



Published in final edited form as:

Front Neuroendocrinol. 2008 May ; 29(2): 313–339. doi:10.1016/j.yfrne.2008.02.001.

Progesterone Receptors: Form and Function in Brain

Roberta Diaz Brinton^{1,2,*}, Richard F. Thompson^{2,3,4}, Michael R. Foy⁶, Michel Baudry^{2,4}, JunMing Wang¹, Caleb E Finch^{2,4,5}, Todd E. Morgan⁵, Frank Z. Stanczyk⁶, Christian J. Pike^{2,5}, and Jon Nilsen¹

¹Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, Los Angeles, California, 90033 and, Loyola Marymount College, Los Angeles, CA, 90045-8405

²Neuroscience Program, University of Southern California, Los Angeles, California, 90033 and, Loyola Marymount College, Los Angeles, CA, 90045-8405

³Psychology Department, University of Southern California, Los Angeles, California, 90033 and, Loyola Marymount College, Los Angeles, CA, 90045-8405

⁴Department of Biological Sciences, University of Southern California, Los Angeles, California, 90033 and, Loyola Marymount College, Los Angeles, CA, 90045-8405

⁵Davis School of Gerontology, University of Southern California, Los Angeles, California, 90033 and, Loyola Marymount College, Los Angeles, CA, 90045-8405

⁶Department of Obstetrics/Gynecology, University of Southern California, Los Angeles, California, 90033 and, Loyola Marymount College, Los Angeles, CA, 90045-8405

Abstract

Emerging data indicate that progesterone has multiple non-reproductive functions in the central nervous system to regulate cognition, mood, inflammation, mitochondrial function, neurogenesis and regeneration, myelination and recovery from traumatic brain injury. Progesterone-regulated neural responses are mediated by an array of progesterone receptors (PR) that include the classic nuclear PRA and PRB receptors and splice variants of each, the seven transmembrane domain 7TMRPβ and the membrane-associated 25-Dx PR (PGRMC1). These PRs induce classic regulation of gene expression while also transducing signaling cascades that originate at the cell membrane and ultimately activate transcription factors. Remarkably, PRs are broadly expressed throughout the brain and can be detected in every neural cell type. The distribution of PRs beyond hypothalamic borders, suggests a much broader role of progesterone in regulating neural function. Despite the large body of evidence regarding progesterone regulation of reproductive behaviors and estrogen-inducible responses as well as effects of progesterone metabolite neurosteroids, much remains to be discovered regarding the functional outcomes resulting from activation of the complex array of PRs in brain by gonadally and / or glial derived progesterone. Moreover, the impact of clinically used progestogens and developing selective PR modulators for targeted outcomes in brain is a critical avenue of investigation as the non-reproductive functions of PRs have far-reaching implications for hormone therapy to maintain neurological health and function throughout menopausal aging.

*Address correspondence to: Roberta Diaz Brinton, Ph.D., Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, School of Pharmacy, 1985 Zonal Avenue, Los Angeles, California, 90089, Tel. 323-442-1428; Fax. 323-442-1489; E-Mail: rbrinton@usc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Progesterone; PR-A; PR-B; 7TMPR; 25-Dx PR; PGRMC1; neurogenesis; inflammation; Alzheimer's disease; hormone therapy

1. Introduction

It has become increasingly evident that the functions of gonadal steroid hormones, such as progesterone (P_4), extend well beyond reproduction. Multiple regions within the central nervous system (CNS) beyond the hypothalamus are targeted by P_4 , including the hippocampus and cortex. In recent years, both of these extrahypothalamic sites have garnered increasing interest from endocrinologists based on accumulating evidence that P_4 has potent and direct neuroprotective and neuroregenerative effects in these brain regions while also regulating estrogen action [1;2;3;4;5;6;7;8;9;10;11;12;13;14;15;16;17;18;19]. The non-reproductive neural effects of P_4 have substantial clinical significance, as progestogens are administered in conjunction with estrogens in hormone therapy to counter the proliferative effect of estrogen on the uterine epithelium. Estrogen, 17β -estradiol (E_2), acts in concert with progesterone to regulate multiple non-reproductive brain functions, such as cognition and neuroprotection [10;20;21;22;23;24;25;26;27;28;29]. Perhaps the best-known neural effect of estrogen is its ability to protect neurons against a wide variety of insults including glutamate excitotoxicity, amyloid beta ($A\beta$), and oxidative stress [10;11;20;21;22;23;24;26;27;28;30;31;32;33;34;35]. On the other hand, the neuroprotective role of P_4 [20;21;22;23;24;26;27;36;37;38;39] is just emerging. From a reproductive gonadal hormone perspective, progesterone always acts in concert with E_2 . However, this is not the case for glial derived progesterone [16;40] or for current and future therapeutic uses of progesterone [16;40;41;42]. Here, we discuss the non-reproductive neural functions of P_4 as well as the possible mechanisms by which P_4 achieves these effects, including 'classical' progesterone receptor-mediated pathways and alternate 'non-genomic' mechanisms.

2. Progesterone Receptors from Membrane to Nucleus

The classical nuclear progesterone receptor (cPR) was first characterized in the 1970s and since this time, has been localized to many regions of the CNS, including the hippocampus, cortex, hypothalamus, and cerebellum [43;44;45;46;47;48;49;50;51;52]. Like most steroids, P_4 exerts its effects by binding and activating specific cellular receptors. According to the common theory of steroid action, P_4 effects are mediated by binding to its cognate receptors (PR), classically defined as ligand-activated transcription factors. In the absence of hormone, PRs are complexed with several chaperone molecules, including heat shock protein (Hsp) 90, hsp70, hsp40. The interaction of PRs with the chaperones is a prerequisite for hormone binding [53;54]. The chaperones also serve to link the PR with protein trafficking systems. Upon P_4 binding, PR undergoes conformational changes, dissociates from the chaperone proteins, dimerizes, and directly interacts with specific response elements (PREs) in the promoters of target genes [55;56;57;58]. When bound to PREs, PRs interact with components of the basal transcription machinery by binding to steroid receptor co-activators. These co-activators bind to PR via a conserved LXXLL amphipathic helix or nuclear-receptor box motifs, which make initial contacts with several helices in the AF-2 (activation function) region of the PR ligand-binding domain [59;60]. Classical PREs are not required for P_4 -induced gene upregulation, as P_4 has been shown to increase expression of genes lacking these elements [61;62;63]. These non-classical responses may occur through alternative genomic mechanisms, such as PR tethering to the SP1 transcription factor [63] or non-genomic mechanisms, such as activation of second messenger signaling cascades [10;11;64].

3. Progesterone Receptors in the Brain: The Case for Multiple Isoforms

Two major isoforms of cPR are known to exist, the full-length B isoform (PRB) and the N-terminal-truncated A isoform (PRA) [65]. Both isoforms are encoded by a single gene (with 8 exons), with translation being initiated from separate start codons (Fig. 1). Like other steroid nuclear receptors, cPR is composed of a variable N-terminal region (encoded by exon 1), a conserved DNA-binding domain (encoded by exons 2 and 3), a variable hinge region (encoded by part of exon 4), and a conserved ligand-binding domain (encoded by exons 4 – 8). The N-terminal 164-amino acids of PRB, known as B-upstream segment or BUS (absent in PRA), constitute an additional activation function (AF-3), which is made up of two LXXLL motifs and a conserved tryptophan residue. Although the role of cPRs in reproductive function has been extensively studied, the receptors that mediate the neuroprotective and neurotrophic effects of P₄ have yet to be identified.

Splice variants other than the classical PRA and PRB variants have been identified [66;67]. These include variants with insertions of ‘intronic’ exons and exon-skipped variants (Fig. 2). The intronic exons T [67] and S can be inserted between exons 3 and 4, while exons i45a and i45b can be inserted between exons 4 and 5 (Fig. 2A). Exon-skipped variants include PR-c, PR-s, and PR-t. These variants are generated through omission of exon 1 (PR-c) or exons 1 – 3 (PR-s and PR-t). Both PR-s and PR-t also retain 5' untranslated exons (Fig. 2B). Other exon-skipped mRNA variants include del 2, del 4, del 6, del 5+6, del 4+6, del 4+5+6, del 3+4, and del 3+4+5+6. Interestingly, some of these variants have a defective DNA-binding domain and also lack the nuclear localization signal (NLS). PR splice variants lacking NLS, which would be expected to result in cytoplasmic localization, and contain the intact proline rich (PXXP) domain, would be expected to displace the intramolecular occupation of SH3 in Src, enabling them to activate Src and mitogen-activated protein kinases (MAPK) in the cytosol. PRs lacking a functional DNA-binding domain and a NLS could serve as a membrane-associated PR.

Although cPR is expressed in the hippocampus and frontal cortex [43;44;45;46;47], P₄ effects have been reported in the CNS of PR knockout mice, indicating that receptors other than cPR may mediate P₄ signaling in the brain [68]. A novel P₄-binding protein that is distinct from the cPR has been identified as a membrane protein, known as 7TMPR for its seven transmembrane domains [69;70], has characteristics of a G protein-coupled receptor. When bound to progestin, this receptor blocks the activity of adenylyl cyclase, the enzyme that catalyzes production of the intracellular second-messenger cAMP [70]. Three isoforms of 7TMPR have been identified— 7TMPR α , β , and γ . The open reading frames (ORFs) of 7TMPR α from human, pig, and mouse are 1038 – 1053 nucleotides in length and encode peptides 346 – 350 amino acids in length. These peptides are similar in size to 7TMPR of spotted seatrout (352 amino acids) and zebrafish (354 amino acids). The ORFs of 7TMPR β from mammals, zebrafish, and *Xenopus laevis* are 1064/1065 nucleotides in length and encode 352–354-amino acid peptides. The 7TMPR γ are slightly shorter, at approximately 330 amino acids.

An additional putative membrane-bound PR has been cloned in multiple different species. The initial isolation and cloning of this protein from pig and rat revealed the presence of a single transmembrane domain. Humans contain two orthologous genes, hpr6.6 (chromosome X, 195 amino acids) and Dg6 (chromosome 4, 223 amino acids). The rat homologue is a 25 kDa, 223-amino acid protein (25-Dx) possessing a hydrophobic domain of 14 residues and a proline-rich domain in the N-terminal region. Independent isolation of rat adrenocortical innerzone-specific antigen (IZAg) by affinity chromatography revealed this protein to be identical to 25-Dx. Overexpression of 25-Dx in CHO cells has been shown to increase P₄ binding to the microsomal fraction [71]. This 25-Dx-enriched microsomal fraction has moderate affinity for testosterone and weak affinity for corticosterone and cortisol. In contrast, it does not bind to either estradiol (E₂) or aldosterone. In human sperm, blockade of 25-Dx with a specific

antibody inhibits P₄-induced increases in intracellular Ca²⁺, providing further support that this protein serves as a membrane PR [71].

4. Localization of Progesterone Receptors in the Brain

Progesterone receptors are broadly expressed throughout the brain, with no apparent restriction to specific cell types. Nevertheless, PR expression may vary depending on the brain region, cell type, or hormonal status (Fig. 3). Both of the classical PR isoforms (PRA and PRB) are expressed in the hippocampus and frontal cortex of the rat (Fig. 3). PR immunoreactivity is especially high within the bed nucleus of the stria terminalis (BST), in particular the medial division of the medial nucleus of the BST (the principal nucleus of the BST). Immunoreactivity is lower in the intermediate and lateral divisions of the medial nucleus of the BST. In the centromedial amygdala, PR expression is prominent in the posterodorsal part of the medial amygdaloid nucleus, but lower in surrounding areas. No sex differences have been observed in PR expression in the BST and centromedial amygdala [72]. In the brainstem, PR immunoreactivity is present in the norepinephrine neurons of the nucleus tractus solitarius, the region from which projections to the hypothalamic supraoptic nuclei arise. Guerra-Araiza *et al.* have used quantitative RT-PCR analysis to characterize sex differences in the regulation of PR isoform expression by sex steroid hormones in the rat cerebellum [44]. PR isoform expression in female rats was not altered by estrogen or P₄, while PRA was selectively induced by estrogens in the male cerebellum. Similarly, in the rat hippocampus and olfactory bulb, E₂ induces PRA isoform expression, whereas P₄ does not affect the expression of any PR isoforms [43;44]. In rodents, PR is present in the ventromedial hypothalamus [73]. Auger *et al.* have reported PR-immunoreactive cells within the preoptic area, the ventromedial and dorsomedial nucleus of the hypothalamus, and the arcuate nucleus of the female rat [72].

Dot blot analysis of 7TMPR expression in human tissue has shown that the α form (from a testicular library) is mainly localized to reproductive tissues such as the placenta, testis, and ovary. In contrast, the γ form (from a colon library) is present in the kidney, fetal kidney, colon, a lung carcinoma, and HeLa 53 cells [70]. The β form (from a brain library) is exclusively localized to neural tissues [70]. These include the cerebral cortex, cerebellum, caudate nucleus, thalamus, pituitary gland, and spinal cord, as shown by dot blot hybridization or tissue array with Northern hybridization.

5. Mechanisms of Progesterone Action

Progesterone produces multiple effects in the brain through three principle mechanisms: regulation of gene expression, modulation of neurotransmitter systems, and activation of signaling cascades. Classification of the determinant pathways and identification of the specific receptors mediating activation of each of these pathways would be expected to uncover new targets and enable development of improved therapeutic strategies. The effects of P₄ are historically thought to be mediated by PRA or PRB-induced gene transcription [55;65;74]. Available evidence suggests that PRA and PRB shuttle between the nucleus and the cytoplasm, with ligand binding inducing interactions between the receptor and nuclear co-activators [55; 74]. PRA and PRB differentially regulate gene transcription, increasing the complexity of this regulatory system [75;76]. For example, PRA is a less potent transactivator than PRB. Also, PRA exerts transrepressional activity on PR-B in a promoter- and cell type-dependent manner [75;76]. The P₄-inducible genetic network is further refined by the expression of PR splice variants with variable ligand affinities and transactivational activities [77].

The effects of P₄ may be attributed to mechanisms apart from the 'classical' gene transcription mediated by PRA and PRB. Recently, P₄ binding sites have been detected at the surface of hypothalamic and spinal neurons [68;78]. These binding sites, identified as 25-Dx (also known as PGRMC1), mediate the antiapoptotic actions of P₄ in granulosa and luteal cells [79;80]. In

the ovary, 25-Dx complexes with the plasminogen activator inhibitor RNA-binding protein 1 (25-Dx/SERBP1) to activate cGMP-dependent protein kinase [81]. P₄ signaling may also be mediated by several putative Src homology domains present in 25-Dx [82]. However, the P₄-activated signaling pathways that are mediated by 25-Dx have yet to be determined in neurons. Like 25-Dx, the seven transmembrane putative progesterone receptor 7TMPR may regulate P₄ signaling. The 7TMPR has been shown to activate a pertussis toxin-sensitive inhibitory G protein, resulting in activation of the MAPK pathway through inhibition of cAMP production [83;84].

Progesterone and its 5 α -reduced derivatives dihydroprogesterone (DHP) and tetrahydroprogesterone (THP or allopregnanolone), can promote Schwann cell proliferation and activation of the myelinating program of these cells [85]. Melcangi and colleagues demonstrated that P₄ and its derivatives increased expression of the transcription factors Sox-10 and Krox-20, both of which play a key role in Schwann cell physiology and in their myelinating program. Western blot analyses indicated that Krox-20 was increased after 3 h of treatment with P₄, dihydroprogesterone, or tetrahydroprogesterone, whereas P₄ or dihydroprogesterone stimulated expression of Sox-10 after 6 h of exposure. Analysis of rat and human promoters for these two transcription factors indicated that putative P₄-response elements are present in the Krox-20 gene but not in Sox-10. These findings suggest that P₄ and its neuroactive derivatives could coordinate the Schwann cell-myelinating program utilizing different intracellular pathways [85].

Although mPR binds P₄ with high selectivity and affinity, it does not bind many of the synthetic progestins including norethisterone, norgestrel, promesgestrone, and demegestone [83]. This differential binding may underlie the distinct differences in neuroprotection and MAPK activation elicited by P₄ and synthetic progestins [10;11]. However, the neuronal expression of mPR has not yet been determined. The various progestin-activated signaling pathways can be combined synergistically or antagonistically in an intriguing number of ways to regulate development, survival, and electrical activity in the CNS. Each step of this signaling network can be influenced in a cell type- or brain region-specific manner through alterations in receptor expression or ligand structure, opening up a wide array of therapeutic possibilities.

The MAPKs modulate cellular differentiation, proliferation, survival, and death. Activation of the MAPK, extracellular signal regulated kinase (ERK), is required for E₂-induced neuroprotection (Figure 4) [33]. We and others have demonstrated that both E₂ and P₄ activate the ERK signaling pathway [10;11;28;33]. However, nuclear activation of ERK is not induced by medroxyprogesterone acetate (MPA), a progestin that lacks neuroprotective effects [11]. Phosphorylation of the MAPK substrate, cAMP response element binding protein (CREB), is associated with increased resistance to ischemic injury [86;87], and CREB is activated in response to ovarian hormones [88;89;90;91;92;93]. CREB, in turn, can upregulate *bcl-2* expression [87;91]. Accordingly, E₂ and P₄ upregulate *bcl-2* in hippocampal neurons [10]. In contrast to P₄, MPA does not activate CREB, nor does it increase *Bcl-2* expression [10]. On the contrary, MPA blocked E₂-induced CREB activation and *Bcl-2* upregulation in primary hippocampal neurons.

