. The vehicle control was 20% DMSO in physiological saline.




## Object Recognition

Object recognition (OR) testing was conducted as we have described previously (Boulware et al., 2013; Fortress et al., 2013a). Sample sizes for all groups tested in object recognition were 8 to 11/group, except for the R5020 groups shown in Figure 1A ($n = 5$/group). The OR task was developed to assess episodic memory in rodents (Ennaceur and Delacour, 1988). In our laboratory, OR assesses nonspatial hippocampal-dependent memory (Baker and Kim, 2002; Fernandez et al., 2008; Cohen et al., 2013). OR testing consisted of three phases conducted over multiple days: habituation (days 1–2), training (day 3), and testing (day 4 or 5). All phases were carried out in a white box (60 cm W × 60 cm L × 47 cm H) in a dimly lit room. For habituation, mice were allowed to explore the empty white box for 5 min/day for 2 days, during which time no data were recorded. Twenty-four hours later, mice were rehabituated in the same box for 2 min, and then placed in a holding cage while two identical objects were placed in the left and right corners (about 5 cm from the walls) of the box. Mice were then placed immediately back into the testing box where they had a maximum of 20 min to

**FIGURE 1. Activation of membrane-associated or intracellular PRs enhances object recognition (OR) memory consolidation. (A) $P_4$, the membrane-restricted BSA-P, and the intracellular PR agonist R5020 enhance OR memory consolidation, as suggested by the fact that mice receiving bilateral dorsal hippocampal infusions of $P_4$ (0.1 μg/hemisphere), BSA-P (36.7 pg/hemisphere), or R5020 (1 pg or 0.1 ng/hemisphere) immediately after training spent significantly more time than chance (dashed line at 15 sec) with the novel object 48 h after training. In contrast, vehicle-infused mice spent similar amounts of time with the novel and familiar objects. Inset shows a Nissl stain of representative cannula placements in the dorsal hippocampus. (B) Mice receiving bilateral dorsal hippocampal infusion of vehicle or one of three doses (0.1, 0.5, or 1.0 μg/hemisphere) of RU486 spent significantly more time than chance exploring the novel object 24 h after training, suggesting that these doses of RU486 did not impair OR memory consolidation on their own. (C) Immediately following OR training, mice received bilateral dorsal hippocampal infusion of vehicle, the ERK activation inhibitor U0126, or the intracellular PR antagonist RU486, followed by an intracerebroventricular (ICV) infusion of vehicle, BSA-P, or R5020. Memory was tested 48 h later. As in (A), vehicle-infused mice did not spend more time than chance with the novel object during testing. Mice receiving ICV infusion of BSA-P (73.4 pg) and DH infusion of either vehicle or RU486 (1.0 μg/hemisphere) spent significantly more time with the novel object than chance, suggesting that the effects of BSA-P on memory were independent of intracellular PRs. In contrast, mice infused with BSA-P and U0126 (0.5 μg/hemisphere) did not display a significant preference for the novel object, suggesting that the memory-enhancing effects of BSA-P require ERK activation. As in (A), mice infused with R5020 (2.0 pg) spent significantly more time than chance exploring the novel object, and this effect was blocked by RU486 (1.0 μg/hemisphere), but not U0126, suggesting that the memory-enhancing effects of intracellular PRs do not require ERK activation. These data also indicate that activation of intracellular PRs is necessary for R5020 to enhance OR memory. Each bar represents the mean ± SEM amount of time spent with each object. $^{***}P \leq 0.001$, $^{**}P \leq 0.01$, and $^{*}P \leq 0.05$, relative to chance in one-sample *t*-tests.**

accumulate 30 sec of time exploring the objects. Time spent exploring an object was recorded when either the front paws or nose made contact with either object. Once 30 sec of exploration was accumulated, the mouse was removed from the box, gently restrained, and immediately infused. After 24 or 48 h, object recognition was tested, using the same procedure as in training except that a novel object was substituted for one of the familiar training objects. The novel object was placed in the corner where the mouse spent the least amount of time to avoid any effect of preference for a particular side of the testing box. Time spent with each object was recorded using ANY-maze software (Stoelting, Wood Dale, IL). Because mice inherently prefer to explore novel objects, more time than chance (15 sec) spent exploring the novel object indicated intact memory for the familiar object. Young ovariectomized mice remember the familiar object after 24 h but not after 48 h (Gresack et al., 2007), allowing impairing effects of drugs to be observed using the 24-h delay and enhancing effects of drugs to be observed using the 48-h delay.

### Western Blotting

Two weeks after the completion of OR training, mice received bilateral dorsal hippocampal and ICV infusions as described above. Sample sizes were 8 to 11/group except for the vehicle group ($n = 19$), which combined vehicle controls from the BSA-P and R5020 groups. Five minutes after infusion, brains were removed, the overlying cortex was dissected away to expose the hippocampus, and a cut was made at a 45° angle at the level of the superior colliculus to isolate the dorsal hippocampus. Tissue samples were immediately frozen on dry ice and stored at −80°C until use. Western blotting was performed once for each sample as described previously (Boulware et al., 2013; Fortress et al., 2013a,b). Briefly, all tissues were homogenized in 1:25 w/v lysis buffer containing 0.1 M Tris pH 7.5, 10 mM EDTA, 10 mM $Na_3VO_4$, 50.3 μM NaPyroPO$_4$, 7.05 μM β-glyceroPO$_4$, 10% v/v Igepal, 1 mM PMSF, and 1× Protease Inhibitor Cocktail (Thermo Scientific) with a probe sonicator (Branson Sonifier 250, Danbury, CT). Twenty micrograms of each sample was electrophoresed on various gradients of Tris-HCl gels (Bio-Rad, Hercules, CA) and transferred to polyvinyldene fluoride (PVDF) membranes using the Trans Blot Turbo system (Bio-Rad). Once transferred, membranes were blocked in 5% milk in tris buffered saline (TBS) for 1 h at room temperature before incubation with the primary antibodies (anti-phospho-ERK [1:2,000], anti-phospho-p70S6k [Thr-421] [1:1,000], anti-phospho-4EBP1 [Thr-37/46] [1:1,000], anti-β-catenin [1:1,000], anti-Cyclin D1 [1:1,000], anti-C-myc [1:1,000], Cell Signaling Technology, Danvers, MA and anti-Wnt7a [1:1,500], Abcam, Cambridge, MA) in 5% bovine serum albumin in TBS-Tween overnight at 4°C. The following day, membranes were incubated with goat anti-rabbit conjugated to horseradish peroxidase (Cell Signaling Technology) for 1 h at room temperature in 5% milk in TBS-Tween. Membranes were incubated in the chemiluminescent substrate West Dura (Thermo Scientific Pierce, Rockford, IL) before imaging, and then imaged on a Bio-Rad ChemiDoc MP. Blots were then stripped and reprobed for the total isoform of the protein (anti-total-ERK [1:2,000], anti-total-p70S6k [1:1,000]) or β-actin (1:5,000) (Cell Signaling Technology). Densitometry was conducted using Molecular Imaging software version 5.3.2 (Carestream Healthcare, Rochester, NY). All proteins were first normalized to their total isoform or β-actin, and then normalized to vehicle and represented as percent of control (100%).

### Statistics

For object recognition data, separate one-sample *t*-tests were performed for each group to determine whether the time spent with the novel object differed from chance (15 sec). This analysis was used because time spent with the objects is not independent; time spent with one object reduces time spent with the other object (Frick and Gresack, 2003). To further demonstrate between group differences, one-way ANOVA was conducted on the time spent with the novel object, followed by Fisher's LSD post hoc tests. Western blot data were analyzed using one-way ANOVA, followed by Fisher's LSD post hoc or a priori *t*-tests compared with vehicle to examine differences between treatment groups and vehicle control.

