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Hippocampal Wnt Signaling: Memory Regulation and Hormone Interactions

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Abstract

Wnt signaling has emerged in recent years as a major player in both nervous system development and adult synaptic plasticity. Of particular relevance to researchers studying learning and memory, Wnt signaling is critical for normal functioning of the hippocampus, a brain region that is essential for many types of memory formation and whose dysfunction is implicated in numerous neurodegenerative and psychiatric conditions. Impaired hippocampal Wnt signaling is implicated in several of these conditions, however, little is known about how Wnt signaling mediates hippocampal memory formation. This review will provide a general overview of Wnt signaling and discuss evidence demonstrating a key role for Wnt signaling in hippocampal memory formation in both normal and disease states. The regulation of Wnt signaling by ovarian sex steroid hormones will also be highlighted, given that the neuroprotection afforded by Wnt-hormone interactions may have significant implications for cognitive function in aging, neurodegenerative disease, and ischemic injury.

Keywords

hippocampus; β -catenin; GSK3 β ; Alzheimer's disease; estradiol; progesterone

Within the past decade, Wnt signaling has emerged as a significant regulator of adult hippocampal plasticity and memory. Wnts have been studied outside of the nervous system for decades as key players in cardiac and bone diseases (Krishnan and others 2006; Marinou and others 2012; Pandey and Chandravati 2013), degenerative skeletal disorders (Church and Francis-West 2002), and cancers (Nusse and Varmus 1982; Nusse and Varmus 2012). In the nervous system, Wnts were initially studied in the context of neural development, where they are necessary for the development of brain regions including the hippocampus (Grove and Tole 1999; Lee and others 2000). Later studies determined that Wnts regulate synaptic plasticity in the adult hippocampus via synapse formation, dendritic morphogenesis, and long-term potentiation (LTP) (Chen and others 2006; Dickins and Salinas 2013; Rosso and Inestrosa 2013). Despite the well-known role of the hippocampus in learning and memory, it has only recently been established that Wnts contribute to hippocampal learning and

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memory, and that hippocampal Wnt signaling is dysregulated in neuropsychiatric and neurodegenerative diseases such as Alzheimer's disease and Down syndrome (Caricasole and others 2004; Contestabile and others 2013). Nevertheless, the specific mechanisms through which Wnts contribute to memory formation remain poorly understood, as do the reasons why Wnt signaling goes awry in various disease states. Unlocking these mysteries may lead to novel therapeutics for a host of neurological and neurodegenerative disorders.

Wnts are evolutionarily conserved across many species and are believed to be at least 600 million years old (MacDonald and others 2009; Nusse and Varmus 2012). Found in a wide array of species from planaria to humans, Wnts play a key role in establishing polarity and determining axis development (Kusserow and others 2005; Petersen and Reddien 2009). The name "Wnt" is derived from the gene *Wg* (wingless) in *Drosophila* and *int1* (integration-1) in rodents. The first Wnt gene, identified at the time as *int1*, was cloned from the mouse genome in 1982 and determined to be a proto-oncogene due to its role in regulating cell cycle progression and oncogenesis (Nusse and Varmus 1982). Interestingly, the Wnt gene was already being investigated under the name *Wg* in *Drosophila* for its role in regulating patterning phenotypes during embryogenesis (Nusslein-Volhard and Wieschaus 1980). In 1987, two independent mapping studies confirmed that *Wg* and *int1* were the same gene (Baker 1987; Rijsewijk and others 1987). Therefore, *Wg* and *int1* were combined in the early 1990s to form *Wnt1* as it is known today (for review, see Nusse and Varmus 2012). A total of 19 Wnt genes have thus far been identified (Niehrs 2012).

Wnt proteins are secreted as lipid-modified glycoproteins of approximately 350 to 400 amino acids in length with a molecular weight of 40 kDa (Coudreuse and Korswagen 2007). All Wnts function as extracellular ligands (Coudreuse and Korswagen 2007; MacDonald and others 2009). Prior to their secretion, Wnts can be regulated in the endoplasmic reticulum by posttranslational modifications to determine the conditions under which a given Wnt will be secreted. The two major types of posttranslational Wnt modifications are lipidation/acylation and N-glycosylation (Tang and others 2012). Although the details of Wnt sorting and secretion are still being elucidated and are discussed elsewhere (Bartscherer and Boutros 2008; Coudreuse and Korswagen 2007; Tang and others 2012), it appears that different posttranslational modifications affect the sorting, secretion, binding, and biological activity of each Wnt protein in unique ways. For example, N-glycosylation of one Wnt protein may enhance secretion of that Wnt, whereas N-glycosylation of another Wnt protein may decrease its expression. Adding to this complexity, Wnts can bind multiple Frizzled receptors, potentially eliciting different responses depending on the cellular environment.

Frizzled (Fzd) receptors are seven transmembrane domain proteins, similar to G-protein-coupled receptors, that are responsible for transducing the effects of Wnt proteins (Dijksterhuis and others 2014). There are currently 10 different known Fzd receptors (Fzd 1–10) to which the 19 Wnt ligands can bind. Fzd receptors facilitate Wnt signaling by interacting with numerous co-receptors including LRP5/6, RYK, ROR1/2 (Niehrs 2012). Regulation of Fzd receptors occurs through posttranslational modifications such as phosphorylation, ubiquitination, and deubiquitination, or through cleavage by metalloproteases (Dijksterhuis and others 2014). However, not all of these modifications have been demonstrated for all Fzd receptors in all species that express Wnts. The role of

Fzd receptors and their co-receptors in adult nervous system function remains relatively unexplored and is likely to offer many new insights into the mechanisms underlying hippocampal function and dysfunction.

Wnt signaling occurs through both autocrine and paracrine mechanisms and can be classified as β -catenin-dependent or β -catenin-independent. β -Catenin-dependent Wnt signaling is referred to as “canonical,” and is the only Wnt pathway mediated by stabilization of β -catenin in the nucleus. β -Catenin-independent Wnt signaling is considered “non-canonical,” and includes both the JNK/Planar Cell Polarity (PCP) and Wnt-Calcium pathways. Each of the Wnt signaling pathways is discussed in turn below (also see Box 1). It should be noted that the same Wnt ligand can activate different Wnt signaling pathways in different cell types or environments. Thus, the various combinations of Wnt ligands and receptor/co-receptors present in a cell will ultimately determine which Wnt pathways are activated (for review, see Niehrs 2012). Additionally, the three Wnt signaling pathways can interact to some extent. For example, activation of the JNK/PCP pathway inhibits the β -catenin-dependent signaling pathway (Sato and others 2010). The complicated interactions among Wnt ligands and Fzd receptors, as well as among Wnt signaling pathways themselves, may explain why alterations in Wnt signaling are linked with numerous neurological diseases (see “Wnt Signaling and Neurological Disease” below).

The Canonical Wnt/ β -Catenin-Dependent Signaling Pathway

Canonical Wnt/ β -catenin-dependent signaling is the most widely studied of the Wnt signaling pathways. The main biological effect of this pathway is to regulate the phosphorylation of β -catenin by GSK3 β (glycogen synthase kinase-3 β ; Fig. 1A). Constitutively active GSK3 β promotes degradation of β -catenin, which then prevents β -catenin from entering the nucleus and acting as a cofactor for TCF (T-cell-specific transcription factor)/LEF (lymphoid enhancer binding factor) transcription factors. The phosphorylation of β -catenin by GSK3 β leads to recognition by β -Trcp (Liu and others 1999), an E3 ubiquitin ligase that targets β -catenin for proteosomal degradation (Stamos and Weis 2013) and prevents the regulation of downstream target genes. Upstream of GSK3 β , canonical Wnt signaling is activated by binding of the Wnt ligand to a Fzd receptor, which then forms a complex with the co-receptor LRP5/6, and recruits Dishevelled (Dvl) to phosphorylate LRP (Bilic and others 2007; Niehrs 2012). The phosphorylation of LRP leads to the dephosphorylation of GSK3 β , which prevents GSK3 β from phosphorylating and degrading β -catenin. Thus, β -catenin is available to enter the nucleus and interact with TCF/LEF transcription factors to facilitate transcription of downstream target genes. However, β -catenin can also interact with cadherins to regulate transsynaptic plasticity and alter spine morphology (Murase and others 2002; Okuda and others 2007; Salinas and Price 2005; Vitvureira and others 2012). This interaction is particularly important for the formation of new synapses in neural development or in response to synaptic events to facilitate synaptic plasticity. Therefore, β -catenin plays a significant role in nervous system function as both a regulator of gene expression and through interactions with cadherins.