Estrogen and P₄ simultaneously activate the MAPK/ERK pathway as well as an alternate pro-survival pathway, the Akt pathway [28]. Activation of Akt by E₂ and P₄ in cortical slice cultures is associated with increase neuronal survival [28]. However, use of mixed cell types in these slice cultures precludes differentiation between direct and indirect effects of the steroids on neural cells. In primary hippocampal neuron cultures, E₂ and P₄ directly activate Akt in neurons. Western blot analysis of whole-cell lysates revealed that E₂ (10 ng/mL) and P₄ (10 ng/mL), either alone or in combination, significantly increased Akt phosphorylation within 20

min of treatment. Treatment of primary hippocampal neurons with MPA did not alter Akt phosphorylation, but blocked E₂-induced Akt phosphorylation.

More recently, it has been recognized that these steroids also regulate metabolic functions sustaining the energetic demands of this neuronal activation [94;95;96;97;98;99;100;101]. Recent findings from Nilsen and colleagues indicate that P₄ significantly increased mitochondrial respiration 24 hrs following a single *in vivo* exposure at a magnitude comparable to E₂ [7]. Consistent with an increase in oxidative respiration, P₄ and E₂ significantly increased COXIV enzyme activity and expression of COXIV mRNA. Both P₄ and E₂ reduced free radical leak indicating greater efficiency of electron transport, which was evidence in a reduced generation of free radicals, P₄ and E₂ induced a significant reduction in mitochondrial lipid peroxidation. The reduction in lipid peroxidation suggests the activation of mechanisms beyond solely mitochondrial efficiency. P₄ induced a significant increase in MnSOD expression as did E₂ and E₂/P₄. In contrast, the expression of peroxiredoxin V was only increased by E₂ but not in the P₄- and P₄ blocked the E₂ induction of peroxiredoxin V. These results indicate that both P₄ and E₂ can promote dismutation of the superoxide anion O₂^{•-} by increasing MnSOD to form H₂O₂ whereas only E₂ induces peroxiredoxin V that promotes clearance of H₂O₂ and prevention of oxidative damage. Further, P₄ and E₂ directly regulate mitochondrial function and are not due to an increase in the number of mitochondria as neither P₄ nor E₂ nor their combination induced evidence for mitochondrial biogenesis. While P₄ was as efficacious as E₂, the combination of P₄ and E₂ led to reduced efficacy. On all outcome measures, the combination of P₄ and E₂ resulted in a substantial decrement in response magnitude.

6. Neuroprotective actions of progesterone and progestin in the CNS

P₄ has established neuroprotective actions that likely involve several different mechanisms. Anxiolytic effects are one way by which P₄ can reduce neural injury. Diverse stimuli including kainate [102], pilocarpine [103], and pentylenetetrazole [104] elicit stereotypic seizure behaviors and within several hours to a few days, significant neuronal loss in select brain regions such as the hippocampus. In these paradigms, P₄ treatment attenuates not only seizure behaviors [105, Rhodes, 2004 #2538; 106], but also neuronal injury [107]. The primary mechanism of neuroprotection in these models appears to involve the P₄ metabolite, allopregnanolone (AP α , also known as 5 α -pregnan-3 α -ol-20-one and 3 α ,5 α -tetrahydroprogesterone). P₄ is metabolized to AP α following the sequential action of 5 α -reductase and 3 α -hydroxysteroid dehydrogenase. AP α acts as potent modulator of gamma-aminobutyric acid subtype A (GABA_A) receptors, increasing chloride conductance evoked by GABA [1;108]. This serves to decrease excitatory signaling and thus, antagonize seizure activity. Support for an AP α -mediated mechanism of P₄ neuroprotection is provided by the finding that i) AP α is as effective as P₄ in seizure paradigms [107;109;110;111;112;113] and ii) molecular [114;115] or pharmacological [109;113;116;117] inhibition of P₄ metabolism to AP α blocks the protective actions of P₄. Interestingly, AP α has also been implicated in the neuroprotective effects of P₄ following oxygen-glucose deprivation of rat Purkinje cells [118] and traumatic injury [23;119;120;121].

P₄ likely triggers multiple neuroprotective mechanisms. For example, in neuronal cultures, P₄ activates MAPK/ERK [10;11;28] and Akt [28] signaling pathways, both of which are associated with neuroprotection [28;33;122]. Recent evidence suggests that, in spinal cord injury models, P₄ neuroprotection is associated with upregulation of brain-derived neurotrophic factor (BDNF) [98;99;100] increased levels and activity of choline acetyltransferase [100], and a reduction in mitochondrial dysfunction [101]. In cerebral ischemia models, the protective effects of P₄ are attributed, in part, to suppression of inflammatory responses and nitric oxide synthase-2 expression [123]. In addition to its direct

effects on neurons, P₄ may exert indirect neuroprotective effects by acting on non-neuronal target cell populations. For example, P₄ reduces blood brain barrier leakage [14], decreases glial activation [124], and increases myelination [125;126].

P₄ and E₂ are known to modulate the activity of one another, sometimes antagonistically. Thus, it is of interest to determine the effect of P₄ and progestogens on the neuroprotective effects of E₂. The antagonistic relationship between P₄ and E₂ is illustrated by the finding that P₄ can block E₂-induced increases in spine density in the hippocampus [18;127]. P₄ can also attenuate E₂-induced upregulation of BDNF, neurotrophin 3, and nerve growth factor in the entorhinal cortex, but not in hippocampus, of female rats [128]. P₄ reverses estrogen-induced enhancement of spatial memory in ovariectomized female rodents (Bimonte-Nelson et al., 2006). Recent results from our group demonstrate that both P₄ and MPA block the neuroprotective effect of E₂ in the hippocampus following kainate lesion in young female rodents [129] and reproductively senescent female rodents (Carroll and Pike, unpublished observations). P₄ treatment decreases the estrogen receptor hybridization signal in monkey brain [130], indicating that P₄ may limit E₂ signaling. However, in both a systemic kainate lesion model [131] and an ischemic stroke model [132], acute P₄ treatment was not neuroprotective and did not significantly affect E₂ neuroprotection.

The neuroprotective action of P₄ in traumatic brain injury has been extensively studied by Stein and colleagues. Results of their extensive body of work indicate that a single injection of P₄ attenuated cerebral edema when administered during the first 24 h after traumatic brain injury (TBI) in rats whereas 5 days of P₄ injection resulted in improved spatial learning performance and reduced sensory neglect [133]. In subsequent analyses, Grossman et al., [124] found that P₄ reduced edema levels, as in previous studies, while increasing the accumulation of activated microglia in traumatic brain injured rat brain [6]. However, a parallel analysis indicated that P₄ and allopregnanolone reduced both IL-1beta and TNF-alpha 3 h post-traumatic brain injury, when the expression of these cytokines peaked in the untreated animals [6]. Progesterone-induced reduction in inflammatory cytokines was also observed in a medial frontal cortex model of traumatic brain injury [12]. Progesterone inhibited the injury-induced rise in complement factor C3, GFAP, and nuclear factor kappa beta (NFkappaB) [12]. The paradox of the dual effect of P₄ to induce accumulation of activated microglia and reduce inflammatory immune cytokines remains unresolved.

The contributions of gender and gonadal hormones in the cascade of events following brain injury were investigated and revealed that normally cycling females exhibited significantly less edema than males following traumatic brain injury and that pseudopregnant females were virtually spared from post-injury edema. Subsequent studies of ovariectomized females, with or without hormone treatment, indicated that the reduction of cerebral edema was associated primarily with the presence of circulating progesterone [134]. The Stein group then went onto to determine whether P₄ metabolite neurosteroids mediated the neuroprotection of exogenous or endogenous P₄. One day after traumatic brain injury, both P₄-treated (16 mg/kg) and allopregnanolone (8 or 16 mg/kg)-treated rats showed less caspase-3 activity, and rats treated with allopregnanolone (16 mg/kg) showed less DNA fragmentation in the lesion area, indicating reduced apoptosis. Nineteen days after the injury, rats treated with P₄ or allopregnanolone (8 or 16 mg/kg) showed no difference in necrotic cavity size but had less cell loss in the medio-dorsal nucleus of the thalamus and less learning and memory impairments compared with the injured vehicle-treated rats. The results from their analyses indicated that P₄ and allopregnanolone had similar neuroprotective efficacy after traumatic brain injury, but that allopregnanolone appeared to be more potent than P₄ in promoting CNS repair [120]. A follow-up study compared the effects of P₄ and its metabolite, allopregnanolone, on the early injury cascade (apoptosis) and long-term functional deficits after traumatic brain injury [119]. Progesterone (16 mg/kg) or allopregnanolone (4, 8, or 16 mg/kg) were injected at 1 h,

6 h, and then for 5 consecutive days after bilateral contusions of the frontal cortex in adult male rats. Within one day after injury, P₄ and allopregnanolone reduced expression of pro-apoptotic proteins caspase-3 and Bax, and apoptotic DNA fragmentation. Progesterone and allopregnanolone also reduced the size of glial fibrillary acid protein (GFAP)-positive astrocytes at the lesion site 24 h after injury. At 19 days post-injury, rats given P₄ or allopregnanolone (8 mg/kg) showed improved performance in a spatial learning task compared to injured rats given only the vehicle [119].

The extensive body of basic science *in vivo* evidence indicating that P₄ was highly efficacious in reducing or preventing the neurological consequences of traumatic brain injury [42], led Stein and colleagues to conduct a clinical trial of P₄ in human victims of moderate to severe coma associated traumatic brain injury [19]. In a phase II, randomized, double-blind, placebo-controlled trial conducted at an urban Level I trauma center with 100 adult trauma patients who arrived within 11 hours of injury with a postresuscitation Glasgow Coma Scale score of 4 to 12 (scores associated with moderate to severe degrees of coma) were enrolled with proxy consent. Neurologic outcome was assessed 30 days postinjury. Seventy-seven patients received progesterone; 23 received placebo. No serious adverse events were attributed to progesterone. Adverse and serious adverse event rates were similar in both groups, except that patients randomized to P₄ had a lower 30-day mortality rate than controls. Thirty days postinjury, the majority of severe traumatic brain injury survivors in both groups had relatively poor Glasgow Outcome Scale-Extended and Disability Rating Scale scores. Moderate traumatic brain injury survivors who received P₄ were more likely to have a moderate to good outcome than those randomized to placebo. The authors concluded that results of this small study, P₄ caused no discernible harm and showed potential benefit [19].

7. Progesterone regulation of memory and neuronal excitability

After more than three decades of research, it is now well established that the ovarian hormone, E₂, exerts a wide variety of effects on neural structures and function, particularly within the hippocampus [135;136;137]. Electrophysiological studies have shown that E₂ enhances hippocampal CA1 synaptic transmission and plasticity by increasing NMDA and AMPA receptor activity, which results in neuronal excitation [138;139;140;141]. While the above studies have focused exclusively on the effect of E₂ on brain structure and function, more recent studies have investigated the effect of P₄ and its neuroactive metabolites AP α and pregnanolone (PREG) on cognitive function and neural excitability.

Long-term potentiation (LTP) is considered to be the best cellular model of memory trace formation in the brain, at least for certain forms of memory in the hippocampus and neocortex [142;143;144]. The phenomenon opposite to LTP is long-term depression (LTD), which was first demonstrated in cerebellar cortex by Ito in 1982 [145]. LTD has also been demonstrated in the hippocampus and neocortex and like LTP, is considered a mechanism for memory storage [146;147]. Although the molecular mechanisms underlying LTP (and LTD) have been extensively investigated, there is a relative paucity of studies demonstrating the critical role of LTP in behavioral learning and memory [148]. Nonetheless, LTP (and LTD) are the best current models of synaptic plasticity, which may underlie memory storage [149]. In the CA1, the most widely studied form of LTP involves glutamate activation of NMDA receptors, which augments AMPA receptor function for the expression and maintenance of LTP. However, this is not the sole form of LTP in the CA1, as Teyler and associates have demonstrated a form of tetanus-induced LTP in the CA1 that is independent of NMDA receptors and involves voltage-dependent calcium channels [150].

Few studies have examined the acute effects of P₄ on synaptic transmission and plasticity, with the results of these studies being mostly contradictory. P₄ (10 μ M) reportedly has no effect on

LTP (CA1 slices from 4 week-old rats), but no non-drug control was used in this study [151]. In another study, P₄ (8 – 10 M, in CA1 slices) significantly enhanced synaptic transmission, as seen by an increased field potential and population spike amplitude; however, following a seizure-induced tetanus, P₄ decreased the field potential, the population spike responses, and the duration of after-discharges [152]. In whole cell patch clamp of pyramidal neurons from slices of prelimbic cortex, P₄ (100 μM) had no effect on the frequency of excitatory postsynaptic currents (EPSCs), but inhibited dopamine-induced increases in EPSCs [153]. P₄ dose-response functions were not obtained in any of these studies. In primary hippocampal neurons, P₄ as well as E₂ enhances glutamate-mediated increases in intracellular calcium, with E₂ having a greater effect. P₄ appears to interfere with E₂ enhancement of synaptic transmission, as seen by the finding that co-treatment with P₄ and E₂ enhances glutamate-mediated increases in intracellular calcium to the same degree as P₄ alone [10].

Clinical investigations have been performed to understand the effect of P₄ on moods associated with premenstrual dysphoric disorder (PMDD) [154]. PMDD is defined in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV, APA 1994) as a cluster of both negative mood symptoms and physical symptoms that occur during the luteal phase of the menstrual cycle (when P₄ and AP α levels are high) and disappear several days following the onset of menstruation (when P₄ and AP α levels are low). The increase in P₄ (and AP α) levels that occurs during the luteal phase of the menstrual cycle is considered to be at least partly responsible for the negative mood changes associated with PMDD [154;155]. Although ovarian steroids are required for onset of premenstrual symptoms, women with PMDD are thought to exhibit an altered GABA receptor sensitivity [156]. Support for the association of P₄ with negative mood symptoms comes from the finding that postmenopausal women exhibiting intermediate AP α plasma concentrations subjectively rated themselves as having significantly more negative mood symptoms during P₄ treatment than during treatment with unopposed E₂ or placebo [157]. P₄ concentrations (measured via radioimmunoassay) in the amygdala, cerebellum, and hypothalamus are significantly higher in fertile women in the luteal phase of menstruation than in postmenopausal controls [158]. Moreover, in fertile women, AP α concentrations are highest in substantia nigra and basal hypothalamus, suggesting that the pattern of steroid secretion during the menstrual cycle is reflected in specific brain tissues [158].

P₄ and AP α are known to regulate cognitive function, particularly those functions related to mood and/ or associated with changes in the menstrual cycle (e.g., postpartum depression, major depression, epilepsy) [159]. The GABAergic system also participates in major depression (for review, see [160]. The GABA_A receptor mediates the majority of rapid (1 – 100 ms) synaptic inhibition in the mammalian brain, and AP α and PREG exert both anxiolytic and anesthetic effects by enhancing GABA-stimulated chloride conductance. This enhanced conductance serves to hyperpolarize postsynaptic membranes and results in neuronal inhibition [161;162]. Recent evidence suggests that specific neurosteroids ‘fine-tune’ neural inhibition via the GABAergic system [163]. In another recent study, the ability of P₄ to influence cognition and memory of biologically salient stimuli was investigated in healthy young women [164]. Here, a single dose of P₄ was orally administered to women who were then asked to memorize and recognize faces while undergoing functional magnetic resonance imaging. The results revealed that P₄ decreases recognition accuracy without affecting reaction times. P₄ also decreased amygdala and fusiform gyrus activity elicited by faces during memory encoding, supporting the conclusion that P₄ alters memory function by influencing amygdala activity [164;165].

In animals, P₄ and its metabolites severely impair learning and memory performance immediately following administration in the Morris water maze test [166;167]. Although the mechanism underlying this impairment is unknown, a recent study demonstrated that pretreatment of rats with AP α induces a partial tolerance against the acute effects of AP α in

the Morris water maze test [168]. These authors suggest that prolonged exposure to AP α in women (e.g., pregnancy, postmenopausal hormone replacement therapy, menstrual cycle) may alter cognitive behavior such as learning and memory, possibly through a GABAergic-dependent mechanism.

Excitatory synapses, which can be approximated by dendritic spine density, serve as the substrate for learning and memory. A significant amount of literature describes the effects of estrogen and P₄ on dendritic spine formation in hippocampal pyramidal neurons [169]. In rats, ovariectomy results in a decrease (over 6 days) in the density of dendritic spines, which can be prevented and reversed by E₂ treatment [18]. Administration of P₄ after estrogen treatment initially augments the effects of E₂ (and sexual behavior such as lordosis). However, 6 h later, P₄ rapidly decreases spine density to the very low levels, which are equivalent to levels seen 18 h after ovariectomy. Indeed, the number of dendritic spines in specific brain regions fluctuates over the 4 – 5 day estrous cycle in accordance with estrogen and P₄ levels [170]. The down regulation of dendritic spines by P₄ is blocked by the P₄ antagonist, RU-38486, consistent with the presence of intracellular PRs in the hippocampus. Electron microscopic studies have demonstrated the presence of non-nuclear PRs in glia and dendritic spines in the hippocampus [135]. Also, P₄ acts directly on GABAergic receptors to enhance GABA inhibition and thus, counter the effects of E₂ [127;171].