## RESULTS

### Progesterone and PR Agonists Facilitate OR Memory Consolidation

To determine the extent to which intracellular and membrane-associated PRs mediate the memory-enhancing effects of $P_4$, mice were trained in the OR task and then immediately received bilateral dorsal hippocampal infusions of vehicle ($n = 13$, HBC Vehicle or 50% EtOH), $P_4$ (0.1 μg/hemisphere, $n = 9$), BSA-P (36.7 pg/hemisphere, $n = 10$), or one of two doses of R5020 (1 pg or 0.1 ng/hemisphere, $n = 5$ each). Forty-eight hours later, vehicle-infused mice did not spend more time than chance (15 sec) with the novel object ($t_{(12)} = 0.47$, $P = 0.64$; Fig. 1A), indicating impaired OR memory consolidation. In contrast, mice infused with $P_4$ spent significantly more time than chance exploring the novel object ($t_{(8)} = 5.39$, $P = 0.0007$; Fig. 1A), thereby replicating our previous findings that $P_4$ facilitates OR memory consolidation (Orr et al., 2009, 2012). Furthermore, both BSA-P and R5020 mimicked the memory-enhancing effects of $P_4$; mice receiving BSA-P ($t_{(9)} = 4.29$, $P = 0.002$), 1 pg/hemisphere R5020 ($t_{(4)} = 3.44$, $P = 0.026$), or 0.1 ng/hemisphere R5020 ($t_{(4)} = 2.75$, $P = 0.05$) spent significantly more time exploring the novel object than chance (15 sec; Fig. 1A). Additionally, a one-way ANOVA revealed a significant main effect of treatment ($F_{(4,37)} = 3.83$, $P = 0.01$), such that mice infused with $P_4$ ($P = 0.0009$), BSA-P ($P = 0.04$), 1 pg/hemisphere R5020 ($P = 0.03$), or 0.1 ng/hemisphere R5020 ($P = 0.02$) spent

significantly more time with the novel object than vehicle-infused controls. These data suggest that intracellular or membrane-associated PRs can independently mediate the mnemonic effects of $P_4$ in the dorsal hippocampus.

## Intracellular and Membrane-Associated PRs Enhance OR Memory Consolidation Using Different Cellular Mechanisms

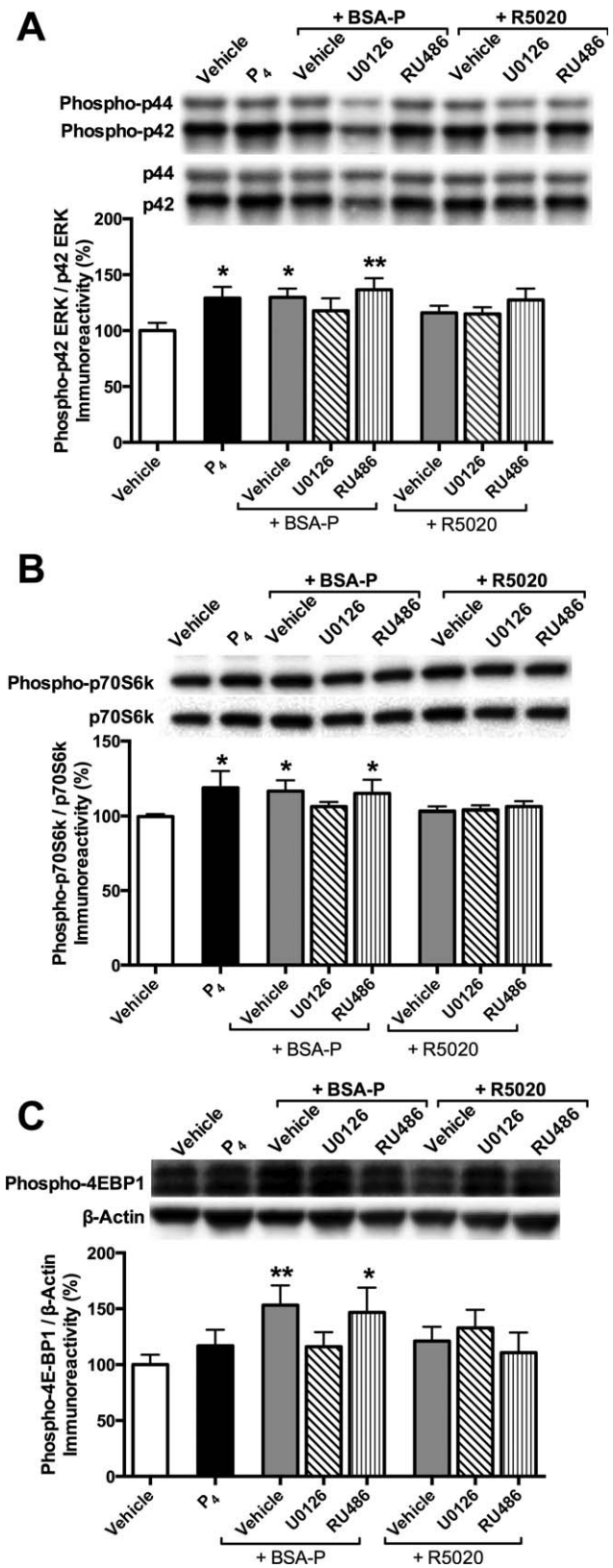
We next sought to determine the neural mechanisms underlying the memory-enhancements produced by activation of intracellular and membrane-associated PRs. To do so, we attempted to block the memory-enhancing effects of BSA-P and R5020. Because no available compounds can block activation of all membrane-associated PRs, we opted to block the presumed intracellular effects of membrane-associated PR activation using the ERK activation inhibitor U0126. We hypothesized that membrane-associated PRs enhance memory by rapidly activating ERK-dependent mTOR signaling. We found previously that the 0.1 μg dose of $P_4$ used above activates p42 ERK and p70S6K (a downstream effector of ERK and mTOR) 5 min after infusion, and that dorsal hippocampal infusion of U0126 prevented ICV-infused 0.1 μg $P_4$ from enhancing OR memory consolidation (Orr et al., 2012). Therefore, if BSA-P or R5020 enhance memory by binding membrane-associated PRs and rapidly activating ERK and/or mTOR signaling, then U0126 should block these effects. To block activation of intracellular PRs, we used the intracellular PR antagonist RU486 (Brogden et al., 1993). However, we first needed to identify a dose of RU486 that did not prevent OR memory consolidation on its own. Therefore, a new set of mice was cannulated and trained in the OR task. Immediately after training, mice received bilateral dorsal hippocampal infusions of vehicle (20% DMSO, $n = 8$) or RU486 in doses of 0.1 μg, 0.5 μg, or 1.0 μg/hemisphere ($n = 8$ each). Memory for the familiar object was tested 24 h later, a time point at which vehicle-infused mice show a preference for the novel object (Gresack et al., 2007; Boulware et al., 2013; Fortress et al., 2013b). As in our previous work, vehicle-infused mice spent significantly more time than chance with the novel object ($t_{(7)} = 4.85$, $P = 0.002$; Fig. 1B), demonstrating a preference for the novel object. No dose of RU486 impaired OR memory consolidation, as mice infused with 0.1 μg ($t_{(7)} = 4.66$, $P = 0.002$), 0.5 μg ($t_{(7)} = 2.46$, $P = 0.04$), and 1.0 μg ($t_{(7)} = 2.66$, $P = 0.03$) demonstrated a significant preference for the novel object relative to chance (15 sec; Fig. 1B). The fact that all groups showed a significant preference for the novel object was also reflected in the one-way ANOVA, in which the main effect of treatment was not significant ($F_{(3,28)} = 2.085$, $P = 0.1248$). As such, the highest ineffective dose (1.0 μg) of RU486 was infused in conjunction with BSA-P and R5020 in the next study.