The JNK/PCP Pathway

The JNK/PCP pathway, classified as non-canonical Wnt signaling, is known for its role in regulating cytoskeletal dynamics. Similar to the β -catenin-dependent pathway, activation of the Fzd receptor typically forms a complex with a co-receptor such as RYK, MUSK, PTK7, Syndecan, Glypican, or ROR1/2, the latter of which is the primary vertebrate co-receptor that transduces the effects of the non-canonical ligand Wnt5a (Niehrs 2012). Activation of the Fzd receptor recruits Dvl to signal to the monomeric GTPases Rho and Rac (Fig. 1B). Rho stimulates ROCK, and Rac activates JNK to regulate activating transcription factor-2 (ATF-2) and downstream target genes that mediate cytoskeletal reorganization (Oliva and others 2013; Vivancos and others 2009) (Fig. 1B). Relevant to memory, the JNK/PCP pathway is an important pathway regulating the dendritic spine formation and stability that leads to synapse formation and hippocampal plasticity during development and adulthood (Luo 2000; Rex and others 2009; Rosso and others 2005; Tashiro and Yuste 2004).

The Wnt/Ca²⁺ Signaling Pathway

The other non-canonical Wnt signaling pathway, Wnt/ Ca²⁺, plays a significant role in axon guidance. This pathway is thought to elicit signaling through a rise in intracellular Ca²⁺ levels resulting from a G-protein-coupled signaling mechanism and activation of Dvl (Montcouquiol and others 2006; Slusarski and others 1997). The generation of diacylglycerol and inositol-(1,4,5)-triphosphate activates protein kinase C (PKC) and increases Ca²⁺ release from the endoplasmic reticulum, respectively. The rise in intracellular Ca²⁺ ultimately increases levels of calcineurin and Ca²⁺/calmodulin dependent protein kinase II (CaMKII), which regulate, respectively, the downstream transcription factors nuclear factor of activated T cells (NFAT) and cAMP response element binding protein (CREB) (Hogan and others 2003; Niehrs 2012; Oliva and others 2013; Rosso and Inestrosa 2013) (Fig. 1B). As such, the Wnt/Ca²⁺ pathway plays a significant role in mediating gene transcription (Hutchins and others 2011).

Wnt Signaling and Hippocampal Function

Some of the first clues that Wnt signaling was important for mammalian neural development came from studies demonstrating that Wnt signaling could regulate neural patterning, axonal remodeling, and synapse formation in the hippocampus and cerebellum (Galceran and others 2000; Grove and Tole 1999; Hall and others 2000; Lee and others 2000; Lucas and Salinas 1997). These findings laid the groundwork for subsequent research demonstrating the importance of Wnt signaling in adulthood for the functioning of brain regions such as the hippocampus. As will be discussed below, Wnt signaling is essential for the development and maintenance of hippocampal function. Therefore, understanding how Wnt signaling regulates hippocampal function may have important therapeutic implications for disorders in which the hippocampus plays a key role.

The involvement of Wnt signaling in the development of the various hippocampal subfields has been illustrated in several mutant mouse lines. For example, *Wnt3a*^{-/-} mice do not develop a dentate gyrus or the CA subfields that are characteristic of a mature, functional

hippocampus (Grove and Tole 1999; Lee and others 2000). Wnt3a regulates cell proliferation, and its loss substantially reduces the population of hippocampal progenitor cells necessary for the proliferation of the cells that comprise the mature hippocampus (Lee and others 2000). Interestingly, the surrounding neocortex and telencephalic choroid plexus epithelium are unaffected by genetic deletion of Wnt3a (Grove and Tole 1999), suggesting a specific role for Wnt3a in regulating the development of the hippocampus. Further evidence for a necessary role of Wnt signaling in hippocampal development comes from studies of mice that cannot express *Lef1*, a nuclear mediator of Wnt signaling (Galceran and others 2000). Specifically, *Lef1* null mutants do not form a dentate gyrus, whereas mice carrying a mutant *Lef1* that also interferes with *Tcf* gene functioning lack the entire hippocampus (Galceran and others 2000). These data illustrate that both Wnt ligands and downstream mediators of Wnt signaling, such as TCF/LEF genes, are critical for the formation of the hippocampus.

Within the normally developed hippocampus, Wnt signaling regulates synapse and circuit formation. At the presynaptic terminal, specific Wnts, such as Wnt3a and Wnt7a, increase the clustering of presynaptic proteins and promote synaptic vesicle release and recycling in cultured hippocampal neurons (Cerpa and others 2008; Varela-Nallar and others 2009). Wnt3a increases clustering of the presynaptic protein bassoon, whereas Wnt7a increases clustering of other presynaptic proteins including vGlut, synaptophysin, and synaptotagmin (Cerpa and others 2008; Ciani and others 2011; Varela-Nallar and others 2009). Pharmacological blockade of canonical Wnt/ β -catenin signaling with the endogenous Wnt inhibitor Dickkopf-1 (Dkk-1) or the secreted frizzled-related protein-1 (sFRP-1) prevents the canonical Wnt ligand Wnt7a from increasing presynaptic protein clustering (Davis and others 2008), suggesting that such clustering may be mediated by β -catenin-dependent signaling (Xu and others 2014). Additionally, Wnt7a potently regulates synaptic vesicle recycling to modulate neurotransmitter release (Cerpa and others 2008). Interestingly, this effect is specific to Wnt7a, as synaptic vesicle recycling was only modestly affected by Wnt3a and was unaffected by Wnt1 or Wnt5a (Cerpa and others 2008). Recombinant Wnt7 has also been shown to increase the number of presynaptic active zones, suggesting that Wnt7 promotes the formation of new presynaptic terminals (Tabatadze and others 2014). The significance of Wnt7a in regulating hippocampal presynaptic plasticity is further demonstrated by evidence that *Wnt7a/Dvl* double mutants exhibit impaired neurotransmitter release, spine morphogenesis, and synaptic transmission in the hippocampus (Ahmad-Annuar and others 2006; Ciani and others 2011). Collectively, this evidence suggests that specific Wnts, particularly Wnt7a, play a critical role in regulating presynaptic function to facilitate hippocampal synapse formation.

Wnt ligands are also essential for postsynaptic assembly and synapse formation. For example, Wnt7a specifically increases the number and strength of excitatory, but not inhibitory, synapses in cultured hippocampal neurons by both increasing the formation and maturation of dendritic spines and promoting excitatory synaptic transmission (Ciani and others 2011). Wnt5a also facilitates a JNK-dependent increase in postsynaptic PSD95 clustering (Farias and others 2009). In contrast to the Wnt7a-induced formation of excitatory synapses (Ciani and others 2011), Wnt5a facilitates the formation of inhibitory synapses by increasing recycling of GABA_A receptors and facilitating GABA receptor currents in a

CaMKII-dependent manner (Cuitino and others 2010). As such, the differential roles of Wnt5a and Wnt7a in excitatory and inhibitory synaptic assembly highlights the specificity with which Wnts regulate synapse development in the hippocampus.

Within the hippocampus, the synthesis and release of Wnt ligands and Fzd receptors appear to be activity-dependent. Early studies in hippocampal neurons demonstrated that potassium-induced depolarization increased dendritic arborization in a manner that requires β -catenin and the release of unspecified Wnt proteins (Yu and Malenka 2003). In 2006, it was determined that Wnt3a is released in the hippocampus following tetanic stimulation in an NMDA-dependent manner (Chen and others 2006). It was proposed that NMDA receptors are activated via an increase in Ca^{2+} that facilitates an increase in Wnt3a release from the synapse, which then allows Wnts to bind to Fzd receptors and activate Wnt signaling (Chen and others 2006). In addition to Wnt release, NMDA receptors can also regulate Wnt synthesis. In particular, Ca^{2+} -dependent activation of ERK through NMDA receptors leads to an increase in CREB at the *Wnt2* promoter to facilitate the transcription of Wnt2 in hippocampal neurons (Wayman and others 2006). Interestingly, at least some Fzd receptors are regulated by synaptic activity as well. In response to high-frequency stimulation or potassium-mediated depolarization, Fzd5 is significantly increased at the cell surface (Sahores and others 2010). Because Fzd5 is believed to mediate the effects of Wnt7a (Sahores and others 2010), the activity-dependent increase in Fzd5 may be one mechanism through which Wnt7a facilitates spinogenesis and the formation of excitatory synapses (Ciani and others 2011).