During the ovulatory cycle, extensive tissue remodeling occurs throughout the body, including certain brain regions. Some of the most profound changes occur in uterine tissue, which undergoes extensive angiogenesis and cell proliferation during the follicular phase. In the absence of a blastocyst, the uterine growth phase is terminated by resorption or exfoliation of epithelial and vascular cells. Numerous inflammatory mediators are cyclically regulated during both the growth and exfoliative phase [172]; [173]; [174]. These cyclically-regulated genes encode diverse proteins, many of which function in apoptosis such as Fas, caspase-3, M30 [175], complement C3 [174] [176], and secretory leukocyte proteinase inhibitor (SLPI) [177]. Progestins regulate the expression of many of these genes. For example, progestins antagonize estrogens in regulation of C3 [174] [176], whereas P₄ acts in synergy with the proinflammatory cytokine, IL-1, to induce expression of SLPI, an antimicrobial peptide important in host defense [177].

In contrast to uterine remodeling during the estrous cycle, brain ‘remodeling’ is more modest and does not involve major changes in the proportions of cell populations. In the rodent hippocampus, certain synaptic beds implicated in declarative memory undergo striking, transient changes during the estrous cycle. The dendritic spine density of CA1 pyramidal cells undergoes cyclic changes, which are strongly correlated with sensitivity to NMDA receptor-mediated synaptic responses [137]; [136]. There is also increased aggregation of MAP2 in apical dendrites [178] as well as an increased expression of syntaxin, synaptophysin (presynaptic), and spinophilin (postsynaptic) [179]. The increase in dendritic spines is driven by elevations in E₂ during the preovulatory follicular phase, whereas the rapid regression of these spines after ovulation depends upon elevation of P₄ from the corpus luteum [18]. Administration of the PR antagonist, RU 486, during proestrus inhibits the decrease in spine density after proestrus [18]. Like the CA1, the hypothalamic arcuate nucleus undergoes remodeling in response to the preovulatory luteinizing hormone surge. In this region, astrocyte volume changes have been associated with altered GABAergic contacts on gonadotropin-releasing hormone axons [180]; [181]. There is now a significant literature concerning the effects of estrogen and progesterone on dendritic spine formation in hippocampal pyramidal neurons [169]. In brief, ovariectomy (in rats) causes a decrease (over 6 days) in the density of dendritic spines, which can be prevented and reversed by estradiol treatment [18]. Progesterone treatment subsequent to estrogen treatment initially augments the effects of estradiol (and sexual behavior, e.g., lordosis) and then (after 6 h) results in a rapid decrease in spine density

to the very low values seen in ovariectomized animals by 18 h. Indeed, the number of dendritic spines in specific brain regions fluctuates over the 4-5 day estrous cycle in accordance with estrogen and progesterone levels [182]. The down regulation of dendritic spines by progesterone is blocked by the progesterone antagonist, RU-38486, consistent with the presence of intracellular progesterone receptors (PR). Electron microscopic studies also indicated the presence of non-nuclear PRs in glia and dendritic spines in hippocampus [135]. Progesterone can also act directly on GABAergic receptors to enhance GABA inhibition, thus countering the effects of E₂ [127;171].

8. Progesterone regulation of glial cell function and response

Progesterone regulates responses in each of the major glial cell types, astrocytes, microglia, oligodendrocytes and Schwann cells. During the estrous cycle astrocyte size varies with CA1 astrocytes shrinking immediately before increases in spine density [183]. Astrocytes also decrease in size in the rostral preoptic location of gonadotropin-releasing hormone cell bodies [184]. Astrocyte size is strongly associated with the expression of glial fibrillary acidic protein (GFAP), which varies during the estrous cycle in the dentate gyrus [185]. Progesterone, and its neurosteroid metabolite dihydroprogesterone induced a significant elevation of GFAP mRNA levels in type 1 astrocytes within hours of exposure with direct administration of dihydroprogesterone inducing an increase within 2 h whereas P₄ required 6 h for increased GFAP expression. These findings suggest that the effect of P₄ is likely due to metabolism to DHP. The requirement for conversion of P₄ to increase GFAP protein level was confirmed through the addition of finasteride (a specific blocker of the 5 alpha-reductase) which completely abolished the effect of P₄ [186].

In astrocytes, P₄ regulates production of multiple proteins including those shown to be involved in regulating synaptic plasticity such as ApoE which is secreted by astrocytes may be an important player in synaptic remodeling, since this protein transports cholesterol and other lipids to outgrowing neurites [187]. Glial apoE mRNA changes cyclically in the CA1 and arcuate nucleus [188]; [189], supporting a role for this protein in synaptic remodeling. During pregnancy, two-fold increases in uterine apoE levels are associated with increased import of maternal lipids [190]. Also, estrogen-dependent sprouting of perforant path fibers to the hippocampus is absent in apoE-knockout mice [191] [189] [192]. Complex heterotypic cellular interactions that occur in response to ovarian steroids extend beyond astrocytic-neuronal interactions. Although ApoE is secreted by astrocytes, astrocytic responses to estrogen require interactions with microglia, as evidenced in monotypic astrocyte cultures which are much less sensitive to estrogen than mixed glial cultures containing microglia [185]. Besides E₂, Premarin[®] also induced ApoE expression in mixed glia [193]. P₄ increased ApoE secretion in macrophages (microglia also express apoE mRNA) by acting on the C-terminal lipid-binding domain of ApoE to block its intracellular degradation [194]. The effect of other clinical progestins on glial apoE expression remains to be determined.

In many organ systems, estrogen actions are attenuated or antagonized by progestins. As discussed above, E₂ drives increases in CA1 spine density (growth phase), while P₄ promotes the regression of dendritic spines (during the proestrus to estrus transition). In mouse hippocampal slice cultures, mossy fiber sprouting into the molecular layer of the dentate gyrus is induced by deafferentation of the entorhinal cortex [192]. In this system, E₂ (100 pM) increases sprouting by 75%. This concentration of E₂ also induces maximal apoE induction in mixed glia [195]. E₂-dependent sprouting can be blocked by P₄ or tamoxifen [192]. P₄ appears to differ from MPA in its ability to regulate synaptic plasticity. Preliminary results from our laboratory show that MPA, but not P₄, inhibits E₂-mediated sprouting. This difference is consistent with recent findings by Nilsen and Brinton that P₄, but not MPA, is neuroprotective against excitotoxicity and stimulates nuclear activation of ERK. Further, MPA, but not P₄,

blocked the neuroprotective effects of E₂ [11]. Additional studies will be necessary to evaluate the differences and similarities of MPA and P₄.

In some models, P₄ has been reported to have anti-inflammatory activities. After stab wounds, P₄ decreased reactive astrocytes to a greater extent than E₂, but less than pregnenolone [196; 197]. In a model of cerebral concussion, male rats given an i.p. injection of P₄ had less edema [124] and lipid peroxidation [14] than their untreated counterparts. On the other hand, P₄ had a negligible effect on glial activation or neuronal loss in this model [124]. In accord with findings from the cerebral concussion model, P₄ inhibited the level of clinical neurogenic edema, possibly by reducing meningeal release of substance P [198]. Of course, P₄ has many complex interactions with neurotransmitters, which may underlie this and other anti-inflammatory effects (e.g., activation of the GABA receptor by P₄ and other C21 steroids [199]).

Progesterone regulation of myelination is now well documented and is a compelling example of the profound direct effects of P₄. The sciatic nerve, and Schwann cells in particular, are capable of synthesizing P₄ and possess the enzymes necessary to convert P₄ to the 5 α -reduced and the 3 α -5 α -reduced derivatives of P₄: dihydroprogesterone and tetrahydroprogesterone [200]. Progesterone receptor has been detected in both sciatic nerve and in Schwann cell cultures. P₄ and its metabolite neurosteroids regulate expression of two major proteins of the peripheral nervous system (PNS): the glycoprotein Po (Po) and peripheral myelin protein 22 (PMP22). Melcangi and colleagues have shown that: (a) dihydroprogesterone enhanced the low mRNA levels of Po in the sciatic nerve of aged male rats; (b) P₄ and its derivatives stimulates the gene expression of Po in the sciatic nerve of adult rats and in Schwann cell cultures; (c) tetrahydroprogesterone increased PMP22 gene expression in the sciatic nerve of adult rats and in Schwann cell cultures. They further demonstrated that P₄ and its derivatives control Po gene expression via the PR, while tetrahydroprogesterone modulated expression of PMP22 through the GABAA receptor [200]. Melcangi and coworkers went on to demonstrate that P₄ and its derivatives regulate other myelin proteins [i.e., myelin-associated glycoprotein (MAG) and myelin and lymphocyte protein (MAL)] in sex-specific cultures of rat Schwann cells [9]. Progesterone or dihydroprogesterone induced a stimulatory effect on P0 mRNA levels in male but not in female Schwann cells. In contrast, treatment with tetrahydroprogesterone increased gene expression of P0 in female derived Schwann cells. A similar sex-difference was also evident for other myelin proteins. PMP22 expression was increased by P₄ in male derived Schwann cell cultures, whereas tetrahydroprogesterone induced an increase of mRNA levels in female derived cells. Moreover, MAG was stimulated by tetrahydroprogesterone treatment in male cultures only, whereas MAL expression was unaffected by neuroactive steroid treatment in both male and female cultures. Collectively these findings indicate that P₄ and its metabolite neuroactive steroids on regulate myelin protein expression in a sexually dimorphic manner. This finding might represent an important background for sex-specific therapies of acquired and inherited peripheral neuropathies [9; 200]. Melcangi and colleagues pursued the relevance of these findings for age-associated myelin loss and morphological alterations of myelinated fibers in the sciatic nerve of 22-24-month-old male rats. The sciatic nerves of untreated old male rats, showed a general disorganization and a significant reduction in the density of myelinated fibers, compared to nerves from 3-month-old male rats. The effect of aging was particularly evident in myelinated fibers of small caliber (<5 micron in diameter). In addition, the sciatic nerves of old rats showed a significant increase in the number of fibers with myelin infoldings in the axoplasm and in the number of fibers with irregular shapes. Treatments of old rats with P, DHP and THP resulted in a significant increase in the number of myelinated fibers of small caliber, a significant reduction in the frequency of myelin abnormalities and a significant increase in the g ratio of small myelinated fibers. Furthermore, P₄ significantly reduced the frequency of myelinated fibers with irregular shapes. Results of these *in vivo* animal studies indicate that in the aged

male rat P₄ and its neuroactive metabolites reduced aging-associated morphological abnormalities of myelin and aging-associated myelin fiber loss in the sciatic nerve[125].

In the central nervous system P₄ and its neurosteroid metabolites were found to promote glial functions, such as the synthesis of myelin proteins. In glial cell cultures prepared from neonatal rat brain, P₄ increased the number of oligodendrocytes expressing myelin basic protein and the 2',3'-cyclic nucleotide-3'-phosphodiesterase (CNPase), the third most abundant myelin protein in the CNS [40]. The role of P₄ in myelination is extensively covered in several excellent reviews by Schumacher and colleagues and the reader is referred to these for further reading [15;16;40].

9. Progesterone Regulation of Meiosis and Mitosis

During development of both vertebrates and invertebrates, P₄ promotes meiosis to generate germ cells [201;202;203]. P₄ induced re-entry into the cell cycle at mediated by a membrane-bound PR [201;204;205;206;207].

P₄ promotion of meiosis is mediated by a rise in intracellular Ca²⁺[208;209;210]. In *Xenopus* oocytes, P₄ induces the resumption of meiosis (maturation) through a nongenomic mechanism involving inhibition of adenylyl cyclase and reduction of intracellular cAMP. However, P₄ action in *Xenopus* oocytes is not blocked by pertussis toxin, indicating that inhibition of the oocyte adenylyl cyclase is not mediated by the α subunit of classical G_i-type G proteins [211]. Subsequent analyses indicate that P₄ is likely inducing maturation by antagonizing constitutive G $\beta\gamma$ -mediated inhibition of cell cycle progression[211].

Intracellular Ca²⁺ influx and inhibition of G $\beta\gamma$ are not the sole requirements for P₄ regulation of meiosis. Multiple laboratories have demonstrated that P₄ activates MAPK signaling pathway in oocytes and that this pathway is required for promotion of meiosis by P₄ [212;213;214]. P₄ activation of MAPK leads to the formation of the M-phase promoting protein complex (CDC2 and cyclin B), which promotes G2 to M phase transition [215;216].

Mitotically, P₄ has a complex function in the uterus and can be both inhibitory and stimulatory for proliferation depending upon cell type (endometrial or stromal) the regimen of treatment, the type of PR that is activated (PRA versus PRB), the dose of E2 and P₄, and when in the cycle P₄ is administered [217;218;219;220;221;222]. In the endometrium, P₄ inhibits proliferation of endometrial cells whereas P₄ is a proliferative agent in the stromal cells of the uterus. Further, P₄ inhibited proliferation in E2 primed uterus but not when administered alone or with low dose E2. A progestinal agent in hormone therapy is added to antagonize endometrial cell proliferation in the uterus [223]. In addition to P₄ action in the uterus, P₄ can promote proliferation in the breast [224;225;226;227].

In the Women's Health Initiative, in which medroxyprogesterone acetate (MPA) was used as the progestinal agent, an increased risk of breast cancer in the hormone therapy (HT) trial was observed which did not occur in the estrogen only therapy (ET) [227;228;229;230;231;232]. The WHI HT trial also revealed an increased risk of invasive ovarian cancer and a reduced risk of endometrial cancer [233;234]. The use of the progestin, MPA, in the WHI HT trials has been proposed to be a major factor contributing to the increased cancer risk seen in this trial. However, the tumorigenic properties of different progestogens have not been systematically studied, and not all progestogen molecules have the same antagonistic or agonistic profiles [235;236;237]. The impact of different progestogens on the proliferation of neural stem cells or neural progenitors is currently unknown.

10. Progesterone and Estrogen Regulation of Neurogenesis and Neural Progenitor Proliferation

As in the uterus [217;238], P₄ regulation of mitosis of neural progenitors in brain has a complex profile. Tanapat *et al.* have shown that ovariectomized rats treated with a high level of E₂ have enhanced hippocampal cell proliferation, whereas subsequent exposure to P₄ resulted in blockade of the E₂-induced enhancement of cell proliferation [239]. In contrast to P₄ regulation of E₂-induced neurogenesis *in vivo*, we have demonstrated that P₄ alone enhances cell proliferation *in vitro* (Wang *et al.*, 2005). More recent results indicate that P₄ induced a dose dependent significant increase in rat neural progenitor cells (rNPC) proliferation as measured by BrdU incorporation over a 24 hr exposure period with an EC₁₀₀ value of 100 pM. Unlike its metabolite steroid AP α , the dose response curve for P₄ was shallow but linear up to 1 μ M whereas the dose response for AP α was steeply linear followed by an inverse function at 1 μ M. Further, when compared to the dose response for E₂-induced rNPC proliferation (EC₁₀₀ of 250 nM), P₄ induced a more consistent enhancement of rNPC proliferation. The time course of P₄-induced rNPC proliferation generated 2 important outcomes. First, P₄-induced DNA synthesis occurred rapidly within the first 1-4 hours of P₄ exposure. Second, it appears that P₄-induced DNA synthesis does not persist beyond 6 hours and that by 8 hrs, P₄ no longer induces DNA synthesis. These data suggest that P₄ is not driving rNPCs into prolonged or uncontrolled proliferation. Lastly, we conducted a steroid specificity analysis which indicates that P₄ and its metabolite AP α are both proliferative agents [240].

Our own work has demonstrated that the neurosteroid P₄ metabolite, AP α is a potent, stereoisomer-specific promoter of neurogenesis of both rat hippocampal neural progenitor cells and human cortical neural stem cells [241]. Allopregnanolone-induced proliferation was isomer and steroid specific, in that the stereoisomer 3 β -hydroxy-5 β -pregnan-20-one and related steroids did not increase 3H-thymidine uptake. Immunofluorescent analyses for the neural progenitor markers, nestin and Tuj1, indicated that newly formed cells were of neuronal lineage. Furthermore, microarray analysis of cell cycle genes and real time RT-PCR and western blot validation revealed that allopregnanolone increased the expression of genes, which promote mitosis and inhibited the expression of genes that repress cell proliferation. Allopregnanolone-induced proliferation was antagonized by the voltage gated L-type calcium channel blocker nifedipine consistent with the finding that allopregnanolone induces a rapid increase in intracellular calcium in hippocampal neurons via a GABA type A receptor activated voltage gated L-type calcium channel [242]. These data demonstrate that AP α significantly increased rNPC and hNSM proliferation with concomitant regulation in mitotic cell cycle genes via a voltage gated L-type calcium channel mechanism.

AP α -induced neurogenesis is a dose-dependent process, with concentrations in the low to mid nanomolar range promoting proliferation and concentrations exceeding 1 μ M significantly inhibiting neurogenesis. The biphasic dose-response profile of AP α -induced neurogenesis could account for the disparity between our *in vitro* data and reports that AP α decreases neurogenesis in the rat dentate gyrus *in vivo*. In these *in vivo* studies, AP α inhibited neurogenesis of rat SVG cells following intracerebral ventricular injection of 7.8 mmoles of AP α [3;186]. Considering that the injected concentration is diluted into the cerebrospinal fluid and the volume of the cerebrospinal fluid in a 300 g rat is ~ 580 μ l [243], the final concentration of AP α would be more than 50 μ M. Thus, inhibition of neurogenesis at micromolar concentrations of AP α in this study is consistent with our dose response data, which demonstrates inhibition of neural progenitor cells proliferation at micromolar concentrations and promotion of neurogenesis at nanomolar concentrations [241]. Griffin and Mellon found that early administration of AP α substantially delays progression and severity of symptoms in

a transgenic mouse model of Niemann-Pick Type C, a disease characterized by disrupted neurosteroidogenesis [244].