Next, a new set of mice was implanted with triple cannulae targeting the dorsal hippocampus and the dorsal third ventricle. BSA-P or R5020 was infused into the dorsal third ventricle to deliver the progestins into the brain, and U0126 or RU486 was infused into the dorsal hippocampus to block ERK-related signaling or intracellular PR activation within this structure (Orr et al., 2012). This is the same approach taken in our previous work which showed that ICV infusion of $P_4$ enhances OR memory consolidation in a manner dependent on dorsal hippocampal ERK and mTOR activation (Orr et al., 2012). Immediately after OR training, mice were infused into the dorsal hippocampus and dorsal third ventricle, respectively, with vehicle + vehicle ($n = 9$), vehicle + BSA-P (36.7 pg, $n = 9$), U0126 (0.5 μg/hemisphere) + BSA-P (73.4 pg, $n = 10$), RU486 (1.0 μg/hemisphere) + BSA-P ($n = 10$), vehicle + R5020 (2.0 pg, $n = 7$), U0126 + R5020 ($n = 7$), or RU486 + R5020 ($n = 8$). Memory was tested 48 h later. As in Figure 1A, vehicle-infused mice did not spend significantly more time than chance with the novel object after 48 h ($t_{(8)} = 2.04$, $P = 0.84$; Fig. 1C), demonstrating impaired memory consolidation. Mice infused with BSA-P ($t_{(8)} = 2.68$, $P = 0.03$) or BSA-P + RU486 ($t_{(9)} = 2.43$, $P = 0.04$) exhibited a significant preference for the novel object relative to chance (Fig. 1C), indicating that blocking intracellular PRs did not prevent BSA-P from enhancing OR. However, mice infused with BSA-P + U0126 did not exhibit a significant preference for the novel object ($t_{(9)} = 0.57$, $P = 0.582$; Fig. 1C), suggesting that the memory-enhancing effects of membrane-associated PRs require ERK activation. As in Figure 1A, mice infused with R5020 + vehicle spent significantly more time than chance with the novel object ($t_{(5)} = 4.48$, $P = 0.007$), and this effect was not blocked by U0126, as suggested by the fact that mice infused with R5020 + U0126 also exhibited a significant preference for the novel object ($t_{(6)} = 2.73$, $P = 0.03$; Fig. 1B). These data indicate that the memory-enhancing effects of intracellular PRs do not require ERK activation. In contrast, mice infused with R5020 + RU486 did not exhibit a significant preference for the novel object ($t_{(7)} = 0.09$, $P = 0.93$; Fig. 1C), suggesting that the memory-enhancing effects of R5020 depend on activation of PR-A and PR-B. Collectively, these data suggest that the memory-enhancing effects of BSA-P require ERK activation, whereas the memory-enhancing effects of R5020 require intracellular PRs. Overall, these findings are supported by results from a one-way ANOVA conducted including all groups, which revealed a significant main effect of treatment ($F_{(6,52)} = 3.5$, $P = 0.001$). Relative to mice receiving vehicle + vehicle, mice infused with BSA-P + vehicle ($P = 0.02$), R5020 + vehicle ($P = 0.01$), or R5020 + U0126 ($P = 0.03$) demonstrated a significant preference for the novel object. In addition, mice infused with BSA-P + U0126 spent significantly less time with the novel object than mice infused with BSA-P + vehicle ($P = 0.01$). Similarly, mice infused with R5020 + RU486 spent significantly less time with the novel object than mice infused with R5020 + vehicle ($P = 0.01$). These findings support the conclusion that intracellular and membrane-associated PRs employ different cellular mechanisms to facilitate memory consolidation.

## Membrane-Associated, but not Intracellular, PRs Promote Dorsal Hippocampal ERK Activation

We next examined effects of $P_4$, BSA-P, and R5020 on rapid activation of ERK in the dorsal hippocampus. A new set of

mice was infused into the dorsal hippocampus and dorsal third ventricle, respectively, with: vehicle + vehicle, vehicle + $P_4$, vehicle + BSA-P, U0126 + BSA-P, or RU486 + BSA-P. Dorsal hippocampal tissue was collected 5 min later to measure phosphorylation of the two ERK isoforms (p42 and p44). We previously found that $P_4$ increases phosphorylation of p42 ERK in the dorsal hippocampus 5 min after dorsal hippocampal infusion (Orr et al., 2012). Similarly, phospho-p42 ERK levels in the present study significantly differed among the groups 5 min after infusion, ($F_{(4,52)} = 3.08$, $P = 0.03$; Fig. 1A). As before (Orr et al., 2012), $P_4$ significantly increased phospho-p42 ERK levels relative to vehicle 5 min after infusion ($P = 0.03$). BSA-P also significantly increased phospho-p42 ERK levels relative to vehicle ($P = 0.02$), and this effect was blocked by U0126 ($P > 0.05$), but not RU486 ($P < 0.01$). These data suggest that membrane-associated PRs activate p42 ERK independent of intracellular PRs. The effects of $P_4$ and BSA-P were restricted to the p42 isoform of ERK, as phospho-p44 ERK was unaffected by any treatment ($F_{(4,53)} = 0.73$, $P = 0.58$, data not shown).




Next, effects of intracellular PR activation on phospho-ERK levels were measured. Additional groups of mice were infused into the dorsal hippocampus and dorsal third ventricle, respectively, with: vehicle + R5020, RU486 + R5020, or U0126 + R5020. As described above, dorsal hippocampal tissues were collected 5 min after infusion, and statistical comparisons were made relative to the vehicle + vehicle group described above. There was no effect of drug treatment on phospho-p42 ERK ($F_{(3,42)} = 2.72$, $P = 0.09$; Fig. 2A) or phospho-p44 ERK ($F_{(3,43)} = 0.4$, $P = 0.75$, data not shown), suggesting that R5020 does not activate dorsal hippocampal ERK. These data are consistent with the behavioral data in Figure 1C showing that blocking ERK activation does not prevent R5020 from enhancing OR memory consolidation.

## Membrane-Associated, but not Intracellular, PRs Promote ERK-Dependent Dorsal Hippocampal mTOR Activation

We next examined the effects of BSA-P and R5020 on ERK-dependent mTOR activation. mTOR signaling is essential

**FIGURE 2. BSA-P, but not R5020, activates ERK and mTOR signaling in the dorsal hippocampus 5 min after infusion. (A) Levels of phospho-p42 ERK were significantly increased relative to vehicle in mice infused with $P_4$. BSA-P also significantly increased levels of phospho-p42 ERK, an effect that was blocked by U0126, but not by RU486. R5020 had no effect on phospho-p42 ERK levels alone or in combination with U0126 or RU486. No effects of any drug were observed on phospho-p44 ERK levels (data not shown). Phospho-ERK protein levels were normalized to total ERK. (B) Levels of phospho-p70S6K were significantly increased in the dorsal hippocampus following infusion of $P_4$ and BSA-P. The effect of BSA-P was blocked by U0126, but not RU486. R5020 had no effect on levels of phospho-p70S6K. Phospho-p70S6K protein levels were normalized to total p70S6K. (C) Although $P_4$ had no significant effect on phospho-4E-BP1, phospho-4EBP1 levels were increased following dorsal hippocampal infusion of BSA-P. Similar to phospho-p70S6K, this effect was blocked by U0126, but not by RU486. R5020 had no effect on phospho-4E-BP1 levels. Phospho-4EBP1 protein levels were normalized to β-Actin. Each bar represents the mean ± SEM percent change from vehicle. Insets show representative Western blots. $^{**}P \leq 0.01$ and $^{*}P \leq 0.05$, relative to vehicle.**

for learning, memory, and dendritic spine morphology, and plays a critical role in new protein synthesis in an ERK-dependent manner (Hoeffer and Klann, 2010; Bhattacharya et al., 2012; Graber et al., 2013). When mTOR becomes phosphorylated by ERK and other upstream kinases, it complexes with other proteins to phosphorylate core components of the protein synthesis machinery, including p70 ribosomal S6 kinase (p70S6K) and eukaryotic initiation factor 4E-binding proteins (4E-BPs) (Hoeffer and Klann, 2010). We have shown that dorsal hippocampal infusion of $P_4$ increases phosphorylation of p70S6K in the dorsal hippocampus within 5 min, and that mTOR signaling is necessary for $P_4$ to facilitate OR memory consolidation in ovariectomized mice (Orr et al., 2012). Furthermore, we have also reported that dorsal hippocampal infusion of U0126 prevents $E_2$ from increasing phosphorylation of p70S6K and 4E-BP1 in the dorsal hippocampus and enhancing OR memory in ovariectomized mice (Fortress et al., 2013b), suggesting that ERK activation triggers the mTOR signaling that is necessary for $E_2$, and potentially $P_4$, to enhance OR memory consolidation. Given that BSA-P, but not R5020, increased p42 ERK phosphorylation and that U0126 blocked the memory enhancing effects of BSA-P only, we hypothesized that BSA-P, but not R5020, would increase phosphorylation of p70S6K and 4E-BP1 in an ERK-dependent manner.

Homogenates assayed for ERK above were subjected to Western blotting for phospho-p70S6K and 4E-BP1. As above, dorsal hippocampal tissue was collected 5 min after infusion with vehicle + vehicle, vehicle + $P_4$, vehicle + BSA-P, U0126 + BSA-P, or RU486 + BSA-P. Although the main effect of treatment for phospho-p70S6K was not significant ($F_{(4,53)} = 2.0$, $P = 0.11$; Fig. 2B), a priori *t*-tests revealed that mice infused with $P_4$ ($t_{(27)} = 2.28$, $P = 0.03$), BSA-P ($t_{(26)} = 3.08$, $P = 0.005$), and BSA-P + RU486 ($t_{(26)} = 2.31$, $P = 0.03$) had significantly higher phospho-p70S6K levels than vehicle-infused controls. Levels of phospho-p70S6K in mice infused with BSA-P + U0126 did not significantly differ from vehicle ($t_{(28)} = 1.94$, $P > 0.05$), suggesting that the increase in phospho-p70S6K induced by BSA-P requires ERK activation. For 4E-BP1, the main effect of treatment was significant ($F_{(4,53)} = 2.53$, $P = 0.05$; Fig. 2C) due to the significantly higher phospho-4E-BP1 levels in the BSA-P ($P = < 0.01$) and BSA-P + RU486 groups ($P = 0.02$) relative to vehicle. As with phospho-p70S6K, U0126 blocked the effects of BSA-P on 4E-BP1, indicating that this increase also requires ERK activation. Interestingly, $P_4$ did not significantly increase phospho-4E-BP1 levels, which may indicate that this protein is more sensitive to the effects of membrane-associated PR activation. The fact that RU486 did not block the effects of BSA-P on phosphorylation of p70S6K or 4E-BP1 indicates that these effects occur independently of intracellular PR activation.