Although much has been learned in the past decade about the effects of various Wnts on hippocampal morphology and plasticity, our understanding of how Wnts regulate hippocampal function remains rudimentary. Much of the work to date has been conducted in vitro or has been limited to an examination of hippocampal morphology and physiology. As such, the functional role of the Wnt signaling pathways in hippocampally-mediated behavior remains poorly understood. Such information is critically important, given evidence that Wnt dysfunction is characteristic of conditions such as Alzheimer's disease (Caricasole and others 2004; Liu and others 2014; Purro and others 2012). To address this issue, a handful of studies have begun to pinpoint the role of Wnt signaling in hippocampal memory formation. This work will be described below.

Wnt Signaling and Hippocampal Memory

Learning-Induced Regulation of Wnts

Several groups have examined whether learning regulates Wnt protein expression in the hippocampus. The first study to address this issue demonstrated that levels of Wnt7 and Wnt5a, but not Wnt3, protein were significantly increased in male rats 7 days after spatial learning in a hippocampal-dependent Morris water maze task (Tabatadze and others 2012). This effect was persistent, as Wnt7 levels remained elevated for 30 days following spatial learning (Tabatadze and others 2012). The learning-induced changes in Wnt7 were observed in the granule cell layer, but not in CA3 (Tabatadze and others 2012), demonstrating subregion specificity of activity-induced changes. Moreover, potassium- or glutamate-induced transport of Wnt7 from cell bodies to neuronal processes was observed in primary

hippocampal neurons, as was activity-induced Wnt7 release (Tabatadze and others 2012; Tabatadze and others 2014), suggesting that neural activity mobilizes Wnt7 to facilitate synaptic plasticity. On a more rapid timescale, an object training protocol that results in dorsal hippocampal-dependent memory consolidation produced a small, yet significant, increase in Wnt7a protein levels in male mice 5 minutes after training (Fortress and others 2013a) (Fig. 2A). This increase was transient, as levels did not differ from vehicle either 30 minutes or 4 hours after training. Also in male mice, contextual fear conditioning, a form of fear conditioning that requires the hippocampus, triggers a specific and significant increase in mRNA and protein levels of Wnt3a in the hippocampus 2 to 3 hours after training (Xu and others 2014). Wnt3a mRNA and protein levels returned to baseline 4 hours after training, suggesting a relatively brief learning-induced elevation of Wnt3a.

Various forms of hippocampal learning also regulate canonical Wnt/ β -catenin signaling proteins, as indicated by increases in phosphorylated GSK3 β and total or active β -catenin within 30 minutes of object training in male mice (Fortress and others 2013a) (Fig. 2B and C), 2 hours of contextual fear conditioning in male mice (Xu and others 2014), or 12 hours of passive avoidance training in male rats (Conboy and others 2007). Object training also produced a rapid and sustained increase in protein levels of the TCF/LEF-induced gene *Cyclin D1*, which was elevated from 5 minutes to 4 hours after training (Fig. 2D). Rapid activation of the Wnt/ Ca^{2+} pathway has also been observed within 15 minutes of contextual fear conditioning in male mice (Xu and others 2014). Together, these findings suggest that β -catenin-dependent and β -catenin-independent signaling can be rapidly activated in the hippocampus following learning.

Wnts as Essential Modulators of Hippocampal Memory

Although learning-induced changes suggest a possible role for Wnt signaling in hippocampal memory, they do not establish that such signaling is required for memory formation. The first study to demonstrate a *necessary* role for Wnt signaling in memory examined amygdala-dependent fear conditioning. This work found that infusion of Wnt1 or the endogenous Wnt/ β -catenin antagonist Dkk-1 into the amygdala of male rats prior to training impaired fear memory consolidation without affecting learning (Maguschak and Ressler 2011). Because of the aforementioned data showing that learning increased Wnt7 levels in the hippocampus (Conboy and others 2007; Fortress and others 2013a; Tabatadze and others 2012), we hypothesized that Wnt signaling may also be necessary for hippocampal memory consolidation. Immediately after training in a hippocampal-dependent object task, we bilaterally infused vehicle or Dkk-1 into the dorsal hippocampus of male mice (Fig. 3A). Three different doses of Dkk-1 impaired object recognition memory consolidation 24 hours later (Fortress and others 2013a) (Fig. 3B), demonstrating that β -catenin-dependent Wnt signaling is necessary for object recognition memory consolidation. Similar findings have recently been reported for contextual fear memory in male mice. In this work, pretraining dorsal hippocampal infusions of Dkk-1 selectively impaired long-term contextual fear memory, suggesting that Wnt/ β -catenin signaling is also critical for contextual fear memory consolidation (Xu and others 2014). In both studies (Fortress and others 2013a; Xu and others 2014), Dkk-1 inhibited canonical Wnt signaling by decreasing phosphorylated GSK3 β and/or β -catenin (Fig. 3C and D). In our object recognition study,

Dkk-1 also reduced protein levels of Wnt7a (Fig. 3E), TCF, LEF, and Cyclin D1 (Fig. 3F) in the dorsal hippocampus (Fortress and others 2013a). In contrast to Dkk-1 treatment, viral deletion of Dkk-1 in older mice enhances hippocampal neurogenesis and spatial working memory and reverses age-related memory consolidation impairments (Seib and others 2013). Together, these data provide persuasive evidence for a central role of Wnt/ β -catenin signaling in hippocampal memory formation.

Another recent study demonstrates that Wnt3a can regulate contextual fear memory via both canonical Wnt/ β -catenin signaling and Wnt/ Ca^{2+} signaling. This study found that decreasing hippocampal Wnt3a expression with sequestering antibodies prevented acquisition and consolidation, but not expression, of contextual fear memory in male mice (Xu and others 2014). Interestingly, the data implicated Wnt/ Ca^{2+} signaling in acquisition and Wnt/ β -catenin in consolidation, indicating differential involvement of Wnt signaling pathways in learning and memory (Xu and others 2014). Accordingly, dorsal hippocampal infusion of exogenous Wnt3a activated both Wnt pathways and enhanced both fear acquisition and consolidation, suggesting that Wnt3a is both necessary and sufficient to regulate contextual fear memory.

The importance of Wnt3a and Wnt5a in facilitating other types of hippocampal memory was shown in another recent study using synthetic small molecules to selectively activate canonical and non-canonical Wnt signaling. Mice were chronically infused into hippocampal CA1 with WASP-1 (Wnt-activating small molecule), which triggers Wnt3a-induced canonical Wnt signaling, or with FOXY-5 (formylated Wnt5a-derived hexapeptide), which mimics Wnt5a-induced JNK/PCP signaling (Vargas and others 2014). Both WASP-1 and FOXY-5 enhanced synaptic plasticity, expression of hippocampal synaptic proteins, spatial memory, and object recognition memory (Vargas and others 2014), suggesting that both Wnt/ β -catenin and JNK/PCP signaling regulate hippocampal spatial and object memory. Moreover, WASP-1 and FOXY-5 enhanced basal synaptic transmission and reversed impairments in both long-term potentiation and object recognition memory in a mouse model of Alzheimer's disease (Vargas and others 2014), suggesting that activation of either Wnt pathway may reverse memory loss in neurodegenerative diseases such as Alzheimer's.

Finally, recent work has suggested that dysfunctional Wnt signaling contributes to age-related hippocampal memory decline. In mice, Dkk-1 expression in the dentate gyrus increases with age, and genetic deletion of Dkk-1 in neural progenitor cells reversed age-related reductions in neurogenesis, TCF/LEF activity, dendritic complexity, and spatial working memory (Seib and others 2013). Other findings demonstrate that conditional deletion of the gene for *Lrp6* in the mouse forebrain significantly decreases dendritic spine density in hippocampal CA1 and cortical pyramidal neurons, reduces hippocampal LTP, and impairs contextual fear conditioning in aged mice (Liu and others 2014). These data suggest that maintaining normal Wnt signaling may be essential for preventing age-related memory decline.