While production of new neurons from proliferating stem/progenitor cells in the SGZ of the dentate gyrus is maintained throughout life in multiple species including humans [245;246], the magnitude of neurogenesis declines with age. Age-associated decline in neurogenic potential in the dentate gyrus has been observed as early as middle age [247;248] and has been proposed to contribute to age-related learning and memory impairments [249;250;251;252]. The mechanism underlying age-associated decline in neurogenesis remains to be fully determined. However, loss of the growth factors FGF-2, IGF-1 and VEGF in the microenvironment of the SGZ is a prime contributor to the reduced neurogenic potential of the SGZ [253]. Recent studies have demonstrated that the levels of these three multiple stem/progenitor cell proliferation factors decline early on during the course of aging in the hippocampus [254;255]. Hippocampal levels of FGF-2, IGF-1, and VEGF are more than 50 – 60% decline lower in adult rats than in young rats [255]. These findings suggest that the dramatic decline in dentate neurogenesis could be linked to reduced concentrations of FGF-2, IGF-1, and VEGF in the hippocampus, as each of these factors can individually influence the proliferation of stem/progenitor cells in the SGZ of the dentate gyrus. For example, FGF-2 enhances dentate neurogenesis in both neonatal and adult brain [255;256;257;258;259;260;261;262], and intracerebroventricular (ICV) infusions of FGF-2 upregulate dentate neurogenesis in the aged brain [247;262;263;264;265]. Likewise, ICV administration of IGF-1 increased dentate neurogenesis in the adult and aged brain [252;266;267]. VEGF can promote dentate neurogenesis in both the intact and the injured adult brain following ICV administration [268;269;270]. During neurogenesis, VEGF may act as a chemoattractant that specifically targets FGF2-stimulated neural progenitors [271].

Growth factors regulate a myriad of cellular processes aside from neurogenesis. For example, EGF has well-characterized proliferative effects on the endometrium. EGF gene expression dramatically increases in the endometrial glands of pregnant mares at approximately 40 days after ovulation [272]. This upregulation is maintained until at least day 250 of gestation and is associated with an increase in EGF receptor binding sites in the endometrium [273]. The expression of both IGF and TGF β 1 is also upregulated during this time [274]. Administration of varying doses and combinations of P₄ and estrogen for 35 days yields negative or only weakly positive EGF expression, whereas administration of only P₄ for 40 days strongly upregulated EGF expression irrespective of additional treatment with estrogen [275]. These findings underscore the importance of P₄ in regulation of growth factors and reveal a mechanism for P₄-associated cell proliferation *in vivo*.

Similar to EGF, IGF and TGF, expression of brain derived neurotrophic factor (BDNF) is positively correlated with E₂ and P₄ levels and negatively correlated with menopausal age [276]. Hormone replacement therapy restored plasma BDNF levels to levels seen in fertile women during the follicular phase [276]. Circulating plasma levels of BDNF change during the menstrual cycle, suggesting that P₄ may regulate neurotrophin expression [276]. Modifications in BDNF circulating levels during the menstrual cycle suggest a potential role for gonadal sex hormones in regulating neurotrophin expression, which has implications for sustaining the regenerative milieu of the brain during the menopausal years.

Regulation of cell cycle entry by progesterone and its neurosteroid metabolites as therapeutic regenerative agents will require careful analysis prior to development for restoration of neurons lost due to neurodegenerative disease. In Alzheimer's disease (AD), cell cycle-specific gene expression is upregulated [277] and, evidence indicates that mitotic signaling is dysregulated [278]. In the aged and AD brain, both the pool of neural stem cells and their proliferative potential are markedly diminished [247;279]. In addition, the level of potential regenerative

factors is reduced in the brains of AD patients compared to age-matched controls [280]. Herrup and colleagues have found that ectopic expression of cell cycle proteins predicts the site of neuronal cell death in the AD brain [281], leading these investigators to propose that dysregulation of various elements of the cell cycle contributes to regionally specific neuronal death in AD. They also found that DNA replication precedes neuronal death in AD brain [282]. More disturbing, cell cycle events precede neuronal death at *all* stages of AD, from mild cognitive impairment to advanced AD [278]. This finding has important implications for strategies targeting neurogenesis in the AD brain. Specifically, it suggests that promoting entry into the cell cycle could potentially be a double-edged sword, with benefit to healthy brains but with exacerbation of ectopic mitosis in brains destined to develop AD or with existing AD.

11. Progesterone and Regulation Alzheimer's Disease Pathology

A key hypothesis linking P₄ to AD posits that P₄ acts as an endogenous regulator of β -amyloid (A β) metabolism. According to the widely but not universally embraced 'amyloid cascade hypothesis,' AD pathogenesis is triggered by any of a number of events that have the final common endpoint of increasing the pool of soluble A β [283;284;285]. In turn, elevated soluble A β leads to the formation of an array of soluble oligomeric, minimally soluble aggregated, and eventually insoluble fibrillar A β species, all of which are linked in a variety of ways to neurodegenerative cascades [286;287;288;289;290;291;292;293]. Thus, factors that have the net effect of reducing the pool of soluble A β are thought to represent potentially powerful strategies for preventing the development of AD [294;295].

Neurosteroids have recently been measured in various brain regions of aged Alzheimer's disease patients and aged non-demented controls by GC/MS [296]. In Alzheimer's patients, there was a general trend toward lower levels of neurosteroids in different brain regions, and neurosteroid levels were negatively correlated with two biochemical markers of Alzheimer's disease, the phosphorylated tau protein and the beta-amyloid peptides [296]. The formation of these metabolites within distinct brain regions negatively correlated with the density of beta-amyloid deposits.

Available evidence suggests that E₂, like P₄, may influence A β levels. In women, E₂ status has been linked to the development of AD [237]. Schonknecht and colleagues found that levels of E₂ in cerebral spinal fluid are lower in AD patients than in non-AD controls and that, in the AD group, E₂ levels are inversely correlated with A β ₁₋₄₂ levels [297]. In accord with this finding, several cell culture studies have shown that E₂ decreases A β production, presumably increasing the non-amyloidogenic cleavage of amyloid precursor protein (APP) via α -secretase [298;299;300;301;302;303]. The physiological significance of this effect has been confirmed by *in vivo* studies investigating the relationship between circulating E₂ concentrations and brain levels of A β . Depletion of endogenous E₂ via ovariectomy results in a significantly increases levels of soluble A β in brains of mice [304] and guinea pigs [305]. Importantly, this effect is partially reversed by E₂ replacement. Furthermore, E₂ reduces pools of soluble [306;307] and deposited A β [307] in mouse models of AD, strongly implicating E₂ as a regulator of AD pathogenesis. Unexpectedly, two recent studies using transgenic mouse models of AD have found weak [308] or absent evidence [309] that E₂ reduces insoluble pools of A β . Several factors may have contributed to these negative results, including the transgenic strains, the relevant pool of A β , and the difference between estrogen levels in circulation and in the brain [310]. Prior investigations of A β regulation by ovarian sex steroids have used ovariectomy models (which depletes endogenous E₂ and P₄) combined with E₂, but not P₄, replacement [305;306;307;308;309].

While E₂ replacement is beneficial in regulation of A β metabolism, the interaction between E₂ with P₄ is more clinically relevant. That is, how are A β levels affected when P₄ acts in

concert with E₂? Our group has recently addressed this question in the 3xTg-AD mouse model of AD. Our results suggest that continuous E₂, but not P₄ treatment attenuates the acceleration of A β accumulation and memory deficits observed in ovariectomized mice. More importantly, in animals receiving both hormones, P₄ blocked the beneficial effect of E₂ on A β accumulation [2].

12. Progestogens, Progestins and Metabolism

Many progestogens are used therapeutically among these, P₄ is the only naturally occurring progestogen (see Table 1). The remainder, which are synthetic, are referred to as progestins (see Table 1)[236]. Progestins are classified on the basis of their chemical structure, since the structures of these molecules vary widely. Progestins can be divided into those related in chemical structure to P₄ and those related chemically to testosterone. The classification scheme and the names of progestins in each of the categories are summarized in Table 1. This classification scheme does not denote the chemical source of the compounds.

Progestins related to P₄ are characterized by the presence or absence of a methyl group on carbon 10, and are subdivided into pregnanes (21 carbons) and 19-norpregnanes (20 carbons) (see Tables 1 and 2) [41]. The pregnanes and 19-norpregnanes can be further separated into compounds with and without an acetyl group. One of the best known and most widely used of these progestins is MPA, which is classified as an acetylated pregnane. All of the 19-norpregnane progestins have been used primarily in Europe and not in the United States.

Unlike the progestins related to P₄, which are first subdivided on the basis of the number of carbons (21 versus 20), those related to testosterone are first subdivided on the basis of whether or not they contain an ethinyl group. The ethinylated progestins are subdivided further into those related to the parent steroid, estrane, and those related to 13-ethylgonane. Both estranes and 13-ethylgonanes lack a methyl group at carbon 10. The estrane group of progestins consists of norethindrone and its prodrugs, namely norethindrone acetate, ethynodiol diacetate, norethynodrel, and lynestrenol. These prodrugs, which are considered part of the norethindrone family, have been widely used for hormone therapy and/or contraception. Although tibolone is also a prodrug and is listed in the estrane category, it is not converted to norethindrone. Instead, it is transformed to other active metabolites.

The 13-ethylgonanes contain an ethyl group on carbon 13 of the basic steroid nucleus (gonane). This category of progestins, sometimes referred to as the levonorgestrel family, consists of levonorgestrel and the levonorgestrel derivatives desogestrel, norgestimate, and gestodene. The latter three progestins are often referred to as the new progestins, as they have been marketed relatively recently. In contrast, levonorgestrel has been used for many years.

Norgestimate and desogestrel, but not gestodene, are prodrugs. However, only norgestimate is converted to levonorgestrel. Norgestimate is also converted to deacetylated norgestimate (levonorgestrel-3-oxime), which has progestational activity. Desogestrel is converted to its active form, 3-ketodesogestrel. Gestodene has inherent progestational activity, but is not approved for use in the United States.

13. Progestogen metabolism

With the exception of P₄, little is known about the metabolism of most progestogens. Baulieu first discovered that P₄ is converted to neuroactive metabolites in the brain [1;15;27]. This has now been well established by many laboratories and documented in multiple species including the rodent and human. Neurosteroids such as AP α are synthesized in the central and peripheral nervous system, primarily by myelinating glial cells, but also by astrocytes and several neuron types [15;311;312;313]. A region-specific expression pattern of P₄-converting enzymes in

brain is evident in both the hippocampus and cortex of rodent and human brain [311;312; 313;314]. The P₄-converting enzymes 5 α -reductase and 3 α -hydroxysteroid dehydrogenase are expressed in the hippocampus of both the rodent and human brain and convert P₄ to its 5 α , 3 α -reduced metabolites (e.g., AP α). Remarkably, these enzymes are also present and functional in pluripotential progenitors [315]. The conversion of P₄ to its 5 α , 3 α -reduced metabolites can be blocked by 5 α -reductase inhibitors, such as finasteride.

Of the progestins, the metabolism of norethindrone and levonorgestrel is the best characterized (see Table 2). These compounds undergo extensive reduction at the double bond between carbons 5 and 6 and the carbonyl group at carbon 3, resulting in the formation of dihydro- and tetrahydroderivatives. They can also undergo hydroxylation. Surprisingly little is known about the metabolism of the most widely used progestin, MPA. This compound, like other progestins structurally related to P₄ or testosterone, contains a double bond between carbons 5 and 6 as well as a ketone group at carbon 3. Therefore, it is likely to undergo extensive reduction at these functional groups. Unlike the neuroactive metabolites of P₄ (e.g., AP α), exceedingly little is known about the neuroactive properties of progestin metabolites.

14. Progesterone and PRs: Translational and therapeutic challenges

Relative to estrogen neurobiology, the non-reproductive neural functions of P₄ and the basic genomic, signaling and cell biology of these processes are just emerging. Progesterone and its neuroactive metabolites can promote the viability of neurons and function of glial cells within both the central and peripheral nervous system. While there is a substantial body of evidence regarding the pleiotropic actions of P₄ [16], much remains to be determined regarding the specific PR required and the associated effector mechanism(s) of action. The use of P₄ as a myelinating agent and as a treatment for traumatic brain injury highlight the basic science and clinical importance of understanding the direct effects of P₄ and other progestogens. It is interesting to note that women have a greater risk of developing the demyelinating disease multiple sclerosis with a frequent onset at menopause [316]. Given the significant impact of P₄ and its metabolite, allopregnanolone (tetrahydroprogesterone) on remyelination following injury and preventing age-associated myelin loss, the potential therapeutic use of P₄ for remyelination is substantial in both men and women. Of particular concern, given the results of the WHI and WHIMS trials, is the impact of clinically used progestins on neurological function. While progestogens in hormone therapy are given to reduce the risk of uterine cancer, these agents potentially exert effects in brain. Collectively, we know little regarding the impact of different progestins on neural function either acutely or chronically. The wide distribution of progesterone receptors in brain suggests that this gonadally and brain derived steroid plays a significant role in neural function, which awaits continued discovery.

Acknowledgements

Development of this work was supported by the National Institute of Aging P01AG026572 on Progesterone in Brain Aging and Alzheimer's Disease to RDB

References

1. Baulieu EE, Schumacher M, Koenig H, Jung-Testas I, Akwa Y. Progesterone as a neurosteroid: actions within the nervous system. *Cell Mol Neurobiol* 1996;16:143–154. [PubMed: 8743966]
2. Carroll JC, Rosario ER, Chang L, Stanczyk FZ, Oddo S, LaFerla FM, Pike CJ. Progesterone and estrogen regulate Alzheimer-like neuropathology in female 3xTg-AD mice. *J Neurosci* 2007;27:13357–65. [PubMed: 18045930]
3. Giachino C, Galbiati M, Fasolo A, Peretto P, Melcangi R. Neurogenesis in the subependymal layer of the adult rat: a role for neuroactive derivatives of progesterone. *Ann N Y Acad Sci* 2003;1007:335–9. [PubMed: 14993066]

4. Gibbs RB. Long-term treatment with estrogen and progesterone enhances acquisition of a spatial memory task by ovariectomized aged rats. *Neurobiology of Aging* 2000;21:106–116.
5. Gonzalez Deniselle MC, Garay L, Gonzalez S, Saravia F, Labombarda F, Guennoun R, Schumacher M, De Nicola AF. Progesterone modulates brain-derived neurotrophic factor and choline acetyltransferase in degenerating Wobbler motoneurons. *Exp Neurol* 2007;203:406–14. [PubMed: 17052708]
6. He J, Evans CO, Hoffman SW, Oyesiku NM, Stein DG. Progesterone and allopregnanolone reduce inflammatory cytokines after traumatic brain injury. *Exp Neurol* 2004;189:404–12. [PubMed: 15380490]
7. Irwin RW, Yao J, Hamilton R, Cadenas E, Brinton RD, Nilsen J. Progesterone and estradiol regulate oxidative metabolism in brain mitochondria. *Endocrinology*. 2008In Press
8. Leonelli E, Bianchi R, Cavaletti G, Caruso D, Crippa D, Garcia-Segura LM, Lauria G, Magnaghi V, Roglio I, Melcangi RC. Progesterone and its derivatives are neuroprotective agents in experimental diabetic neuropathy: a multimodal analysis. *Neuroscience* 2007;144:1293–304. [PubMed: 17187935]
9. Magnaghi V, Veiga S, Ballabio M, Gonzalez LC, Garcia-Segura LM, Melcangi RC. Sex-dimorphic effects of progesterone and its reduced metabolites on gene expression of myelin proteins by rat Schwann cells. *J Peripher Nerv Syst* 2006;11:111–8. [PubMed: 16787508]
10. Nilsen J, Brinton RD. Impact of progestins on estrogen-induced neuroprotection: synergy by progesterone and 19-norprogesterone and antagonism by medroxyprogesterone acetate. *Endocrinology* 2002;143:205–12. [PubMed: 11751611]
11. Nilsen J, Brinton RD. Divergent impact of progesterone and medroxyprogesterone acetate (Provera) on nuclear mitogen-activated protein kinase signaling. *Proc Natl Acad Sci U S A* 2003;100:10506–11. [PubMed: 12925744]
12. Pettus EH, Wright DW, Stein DG, Hoffman SW. Progesterone treatment inhibits the inflammatory agents that accompany traumatic brain injury. *Brain Res* 2005;1049:112–9. [PubMed: 15932748]
13. Roof RL, Duvdevani R, Heyburn JW, Stein DG. Progesterone rapidly decreases brain edema: treatment delayed up to 24 hours is still effective. *Exp Neurol* 1996;138:246–251. [PubMed: 8620923]
14. Roof RL, Hoffman SW, Stein DG. Progesterone protects against lipid peroxidation following traumatic brain injury in rats. *Mol Chem Neuropathol* 1997;31:1–11. [PubMed: 9271001]
15. Schumacher M, Guennoun R, Ghomari A, Massaad C, Robert F, El-Etr M, Akwa Y, Rajkowski K, Baulieu EE. Novel perspectives for progesterone in hormone replacement therapy, with special reference to the nervous system. *Endocr Rev* 2007;28:387–439. [PubMed: 17431228]
16. Schumacher M, Guennoun R, Stein DG, De Nicola AF. Progesterone: therapeutic opportunities for neuroprotection and myelin repair. *Pharmacol Ther* 2007;116:77–106. [PubMed: 17659348]
17. Shear DA, Galani R, Hoffman SW, Stein DG. Progesterone protects against necrotic damage and behavioral abnormalities caused by traumatic brain injury. *Exp Neurol* 2002;178:59–67. [PubMed: 12460608]
18. Woolley CS, McEwen BS. Roles of estradiol and progesterone in regulation of hippocampal dendritic spine density during the estrous cycle in the rat. *J Comp Neurol* 1993;336:293–306. [PubMed: 8245220]
19. 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. ProTECT: a randomized clinical trial of progesterone for acute traumatic brain injury. *Ann Emerg Med* 2007;49:391–402. [PubMed: 17011666]402 e1-2
20. Callier S, Morissette M, Grandbois M, Pelaprat D, Di Paolo T. Neuroprotective properties of 17beta-estradiol, progesterone, and raloxifene in MPTP C57Bl/6 mice. *Synapse* 2001;41:131–8. [PubMed: 11400179]
21. Gonzalez Deniselle MC, Lopez Costa JJ, Gonzales SL, Labombarda F, Garay L. Basis of progesterone protection in spinal cord neurodegeneration. *Journal of Steroid Biochemistry and Molecular Biology* 2002;83:199–209. [PubMed: 12650717]
22. Gonzalez Deniselle MC, Lopez-Costa JJ, Saavedra JP, Pietranera L, Gonzalez SL, Garay L, Guennoun R, Schumacher M, De Nicola AF. Progesterone neuroprotection in the Wobbler mouse, a genetic model of spinal cord motor neuron disease. *Neurobiol Dis* 2002;11:457–68. [PubMed: 12586554]