We next turned our attention to R5020 and assayed for phospho-p70S6K and 4E-BP1 levels in homogenates assayed for ERK from mice infused with vehicle + vehicle, vehicle + R5020, RU486 + R5020, or U0126 + R5020. There was no main effect of treatment on phospho-p70S6K ($F_{(3,42)} = 1.26$, $P = 0.30$; Fig. 2B) or phospho-4E-BP1 ($F_{(3,42)} = 1.27$, $P = 0.30$; Fig. 2C), nor were any a priori planned comparisons with the vehicle group significant. These data show that R5020 does not activate mTOR signaling, which is consistent with its lack of effect on ERK phosphorylation (Fig. 2A) and the inability of U0126 to prevent R5020 from enhancing OR memory consolidation (Fig. 2B). As such, these data suggest that intracellular PRs do not enhance OR memory by activating ERK or downstream mTOR signaling. But given that the effects of intracellular PR activation on memory are relatively rapid, we next wondered if the canonical Wnt cell-signaling pathway might instead contribute to the memory-enhancing effects of R5020.

## Intracellular PRs Activate Canonical Wnt Signaling in the Dorsal Hippocampus

To examine the effects of $P_4$ and PR agonists on canonical Wnt signaling, we measured levels of Wnt 7a and β-Catenin protein in the dorsal hippocampal samples assayed for ERK and mTOR signaling. We first analyzed the tissues of mice infused into the dorsal hippocampus and dorsal third ventricle with vehicle + vehicle, vehicle + $P_4$, vehicle + BSA-P, U0126 + BSA-P, or RU486 + BSA-P. The main effect of treatment was significant for Wnt 7a protein levels ($F_{(4,54)} = 13.53$, $P < 0.0001$; Fig. 3A), reflecting a robust and significant increase in Wnt 7a induced by $P_4$ infusion relative to all other groups ($P$s $< 0.0001$). BSA-P, alone or in combination with U0126 or RU486, had no effect on Wnt 7a levels relative to vehicle ($P$s $> 0.05$). Similarly, the main effect of treatment was significant for β-Catenin ($F_{(4,50)} = 2.85$, $P = 0.03$; Fig. 3B) due to a significant increase in β-Catenin induced by $P_4$ infusion ($P < 0.01$ relative to vehicle). As with Wnt 7a, β-Catenin levels were not altered by any BSA-P treatment ($P$s $> 0.05$). Next, we analyzed tissue from mice receiving vehicle + vehicle, vehicle + R5020, or RU486 + R5020. We found a significant main effect of treatment for Wnt 7a ($F_{(2,34)} = 3.79$, $P < 0.033$; Fig. 3A) and β-Catenin ($F_{(2,33)} = 4.23$, $P = 0.02$; Fig. 3B), driven by a significant increase in levels of both proteins in mice infused with R5020 compared with vehicle ($P \leq 0.01$). RU486 partially blocked these increases, as levels of both proteins in the R5020 + RU486 groups were not significantly different from vehicle ($P > 0.05$). Collectively, our findings showing that R5020, but not BSA-P, increased Wnt 7a and β-Catenin levels suggest that the $P_4$-induced increase of these canonical Wnt proteins is due to activation of intracellular PRs rather than membrane PRs.

When unphosphorylated, β-Catenin is free to translocate into the nucleus and initiate gene transcription (MacDonald et al., 2009). Therefore, to determine if the increase in total β-Catenin protein was associated with an increase in protein levels associated with downstream gene targets, we also measured protein levels of the canonical Wnt downstream target genes *c-myc* and *Cyclin-D1*. As with Wnt 7a and β-Catenin, mice receiving $P_4$, but not any BSA-P treatment, displayed significantly increased c-myc ($F_{(4,45)} = 6.74$, $P < 0.001$; Fig. 3C) and Cyclin D1 ($F_{(4,54)} = 3.53$, $P = 0.013$; Fig. 3D) protein.

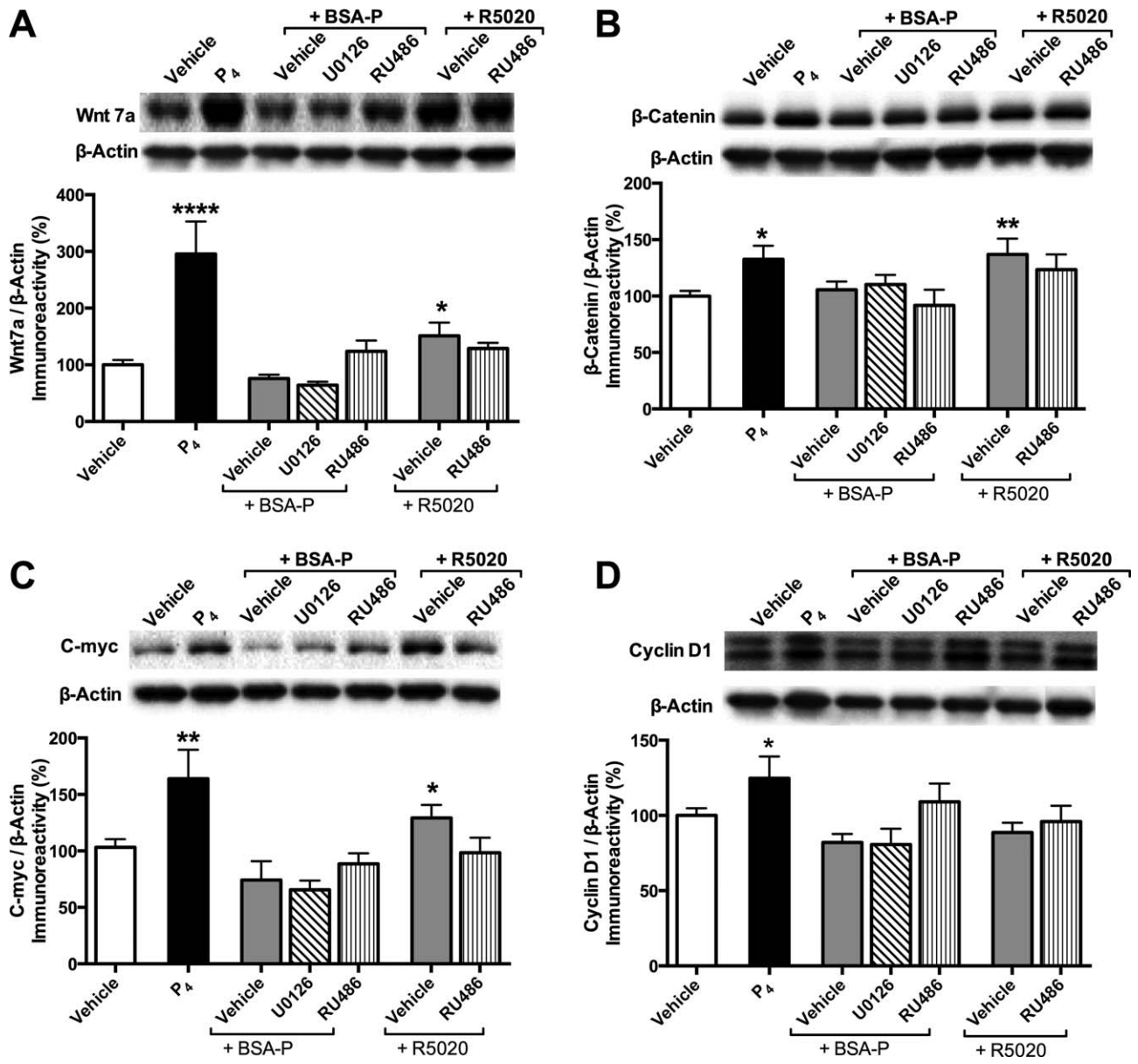



**FIGURE 3. $P_4$ and R5020 activate canonical Wnt signaling in the dorsal hippocampus 5 min after infusion. (A) Wnt7a and (B) β-catenin protein levels were significantly increased 5 min after infusion of $P_4$ or R5020 into the dorsal hippocampus. BSA-P had no effect on Wnt 7a or β-catenin protein expression. (C) c-myc and (D) Cyclin D1 protein levels were significantly increased 5 min after infusion of $P_4$ in the dorsal hippocampus. Neither BSA-P nor R5020 affected Cyclin D1 protein levels at this time point, but R5020 increased c-myc levels. Protein levels in all panels were normalized to β-Actin. Each bar represents the mean ± SEM percent change from vehicle. Insets show representative Western blots. $^{**}P \leq 0.01$ and $^{*}P \leq 0.05$, relative to vehicle.**

Post hoc analyses revealed that c-myc and Cyclin D1 protein levels were significantly increased by $P_4$ relative to vehicle ($P \leq 0.05$). In the analysis of the R5020 samples, the main effect of treatment was not significant for either protein, but an a priori *t*-test indicated a significant increase in c-myc following R5020 treatment relative to vehicle ($t_{(23)} = 1.98$, $P = 0.03$; Fig. 3C). Together, these data suggest that $P_4$ activates the transcription of canonical Wnt target genes, perhaps by binding to intracellular PRs.