Collectively, several findings published in just the past 3 years demonstrate that multiple Wnt signaling pathways can regulate various forms of hippocampal memory in rodents.

These results have significant implications for understanding the neural mechanisms underlying learning and memory, as the many Wnt ligands, Fzd receptors, and Wnt signaling pathways appear capable of modulating hippocampal function in myriad ways. Because hippocampal dysfunction is implicated in the etiology or symptomatology of conditions characterized by memory impairment, such as Alzheimer's disease, Down syndrome, ischemia, stress, and aging (Box 2) (Busceti and others 2008; Cramer and Galdzicki 2012; Fjell and others 2014; Matrisciano and others 2011; Nikonenko and others 2009; Pandey and others 2014; Wingenfeld and Wolf 2014), better understanding how Wnt signaling regulates hippocampal memory may reveal new avenues for therapeutics to prevent or reverse memory loss in these conditions. Data linking Wnt signaling to neurological diseases will be discussed in the next section.

Wnt Signaling in Neurological Disease

A link between dysfunctional Wnt signaling and Alzheimer's disease was established about a decade before the necessity of Wnt signaling for memory formation was demonstrated. Alzheimer's disease is the most common form of dementia and is characterized by widespread deterioration in the brain, particularly in the hippocampus, medial temporal cortex, and prefrontal cortex. In addition to substantial neuron loss, the brains of Alzheimer's patients are riddled with amyloid plaques, containing toxic beta-amyloid ($A\beta$) proteins, and neurofibrillary tangles, comprised of hyperphosphorylated tau filaments. Consistent with a beneficial role of Wnt signaling in memory, LRP6-mediated Wnt signaling is down-regulated and negatively correlated with neurotoxic $A\beta_{40}$ and $A\beta_{42}$ peptides in the temporal cortex of Alzheimer's patients (Liu and others 2014). Levels of β -catenin are also reduced in the Alzheimer's brain, and the destabilization of β -catenin by presenilin-1, mutations of which are associated with early-onset Alzheimer's, increases $A\beta$ -induced apoptosis (Zhang and others 1998). Interestingly, GSK3 β actively hyperphosphorylates tau in neurons, as demonstrated by studies in which GSK3 β inhibitors blocked $A\beta$ -induced tau hyperphosphorylation (Caricasole and others 2004). The endogenous Wnt inhibitor Dkk-1 is also necessary for tau phosphorylation in $A\beta$ -treated neurons, and expression of Dkk-1 is increased in Alzheimer's brains relative to controls (Caricasole and others 2004; Liu and others 2014). In keeping with a role for Dkk-1 in tau phosphorylation, this increase occurs specifically in degenerating neurons and colocalizes with hyperphosphorylated tau, neurofibrillary tangles, and dystrophic neurites (Caricasole and others 2004). Dkk-1 is also required for the synaptic loss induced by $A\beta$ in cultured rat hippocampal neurons (Purro and others 2012), suggesting that suppression of Wnt signaling by Dkk-1 increases the risk of Alzheimer's pathology.

In contrast, activation of Wnt signaling can prevent the neurotoxic effects of $A\beta$. For example, activation of protein kinase C protected rat hippocampal neurons from $A\beta$ -induced neurotoxicity by decreasing GSK3 β activity and increasing β -catenin-induced gene expression (Garrido and others 2002). Similar effects were observed after treatment with Wnt3a (Garrido and others 2002). The benefits of Wnt3a were also shown in another study in which Wnt3a protected rat hippocampal neurons from $A\beta$ -induced apoptosis by decreasing the phosphorylation of tau and GSK3 β , increasing β -catenin, and enhancing cell survival (Alvarez and others 2004). Interestingly, the FDA-approved mood stabilizing agent

lithium activates Wnt/ β -catenin signaling by increasing GSK3 β phosphorylation, and in vitro findings indicate that it also blocks the neurotoxic effects of A β in rat hippocampal neurons (Garrido and others 2002). In vivo, lithium reverses and prevents the neurodegenerative and memory-impairing effects of A β fibrils injected into the hippocampus of adult rats (De Ferrari and others 2003). Dietary lithium also reduces amyloid plaque formation and enhances hippocampal neurogenesis and hippocampal-dependent spatial memory in mice overexpressing amyloid precursor protein (TgCRND8) (Fiorentini and others 2010), supporting a key role for Wnt/ β -catenin signaling in protecting hippocampal neurons from amyloid toxicity. Combined, the findings from Alzheimer's patients and rodent models suggest that Wnt signaling is a key regulator of Alzheimer's neuropathology and can protect against the cellular hallmarks of the disease.

Wnt signaling is also altered in numerous other conditions, including Down syndrome, Williams syndrome, ischemia, chronic stress, and drug use (Busceti and others 2008; Contestabile and others 2013; Mohn and others 2014; O'Brien and others 2004; Pandey and others 2014; Zhao and others 2005). For example, the Fzd9 receptor is mutated in Williams syndrome, and its deletion in mice causes hippocampal neuron loss and spatial memory deficits (Zhao and others 2005). In a mouse model of Down syndrome, lithium restores normal hippocampal neurogenesis and memory function by activating Wnt/ β -catenin signaling (Contestabile and others 2013; Zhao and others 2005). Moreover, Dkk-1 is induced in the rodent hippocampus after cerebral ischemia (Cappuccio and others 2005), acute or chronic stress (Matrisciano and others 2011), or short-term treatment with ecstasy (MDMA) (Busceti and others 2008). The ischemia-induced increase in Dkk-1 was blocked by pretreatment with lithium or Dkk-1 antisense oligonucleotides (Cappuccio and others 2005). The ecstasy-induced increase in Dkk-1 was also accompanied by increased hippocampal tau hyperphosphorylation, reduced canonical Wnt/ β -catenin signaling, and impaired spatial learning (Busceti and others 2008). Collectively, these studies suggest that impaired hippocampal Wnt signaling, particularly Wnt/ β -catenin signaling, may contribute to the neuropathology and/or symptomatology of various neurological or neuropsychiatric conditions.

Wnt-Hormone Interactions and Hippocampal Function

Another way in which Wnts may mediate memory formation is by interacting with sex-steroid hormones, which are key modulators of hippocampal memory formation in their own right. Some neurodegenerative diseases (e.g., Alzheimer's) and neuropsychiatric conditions (e.g., depression, anxiety disorders) are more prevalent in women than in men (Alzheimer's Association 2011; Kessler and others 2005), potentially due to sex differences in circulating estrogens and progestins. Of particular relevance to hippocampal memory is the elevated risk of Alzheimer's disease faced by menopausal women (Yaffe and others 1998; Zandi and others 2002). This risk is thought to be due to the precipitous drop in circulating estrogens and progestins that occurs at menopause (Sherwin and Henry 2008; Yaffe and others 2007). Given the importance of Wnt signaling to hippocampal memory and the ability of Wnt signaling to protect from A β neurotoxicity, interactions among estrogens, progestins, and Wnts may contribute to the etiology and/or symptomatology of Alzheimer's. Understanding the extent to which these potential interactions influence hippocampal functioning could

provide vital new insights into this disease. As such, Wnt-hormone interactions will be discussed in this section.

The hippocampus is exquisitely sensitive to sex-steroid hormones, such as 17 β -estradiol (E₂) and progesterone (P₄). Both E₂ and P₄ facilitate hippocampal dendritic spinogenesis, synaptic transmission, synaptic plasticity, and neurogenesis (Foy and others 2010; Kato and others 2013). Moreover, these hormones regulate the same types of hippocampal memory modulated by Wnt signaling (Luine 2014; Tuscher and others 2014). Because numerous reviews have discussed the molecular mechanisms through which E₂ and P₄ mediate hippocampal memory formation (Brinton and others 2008; Fortress and Frick 2014; Frick 2009; Sellers and others 2014; Singh and others 2013; Tuscher and others 2014), this information will be reviewed only briefly here. Both E₂ and P₄ are synthesized from a cholesterol precursor in the gonads and de novo in brain areas including the hippocampus (Cui and others 2013; Kato and others 2013). E₂ binds to intracellular estrogen receptors (ERs) ER α and ER β located in the cytoplasm, or to membrane-associated receptors such as GPER or Gq-mER. P₄ binds to the intracellular receptors PR-A and PR-B, and to the membrane-associated receptors PGRMC1/2 and mPR α - ϵ . Both E₂ and P₄ can induce either a classical genomic response, where receptor homo- or heterodimers act as transcription factors at the hormone response element on DNA, or a non-classical response that triggers rapid changes in cell-signaling mechanisms. For example, both E₂ and P₄ induce rapid changes in dorsal hippocampal ERK and mTOR signaling that are necessary for each hormone to enhance object recognition memory consolidation in female mice (Fig. 4) (Fernandez and others 2008; Fortress and others 2013b; Orr and others 2012). Furthermore, evidence from our laboratory suggests that E₂ and P₄ each rapidly regulate epigenetic processes, such as histone acetylation (Fig. 4), in the dorsal hippocampus to facilitate memory formation (Fortress and others 2014b; Heisler and others 2012; Zhao and others 2010; Zhao and others 2012).