23. He J, Hoffman SW, Stein DG. Allopregnanolone, a progesterone metabolite, enhances behavioral recovery and decreases neuronal loss after traumatic brain injury. *Restor Neurol Neurosci* 2004;22:19–31. [PubMed: 15096691]
24. Labombarda F, Gonzalez SL, Gonzalez DM, Guennoun R, Schumacher M, de Nicola AF. Cellular basis for progesterone neuroprotection in the injured spinal cord. *J Neurotrauma* 2002;19:343–55. [PubMed: 11939502]
25. Nilsen J, Chen S, Brinton RD. Dual action of estrogen on glutamate-induced calcium signaling: mechanisms requiring interaction between estrogen receptors and src/mitogen activated protein kinase pathway. *Brain Res* 2002;930:216–34. [PubMed: 11879813]
26. Roof RL, Hall ED. Gender differences in acute CNS trauma and stroke: neuroprotective effects of estrogen and progesterone. *J Neurotrauma* 2000;17:367–88. [PubMed: 10833057]
27. Schumacher M, Guennoun R, Robert F, Carelli C, Gago N, Ghomari A, Gonzalez Deniselle MC, Gonzalez SL, Ibanez C, Labombarda F, Coirini H, Baulieu EE, De Nicola AF. Local synthesis and dual actions of progesterone in the nervous system: neuroprotection and myelination. *Growth Horm IGF Res* 2004;14:S18–33. [PubMed: 15135772]
28. Singh M. Ovarian hormones elicit phosphorylation of Akt and extracellular-signal regulated kinase in explants of the cerebral cortex. *Endocrine* 2001;14:407–15. [PubMed: 11444439]
29. Gibbs RB. Oestrogen and the cholinergic hypothesis: implications for oestrogen replacement therapy in postmenopausal women. *Novartis Found Symp* 2000;230:94–107. [PubMed: 10965504] discussion 107-111
30. Brinton RD, Chen S, Montoya M, Hsieh D, Minaya J. The estrogen replacement therapy of the Women's Health Initiative promotes the cellular mechanisms of memory and neuronal survival in neurons vulnerable to Alzheimer's disease. *Maturitas* 2000;34:S35–52. [PubMed: 10915920]
31. Brinton RD. Cellular and molecular mechanisms of estrogen regulation of memory formation and neuroprotection against Alzheimer's disease: Recent insights and remaining challenges. *Learning and Memory* 2001;8:121–133. [PubMed: 11390632]
32. Dubal DB, Shughrue PJ, Wilson ME, Merchenthaler I, Wise PM. Estradiol modulates bcl-2 in cerebral ischemia: a potential role for estrogen receptors. *J Neurosci* 1999;19:6385–93. [PubMed: 10414967]
33. Singer CA, Figueroa-Masot XA, Batchelor RH, Dorsa DM. The mitogen-activated protein kinase pathway mediates estrogen neuroprotection after glutamate toxicity in primary cortical neurons. *J Neurosci* 1999;19:2455–63. [PubMed: 10087060]
34. Wise PM. Estrogens and neuroprotection. *Trends Endocrinol Metab* 2002;13:229–30. [PubMed: 12128278]
35. Yang SH, Liu R, Wu SS, Simpkins JW. The use of estrogens and related compounds in the treatment of damage from cerebral ischemia. *Ann N Y Acad Sci* 2003;1007:101–7. [PubMed: 14993044]
36. Gonzalez Deniselle MC, Garay L, Lopez-Costa JJ, Gonzalez S, Mougel A, Guennoun R, Schumacher M, De Nicola AF. Progesterone treatment reduces NADPH-diaphorase/nitric oxide synthase in Wobbler mouse motoneuron disease. *Brain Res* 2004;1014:71–9. [PubMed: 15212993]
37. Labombarda F, Gonzalez SL, Deniselle MC, Vinson GP, Schumacher M, De Nicola AF, Guennoun R. Effects of injury and progesterone treatment on progesterone receptor and progesterone binding protein 25-Dx expression in the rat spinal cord. *J Neurochem* 2003;87:902–13. [PubMed: 14622121]
38. Koski CL, Hila S, Hoffman GE. Regulation of cytokine-induced neuron death by ovarian hormones: involvement of antiapoptotic protein expression and c-JUN N-terminal kinase-mediated proapoptotic signaling. *Endocrinology* 2004;145:95–103. [PubMed: 14512437]
39. Gonzalez Deniselle MC, Gonzalez SL, De Nicola AF. Cellular basis of steroid neuroprotection in the wobbler mouse, a genetic model of motoneuron disease. *Cell Mol Neurobiol* 2001;21:237–54. [PubMed: 11569536]
40. Baulieu E, Schumacher M. Progesterone as a neuroactive neurosteroid, with special reference to the effect of progesterone on myelination. *Steroids* 2000;65:605–12. [PubMed: 11108866]
41. Stanczyk FZ. All progestins are not created equal. *Steroids* 2003;68:879–90. [PubMed: 14667980]
42. Stein DG. Brain damage, sex hormones and recovery: a new role for progesterone and estrogen? *Trends Neurosci* 2001;24:386–91. [PubMed: 11410269]

43. Guerra-Araiza C, Villamar-Cruz O, Gonzalez-Arenas A, Chavira R, Camacho-Arroyo I. Changes in progesterone receptor isoforms content in the rat brain during the oestrous cycle and after oestradiol and progesterone treatments. *J Neuroendocrinol* 2003;15:984–90. [PubMed: 12969244]
44. Guerra-Araiza C, Coyoy-Salgado A, Camacho-Arroyo I. Sex differences in the regulation of progesterone receptor isoforms expression in the rat brain. *Brain Res Bull* 2002;59:105–9. [PubMed: 12379440]
45. Guerra-Araiza C, Reyna-Neyra A, Salazar AM, Cerbon MA, Morimoto S, Camacho-Arroyo I. Progesterone receptor isoforms expression in the prepuberal and adult male rat brain. *Brain Res Bull* 2001;54:13–7. [PubMed: 11226710]
46. Guerra-Araiza C, Cerbon MA, Morimoto S, Camacho-Arroyo I. Progesterone receptor isoforms expression pattern in the rat brain during the estrous cycle. *Life Sci* 2000;66:1743–52. [PubMed: 10809171]
47. Camacho-Arroyo I, Guerra-Araiza C, Cerbon MA. Progesterone receptor isoforms are differentially regulated by sex steroids in the rat forebrain. *Neuroreport* 1998;9:3993–6. [PubMed: 9926835]
48. Kato J, Hirata S, Nozawa A, Mouri N. The ontogeny of gene expression of progesterone receptors in the female rat brain. *J Steroid Biochem Mol Biol* 1993;47:173–82. [PubMed: 8274433]
49. Hagihara K, Hirata S, Osada T, Hirai M, Kato J. Distribution of cells containing progesterone receptor mRNA in the female rat di- and telencephalon: an in situ hybridization study. *Brain Res Mol Brain Res* 1992;14:239–49. [PubMed: 1331652]
50. Hagihara K, Hirata S, Osada T, Hirai M, Kato J. Expression of progesterone receptor in the neonatal rat brain cortex: detection of its mRNA using reverse transcription-polymerase chain reaction. *J Steroid Biochem Mol Biol* 1992;41:637–40. [PubMed: 1373302]
51. Kato J, Onouchi T. Progesterone receptors in female rat brain and hypophysis in the development from fetal to postnatal stages. *Endocrinology* 1983;113:29–36. [PubMed: 6861703]
52. Kato J, Onouchi T, Takamatsu M. Decreased progesterone receptors in the cerebral cortex of hypothyroid postnatal rats. *J Steroid Biochem* 1984;20:817–9. [PubMed: 6708554]
53. Pratt WB. The hsp90-based chaperone system: involvement in signal transduction from a variety of hormone and growth factor receptors. *Proc Soc Exp Biol Med* 1998;217:420–34. [PubMed: 9521088]
54. Pratt WB, Toft DO. Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. *Exp Biol Med (Maywood)* 2003;228:111–33. [PubMed: 12563018]
55. Leonhardt SA, Boonyaratanakornkit V, Edwards DP. Progesterone receptor transcription and non-transcription signaling mechanisms. *Steroids* 2003;68:761–70. [PubMed: 14667966]
56. DeMarzo AM, Beck CA, Onate SA, Edwards DP. Dimerization of mammalian progesterone receptors occurs in the absence of DNA and is related to the release of the 90-kDa heat shock protein. *Proc Natl Acad Sci U S A* 1991;88:72–6. [PubMed: 1986383]
57. Edwards DP, DeMarzo AM, Onate SA, Beck CA, Estes PA, Nordeen SK. Mechanisms controlling steroid receptor binding to specific DNA sequences. *Steroids* 1991;56:271–8. [PubMed: 1652169]
58. Allan GF, Leng X, Tsai SY, Weigel NL, Edwards DP, Tsai MJ, O'Malley BW. Hormone and antihormone induce distinct conformational changes which are central to steroid receptor activation. *J Biol Chem* 1992;267:19513–20. [PubMed: 1326555]
59. McKenna NJ, O'Malley BW. Minireview: nuclear receptor coactivators--an update. *Endocrinology* 2002;143:2461–5. [PubMed: 12072374]
60. Westin S, Rosenfeld MG, Glass CK. Nuclear receptor coactivators. *Adv Pharmacol* 2000;47:89–112. [PubMed: 10582085]
61. Groshong SD, Owen GI, Grimison B, Schauer IE, Todd MC, Langan TA, Sclafani RA, Lange CA, Horwitz KB. Biphasic regulation of breast cancer cell growth by progesterone: role of the cyclin-dependent kinase inhibitors, p21 and p27(Kip1). *Mol Endocrinol* 1997;11:1593–607. [PubMed: 9328342]
62. Richer JK, Lange CA, Manning NG, Owen G, Powell R, Horwitz KB. Convergence of progesterone with growth factor and cytokine signaling in breast cancer. Progesterone receptors regulate signal transducers and activators of transcription expression and activity. *J Biol Chem* 1998;273:31317–26. [PubMed: 9813040]

63. Owen GI, Richer JK, Tung L, Takimoto G, Horwitz KB. Progesterone regulates transcription of the p21(WAF1) cyclin- dependent kinase inhibitor gene through Sp1 and CBP/p300. *J Biol Chem* 1998;273:10696–701. [PubMed: 9553133]
64. Lange CA, Richer JK, Shen T, Horwitz KB. Convergence of progesterone and epidermal growth factor signaling in breast cancer. Potentiation of mitogen-activated protein kinase pathways. *J Biol Chem* 1998;273:31308–16. [PubMed: 9813039]
65. Conneely OM, Maxwell BL, Toft DO, Schrader WT, O'Malley BW. The A and B forms of the chicken progesterone receptor arise by alternate initiation of translation of a unique mRNA. *Biochem Biophys Res Commun* 1987;149:493–501. [PubMed: 3426587]
66. Hirata S, Shoda T, Kato J, Hoshi K. Isoform/variant mRNAs for sex steroid hormone receptors in humans. *Trends Endocrinol Metab* 2003;14:124–9. [PubMed: 12670738]
67. Hirata S, Shoda T, Kato J, Hoshi K. Novel isoforms of the mRNA for human female sex steroid hormone receptors. *J Steroid Biochem Mol Biol* 2002;83:25–30. [PubMed: 12650698]
68. Krebs CJ, Jarvis ED, Chan J, Lydon JP, Ogawa S, Pfaff DW. A membrane-associated progesterone-binding protein, 25-Dx, is regulated by progesterone in brain regions involved in female reproductive behaviors. *Proc Natl Acad Sci U S A* 2000;97:12816–21. [PubMed: 11070092]
69. Zhu Y, Rice CD, Pang Y, Pace M, Thomas P. Cloning, expression, and characterization of a membrane progesterin receptor and evidence it is an intermediary in meiotic maturation of fish oocytes. *Proc Natl Acad Sci U S A* 2003;100:2231–6. [PubMed: 12574519]
70. Zhu Y, Bond J, Thomas P. Identification, classification, and partial characterization of genes in humans and other vertebrates homologous to a fish membrane progesterin receptor. *Proc Natl Acad Sci U S A* 2003;100:2237–42. [PubMed: 12601167]
71. Falkenstein E, Heck M, Gerdes D, Grube D, Christ M, Weigel M, Buddhikot M, Meizel S, Wehling M. Specific progesterone binding to a membrane protein and related nongenomic effects on Ca²⁺-fluxes in sperm. *Endocrinology* 1999;140:5999–6002. [PubMed: 10579369]
72. Auger CJ, De Vries GJ. Progesterin receptor immunoreactivity within steroid-responsive vasopressin-immunoreactive cells in the male and female rat brain. *J Neuroendocrinol* 2002;14:561–567. [PubMed: 12121493]
73. Kato J, Hirata S, Nozawa A, Yamada-Mouri N. Gene expression of progesterone receptor isoforms in the rat brain. *Horm Behav* 1994;28:454–63. [PubMed: 7729814]
74. Allan GF, Tsai SY, Tsai MJ, O'Malley BW. Ligand-dependent conformational changes in the progesterone receptor are necessary for events that follow DNA binding. *Proc Natl Acad Sci U S A* 1992;89:11750–4. [PubMed: 1465392]
75. Aupperlee MD, Haslam SZ. Differential hormonal regulation and function of progesterone receptor isoforms in normal adult mouse mammary gland. *Endocrinology* 2007;148:2290–300. [PubMed: 17317767]
76. Brayman MJ, Julian J, Mulac-Jericevic B, Conneely OM, Edwards DP, Carson DD. Progesterone receptor isoforms A and B differentially regulate MUC1 expression in uterine epithelial cells. *Mol Endocrinol* 2006;20:2278–91. [PubMed: 16740655]
77. Marshburn PB, Zhang J, Bahrani-Mostafavi Z, Matthews ML, White J, Hurst BS. Variant progesterone receptor mRNAs are co-expressed with the wild-type progesterone receptor mRNA in human endometrium during all phases of the menstrual cycle. *Mol Hum Reprod* 2005;11:809–15. [PubMed: 16339776]
78. Sakamoto H, Ukena K, Takemori H, Okamoto M, Kawata M, Tsutsui K. Expression and localization of 25-Dx, a membrane-associated putative progesterone-binding protein, in the developing Purkinje cell. *Neuroscience* 2004;126:325–34. [PubMed: 15207350]
79. Peluso JJ, Romak J, Liu X. Progesterone Receptor Membrane Component-1 (PGRMC1) is the Mediator of Progesterone's Anti-apoptotic Action in Spontaneously Immortalized Granulosa Cells as Revealed by PGRMC1 siRNA Treatment and Functional Analysis of PGRMC1 Mutations. *Endocrinology*. 2007
80. Peluso JJ, Pappalardo A, Losel R, Wehling M. Progesterone membrane receptor component 1 expression in the immature rat ovary and its role in mediating progesterone's antiapoptotic action. *Endocrinology* 2006;147:3133–40. [PubMed: 16513825]