## DISCUSSION

Although previous studies have demonstrated that $P_4$ can enhance hippocampal memory consolidation, the PR and cell signaling mechanisms through which this facilitation occurs have remained largely unknown. The current study is the first to determine the roles of specific classes of PRs in mediating hippocampal memory and to identify cell-signaling pathways

activated by the various types of PRs in the hippocampus. Our findings suggest that membrane-associated and intracellular PRs in the dorsal hippocampus independently enhance object recognition memory consolidation via different cell-signaling mechanisms. Specifically, we found that membrane-associated PRs enhance memory by activating ERK and mTOR signaling pathways, whereas intracellular PRs may influence memory by activating canonical Wnt signaling.

Our data showing that dorsal hippocampal infusion of $P_4$ enhances object recognition memory consolidation is consistent with previous reports that post-training administration of $P_4$ systemically or directly into the dorsal hippocampus or dorsal third ventricle facilitates OR memory consolidation in ovariectomized rats and mice (Walf et al., 2006; Frye and Walf, 2008; Orr et al., 2009; Orr et al., 2012). Here, we extend these findings to present the first evidence that activation of membrane-associated or intracellular PRs in the dorsal hippocampus also enhances object recognition memory consolidation. Importantly, the memory-enhancing effects of BSA-P and R5020 were similar when infused into the dorsal hippocampus (Fig. 1A) or dorsal third ventricle (Fig. 1C), demonstrating that the dorsal third ventricle infusion is an effective way to target PRs in the dorsal hippocampus while simultaneously allowing us to inhibit processes in the dorsal hippocampus. Because compounds are not yet available to target specific PRs, we cannot more definitively pinpoint which PRs are responsible for these effects at the present time. Nevertheless, the data provide essential new information about how the distinct classes of PRs influence hippocampal memory formation. The effects of BSA-P and R5020 on object recognition, and most other forms of hippocampal-dependent memory, have not been previously examined, so there is scant literature with which our data can be compared. However, one study that administered R5020 systemically before training found that both R5020 and $P_4$ impaired social recognition in male rats (Bychowski and Auger, 2012). Although this study would seem to contradict our findings, it is important to remember that $P_4$ given before training has been shown to impair memory in some tasks (Farr et al., 1995; Bimonte-Nelson et al., 2004). Moreover, as it did in our study, R5020 mimicked the effects of $P_4$ on social recognition, suggesting similar effects of the hormone and its intracellular receptors on memory regardless of whether the effect impairs or enhances memory. One potential concern about our use of BSA-P is that some of its memory-enhancing effects could have been caused by free $P_4$ that came unbound from the BSA molecule. As such, it is worth noting that our BSA-P was ultrafiltered before use, and therefore, the effects of BSA-P on memory are unlikely to have been caused by free $P_4$. Furthermore, free $P_4$ would have also bound to intracellular PRs, so it is unlikely that we would have observed such a striking dissociation in the cell signaling pathways underlying the object recognition memory enhancements of BSA-P and R5020. Collectively, the data support the notion that independently activating either membrane-associated or intracellular PRs in the dorsal hippocampus is sufficient to enhance object recognition memory consolidation, suggesting that both classes of PRs serve an important physiological role in the hippocampus.

Activation of membrane-associated PRs in the dorsal hippocampus by BSA-P enhanced object recognition memory consolidation via an ERK-dependent mechanism, as suggested by the fact that the effects of BSA-P on object recognition and phosphorylation of p42 ERK, p70S6K, and 4E-BP1 were blocked by U0126. These findings are consistent with previous work from our laboratory demonstrating that dorsal hippocampal ERK or mTOR activation is necessary for dorsal hippocampal infusion of $P_4$ to enhance object recognition memory consolidation in ovariectomized mice (Orr et al., 2012). Here, the ERK inhibitor U0126 prevented BSA-P from enhancing object recognition and activating hippocampal ERK and mTOR signaling, which is consistent with other findings from our laboratory demonstrating that $E_2$ requires ERK-dependent mTOR signaling to enhance object recognition memory consolidation (Fortress et al., 2013b). Because the mTOR pathway regulates local protein synthesis and spine density and is a critical regulator of memory (Richter and Klann, 2009), changes in spine density may be one mechanism by which PRs facilitate memory consolidation. Indeed, $P_4$ increases spine density in acute hippocampal slices (Kato et al., 2013). Although we cannot specify a role for any particular membrane-associated PR, PGRMC1 may be responsible for the ERK-dependent memory-enhancing effects of BSA-P. $P_4$ requires PGRMC1 and ERK activation in rat neural precursor cells to promote neural progenitor cell proliferation (Liu et al., 2009), suggesting that PGRMC1 may mediate the ERK-dependent memory enhancing effects of BSA-P by facilitating neurogenesis. This hypothesis is substantiated by a study in which $P_4$ increased hippocampal neurogenesis and spatial memory in male rats, and the $P_4$-induced increase in neurogenesis was completely blocked by U0126 (Zhang et al., 2010). These findings suggest a potentially important role for PGRMC1 in the memory-enhancing effects of $P_4$. Because this study has demonstrated that membrane-associated PRs contribute to memory consolidation in the dorsal hippocampus, future studies should extend this work using non-pharmacological methods to further define the role of specific PRs, such as PGRMC1, in $P_4$-mediated object recognition memory consolidation.

We also found that the intracellular PR agonist R5020 enhanced object recognition memory consolidation. Interestingly, $P_4$ and R5020, but not BSA-P, increased levels of Wnt7a, β-Catenin, and c-myc protein in the dorsal hippocampus five minutes after infusion. Unlike BSA-P, R5020 did not increase phospho-p42 ERK levels, nor did U0126 block the effects of R5020 on object recognition or Wnt proteins. As such, these data provide the first evidence that activation of intracellular PRs by $P_4$ can rapidly activate canonical Wnt signaling, which may contribute to the memory-enhancing effects of $P_4$. Evidence for $P_4$-mediated regulation of Wnt signaling has been reported in reproductive tissues such as uterus and breast (Robinson et al., 2000; Faivre and Lange, 2007; Lamb et al., 2007). In cancer cells, $P_4$ binds to intracellular PRs to promote cell proliferation by increasing the expression of Wnt 1, which is necessary for activation of c-src/ERK signaling 6 to 72 h later (Skildum et al., 2005; Faivre and Lange, 2007). In

the current study, we did not find that activation of intracellular PRs increased ERK at the 5-min time point examined, consistent with the timing reported in cancer cells, however, we cannot rule out an effect at later time points. Our evidence for a $P_4$-mediated increase in canonical Wnt signaling presents a novel and plausible mechanism through which $P_4$ could regulate hippocampal memory. Canonical Wnt signaling plays a critical role nervous system development, structure, and function (Ciani and Salinas, 2005; Inestrosa and Arenas, 2010; Oliva et al., 2013; Ortiz-Matamoros et al., 2013). In the adult hippocampus, canonical Wnt signaling facilitates synaptogenesis (Sahores et al., 2010), neurogenesis (Qu et al., 2013), and long-term potentiation (Chen et al., 2006). Moreover, canonical Wnt proteins are increased in the hippocampus following environmental enrichment (Gogolla et al., 2009) and spatial learning tasks (Tabatadze et al., 2012). Canonical Wnt signaling is dysregulated in multiple neurological and neuropsychiatric conditions in which hormones are implicated in the disorder's etiology or symptomatology, such as Alzheimer's disease (Purro et al., 2014), bipolar disorder (Meffre et al., 2014), and depression (Sani et al., 2012). We recently demonstrated in male mice that post-training dorsal hippocampal infusions of the endogenous canonical Wnt inhibitor Dkk-1 impairs object recognition memory, suggesting that canonical Wnt signaling is necessary for object recognition memory consolidation (Fortress et al., 2013a). As such, activation of canonical Wnt signaling by $P_4$ may play an important role in mediating object recognition memory consolidation in females. Interestingly, this involvement would appear to be mediated by intracellular PRs, rather than membrane-associated PRs. The requirement for canonical Wnt signaling in the effects of $P_4$ will need to be examined in future studies.