Estradiol/Wnt Interactions

Although no study has specifically tested the importance of Wnt-hormone interactions for hippocampal memory, the ability of E₂ to regulate Wnt signaling in the hippocampus and cortex has been well established (Scott and Brann 2013). For example, E₂ induces a long-term reduction in GSK3 β activity and tau phosphorylation in primary hippocampal neurons (Cardona-Gomez and others 2004). In primary cortical neurons, E₂ reduces GSK3 β activity, increases the amount and stabilization of β -catenin, and increases TCF/LEF-induced gene transcription (Varea and others 2009). This gene transcription is blocked by an estrogen receptor antagonist and increased by estrogen receptor agonists (Varea and others 2009), suggesting that estrogen receptor activation influences Wnt/ β -catenin-induced gene transcription. Indeed, in primary hippocampal cultures, β -catenin complexes with ER α and GSK3 β , and E₂ causes β -catenin to dissociate from this complex (Cardona-Gomez and others 2004), thereby allowing it to interact with other molecules. Moreover, reproductively senescent aged female rats exhibit higher hippocampal levels of Dkk-1 and β -catenin phosphorylation than young females, as well as a reduced ability of E₂ to decrease Dkk-1 levels (Scott and others 2013), suggesting that long-term E₂ deprivation may promote basal Wnt/ β -catenin dysfunction in aging females.

Consistent with an increased risk of Alzheimer's in postmenopausal females, E₂ reduces degeneration associated with A β and tau hyperphosphorylation. In vitro, pretreatment of PC12 cells with E₂ prevents an A β -induced activation of GSK3 β and decrease in phosphorylation of the transcription factor CREB (Chen and others 2013), suggesting that regulation of GSK3 β may be a key mechanism through which E₂ protects against A β neurotoxicity. Similarly, E₂ in PC12 and N2a cells rapidly phosphorylates GSK3 β in an Akt-dependent manner, leading to a reduction in tau phosphorylation (Chen and others 2013; Shi and others 2008). In primary hippocampal neurons, the E₂-induced phosphorylation of GSK3 β decreases its intrinsic kinase activity, increases β -catenin stabilization, and increases the expression of Wnt7a and Wnt5a (Quintanilla and others 2005). The increased expression of Wnt7a and Wnt5a suggests that E₂ may drive a positive Wnt feedback loop in which repression of GSK3 β increases expression of Wnt ligands, thereby potentiating additional Wnt signaling. Such positive feedback could facilitate hippocampal function and protect hippocampal neurons from A β - and tau-induced neurodegeneration.

E₂ is also neuroprotective in rodent models of ischemia. The negative consequences of ischemic injury, such as size of infarct, memory impairments, and hippocampal cell death, are more pronounced when E₂ levels are low, such as after menopause (Koellhoffer and McCullough 2013; Zuo and others 2013). In ovariectomized rats, E₂ protects hippocampal CA1 neurons from degeneration after global cerebral ischemia by suppressing Dkk-1 expression and tau hyperphosphorylation, and increasing β -catenin and Wnt3 levels (Scott and others 2013; Zhang and others 2008). Interestingly, the ability of E₂ to promote Wnt/ β -catenin signaling and protect against ischemic injury is lost 10 weeks after ovariectomy (Scott and others 2013), suggesting that long-term estrogen deprivation diminishes the neuroprotective effects of E₂. Similar detrimental effects of long-term estrogen deprivation have been reported in studies showing that E₂ loses its ability to enhance hippocampal LTP and memory formation in rats ovariectomized for several months prior to treatment (Daniel 2006; Smith and others 2010; Vedder and others 2014). These findings may help explain why postmenopausal women, who are estrogen deprived, are particularly susceptible to Alzheimer's disease.

Progesterone/Wnt Interactions

Although most studies have focused on the role of E₂ in regulating Wnt signaling, new evidence indicates that P₄ robustly activates canonical Wnt/ β -catenin signaling in the dorsal hippocampus. As described above, P₄ requires ERK and mTOR signaling to facilitate object recognition memory consolidation in ovariectomized female mice (Orr and others 2012). Our laboratory recently reported that the P₄-induced activation of ERK and mTOR is mediated by membrane-associated progesterone receptors (PRs) in the dorsal hippocampus (Fortress and others 2014a). In contrast, intracellular PRs activated Wnt/ β -catenin signaling instead of ERK or mTOR signaling (Fortress and others 2014a) (Fig. 5). Specifically, infusion of the intracellular PR-A and PR-B agonist R5020 into the dorsal hippocampus increased protein levels of Wnt7a, β -catenin, and the β -catenin target gene *c-myc* in this brain region (Fig. 6A–C). The effects of R5020 were attenuated by the intracellular PR antagonist RU486 (Fig. 6A–C), supporting a role for activation of Wnt/ β -catenin signaling by PR-A and PR-B (Fortress and others 2014a). Given that R5020 also enhanced object

recognition memory consolidation, these data suggest that P₄ may facilitate memory by activating Wnt/ β -catenin signaling. Such an interaction is consistent with data from the uterus and mammary gland, where PR-A and PR-B require β -catenin-dependent signaling to promote the development of those organs (Boras-Granic and Hamel 2013; Robinson and others 2000; Satterfield and others 2008). Given that both P₄ and β -catenin-dependent signaling contribute to hippocampal memory consolidation (Fortress and others 2013a; Xu and others 2014), our data suggest that P₄-induced Wnt/ β -catenin signaling may modulate hippocampal memory formation.

Conclusions

As this review has illustrated, the multitude of ligands, receptors, and pathways that fall under the umbrella of “Wnt signaling” can have profound effects on the functioning of the hippocampus. Not only do Wnts direct hippocampal cytoarchitecture during embryogenesis, but they also regulate synapse formation and function in adulthood. As a result, hippocampal memory consolidation requires at least some forms of Wnt signaling. Wnts may be involved in the powerful modulatory effects of sex steroid hormones on hippocampal function, particularly in relation to age-related memory decline. The important role of Wnt signaling in protecting hippocampal neurons from A β - and ischemia-induced neurotoxicity, and regulating tau hyperphosphorylation, also positions Wnts as key players in neurodegenerative disease. Better understanding how the Wnt signaling pathways work and interact with other modulatory factors in the hippocampus should lead to important new insights into memory function and dysfunction in the coming decades.