81. Engmann L, Losel R, Wehling M, Peluso JJ. Progesterone regulation of human granulosa/luteal cell viability by an RU486-independent mechanism. *J Clin Endocrinol Metab* 2006;91:4962–8. [PubMed: 16984987]
82. Peluso JJ. Multiplicity of progesterone's actions and receptors in the mammalian ovary. *Biol Reprod* 2006;75:2–8. [PubMed: 16452458]
83. Thomas P, Pang Y, Dong J, Groenen P, Kelder J, de Vlieg J, Zhu Y, Tubbs C. Steroid and G protein binding characteristics of the seatrout and human progestin membrane receptor alpha subtypes and their evolutionary origins. *Endocrinology* 2007;148:705–18. [PubMed: 17082257]
84. Zhu Y, Rice CD, Pang Y, Pace M, Thomas P. Cloning, expression, and characterization of a membrane progestin receptor and evidence it is an intermediary in meiotic maturation of fish oocytes. *Proc Natl Acad Sci U S A* 2003;100:2231–6. [PubMed: 12574519]
85. Magnaghi V, Ballabio M, Roglio I, Melcangi RC. Progesterone derivatives increase expression of Krox-20 and Sox-10 in rat Schwann cells. *J Mol Neurosci* 2007;31:149–57. [PubMed: 17478888]
86. Finkbeiner S. CREB couples neurotrophin signals to survival messages. *Neuron* 2000;25:11–4. [PubMed: 10707967]
87. Freeland K, Boxer LM, Latchman DS. The cyclic AMP response element in the Bcl-2 promoter confers inducibility by hypoxia in neuronal cells. *Brain Research Molecular Brain Research* 2001;92:98–106. [PubMed: 11483246]
88. Gu G, Rojo AA, Zee MC, Yu J, Simerly RB. Hormonal regulation of CREB phosphorylation in the anteroventral periventricular nucleus. *J Neurosci* 1996;16:3035–44. [PubMed: 8622133]
89. Lee SJ, Campomanes CR, Sikat PT, Greenfield AT, Allen PB, McEwen BS. Estrogen induces phosphorylation of cyclic AMP response element binding (pCREB) in primary hippocampal cells in a time-dependent manner. *Neuroscience* 2004;124:549–560. [PubMed: 14980726]
90. Wade CB, Dorsa DM. Estrogen activation of cyclic adenosine 5'-monophosphate response element-mediated transcription requires the extracellularly regulated kinase/mitogen-activated protein kinase pathway. *Endocrinology* 2003;144:832–8. [PubMed: 12586759]
91. Wu TW, Wang JM, Chen S, Brinton RD. 17Beta-Estradiol induced Ca²⁺ influx via L-type calcium channels activates the Src/Erk/CREB signal pathway and bcl-2 expression in rat hippocampal neurons: A potential initiation mechanism for estrogen-induced neuroprotection. *Neuroscience*. 2004submitted to
92. Zhao L, Chen S, Wang JM, Brinton RD. 17β -Estradiol induces Ca²⁺ influx, dendritic and nuclear Ca²⁺ rise and subsequent CREB activation in hippocampal neurons: A potential initiation mechanism for estrogen neurotrophism. *Neuroscience*. 2004In Press
93. Zhou Y, Watters JJ, Dorsa DM. Estrogen rapidly induces the phosphorylation of the cAMP response element binding protein in rat brain. *Endocrinology* 1996;137:2163–6. [PubMed: 8612562]
94. Nilsen J, Irwin RW, Gallaher TK, Brinton RD. Estradiol in vivo regulation of brain mitochondrial proteome. *J Neurosci* 2007;27:14069–77. [PubMed: 18094246]
95. Nilsen, J.; Irwin, RW.; Masri, R.; Brinton, RD. Hormone therapy enhances functional efficiency of brain mitochondria. *Endocrine Society Annual Meeting*; Boston, MA. 2006.
96. Nilsen, J.; Irwin, RW.; Yao, J.; Brinton, RD. Nuclear receptors and brain metabolism: Regulation of mitochondrial energetics by ovarian hormones, *Keystone Symposia: Nuclear Receptor Pathways to Metabolic Regulation*; Steamboat Springs, CO. 2007.
97. Nilsen, JaB, RD. Mitochondria as therapeutic targets of estrogen action in the central nervous system. *Current Drug Targets - CNS & Neurological Disorders*. 2004In Press
98. Wang J, Green PS, Simpkins JW. Estradiol protects against ATP depletion, mitochondrial membrane potential decline and the generation of reactive oxygen species induced by 3-nitropropionic acid in SK-N-SH human neuroblastoma cells. *J Neurochem* 2001;77:804–11. [PubMed: 11331409]
99. Borras C, Sastre J, Garcia-Sala D, Lloret A, Pallardo FV, Vina J. Mitochondria from females exhibit higher antioxidant gene expression and lower oxidative damage than males. *Free Radic Biol Med* 2003;34:546–52. [PubMed: 12614843]
100. Simpkins JW, Dykens JA. Mitochondrial mechanisms of estrogen neuroprotection. *Brain Res Rev*. 2007

101. Robertson CL, Puskar A, Hoffman GE, Murphy AZ, Saraswati M, Fiskum G. Physiologic progesterone reduces mitochondrial dysfunction and hippocampal cell loss after traumatic brain injury in female rats. *Exp Neurol* 2006;197:235–43. [PubMed: 16259981]
102. Ben-Ari Y. Limbic seizure and brain damage produced by kainic acid: Mechanisms and relevance to human temporal lobe epilepsy. *Neuroscience* 1985;14:375–403. [PubMed: 2859548]
103. Turski L, Ikonomidou C, Turski WA, Bortolotto ZA, Cavalheiro EA. Review: cholinergic mechanisms and epileptogenesis. The seizures induced by pilocarpine: a novel experimental model of intractable epilepsy. *Synapse* 1989;3:154–171. [PubMed: 2648633]
104. Jung ME, Lal H, Gatch MB. The discriminative stimulus effects of pentylenetetrazol as a model of anxiety: recent developments. *Neurosci Biobehav Rev* 2002;26:429–439. [PubMed: 12204190]
105. Hoffman GE, Moore N, Fiskum G, Murphy AZ. Ovarian steroid modulation of seizure severity and hippocampal cell death after kainic acid treatment. *Exp Neurol* 2003;182:124–134. [PubMed: 12821382]
106. Rhodes ME, Frye CA. Actions at GABA(A) receptors in the hippocampus may mediate some antiseizure effects of progestins. *Epilepsy Behav* 2005;6:320–327. [PubMed: 15820338]
107. Ciriza I, Azcoitia I, Garcia-Segura LM. Reduced progesterone metabolites protect rat hippocampal neurons from kainic acid excitotoxicity in vivo. *J Neuroendocrinol* 2004;16:58–63. [PubMed: 14962077]
108. Lephart ED, Husmann DA. Altered brain and pituitary androgen metabolism by prenatal, perinatal or pre- and postnatal finasteride, flutamide or dihydrotestosterone treatment in juvenile male rats. *Prog Neuropsychopharmacol Biol Psychiatry* 1993;17:991–1003. [PubMed: 8278608]
109. Rhodes ME, Frye CA. Progestins in the hippocampus of female rats have antiseizure effects in a pentylenetetrazole seizure model. *Epilepsia* 2004;45:1531–1538. [PubMed: 15571511]
110. Beyenburg S, Stoffel-Wagner B, Bauer J, Watzka M, Blumcke I, Bidlingmaier F, Elger CE. Neuroactive steroids and seizure susceptibility. *Epilepsy Res* 2001;44:141–153. [PubMed: 11325570]
111. Frye CA, Scalise TJ. Anti-seizure effects of progesterone and 3alpha,5alpha-THP in kainic acid and perforant pathway models of epilepsy. *Psychoneuroendocrinology* 2000;25:407–20. [PubMed: 10725616]
112. Frye CA. The neurosteroid 3 alpha, 5 alpha-THP has antiseizure and possible neuroprotective effects in an animal model of epilepsy. *Brain Res* 1995;696:113–20. [PubMed: 8574658]
113. Frye CA, Bayon LE. Seizure activity is increased in endocrine states characterized by decline in endogenous levels of the neurosteroid 3 alpha,5 alpha-THP. *Neuroendocrinology* 1998;68:272–80. [PubMed: 9772342]
114. Frye CA, Rhodes ME, Walf A, Harney J. Progesterone reduces pentylenetetrazol-induced ictal activity of wild-type mice but not those deficient in type I 5alpha-reductase. *Epilepsia* 2002;43:14–17. [PubMed: 12121288]
115. Reddy DS, Castaneda DC, O'Malley BW, Rogawski MA. Anticonvulsant activity of progesterone and neurosteroids in progesterone receptor knockout mice. *J Pharmacol Exp Ther* 2004;310:230–9. [PubMed: 14982969]
116. Kokate TG, Banks MK, Magee T, Yamaguchi S, Rogawski MA. Finasteride, a 5alpha-reductase inhibitor, blocks the anticonvulsant activity of progesterone in mice. *J Pharmacol Exp Ther* 1999;288:679–684. [PubMed: 9918575]
117. Ugale RR, Mittal N, Hirani K, Chopde CT. Essentiality of central GABAergic neuroactive steroid allopregnanolone for anticonvulsant action of fluoxetine against pentylenetetrazole-induced seizures in mice. *Brain Res* 2004;1023:102–111. [PubMed: 15364024]
118. Ardeshiri A, Kelley MH, Korner IP, Hurn PD, Herson PS. Mechanism of progesterone neuroprotection of rat cerebellar Purkinje cells following oxygen-glucose deprivation. *Eur J Neurosci* 2006;24:2567–74. [PubMed: 17100844]
119. Djebaili M, Guo Q, Pettus EH, Hoffman SW, Stein DG. The neurosteroids progesterone and allopregnanolone reduce cell death, gliosis, and functional deficits after traumatic brain injury in rats. *J Neurotrauma* 2005;22:106–18. [PubMed: 15665606]

120. Djebaili M, Hoffman SW, Stein DG. Allopregnanolone and progesterone decrease cell death and cognitive deficits after a contusion of the rat pre-frontal cortex. *Neuroscience* 2004;123:349–59. [PubMed: 14698743]
121. Roof RL, Duvdevani R, Braswell L, Stein DG. Progesterone facilitates cognitive recovery and reduces secondary neuronal loss caused by cortical contusion injury in male rats. *Exp Neurol* 1994;129:64–9. [PubMed: 7925843]
122. Zhu Y, Culmsee C, Klumpp S, Kriegelstein J. Neuroprotection by transforming growth factor-beta1 involves activation of nuclear factor-kappaB through phosphatidylinositol-3-OH kinase/Akt and mitogen-activated protein kinase-extracellular-signal regulated kinase1,2 signaling pathways. *Neuroscience* 2004;123:897–906. [PubMed: 14751283]
123. Gibson CL, Constantin D, Prior MJ, Bath PM, Murphy SP. Progesterone suppresses the inflammatory response and nitric oxide synthase-2 expression following cerebral ischemia. *Exp Neurol* 2005;193:522–530. [PubMed: 15869954]
124. Grossman KJ, Goss CW, Stein DG. Effects of progesterone on the inflammatory response to brain injury in the rat. *Brain Res* 2004;1008:29–39. [PubMed: 15081379]
125. Azcoitia I, Leonelli E, Magnaghi V, Veiga S, Garcia-Segura LM, Melcangi RC. Progesterone and its derivatives dihydroprogesterone and tetrahydroprogesterone reduce myelin fiber morphological abnormalities and myelin fiber loss in the sciatic nerve of aged rats. *Neurobiol Aging* 2003;24:853–60. [PubMed: 12927767]
126. Koenig HL, Schumacher M, Ferzaz B, Thi AN, Ressouches A, Guennoun R, Jung-Testas I, Robel P, Akwa Y, Baulieu EE. Progesterone synthesis and myelin formation by Schwann cells. *Science* 1995;268:1500–3. [PubMed: 7770777]
127. Murphy DD, Segal M. Progesterone prevents estradiol-induced dendritic spine formation in cultured hippocampal neurons. *Neuroendocrinology* 2000;72:133–143. [PubMed: 11025407]
128. Bimonte-Nelson HA, Nelson ME, Granholm AC. Progesterone counteracts estrogen-induced increases in neurotrophins in the aged female rat brain. *Neuroreport* 2004;15:2659–2663. [PubMed: 15570173]
129. Rosario ER, Ramsden M, Pike CJ. Progestins inhibit the neuroprotective effects of estrogen in rat hippocampus. *Brain Res* 2006;1099:206–10. [PubMed: 16793026]
130. Gundlach C, Kohama SG, Mirkes SJ, Garyfallou VT, Urbanski HF, Bethea CL. Distribution of estrogen receptor beta (ERbeta) mRNA in hypothalamus, midbrain and temporal lobe of spayed macaque: continued expression with hormone replacement. *Brain Res Mol Brain Res* 2000;76:191–204. [PubMed: 10762694]
131. Azcoitia I, Fernandez-Galaz C, Sierra A, Garcia-Segura LM. Gonadal hormones affect neuronal vulnerability to excitotoxin-induced degeneration. *J Neurocytol* 1999;28:699–710. [PubMed: 10859573]
132. Toung TJ, Chen TY, Littleton-Kearney MT, Hurn PD, Murphy SJ. Effects of combined estrogen and progesterone on brain infarction in reproductively senescent female rats. *J Cereb Blood Flow Metab* 2004;24:1160–1166. [PubMed: 15529016]
133. Shear DA, Galani R, Hoffman SW, Stein DG. Progesterone protects against necrotic damage and behavioral abnormalities caused by traumatic brain injury. *Exp Neurol* 2002;178:59–67. [PubMed: 12460608]
134. Roof RL, Duvdevani R, Stein DG. Gender influences outcome of brain injury: progesterone plays a protective role. *Brain Res* 1993;607:333–6. [PubMed: 8481809]
135. McEwen B, Akama K, Alves S, Brake WG, Bulloch K. Tracking the estrogen receptor in neurons: Implications for estrogen-induced synapse formation. *Proceedings of the National Academy of Sciences* 2001;98:7093–7100.
136. Woolley CS, Weiland NG, McEwen BS, Schwartzkroin PA. Estradiol increases the sensitivity of hippocampal CA1 pyramidal cells to NMDA receptor-mediated synaptic input: correlation with dendritic spine density. *J Neurosci* 1997;17:1848–59. [PubMed: 9030643]
137. Woolley CS, McEwen BS. Estradiol regulates hippocampal dendritic spine density via an N-methyl-D-aspartate receptor-dependent mechanism. *J Neurosci* 1994;14:7680–7. [PubMed: 7996203]
138. Teyler TJ, Vardaris RM, Lewis D, Rawitch AB. Gonadal steroids: Effect on excitability of hippocampal pyramidal cells. *Science* 1980;209:1017–1019. [PubMed: 7190730]

139. Foy MR, Xu J, Xie X, Brinton RD, Thompson RF, Berger TW. 17 β -estradiol enhances NMDA receptor-mediated EPSPs and long-term potentiation. *Journal of Neurophysiology* 1999;81:925–929. [PubMed: 10036289]
140. Wong M, Moss RL. Electrophysiological evidence for a rapid membrane action of the gonadal steroid, 17 β -estradiol, on CA1 pyramidal neurons of the rat hippocampus. *Brain Research Bulletin* 1991;54:148–152.
141. Wong M, Moss RL. Long-term and short-term electrophysiological effects of estrogen on the synaptic properties of hippocampal CA1 neurons. *Journal of Neuroscience* 1992;12:3217–3225. [PubMed: 1353794]
142. Baudry, M.; Davis, JL.; Thompson, RF. *Advances in Synaptic Plasticity*. The MIT Press; Cambridge, MA: 2000.
143. Bliss TVP, Collingridge GL. A synaptic model of memory: Long-term potentiation in the hippocampus. *Nature* 1993;361:31–39. [PubMed: 8421494]
144. Landfield, PW.; Deadwyler, SA. *Long-term potentiation: From biophysics to behavior*. Alan R. Liss, Inc.; New York: 1988.
145. Ito, M. *The cerebellum and neural control*. Raven Press; New York: 1984.
146. Bear MF, Malenka RC. Synaptic plasticity: LTP and LTD. *Current Opinion in Neurobiology* 1994;4:389–399. [PubMed: 7919934]
147. Dudek SM, Bear MF. Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. *Proceedings of the National Academy of Sciences* 1992;89:4363–4367.
148. Shors TJ, Matzel LD. Long-term potentiation: What's learning got to do with it? *Brain and Behavioral Sciences* 1997;20:597–614.
149. Bi GQ, Poo MM. Synaptic modification by correlated activity: Hebb's postulate revisited. *Annual Review of Neuroscience* 2001;24:139–166.
150. Grover LM, Teyler TJ. Two components of long-term potentiation induced by different patterns of afferent activation. *Nature* 1990;347:477–479. [PubMed: 1977084]
151. Ito KI, Skinkle KL, Hicks TP. Age-dependent, steroid-specific effects of oestrogen on long-term potentiation in rat hippocampal slices. *Journal of Physiology* 1999;515:209–220. [PubMed: 9925890]
152. Edwards HE, Errps T, Carlen PLJ, MacLusky N. Progesterone receptors mediate progesterone suppression of epileptiform activity in tetanized hippocampal slices in vitro. *Neuroscience* 2000;101:895–906. [PubMed: 11113338]
153. Feng XQ, Dong Y, Fu YM, Zhu YH, Sun JL. Progesterone inhibition of dopamine-induced increase in frequency of spontaneous excitatory postsynaptic currents in rat prelimbic cortical neurons. *Neuropharmacology* 2004;46:211–222. [PubMed: 14680759]
154. Wihlback A, Sundstrom-Poromaa I, Backstrom T. Action by and sensitivity to neuroactive steroids in menstrual cycle related CNS disorders. *Psychopharmacology (Berl)* 2006;186:388–401. [PubMed: 16362406]
155. Backstrom T, Sanders D, Leask R, Davidson D, Warner P, Bancroft J. Mood, sexuality, hormones, and the menstrual cycle. II. Hormone levels and their relationship to the premenstrual syndrome. *Psychosom Med* 1983;45:503–7. [PubMed: 6686333]
156. Sundstrom I, Andersson A, Nyberg S, Ashbrook D, Purdy RH, Backstrom T. Patients with premenstrual syndrome have a different sensitivity to a neuroactive steroid during the menstrual cycle compared to control subjects. *Neuroendocrinology* 1998;67:126–38. [PubMed: 9508043]
157. Andreen L, Sundstrom-Poromaa I, Bixo M, Andersson A, Nyberg S, Backstrom T. Relationship between allopregnanolone and negative mood in postmenopausal women taking sequential hormone replacement therapy with vaginal progesterone. *Psychoneuroendocrinology* 2005;30:212–24. [PubMed: 15471618]
158. Bixo M, Andersson A, Winblad B, Purdy RH, Backstrom T. Progesterone, 5 α -pregnane-3,20-dione and 3 α -hydroxy-5 α -pregnane-20-one in specific regions of the human female brain in different endocrine states. *Brain Res* 1997;764:173–8. [PubMed: 9295207]