Interestingly, infusion of $P_4$ alone activated both the mTOR and canonical Wnt signaling pathways, suggesting the possibility that these intracellular- and membrane-initiated signaling pathways could converge to facilitate memory consolidation. Wnt signaling can activate the mTOR pathway in non-neural tissues (Inoki et al., 2006), suggesting that $P_4$-induced memory enhancement may involve an interaction between these pathways. On the other hand, the fact that mTOR activation triggers spinogenesis (Richter and Klann, 2009) and canonical Wnt signaling leads to neurogenesis (Varela-Nallar and Inestrosa, 2013) suggests that mTOR and canonical Wnt signaling could act independently to facilitate the effects of $P_4$ on memory consolidation. Unfortunately, the present data do not allow us to differentiate between these possibilities, but this issue should be addressed in future studies.

It is important to note that $P_4$ may influence memory via mechanisms other than intracellular and membrane-associated PRs. $P_4$ is the obligatory precursor for all corticosteroids, androgens, and estrogens, as well as progestin neurosteroids including 3α,5α-tetrahydroprogesterone (3α,5α-THP or allopregnanolone) (Compagnone and Mellon, 2000; Melcangi et al., 2014). Each of these classes of steroid hormones has their own well-documented effects on hippocampal memory (Cherrier, 2009; de Quervain et al., 2009; Tuscher et al., in press) and, therefore, the effects on memory of $P_4$ conversion to these other hormones cannot be entirely ruled out here. Furthermore, it should also be mentioned that RU486 functions as an antagonist for glucocorticoid receptors in addition to intracellular PRs (Chen et al., 2012; Yoshiya et al., 2013). Thus, it is possible that RU486 blocked both GRs and PRs in our studies. However, we believe that the effects reported here can be attributed to PRs for several reasons. First, competitive binding assays in uterine tissue indicate that $P_4$, R5020, and RU486 all compete for access to intracellular PRs (Hurd and Moudgil, 1988), suggesting some specificity and/or affinity of R5020 and RU486 for PRs. Second, we observed effects on protein levels five minutes after infusion, and think it unlikely that sufficient conversion to other metabolites in this time frame could account for these rapid effects. Third, it is doubtful that the $P_4$ conjugated to BSA would have been metabolized so rapidly in the extracellular space that a neurosteroid metabolite like allopregnanolone could have bound to its receptor ($GABA_A$) to regulate memory consolidation. Moreover, the BSA conjugation restricts effects of $P_4$ to the plasma membrane, thereby decreasing possible interactions with intracellular steroid receptors. Fourth, R5020 activates PR-A and PR-B in a manner that is RU486- dependent (Molenda-Figueira et al., 2008), so it is unlikely to interact with other intracellular steroid receptors. Finally, RU486 blocked the effects of R5020, but not BSA-P, suggesting effects specific to intracellular PRs. Nevertheless, we cannot definitively exclude these other mechanisms here, and will need to explore this issue in future studies with antagonists for other steroids and neurosteroid receptors.

In sum, this study provides the first evidence that $P_4$ can facilitate object recognition memory consolidation by activating either intracellular PRs or membrane-associated PRs, and that these two classes of PRs activate different cell-signaling pathways in the dorsal hippocampus. Specifically, our findings demonstrate that the memory-enhancing effects of membrane PRs are dependent on ERK-dependent mTOR signaling in the dorsal hippocampus. Moreover, activation of intracellular PRs enhances object recognition memory consolidation in a manner independent of membrane-associated PRs via mechanisms that may include activation of canonical Wnt signaling in the dorsal hippocampus. Our findings provide much needed insight into the molecular mechanisms through which $P_4$ regulates memory formation. Because $P_4$ exerts significant neuroprotective effects in models of brain injury, stroke, and neurodegenerative disease (Brinton et al., 2008; Sayeed and Stein, 2009; Feeser and Loria, 2011; Yousuf et al., 2014), our data could have important implications for the development of drugs to target specific types of PRs without the side effects of current progestin therapies.

## Acknowledgments

The authors thank Andrew Kozlowski and Julia Szinte for assistance with behavioral testing, Jennifer Tuscher for critical comments on this article, and Brett Fulleylove-Krause from Dr. James Moyer's laboratory for assistance with histological imaging.

## REFERENCES


Baker KB, Kim JJ. 2002. Effects of stress and hippocampal NMDA receptor antagonism on recognition memory in rats. Learn Mem 9:58–65.

Barron AM, Pike CJ. 2012. Sex hormones, aging, and Alzheimer's disease. Front Biosci (Elite Ed) 4:976–997.

Bhattacharya A, Kaphzan H, Alvarez-Dieppa AC, Murphy JP, Pierre P, Klann E. 2012. Genetic removal of p70 S6 kinase 1 corrects molecular, synaptic, and behavioral phenotypes in fragile X syndrome mice. Neuron 76:325–337.

Bimonte-Nelson HA, Singleton RS, Williams BJ, Granholm A-CE. 2004. Ovarian hormones and cognition in the aged female rat: II. Progesterone supplementation reverses the cognitive enhancing effects of ovariectomy. Behav Neurosci 118:707–714.

Bitran D, Hilvers RJ, Kellogg CK. 1991a. 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 561:157–161.

Bitran D, Hilvers RJ, Kellogg CK. 1991b. Ovarian endocrine status modulates the anxiolytic potency of diazepam and the efficacy of gamma-aminobutyric acid-benzodiazepine receptor-mediated chloride ion transport. Behav Neurosci 105:653–662.

Boras-Granic K, Hamel PA. 2013. Wnt-signalling in the embryonic mammary gland. J Mammary Gland Biol Neoplasia 18:155–163.

Boulware MI, Heisler JD, Frick KM. 2013. The memory-enhancing effects of hippocampal estrogen receptor activation involve metabotropic glutamate receptor signaling. J Neurosci 33:15184–15194.

Bove R, Secor E, Chibnik LB, Barnes LL, Schneider JA, Bennett DA, De Jager PL. 2014. Age at surgical menopause influences cognitive decline and Alzheimer pathology in older women. Neurology 82: 222–229.

Brinton RD, Thompson RF, Foy MR, Baudry M, Wang J, Finch CE, Morgan TE, Pike CJ, Mack WJ, Stanczyk FZ, Nilsen J. 2008. Progesterone receptors: Form and function in brain. Front Neuroendocrinol 29:313–339.

Brogden RN, Goa KL, Faulds D. 1993. Mifepristone. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential. Drugs 45:384–409.

Bychowski ME, Auger CJ. 2012. Progesterone impairs social recognition in male rats. Horm Behav 61:598–604.

Camacho-Arroyo I, Guerra-Araiza C, Cerbón MA. 1998. Progesterone receptor isoforms are differentially regulated by sex steroids in rat forebrain. Neuroreport 9:3993–3996.

Chen DY, Bambah-Mukku D, Pollonini G, Alberini CM. 2012. Glucocorticoid receptors recruit the CaMKIIalpha-BDNF-CREB pathways to mediate memory consolidation. Nat Neurosci 15:1707–1714.

Chen J, Park CS, Tang SJ. 2006. Activity-dependent synaptic Wnt release regulates hippocampal long term potentiation. J Biol Chem 281:11910–11916.

Cherrier MM. 2009. Testosterone effects on cognition in health and disease. Front Horm Res 37:150–162.

Chesler EJ, Juraska JM. 2000. Acute administration of estrogen and progesterone impairs the acquisition of the spatial Morris water maze in ovariectomized rats. Horm Behav 38:234–242.

Ciani L, Salinas PC. 2005. WNTs in the vertebrate nervous system: From patterning to neuronal connectivity. Nat Rev Neurosci 6: 351–362.

Cohen SJ, Munchow AH, Rios LM, Zhang G, Asgeirsdottir HN, Stackman RW, Jr. 2013. The rodent hippocampus is essential for nonspatial object memory. Curr Biol 23:1685–1690.