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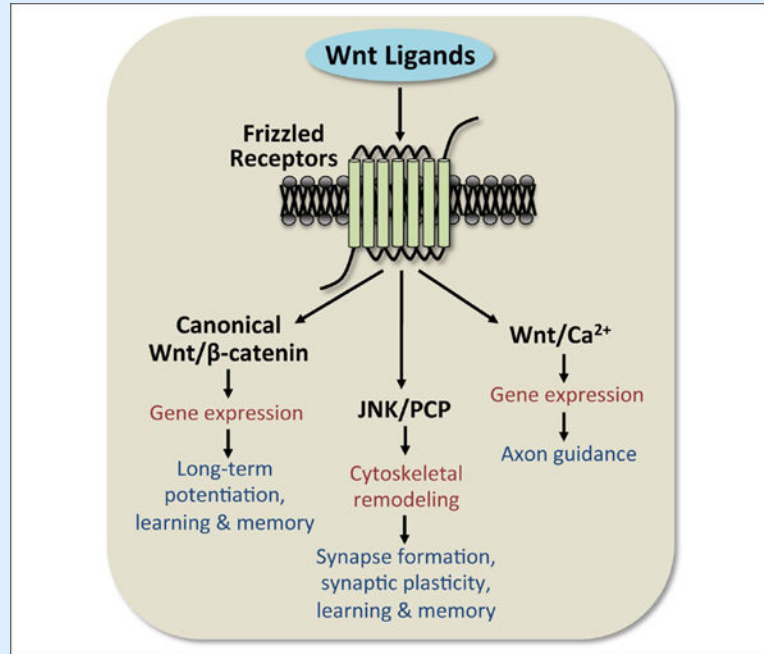
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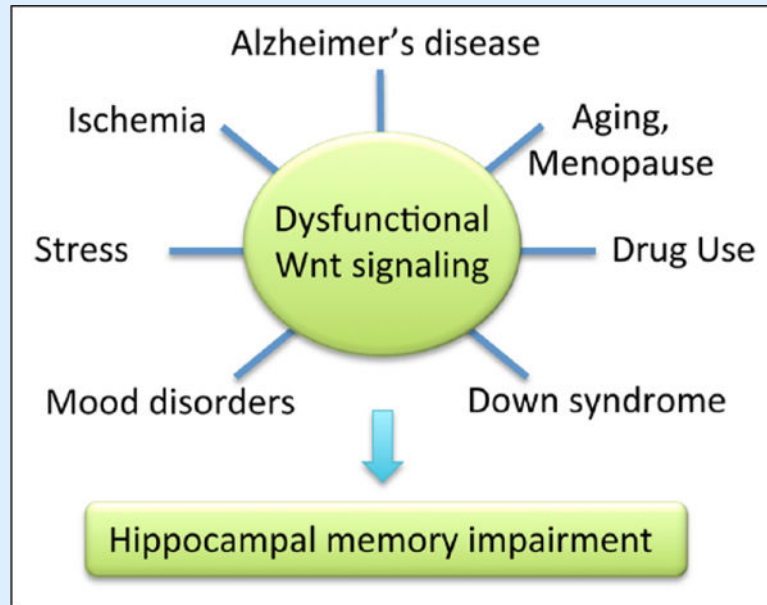
Box 1.

General overview of the three Wnt signaling pathways. A total of 19 Wnt ligands bind to 10 different Frizzled receptors. Activation of Wnt signaling occurs through one of three pathways: the canonical Wnt/ β -catenin-dependent pathway or the non-canonical JNK/PCP or Wnt/ Ca^{2+} pathways. Each Wnt pathway has general effects on cellular function (red), but also contributes in specific ways to regulating hippocampal structure and function (dark blue).



Box 2.

Dysfunctional Wnt signaling is associated with the etiology or symptomatology of multiple neurodegenerative and neuropsychiatric disorders, including Alzheimer's disease, ischemia, drug use, Down syndrome, and mood disorders (depression, bipolar disorder). Aberrant Wnt signaling is also observed in aging and menopause, and after chronic stress. Each of these conditions is associated with hippocampal memory impairments, which may be the result of perturbations in Wnt signaling.



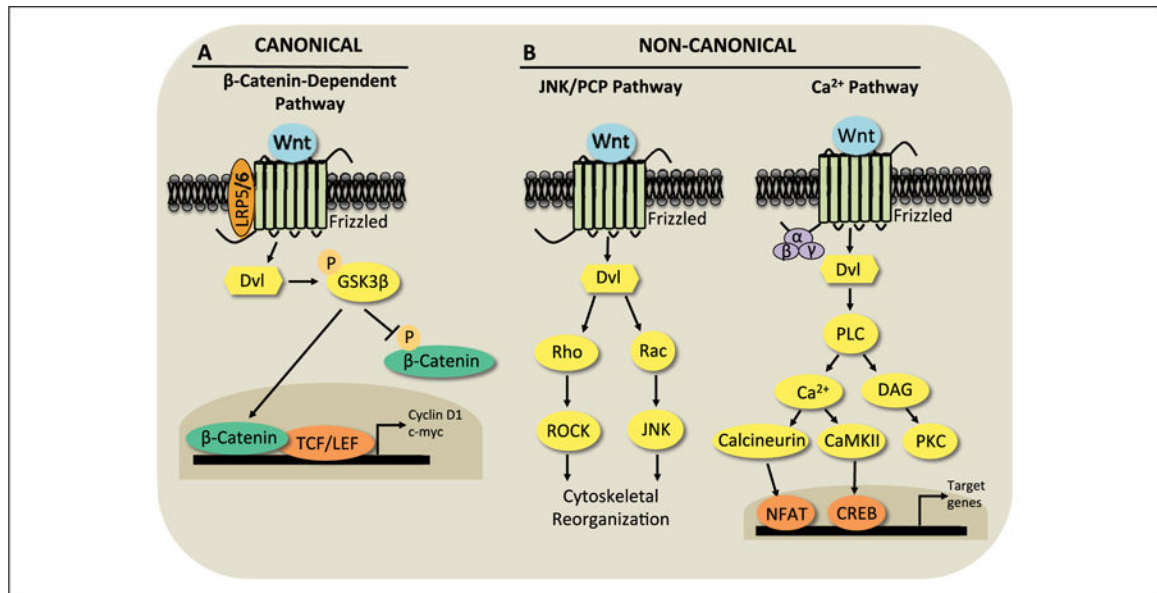


Figure 1.

Wnt signaling is mediated by canonical and non-canonical pathways. (A) Canonical Wnt/ β -catenin signaling is activated when a canonical Wnt ligand binds to a frizzled (Fzd) receptor and its co-receptor LRP 5/6. This leads Dishevelled (Dvl) to phosphorylate GSK3 β on Serine 9, which inactivates GSK3 β . This inactivation decreases phosphorylation of the transcriptional activator β -catenin and increases nuclear translocation of β -catenin, which then interacts with TCF/LEF transcriptional complexes to increase expression of downstream target genes such as Cyclin D1 and c-myc. (B) Wnt ligands binding to Fzd receptors can also activate the non-canonical JNK/planar cell polarity (PCP) or the Wnt/ Ca^{2+} pathways. In the JNK/PCP pathway, activation of Dvl signals to Rho and Rac GTPases to activate ROCK and JNK, respectively, to regulate cytoskeletal reorganization. In the Wnt/ Ca^{2+} pathway, G-protein mediated activation of phospholipase C increases intracellular Ca^{2+} and diacylglycerol (DAG). The rise in intracellular Ca^{2+} activates CaMKII and calcineurin to modulate transcriptional activity through regulation of CREB and NFAT, respectively. DAG activates the enzyme protein kinase C (PKC).