159. Backstrom T, Andreen L, Birzniece V, Bjorn I, Johansson IM, Nordenstam-Haghjo M, Nyberg S, Sundstrom-Poromaa I, Wahlstrom G, Wang M, Zhu D. The role of hormones and hormonal treatments in premenstrual syndrome. *CNS Drugs* 2003;17:325–42. [PubMed: 12665391]
160. Brambilla P, Perez J, Barale F, Schettini G, Soares JC. GABAergic dysfunction in mood disorders. *Mol Psychiatry* 2003;8:721–37. 715. [PubMed: 12888801]
161. Majewska MD, Harrison NL, Schwartz RD, Barker JL, Paul SM. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science* 1986;232:1004–7. [PubMed: 2422758]
162. Majewska MD. Neurosteroids: endogenous bimodal modulators of the GABAA receptor. Mechanism of action and physiological significance. *Prog Neurobiol* 1992;38:379–95. [PubMed: 1349441]
163. Mitchell EA, Herd MB, Gunn BG, Lambert JJ, Belelli D. Neurosteroid modulation of GABA(A) receptors: Molecular determinants and significance in health and disease. *Neurochem Int.* 2007
164. van Wingen G, van Broekhoven F, Verkes RJ, Petersson KM, Backstrom T, Buitelaar J, Fernandez G. How progesterone impairs memory for biologically salient stimuli in healthy young women. *J Neurosci* 2007;27:11416–23. [PubMed: 17942736]
165. van Wingen G, van Broekhoven F, Verkes RJ, Petersson KM, Backstrom T, Buitelaar JK, Fernandez G. Progesterone selectively increases amygdala reactivity in women. *Mol Psychiatry.* 2007
166. Johansson IM, Birzniece V, Lindblad C, Olsson T, Backstrom T. Allopregnanolone inhibits learning in the Morris water maze. *Brain Research* 2002;934:125–131. [PubMed: 11955475]
167. Silvers JM, Tokunaga S, Berry RB, White AM, Matthews DB. Impairments in spatial learning and memory: ethanol, allopregnanolone, and the hippocampus. *Brain Res Brain Res Rev* 2003;43:275–84. [PubMed: 14629930]
168. Turkmen S, Lofgren M, Birzniece V, Backstrom T, Johansson IM. Tolerance development to Morris water maze test impairments induced by acute allopregnanolone. *Neuroscience* 2006;139:651–9. [PubMed: 16457954]
169. Gould E, Woolley CS, Frankfurt M, McEwen BS. Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. *J Neurosci* 1990;10:1286–91. [PubMed: 2329377]
170. Frankfurt M, Gould E, Woolley CS, McEwen BS. Gonadal steroids modify dendritic spine density in ventromedial hypothalamic neurons: a Golgi study in the adult rat. *Neuroendocrinology* 1990;51:530–5. [PubMed: 2112730]
171. Wilson MA. GABA physiology: Modulation by benzodiazepines and hormones. *Critical Review of Neurobiology* 1996;10:1–37.
172. Finn CA. Menstruation: a nonadaptive consequence of uterine evolution. *Q Rev Biol* 1998;73:163–73. [PubMed: 9618925]
173. Krzymowski T, Stefanczyk-Krzybowska S. Uterine blood supply as a main factor involved in the regulation of the estrous cycle—a new theory. *Reprod Biol* 2002;2:93–114. [PubMed: 14666152]
174. Lundeen SG, Zhang Z, Zhu Y, Carver JM, Winneker RC. Rat uterine complement C3 expression as a model for progesterone receptor modulators: characterization of the new progestin trimegestone. *J Steroid Biochem Mol Biol* 2001;78:137–43. [PubMed: 11566438]
175. Atasoy P, Bozdogan O, Erekul S, Bozdogan N, Bayram M. Fas-mediated pathway and apoptosis in normal, hyperplastic, and neoplastic endometrium. *Gynecol Oncol* 2003;91:309–17. [PubMed: 14599860]
176. Li SH, Huang HL, Chen YH. Ovarian steroid-regulated synthesis and secretion of complement C3 and factor B in mouse endometrium during the natural estrous cycle and pregnancy period. *Biol Reprod* 2002;66:322–32. [PubMed: 11804945]
177. King AE, Morgan K, Sallenave JM, Kelly RW. Differential regulation of secretory leukocyte protease inhibitor and elafin by progesterone. *Biochem Biophys Res Commun* 2003;310:594–9. [PubMed: 14521952]
178. Reyna-Neyra A, Arias C, Ferrera P, Morimoto S, Camacho-Arroyo I. Changes in the content and distribution of microtubule associated protein 2 in the hippocampus of the rat during the estrous cycle. *J Neurobiol* 2004;60:473–80. [PubMed: 15307151]
179. Choi JM, Romeo RD, Brake WG, Bethea CL, Rosenwaks Z, McEwen BS. Estradiol increases pre- and post-synaptic proteins in the CA1 region of the hippocampus in female rhesus macaques (*Macaca mulatta*). *Endocrinology* 2003;144:4734–8. [PubMed: 12960039]

180. Hung AJ, Stanbury MG, Shanabrough M, Horvath TL, Garcia-Segura LM, Naftolin F. Estrogen, synaptic plasticity and hypothalamic reproductive aging. *Exp Gerontol* 2003;38:53–9. [PubMed: 12543261]
181. Mydlarski MB, Liberman A, Schipper HM. Estrogen induction of glial heat shock proteins: implications for hypothalamic aging. *Neurobiol Aging* 1995;16:977–81. [PubMed: 8622790]
182. Woolley CS, Gould E, Frankfurt M, McEwen BS. Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. *J Neurosci* 1990;10:4035–9. [PubMed: 2269895]
183. Klintsova A, Levy WB, Desmond NL. Astrocytic volume fluctuates in the hippocampal CA1 region across the estrous cycle. *Brain Res* 1995;690:269–74. [PubMed: 8535849]
184. Cashion AB, Smith MJ, Wise PM. The morphometry of astrocytes in the rostral preoptic area exhibits a diurnal rhythm on proestrus: relationship to the luteinizing hormone surge and effects of age. *Endocrinology* 2003;144:274–80. [PubMed: 12488355]
185. Stone DJ, Song Y, Anderson CP, Krohn KK, Finch CE, Rozovsky I. Bidirectional transcription regulation of glial fibrillary acidic protein by estradiol in vivo and in vitro. *Endocrinology* 1998;139:3202–9. [PubMed: 9645694]
186. Giachino C, Galbiati M, Fasolo A, Peretto P, Melcangi RC. Effects of progesterone derivatives, dihydroprogesterone and tetrahydroprogesterone, on the subependymal layer of the adult rat. *J Neurobiol* 2004;58:493–502. [PubMed: 14978726]
187. Mahley RW, Rall SC Jr. Apolipoprotein E: far more than a lipid transport protein. *Annu Rev Genomics Hum Genet* 2000;1:507–37. [PubMed: 11701639]
188. Stone DJ, Rozovsky I, Morgan TE, Anderson CP, Hajian H, Finch CE. Astrocytes and microglia respond to estrogen with increased apoE mRNA in vivo and in vitro. *Exp Neurol* 1997;143:313–8. [PubMed: 9056393]
189. Nathan BP, Barsukova AG, Shen F, McAsey M, Struble RG. Estrogen facilitates neurite extension via apolipoprotein E in cultured adult mouse cortical neurons. *Endocrinology* 2004;145:3065–73. [PubMed: 15033916]
190. Umans L, Overbergh L, Serneels L, Tesseur I, Van Leuven F. Analysis of expression of genes involved in apolipoprotein E-based lipoprotein metabolism in pregnant mice deficient in the receptor-associated protein, the low density lipoprotein receptor, or apolipoprotein E. *Biol Reprod* 1999;61:1216–25. [PubMed: 10529267]
191. Stone DJ, Rozovsky I, Morgan TE, Anderson CP, Finch CE. Increased synaptic sprouting in response to estrogen via an apolipoprotein E-dependent mechanism: implications for Alzheimer's disease. *J Neurosci* 1998;18:3180–5. [PubMed: 9547226]
192. Teter B, Harris-White ME, Frautschy SA, Cole GM. Role of apolipoprotein E and estrogen in mossy fiber sprouting in hippocampal slice cultures. *Neuroscience* 1999;91:1009–16. [PubMed: 10391478]
193. Rozovsky I, Wei M, Stone DJ, Zanjani H, Anderson CP, Morgan TE, Finch CE. Estradiol (E2) enhances neurite outgrowth by repressing glial fibrillary acidic protein expression and reorganizing laminin. *Endocrinology* 2002;143:636–46. [PubMed: 11796520]
194. Duan H, Gu D, Mazzone T. Sterols and inhibitors of sterol transport modulate the degradation and secretion of macrophage ApoE: requirement for the C-terminal domain. *Biochim Biophys Acta* 2000;1484:142–50. [PubMed: 10760464]
195. Rozovsky I, Hoving S, Anderson CP, O'Callaghan J, Finch CE. Equine estrogens induce apolipoprotein E and glial fibrillary acidic protein in mixed glial cultures. *Neurosci Lett* 2002;323:191–4. [PubMed: 11959417]
196. Garcia-Estrada J, Luquin S, Fernandez AM, Garcia-Segura LM. Dehydroepiandrosterone, pregnenolone and sex steroids down-regulate reactive astroglia in the male rat brain after a penetrating brain injury. *Int J Dev Neurosci* 1999;17:145–51. [PubMed: 10221674]
197. Garcia-Estrada J, Del Rio JA, Luquin S, Soriano E, Garcia-Segura LM. Gonadal hormones down-regulate reactive gliosis and astrocyte proliferation after a penetrating brain injury. *Brain Res* 1993;628:271–8. [PubMed: 8313156]
198. Gruber CJ, Huber JC. Differential effects of progestins on the brain. *Maturitas* 2003;46:S71–5. [PubMed: 14670648]

199. Frye CA. The role of neurosteroids and non-genomic effects of progestins and androgens in mediating sexual receptivity of rodents. *Brain Res Brain Res Rev* 2001;37:201–22. [PubMed: 11744087]
200. Martini L, Magnaghi V, Melcangi RC. Actions of progesterone and its 5alpha-reduced metabolites on the major proteins of the myelin of the peripheral nervous system. *Steroids* 2003;68:825–9. [PubMed: 14667974]
201. Baulieu EE, Godeau F, Schorderet M, Schorderet-Slatkine S. Steroid-induced meiotic division in *Xenopus laevis* oocytes: surface and calcium. *Nature* 1978;275:593–8. [PubMed: 30046]
202. Channing CP, Hillensjo T, Schaerf FW. Hormonal control of oocyte meiosis, ovulation and luteinization in mammals. *Clin Endocrinol Metab* 1978;7:601–24. [PubMed: 215357]
203. Schorderet-Slatkine S, Schorderet M, Baulieu EE. Progesterone-induced meiotic reinitiation in vitro in *Xenopus laevis* oocytes: a role for the displacement of membrane-bound calcium. *Differentiation* 1977;9:67–76. [PubMed: 73488]
204. Baulieu EE. Cell membrane, a target for steroid hormones. *Mol Cell Endocrinol* 1978;12:247–54. [PubMed: 367849]
205. Bayaa M, Booth RA, Sheng Y, Liu XJ. The classical progesterone receptor mediates *Xenopus* oocyte maturation through a nongenomic mechanism. *Proc Natl Acad Sci U S A* 2000;97:12607–12. [PubMed: 11050156]
206. Thomas P, Zhu Y, Pace M. Progesterone membrane receptors involved in the meiotic maturation of teleost oocytes: a review with some new findings. *Steroids* 2002;67:511–7. [PubMed: 11960629]
207. Hammes SR, Levin ER. Extranuclear steroid receptors: nature and actions. *Endocr Rev* 2007;28:726–41. [PubMed: 17916740]
208. Bement WM, Capco DG. Intracellular signals trigger ultrastructural events characteristic of meiotic maturation in oocytes of *Xenopus laevis*. *Cell Tissue Res* 1989;255:183–91. [PubMed: 2544275]
209. Moreau M, Vilain JP, Guerrier P. Free calcium changes associated with hormone action in amphibian oocytes. *Dev Biol* 1980;78:201–14. [PubMed: 6249687]
210. Wasserman WJ, Pinto LH, O'Connor CM, Smith LD. Progesterone induces a rapid increase in $[Ca^{2+}]_i$ in *Xenopus laevis* oocytes. *Proc Natl Acad Sci U S A* 1980;77:1534–6. [PubMed: 6929506]
211. Lutz LB, Kim B, Jahani D, Hammes SR. G protein beta gamma subunits inhibit nongenomic progesterone-induced signaling and maturation in *Xenopus laevis* oocytes. Evidence for a release of inhibition mechanism for cell cycle progression. *J Biol Chem* 2000;275:41512–20. [PubMed: 11018039]
212. Fabian JR, Morrison DK, Daar IO. Requirement for Raf and MAP kinase function during the meiotic maturation of *Xenopus* oocytes. *J Cell Biol* 1993;122:645–52. [PubMed: 8335690]
213. Karaiskou A, Dupre A, Haccard O, Jessus C. From progesterone to active Cdc2 in *Xenopus* oocytes: a puzzling signalling pathway. *Biology of the Cell* 2001;93:35–46. [PubMed: 11730320]
214. Fisher DL, Brassac T, Galas S, Doree M. Dissociation of MAP kinase activation and MPF activation in hormone-stimulated maturation of *Xenopus* oocytes. *Development* 1999;126:4537–46. [PubMed: 10498688]
215. Lenormand JL, Dellinger RW, Knudsen KE, Subramani S, Donoghue DJ. Speedy: a novel cell cycle regulator of the G2/M transition. *EMBO Journal* 1999;18:1869–77. [PubMed: 10202150]
216. Masui Y. From oocyte maturation to the in vitro cell cycle: the history of discoveries of Maturation-Promoting Factor (MPF) and Cytostatic Factor (CSF). *Differentiation* 2001;69:1–17. [PubMed: 11776390]
217. Conneely OM, Mulac-Jericevic B, DeMayo F, Lydon JP, O'Malley BW. Reproductive functions of progesterone receptors. *Recent Prog Horm Res* 2002;57:339–55. [PubMed: 12017551]
218. Curtis SW, Clark J, Myers P, Korach KS. Disruption of estrogen signaling does not prevent progesterone action in the estrogen receptor alpha knockout mouse uterus. *Proc Natl Acad Sci U S A* 1999;96:3646–51. [PubMed: 10097091]
219. Dai D, Wolf DM, Litman ES, White MJ, Leslie KK. Progesterone inhibits human endometrial cancer cell growth and invasiveness: down-regulation of cellular adhesion molecules through progesterone B receptors. *Cancer Res* 2002;62:881–6. [PubMed: 11830547]
220. Glasser SR, Clark JH. A determinant role for progesterone in the development of uterine sensitivity to decidualization and ovo-implantation. *Symp Soc Dev Biol* 1975:311–45. [PubMed: 168660]