Compagnone NA, Mellon SH. 2000. Neurosteroids: Biosynthesis and function of these novel neuromodulators. Front Neuroendocrinol 21:1–56.

Cooke PS, Spencer TE, Bartol FF, Hayashi K. 2013. Uterine glands: Development, function and experimental model systems. Mol Hum Reprod 19:547–558.

de Quervain DJ, Aerni A, Schelling G, Roozendaal B. 2009. Glucocorticoids and the regulation of memory in health and disease. Front Neuroendocrinol 30:358–370.

Dong Z, Han H, Wang M, Xu L, Hao W, Cao J. 2006. Morphine conditioned place preference depends on glucocorticoid receptors in both hippocampus and nucleus accumbens. Hippocampus 16: 809–813.

Ennaceur A, Delacour J. 1988. A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behav Brain Res 31:47–59.

Faivre EJ, Lange CA. 2007. Progesterone receptors upregulate Wnt-1 to induce epidermal growth factor receptor transactivation and c-Src-dependent sustained activation of Erk1/2 mitogen-activated protein kinase in breast cancer cells. Mol Cell Biol 27:466–480.

Farr SA, Flood JF, Scherrer JF, Kaiser FE, Taylor GT, Morley JE. 1995. Effect of ovarian steroids on footshock avoidance learning and retention in female mice. Physiol Behav 58:715–723.

Feeser VR, Loria RM. 2011. Modulation of traumatic brain injury using progesterone and the role of glial cells on its neuroprotective actions. J Neuroimmunol 237:4–12.

Fernandez SM, Lewis MC, Pechenino AS, Harburger LL, Orr PT, Gresack JE, Schafe GE, Frick KM. 2008. Estradiol-induced enhancement of object memory consolidation involves hippocampal ERK activation and membrane-bound estrogen receptors. J Neurosci 28:8660–8667.

Fortress AM, Frick KM. 2014. Epigenetic regulation of estrogen-dependent memory. Front Neuroendocrinol 35:530–549.

Fortress AM, Schram SL, Tuscher JJ, Frick KM. 2013a. Canonical Wnt signaling is necessary for object recognition memory consolidation. J Neurosci 33:12619–12626.

Fortress AM, Fan L, Orr PT, Zhao Z, Frick KM. 2013b. Estradiol-induced object recognition memory consolidation is dependent on activation of mTOR signaling in dorsal hippocampus. Learn Mem 20:147–155.

Foy MR. 2011. Ovarian hormones, aging and stress on hippocampal synaptic plasticity. Neurobiol Learn Mem 95:134–144.

Frick KM, Gresack JE. 2003. Sex differences in the behavioral response to spatial and object novelty in adult C57BL/6 mice. Behav Neurosci 117:1283–1291.

Frye CA, Walf AA. 2008. 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. Neurobiol Learn Mem 90:171–177.

Frye CA, Walf AA. 2010. Progesterone enhances learning and memory of aged wildtype and progestin receptor knockout mice. Neurosci Lett 472:38–42.

Frye CA, Duffy CK, Walf AA. 2007. Estrogens and progestins enhance spatial learning of intact and ovariectomized rats in the object placement task. Neurobiol Learn Mem 88:208–216.

Frye CA, Koonce CJ, Walf AA. 2013. Progesterone, compared to medroxyprogesterone acetate, to C57BL/6, but not 5alpha-reductase mutant, mice enhances object recognition and placement memory and is associated with higher BDNF levels in the hippocampus and cortex. Neurosci Lett 551:53–57.

Gogolla N, Galimberti I, Deguchi Y, Caroni P. 2009. Wnt signaling mediates experience-related regulation of synapse numbers and mossy fiber connectivities in the adult hippocampus. Neuron 62: 510–525.

Graber TE, McCamphill PK, Sossin WS. 2013. A recollection of mTOR signaling in learning and memory. Learn Mem 20:518–530.

Gregory CW, Johnson RT, Jr., Presnell SC, Mohler JL, French FS. 2001. Androgen receptor regulation of G1 cyclin and cyclin-

dependent kinase function in the CWR22 human prostate cancer xenograft. J Androl 22:537–548.
Gresack JE, Kerr KM, Frick KM. 2007. Life-long environmental enrichment differentially affects the mnemonic response to estrogen in young, middle-aged, and aged female mice. Neurobiol Learn Mem 88:393–408.
Guennoun R, Labombarda F, Gonzalez Deniselle MC, Liere P, De Nicola AF, Schumacher M. Progesterone and allopregnanolone in the central nervous system: Response to injury and implication for neuroprotection. J Steroid Biochem Mol Biol (in press).
Guerra-Araiza C, Coyoy-Salgado A, Camacho-Arroyo I. 2002. Sex differences in the regulation of progesterone receptor isoforms expression in the rat brain. Brain Res Bull 59:105–109.
Guerra-Araiza C, Villamar-Cruz O, González-Arenas A, Chavira R, Camacho-Arroyo I. 2003. Changes in progesterone receptor isoforms content in the rat brain during the oestrous cycle and after oestradiol and progesterone treatments. J Endocrinol 15:984–990.
Hagan CR, Daniel AR, Dressing GE, Lange CA. 2012. Role of phosphorylation in progesterone receptor signaling and specificity. Mol Cell Endocrinol 357:43–49.
Harburger LL, Pechenino AS, Saadi A, Frick KM. 2008. Post-training progesterone dose-dependently enhances object, but not spatial, memory consolidation. Behav Brain Res 194:174–180.
Harburger LL, Saadi A, Frick KM. 2009. Dose-dependent effects of post-training estradiol plus progesterone treatment on object memory consolidation and hippocampal extracellular signal-regulated kinase activation in young ovariectomized mice. Neuroscience 160: 6–12.
He L, Yang H, Zhai LD, Shao H, Li YS. 2011. A preliminary study on progesterone antioxidation in promoting learning and memory of young ovariectomized mice. Arch Med Sci 7:397–404.
Hoeffer CA, Klann E. 2010. mTOR signaling: At the crossroads of plasticity, memory and disease. Trends Neurosci 33:67–75.
Hurd C, Moudgil VK. 1988. Characterization of R5020 and RU486 binding to progesterone receptor from calf uterus. Biochemistry 27:3618–3623.
Inestrosa NC, Arenas E. 2010. Emerging roles of Wnts in the adult nervous system. Nat Rev Neurosci 11:77–86.
Inoki K, Ouyang H, Zhu T, Lindvall C, Wang Y, Zhang X, Yang Q, Bennett C, Harada Y, Stankunas K, Wang CY, He X, MacDougald OA, You M, Williams BO, Guan KL. 2006. TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. Cell 126:955–968.
Josefsberg Ben-Yehoshua L, Lewellyn AL, Thomas P, Maller JL. 2007. The role of Xenopus membrane progesterone receptor beta in mediating the effect of progesterone on oocyte maturation. Mol Endocrinol 21:664–673.
Karteris E, Zervou S, Pang Y, Dong J, Hillhouse EW, Randeva HS, Thomas P. 2006. Progesterone signaling in human myometrium through two novel membrane G protein-coupled receptors: Potential role in functional progesterone withdrawal at term. Mol Endocrinol 20:1519–1534.
Kato A, Hojo Y, Higo S, Komatsuzaki Y, Murakami G, Yoshino H, Uebayashi M, Kawato S. 2013. Female hippocampal estrogens have a significant correlation with cyclic fluctuation of hippocampal spines. Front Neural Circuits 7:149.
Kato J, Hirata S, Nozawa A, Yamada-Mouri N. 1994. Gene expression of progesterone receptor isoforms in the rat brain. Horm Behav 28:454–463.
Lamb R, Harrison H, Clarke RB. 2007. Mammary development, carcinomas and progesterone: Role of Wnt signalling. Ernst Schering Found Symp Proc 1:1–23.
Liu L, Wang J, Zhao L, Nilsen J, McClure K, Wong K, Brinton RD. 2009. Progesterone increases rat neural progenitor cell cycle gene expression and proliferation via extracellularly regulated kinase and progesterone receptor membrane components 1 and 2. Endocrinology 150:3186–3196.
Luine VN, Jacome LF, MacLusky NJ. 2003. Rapid enhancement of visual and place memory by estrogens in rats. Endocrinology 144: 2836–2844.
MacDonald BT, Tamai K, He X. 2009. Wnt/beta-catenin signaling: Components, mechanisms, and diseases. Dev Cell 17:9–26.
Meffre D, Grenier J, Bernard S, Courtin F, Dudev T, Shackleford G, Jafarian-Tehrani M, Massaad C. 2014. Wnt and lithium: A common destiny in the therapy of nervous system pathologies? Cel Mol Life Sci 71:1123–1148.
Melcangi RC, Giatti S, Calabrese D, Pesaresi M, Cermenati G, Mitro N, Viviani B, Garcia-Segura LM, Caruso D. 2014. Levels and actions of progesterone and its metabolites in the nervous system during physiological and pathological conditions. Prog Neurobiol 113:56–69.
Mitterling KL, Spencer JL, Dziedzic N, Shenoy S, McCarthy K, Waters EM, McEwen BS, Milner TA. 2010. Cellular and subcellular localization of estrogen and progestin receptor immunoreactivities in the mouse hippocampus. J Comp Neurol 518:2729–2743.
Molenda-Figueira HA, Murphy SD, Shea KL, Siegal NK, Zhao Y, Chadwick JG Jr, Denner LA, Tetel MJ. 2008. Steroid receptor coactivator-1 from brain physically interacts differentially with steroid receptor subtypes. Endocrinology 149:5272–5279.
Moore MR, Zhou JL, Blankenship KA, Strobl JS, Edwards DP, Gentry RN. 1997. A sequence in the 5' flanking region confers progestin responsiveness on the human c-myc gene. J Steroid Biochem Mol Biol 62:243–252.
Oliva CA, Vargas JY, Inestrosa NC. 2013. Wnt signaling: Role in LTP, neural networks and memory. Ageing Res Rev 12:786–800.
Orr PT, Lewis MC, Frick KM. 2009. Dorsal hippocampal progesterone infusions enhance object recognition in young female mice. Pharmacol Biochem Behav 93:177–182.
Orr PT, Rubin AJ, Fan L, Kent BA, Frick KM. 2012. The progesterone-induced enhancement of object recognition memory consolidation involves activation of the extracellular signal-regulated kinase (ERK) and mammalian target of rapamycin (mTOR) pathways in the dorsal hippocampus. Horm Behav 61:487–495.
Ortiz-Matamoros A, Salcedo-Tello P, Avila-Munoz E, Zepeda A, Arias C. 2013. Role of wnt signaling in the control of adult hippocampal functioning in health and disease: Therapeutic implications. Curr Neuropharmacol 11:465–476.
Phan A, Gabor CS, Favaro KJ, Kaschack S, Armstrong JN, MacLusky NJ, Choleris E. 2012. Low doses of 17beta-estradiol rapidly improve learning and increase hippocampal dendritic spines. Neuropsychopharmacology 37:2299–2309.
Pitha J, Harman SM, Michel ME. 1986. Hydrophilic cyclodextrin derivatives enable effective oral administration of steroidal hormones. J Pharm Sci 75:165–167.
Proietti C, Salatino M, Rosemblit C, Carnevale R, Pecci A, Kornblihtt AR, Molinolo AA, Frahm I, Charreau EH, Schillaci R, Elizalde PV. 2005. Progestins induce transcriptional activation of signal transducer and activator of transcription 3 (Stat3) via a Jak- and Src-dependent mechanism in breast cancer cells. Mol Cell Biol 25: 4826–4840.
Purro SA, Galli S, Salinas PC. 2014. Dysfunction of Wnt signaling and synaptic disassembly in neurodegenerative diseases. J Mol Cell Biol 6:75–80.
Qu Q, Sun G, Murai K, Ye P, Li W, Asuelime G, Cheung K, Shi Y. 2013. Wnt7a regulates multiple steps of neurogenesis. Mol Cell Biol 33:2551–2559.
Richter JD, Klann E. 2009. Making synaptic plasticity and memory last: Mechanisms of translational regulation. Genes Dev 23:1–11.
Robinson GW, Hennighausen L, Johnson PF. 2000. Side-branching in the mammary gland: The progesterone-Wnt connection. Genes Dev 14:889–894.
Sahores M, Gibb A, Salinas PC. 2010. Frizzled-5, a receptor for the synaptic organizer Wnt7a, regulates activity-mediated synaptogenesis. Development 137:2215–2225.