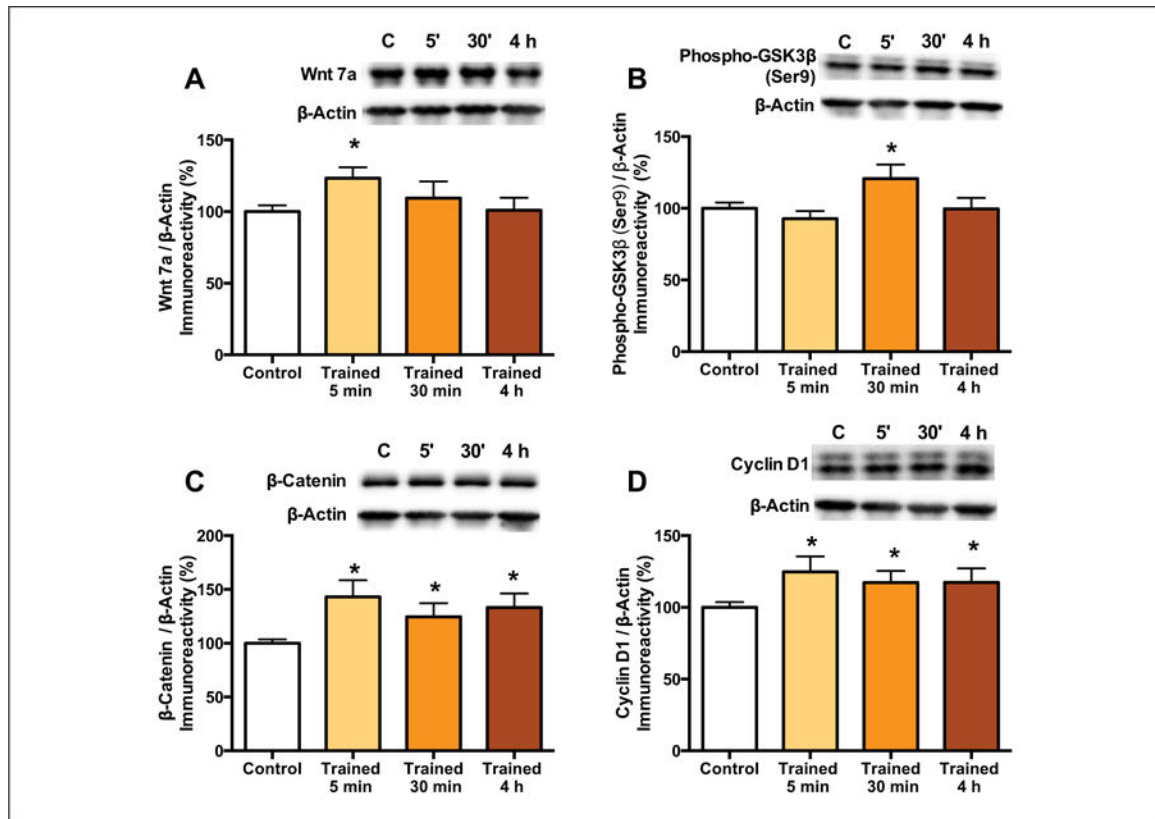
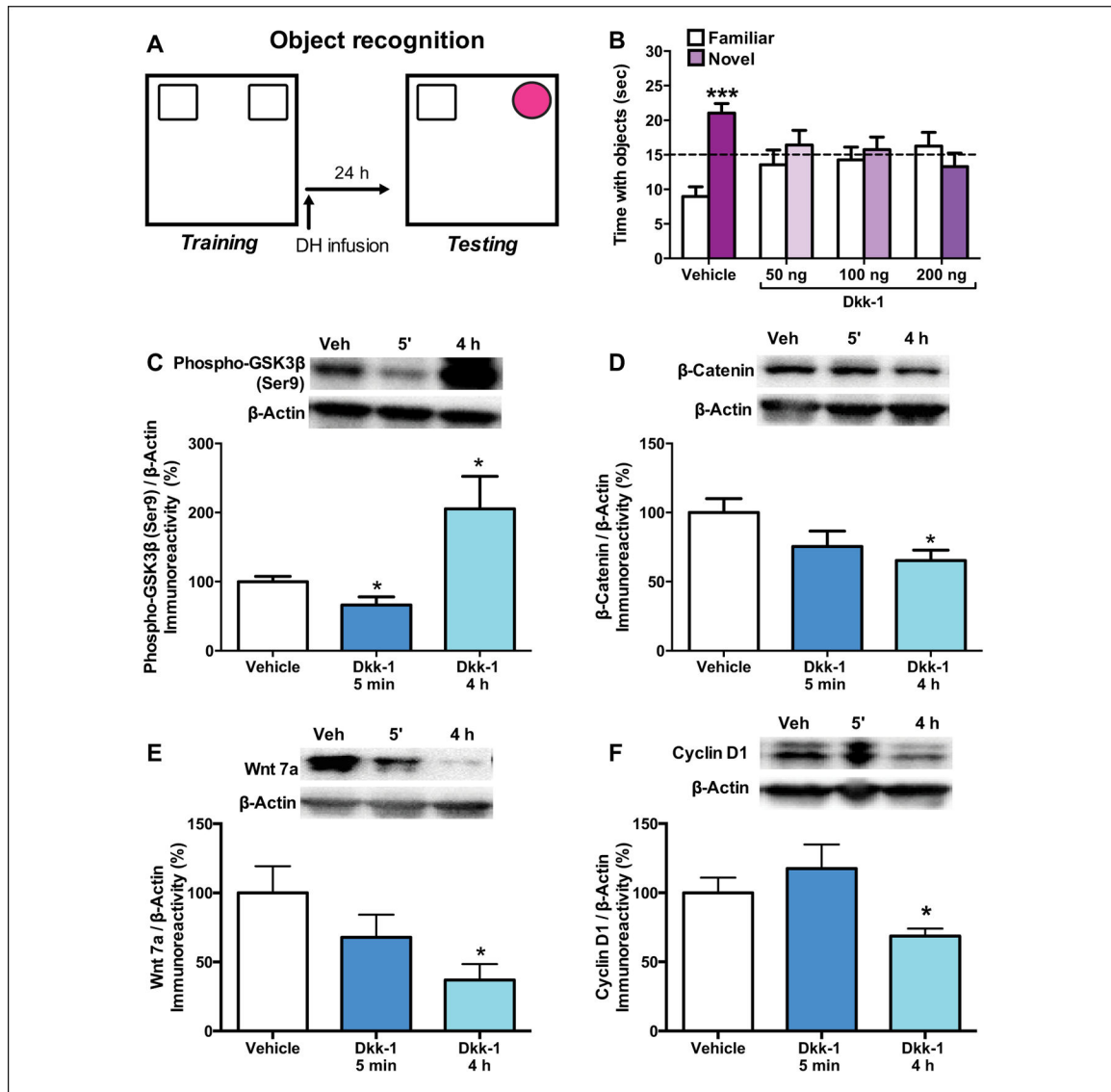


Figure 2.

Object training activates canonical Wnt/ β -catenin-dependent signaling in the dorsal hippocampus of male mice. (A) Protein levels of Wnt7a were significantly increased relative to controls 5 minutes after training. (B) Phospho-GSK3 β protein levels were significantly increased relative to controls 30 minutes after training. (C, D) At all time points following training, protein levels of β -catenin (C) and Cyclin D1 (D) were significantly increased relative to controls. Protein levels were normalized to β -actin. Each bar represents the mean \pm SEM percent change from vehicle ($*P < 0.05$ relative to controls). Insets show representative Western blots. Adapted with permission from Fortress and others (2013a).

**Figure 3.**

Inhibition of canonical Wnt/ β -catenin-dependent signaling in the dorsal hippocampus prevents object recognition memory consolidation and downstream cell signaling. (A) Behavioral paradigm used to test object recognition. Immediately after accumulating 30 seconds exploring two identical objects (training), mice received bilateral dorsal hippocampal infusions of vehicle or one of three doses of the canonical Wnt/ β -catenin inhibitor Dkk-1. Twenty-four hours later, a time at which vehicle mice remember the familiar object, memory for the familiar object was tested by allowing mice to accumulate 30 seconds of time with a familiar and novel object. Because mice are drawn to novelty, they will explore the novel object more than chance (15 seconds) if they remember the familiar training objects. (B) Twenty-four hours after training, mice infused with vehicle, but not any dose of Dkk-1 (50 ng, 100 ng, 200 ng/hemisphere), spent significantly more time than chance (dashed line at 15 seconds) with the novel object (** $P < 0.01$ compared to chance). These data indicate that inhibition of Wnt signaling impaired memory consolidation.

Bilateral dorsal hippocampal infusion of 50 ng Dkk-1 significantly decreased dorsal hippocampal Wnt7a (C) 5 minutes after infusion and increased GSK3 β (D) protein levels 4 hours after infusion (* P 0.05 relative to controls). Similarly, bilateral dorsal hippocampal infusion of 50 ng of Dkk-1 significantly decreased β -Catenin (E) protein levels in the dorsal hippocampus 4 hours after infusion. Protein levels were normalized to β -actin. Each bar represents the mean \pm SEM percent change from vehicle (* P 0.05 relative to vehicle-infused mice). Insets show representative Western blots. Adapted with permission from Fortress and others (2013a).

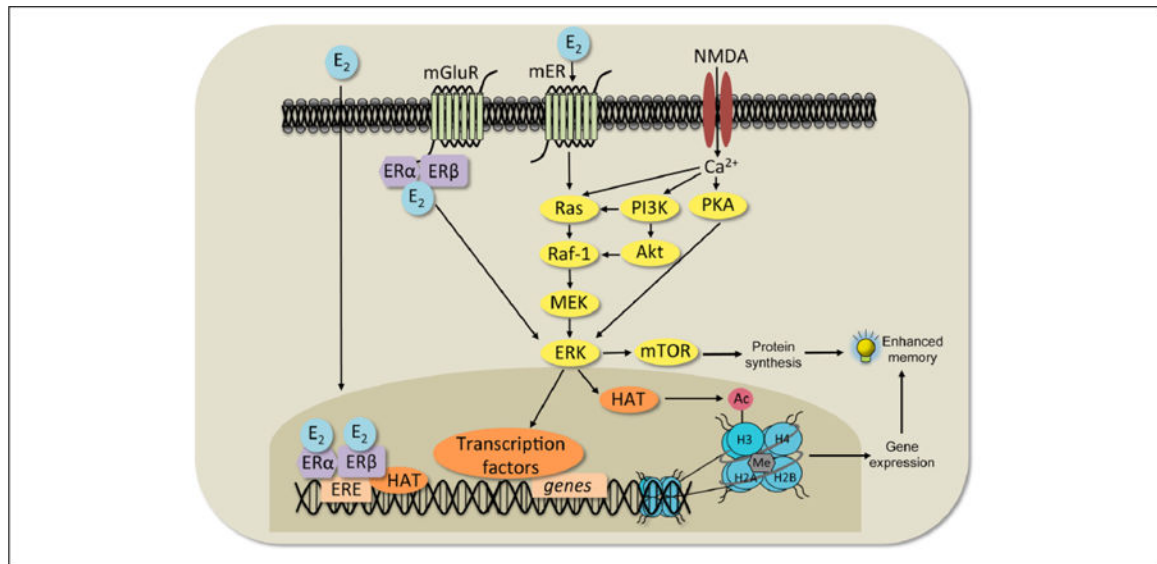


Figure 4.

Classical and non-classical 17β-estradiol (E₂) signaling mechanisms. In the classical response (left), E₂ binds ERα and ERβ, which then translocate into the nucleus, bind to the estrogen response element (ERE) on DNA, and interact with co-regulatory proteins (including histone acetyltransferases, HAT) to influence transcription. In a non-classical response (center), E₂ may affect cell signaling in several ways. It can bind to ERs that interact with metabotropic glutamate receptors (mGluRs) at the membrane and activate extracellular regulated kinase (ERK) signaling. E₂ can also interact with NMDA receptors and membrane-bound ERs (mER) to activate the protein kinase A (PKA), phosphoinositol-3-kinase (PI3K), and mammalian target of rapamycin (mTOR) signaling pathways. mTOR signaling regulates the protein synthesis necessary for memory formation. Activation of ERK increases histone H3 acetylation (Ac). Both H3 acetylation and DNA methylation (Me) are necessary for E₂ to enhance memory consolidation. Adapted with permission from Fortress and Frick (2014).

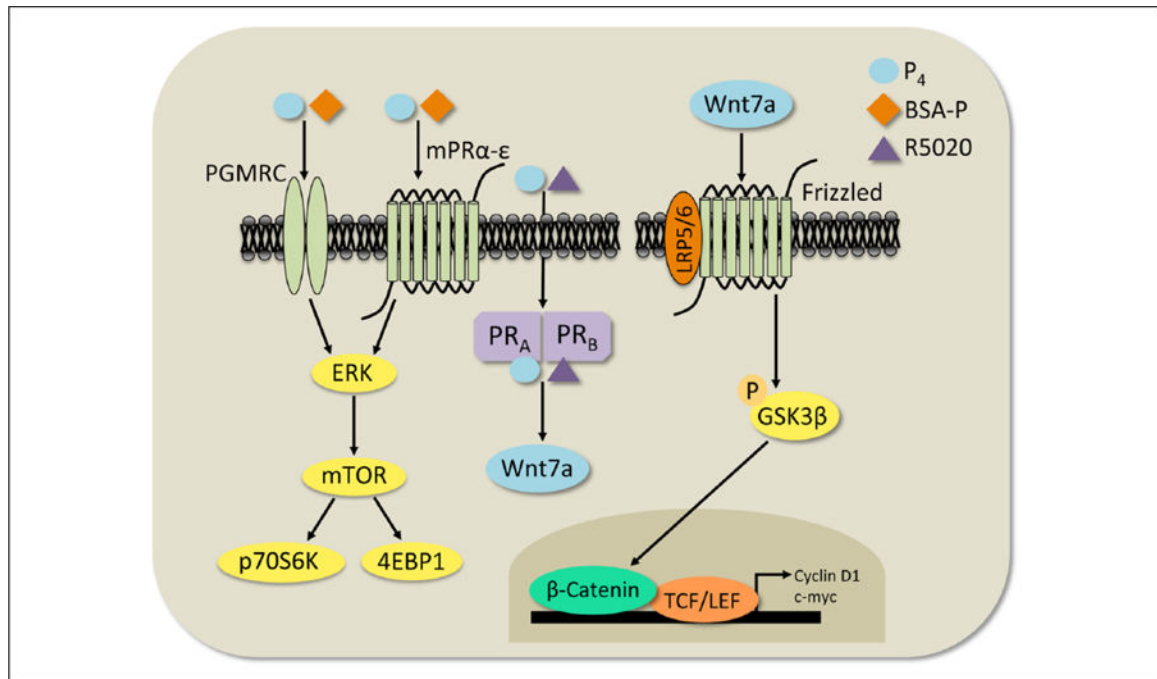
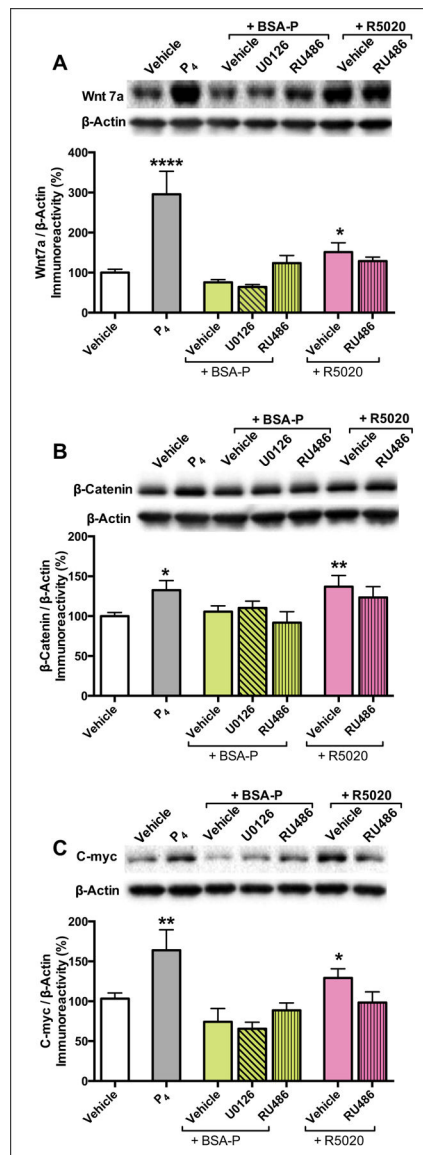


Figure 5.

Diagram of hypothesized progesterone (P_4)-mediated cell signaling mechanisms in the dorsal hippocampus. Our data suggest that P_4 activates membrane or intracellular receptors to activate two different signaling cascades. P_4 , or the membrane-associated progesterone receptor (PR) agonist BSA-P, binds to PRs such as the PGMRCs or the $mPR\alpha-\epsilon$ to trigger signaling through the ERK-dependent mTOR pathway (left). Alternatively, P_4 , or the intracellular agonist R5020, can bind to PR-A and PR-B to increase Wnt7a protein levels (center). The rapid rise in Wnt7a protein levels may then activate downstream canonical Wnt/ β -catenin-dependent signaling (right).

**Figure 6.**

Progesterone (P₄)-mediated activation of canonical Wnt/β-catenin-dependent signaling. Bilateral dorsal hippocampal infusion of P₄ or the intracellular PR agonist R5020 significantly increased Wnt7a (A) and β-catenin (B) protein levels 5 minutes after infusion. Protein levels of Wnt7a and β-catenin were not affected by the membrane-associated PR agonist BSA-P, suggesting a role of intracellular PRs in activating Wnt signaling. Bilateral dorsal hippocampal infusion of P₄ also significantly increased c-myc (C) protein levels 5 minutes after infusion. Protein levels were normalized to β-actin. Each bar represents the mean ± SEM percent change from vehicle (**P* 0.05 relative to vehicle-infused mice). Insets show representative Western blots. Adapted with permission from Fortress and others (2014a).