Sani G et al. 2012. The wnt pathway in mood disorders. Curr Neuropharmacol 10:239–253.

Satterfield MC, Song G, Hayashi K, Bazer FW, Spencer TE. 2008. Progesterone regulation of the endometrial WNT system in the ovine uterus. Reprod Fertil Dev 20:935–946.

Sayeed I, Stein DG. 2009. Progesterone as a neuroprotective factor in traumatic and ischemic brain injury. Prog Brain Res 175:219–237.

Scott EL, Brann DW. 2013. Estrogen regulation of Dkk1 and Wnt/beta-Catenin signaling in neurodegenerative disease. Brain Res 1514:63–74.

Singh M, Su C, Ng S. 2013. Non-genomic mechanisms of progesterone action in the brain. Front Neurosci 7:159.

Skildum A, Faivre E, Lange CA. 2005. Progesterone receptors induce cell cycle progression via activation of mitogen-activated protein kinases. Mol Endocrinol 19:327–339.

Sweatt JD. 2004. Mitogen-activated protein kinases in synaptic plasticity and memory. Curr Opin Neurobiol 14:311–317.

Tabatadze N, Tomas C, McGonigal R, Lin B, Schook A, Routtenberg A. 2012. Wnt transmembrane signaling and long-term spatial memory. Hippocampus 22:1228–1241.

Tsokas P, Ma T, Iyengar R, Landau EM, Blitzer RD. 2007. Mitogen-activated protein kinase upregulates the dendritic translation machinery in long-term potentiation by controlling the mammalian target of rapamycin pathway. J Neurosci 27:5885–5894.

Tuscher JJ, Fortress AM, Kim J, Frick KM. Regulation of novel object recognition and object placement by ovarian sex steroid hormones. Behav Brain Res (in press).

Varea O, Garrido JJ, Dopazo A, Mendez P, Garcia-Segura LM, Wandosell F. 2009. Estradiol activates beta-catenin dependent transcription in neurons. PloS One 4:e5153.

Varela-Nallar L, Inestrosa NC. 2013. Wnt signaling in the regulation of adult hippocampal neurogenesis. Front Cell Neurosci 7:100.

Vargas JY, Fuenzalida M, Inestrosa NC. 2014. In vivo activation of Wnt signaling pathway enhances cognitive function of adult mice and reverses cognitive deficits in an Alzheimer's disease model. J Neurosci 34:2191–2202.

Walf AA, Rhodes ME, Frye CA. 2006. Ovarian steroids enhance object recognition in naturally cycling and ovariectomized, hormone-primed rats. Neurobiol Learn Mem 86:35–46.

Woolley CS, McEwen BS. 1993. Roles of estradiol and progesterone in regulation of hippocampal dendritic spine density during the estrous cycle in the rat. J Comp Neurol 336:293–306.

Wright DW, Kellermann AL, Hertzberg VS, Clark PL, Frankel M, Goldstein FC, Salomone JP, Dent LL, Harris OA, Ander DS, Lowery DW, Patel MM, Denson DD, Gordon AB, Wald MM, Gupta S, Hoffman SW, Stein DG. 2007. ProTECT: A randomized clinical trial of progesterone for acute traumatic brain injury. Ann Emerg Med 49:391–402, 402 e391-e392.

Xiao G, Wei J, Yan W, Wang W, Lu Z. 2008. Improved outcomes from the administration of progesterone for patients with acute severe traumatic brain injury: A randomized controlled trial. Crit Care 12:R61.

Yoshiya M, Komatsuzaki Y, Hojo Y, Ikeda M, Mukai H, Hatanaka Y, Murakami G, Kawata M, Kimoto T, Kawato S. 2013. Corticosterone rapidly increases thorns of CA3 neurons via synaptic/extranuclear glucocorticoid receptor in rat hippocampus. Front Neural Circuits 7:191.

Yousuf S, Atif F, Sayeed I, Tang H, Stein DG. 2014. Progesterone in transient ischemic stroke: A dose-response study. Psychopharmacology 231:3313–3323.

Zhang QG, Wang R, Khan M, Mahesh V, Brann DW. 2008. Role of Dickkopf-1, an antagonist of the Wnt/beta-catenin signaling pathway, in estrogen-induced neuroprotection and attenuation of tau phosphorylation. J Neurosci 28:8430–8441.

Zhang Z, Yang R, Zhou R, Li L, Sokabe M, Chen L. 2010. Progesterone promotes the survival of newborn neurons in the dentate gyrus of adult male mice. Hippocampus 20:402–412.

Zhao Z, Fan L, Fortress AM, Boulware MI, Frick KM. 2012. Hippocampal histone acetylation regulates object recognition and the estradiol-induced enhancement of object recognition. J Neurosci 32:2344–2351.