221. Hsueh AJ, Peck EJ Jr, Clark JH. Progesterone antagonism of the oestrogen receptor and oestrogen-induced uterine growth. *Nature* 1975;254:337–9. [PubMed: 163981]
222. Kirkland JL, Murthy L, Stancel GM. Progesterone inhibits the estrogen-induced expression of c-fos messenger ribonucleic acid in the uterus. *Endocrinology* 1992;130:3223–30. [PubMed: 1375896]
223. Shoupe D, Meme D, Mezrow G, Lobo RA. Prevention of endometrial hyperplasia in postmenopausal women with intrauterine progesterone. *N Engl J Med* 1991;325:1811–2. [PubMed: 1944490]
224. Kiss R, Paridaens RJ, Heuson JC, Danguy AJ. Effect of progesterone on cell proliferation in the MXT mouse hormone-sensitive mammary neoplasm. *J Natl Cancer Inst* 1986;77:173–8. [PubMed: 3459912]
225. Pike MC, Ross RK. Progestins and menopause: epidemiological studies of risks of endometrial and breast cancer. *Steroids* 2000;65:659–64. [PubMed: 11108873]
226. Bernstein L. Epidemiology of endocrine-related risk factors for breast cancer. *J Mammary Gland Biol Neoplasia* 2002;7:3–15. [PubMed: 12160084]
227. Chlebowski RT, Hendrix SL, Langer RD, Stefanick ML, Gass M, Lane D, Rodabough RJ, Gilligan MA, Cyr MG, Thomson CA, Khandekar J, Petrovitch H, McTiernan A. Influence of estrogen plus progestin on breast cancer and mammography in healthy postmenopausal women: the Women's Health Initiative Randomized Trial. *Jama* 2003;289:3243–53. [PubMed: 12824205]
228. Writing Group for the Women's Health Initiative Investigators. Risks and Benefits of Estrogen Plus Progestin in Healthy Postmenopausal Women: Principal Results From the Women's Health Initiative Randomized Controlled Trial. *JAMA* 2002;288:321–333. [PubMed: 12117397]
229. Weiss LK, Burkman RT, Cushing-Haugen KL, Voigt LF, Simon MS, Daling JR, Norman SA, Bernstein L, Ursin G, Marchbanks PA, Strom BL, Berlin JA, Weber AL, Doody DR, Wingo PA, McDonald JA, Malone KE, Folger SG, Spirtas R. Hormone replacement therapy regimens and breast cancer risk(1). *Obstet Gynecol* 2002;100:1148–58. [PubMed: 12468157]
230. Braunsberg H, Coldham NG, Leake RE, Cowan SK, Wong W. Actions of a progestogen on human breast cancer cells: mechanisms of growth stimulation and inhibition. *Eur J Cancer Clin Oncol* 1987;23:563–71. [PubMed: 2958286]
231. The Women's Health Initiative Steering Committee. Effects of Conjugated Equine Estrogen in Postmenopausal Women With Hysterectomy: The Women's Health Initiative Randomized Controlled Trial. *JAMA* 2004;291:1701–1712. [PubMed: 15082697]
232. Gann PH, Morrow M. Combined Hormone Therapy and Breast Cancer: A Single-Edged Sword. *JAMA* 2003;289:3304–3306. [PubMed: 12824214]
233. Anderson GL, Judd HL, Kaunitz AM, Barad DH, Beresford SA, Pettinger M, Liu J, McNeeley SG, Lopez AM. Effects of estrogen plus progestin on gynecologic cancers and associated diagnostic procedures: the Women's Health Initiative randomized trial. *Jama* 2003;290:1739–48. [PubMed: 14519708]
234. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *Jama* 2002;288:321–33. [PubMed: 12117397]
235. Stanczyk FZ. Pharmacokinetics of progesterone administered by the oral and parenteral routes. *J Reprod Med* 1999;44:141–7. [PubMed: 11392023]
236. Stanczyk FZ. Pharmacokinetics and potency of progestins used for hormone replacement therapy and contraception. *Rev Endocr Metab Disord* 2002;3:211–24. [PubMed: 12215716]
237. Baker LD, Sambamurti K, Craft S, Cherrier M, Raskind MA, Stanczyk FZ, Plymate SR, Asthana S. 17 β -Estradiol reduces plasma A β 40 for HRT-naive postmenopausal women with Alzheimer disease: a preliminary study. *Am J Geriatr Psychiatry* 2003;11:239–244. [PubMed: 12611754]
238. Graham JD, Clarke CL. Physiological action of progesterone in target tissues. *Endocr Rev* 1997;18:502–19. [PubMed: 9267762]
239. Tanapat P, Hastings NB, Gould E. Ovarian steroids influence cell proliferation in the dentate gyrus of the adult female rat in a dose- and time-dependent manner. *Journal of Comparative Neurology* 2004;481:252–265. [PubMed: 15593136]
240. Brinton, RD.; Wang, J.; Liu, L. Progesterone regulation of neural progenitor proliferation. Society for Neuroscience Annual Meeting; San Diego, CA. 2007. p. 56p. 16

241. Wang JM, Johnston P, Ball B, Brinton RD. The neurosteroid allopregnanolone promotes proliferation of rodent and human neural progenitor and regulates cell-cycle gene and protein expression. *Journal of Neuroscience* 2005;25:4706–4718. [PubMed: 15888646]
242. Wang, JaB, RD. Allopregnanolone induces a rapid transient rise in intracellular calcium in embryonic hippocampal neurons via L-type calcium channel dependent mechanism. *Current Alzheimer Research*. 2008In Press
243. Lai YL, Smith PM, Lamm WJ, Hildebrandt J. Sampling and analysis of cerebrospinal fluid for chronic studies in awake rats. *J Appl Physiol* 1983;54:1754–7. [PubMed: 6409862]
244. Griffin LD, Gong W, Verot L, Mellon SH. Niemann-Pick type C disease involves disrupted neurosteroidogenesis and responds to allopregnanolone. *Nat Med* 2004;10:704–11. [PubMed: 15208706]
245. Gould E, Gross CG. Neurogenesis in adult mammals: some progress and problems. *Journal of Neuroscience* 2002;22:619–23. [PubMed: 11826089]
246. Gage FH. Neurogenesis in the Adult Brain. *J Neurosci* 2002;22:612–613. [PubMed: 11826087]
247. Kuhn HG, Dickinson-Anson H, Gage FH. Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *J Neurosci* 1996;16:2027–33. [PubMed: 8604047]
248. Rao MS, Hattiangady B, Abdel-Rahman A, Stanley DP, Shetty AK. Newly born cells in the ageing dentate gyrus display normal migration, survival and neuronal fate choice but endure retarded early maturation. *Eur J Neurosci* 2005;21:464–76. [PubMed: 15673445]
249. Dobrossy MD, Drapeau E, Aourousseau C, Le Moal M, Piazza PV, Abrous DN. Differential effects of learning on neurogenesis: learning increases or decreases the number of newly born cells depending on their birth date. *Mol Psychiatry* 2003;8:974–82. [PubMed: 14647395]
250. Gage FH. Brain, repair yourself. *Sci Am* 2003;289:46–53. [PubMed: 12951827]
251. Geinisman Y, Ganeshina O, Yoshida R, Berry RW, Disterhoft JF, Gallagher M. Aging, spatial learning, and total synapse number in the rat CA1 stratum radiatum. *Neurobiol Aging* 2004;25:407–16. [PubMed: 15123345]
252. Aberg MA, Aberg ND, Hedbacker H, Oscarsson J, Eriksson PS. Peripheral infusion of IGF-I selectively induces neurogenesis in the adult rat hippocampus. *Journal of Neuroscience* 2000;20:2896–903. [PubMed: 10751442]
253. Hallbergson AF, Gnatenco C, Peterson DA. Neurogenesis and brain injury: managing a renewable resource for repair. *J Clin Invest* 2003;112:1128–33. [PubMed: 14561695]
254. Bhatnagar M, Cintra A, Chadi G, Lindberg J, Oitzl M, De Kloet ER, Moller A, Agnati LF, Fuxe K. Neurochemical changes in the hippocampus of the brown Norway rat during aging. *Neurobiol Aging* 1997;18:319–27. [PubMed: 9263198]
255. Shetty AK, Hattiangady B, Shetty GA. Stem/progenitor cell proliferation factors FGF-2, IGF-1, and VEGF exhibit early decline during the course of aging in the hippocampus: Role of astrocytes. *Glia*. 2005
256. Palmer TD, Markakis EA, Willhoite AR, Safar F, Gage FH. Fibroblast growth factor-2 activates a latent neurogenic program in neural stem cells from diverse regions of the adult CNS. *Journal of Neuroscience* 1999;19:8487–97. [PubMed: 10493749]
257. Reuss B, von Bohlen und Halbach O. Fibroblast growth factors and their receptors in the central nervous system. *Cell Tissue Res* 2003;313:139–57. [PubMed: 12845521]
258. Tropepe V, Sibilio M, Ciruna BG, Rossant J, Wagner EF, van der Kooy D. Distinct neural stem cells proliferate in response to EGF and FGF in the developing mouse telencephalon. *Developmental Biology* 1999;208:166–88. [PubMed: 10075850]
259. Cheng Y, Tao Y, Black IB, DiCicco-Bloom E. A single peripheral injection of basic fibroblast growth factor (bFGF) stimulates granule cell production and increases cerebellar growth in newborn rats. *J Neurobiol* 2001;46:220–9. [PubMed: 11169507]
260. Cheng Y, Black IB, DiCicco-Bloom E. Hippocampal granule neuron production and population size are regulated by levels of bFGF. *Eur J Neurosci* 2002;15:3–12. [PubMed: 11860501]
261. Wagner JP, Black IB, DiCicco-Bloom E. Stimulation of neonatal and adult brain neurogenesis by subcutaneous injection of basic fibroblast growth factor. *J Neurosci* 1999;19:6006–16. [PubMed: 10407038]

262. Gage FH, Kempermann G, Palmer TD, Peterson DA, Ray J. Multipotent progenitor cells in the adult dentate gyrus. *Journal of Neurobiology* 1998;36:249–66. [PubMed: 9712308]
263. Horner PJ, Gage FH. Regeneration in the adult and aging brain. *Arch Neurol* 2002;59:1717–20. [PubMed: 12433257]
264. Jin K, Sun Y, Xie L, Batteur S, Mao XO, Smelick C, Logvinova A, Greenberg DA. Neurogenesis and aging: FGF-2 and HB-EGF restore neurogenesis in hippocampus and subventricular zone of aged mice. *Aging Cell* 2003;2:175–83. [PubMed: 12882410]
265. Kuhn HG, Winkler J, Kempermann G, Thal LJ, Gage FH. Epidermal Growth Factor and Fibroblast Growth Factor-2 Have Different Effects on Neural Progenitors in the Adult Rat Brain. *J Neurosci* 1997;17:5820–5829. [PubMed: 9221780]
266. Aberg MA, Aberg ND, Palmer TD, Alborn AM, Carlsson-Skewir C, Bang P, Rosengren LE, Olsson T, Gage FH, Eriksson PS. IGF-I has a direct proliferative effect in adult hippocampal progenitor cells. *Mol Cell Neurosci* 2003;24:23–40. [PubMed: 14550766]
267. Lichtenwalner RJ, Forbes ME, Bennett SA, Lynch CD, Sonntag WE, Riddle DR. Intracerebroventricular infusion of insulin-like growth factor-I ameliorates the age-related decline in hippocampal neurogenesis. *Neuroscience* 2001;107:603–13. [PubMed: 11720784]
268. Sun Y, Jin K, Xie L, Childs J, Mao XO, Logvinova A, Greenberg DA. VEGF-induced neuroprotection, neurogenesis, and angiogenesis after focal cerebral ischemia. *J Clin Invest* 2003;111:1843–51. [PubMed: 12813020]
269. Jin K, Mao XO, Sun Y, Xie L, Jin L, Nishi E, Klagsbrun M, Greenberg DA. Heparin-binding epidermal growth factor-like growth factor: hypoxia-inducible expression in vitro and stimulation of neurogenesis in vitro and in vivo. *J Neurosci* 2002;22:5365–73. [PubMed: 12097488]
270. Jin K, Zhu Y, Sun Y, Mao XO, Xie L, Greenberg DA. Vascular endothelial growth factor (VEGF) stimulates neurogenesis in vitro and in vivo. *Proc Natl Acad Sci U S A* 2002;99:11946–50. [PubMed: 12181492]
271. Zhang H, Vutskits L, Pepper MS, Kiss JZ. VEGF is a chemoattractant for FGF-2-stimulated neural progenitors. *J Cell Biol* 2003;163:1375–84. [PubMed: 14691144]
272. Stewart F, Power CA, Lennard SN, Allen WR, Amet L, Edwards RM. Identification of the horse epidermal growth factor (EGF) coding sequence and its use in monitoring EGF gene expression in the endometrium of the pregnant mare. *J Mol Endocrinol* 1994;12:341–50. [PubMed: 7916972]
273. Lennard SN, Gerstenberg C, Allen WR, Stewart F. Expression of epidermal growth factor and its receptor in equine placental tissues. *J Reprod Fertil* 1998;112:49–57. [PubMed: 9538329]
274. Lennard SN, Stewart F, Allen WR. Insulin-like growth factor II gene expression in the fetus and placenta of the horse during the first half of gestation. *J Reprod Fertil* 1995;103:169–79. [PubMed: 7707294]
275. Gerstenberg C, Allen WR, Stewart F. Factors controlling epidermal growth factor (EGF) gene expression in the endometrium of the mare. *Mol Reprod Dev* 1999;53:255–65. [PubMed: 10369386]
276. Begliuomini S, Casarosa E, Pluchino N, Lenzi E, Centofanti M, Freschi L, Pieri M, Genazzani AD, Luisi S, Genazzani AR. Influence of endogenous and exogenous sex hormones on plasma brain-derived neurotrophic factor. *Hum Reprod* 2007;22:995–1002. [PubMed: 17251358]
277. Blalock EM, Geddes JW, Chen KC, Porter NM, Markesbery WR, Landfield PW. Incipient Alzheimer's disease: microarray correlation analyses reveal major transcriptional and tumor suppressor responses. *Proc Natl Acad Sci U S A* 2004;101:2173–8. [PubMed: 14769913]
278. Yang Y, Mufson EJ, Herrup K. Neuronal cell death is preceded by cell cycle events at all stages of Alzheimer's disease. *J Neurosci* 2003;23:2557–63. [PubMed: 12684440]
279. Lie DC, Song H, Colamarino SA, Ming GL, Gage FH. Neurogenesis in the adult brain: New Strategies for Central Nervous System Diseases. *Annu Rev Pharmacol Toxicol* 2004;44:399–421. [PubMed: 14744252]
280. Weill-Engerer S, David JP, Sazdovitch V, Liere P, Eychenne B, Pianos A, Schumacher M, Delacourte A, Baulieu EE, Akwa Y. Neurosteroid quantification in human brain regions: comparison between Alzheimer's and nondemented patients. *J Clin Endocrinol Metab* 2002;87:5138–43. [PubMed: 12414884]

281. Busser J, Geldmacher DS, Herrup K. Ectopic Cell Cycle Proteins Predict the Sites of Neuronal Cell Death in Alzheimer's Disease Brain. *J Neurosci* 1998;18:2801–2807. [PubMed: 9525997]
282. Yang Y, Geldmacher DS, Herrup K. DNA replication precedes neuronal cell death in Alzheimer's disease. *J Neurosci* 2001;21:2661–8. [PubMed: 11306619]
283. Hardy J, Duff K, Hardy KG, Perez-Tur J, Hutton M. Genetic dissection of Alzheimer's disease and related dementias: amyloid and its relationship to tau. *Nat Neurosci* 1998;1:355–358. [PubMed: 10196523]
284. Hardy JA, Higgins GA. Alzheimer's disease: The amyloid cascade hypothesis. *Science* 1992;256:184–185. [PubMed: 1566067]
285. Sommer B. Alzheimer's disease and the amyloid cascade hypothesis: ten years on. *Curr Opin Pharmacol* 2002;2:87–92. [PubMed: 11786314]
286. Cleary JP, Walsh DM, Hofmeister JJ, Shankar GM, Kuskowski MA, Selkoe DJ, Ashe KH. Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function. *Nat Neurosci* 2005;8:79–84. [PubMed: 15608634]
287. Pike CJ, Burdick D, Walencewicz AJ, Glabe CG, Cotman CW. Neurodegeneration induced by β -amyloid peptides *in vitro*: the role of peptide assembly state. *J Neurosci* 1993;13:1676–1687. [PubMed: 8463843]
288. Pike CJ, Walencewicz AJ, Glabe CG, Cotman CW. Aggregation-related toxicity of synthetic β -amyloid protein in hippocampal cultures. *Euro J Pharm* 1991;207:367–368.
289. Selkoe DJ. Amyloid β -protein and the genetics of Alzheimer's disease. *J Biol Chem* 1996;271:18295–18298. [PubMed: 8756120]
290. Lorenzo A, Yankner BA. β -Amyloid neurotoxicity requires fibril formation and is inhibited by congo red. *Proc Natl Acad Sci USA* 1994;91:12243–12247. [PubMed: 7991613]
291. Mattson MP, Barger SW, Cheng B, Lieberburg I, Smith-Swintosky VL, Rydel RE. β -Amyloid precursor protein metabolites and loss of neuronal Ca^{2+} homeostasis in Alzheimer's disease. *Trends Neurosci* 1993;16:409–414. [PubMed: 7504356]
292. Simmons LK, May PC, Tomaselli KJ, Rydel RE, Fuson KS, Brigham EF, Wright S, Lieberburg I, Becker GW, Brems DN, Li WY. Secondary structure of amyloid b peptide correlates with neurotoxic activity *in vitro*. *Mol Pharmacol* 1994;45:373–379. [PubMed: 8145724]
293. Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Trommer B, Viola KL, Wals P, Zhang C, Finch CE, Krafft GA, Klein WL. Diffusible, nonfibrillar ligands derived from A β 1–42 are potent central nervous system neurotoxins. *Proc Natl Acad Sci USA* 1998;95:6448–6453. [PubMed: 9600986]
294. Golde TE. The A β hypothesis: leading us to rationally-designed therapeutic strategies for the treatment or prevention of Alzheimer disease. *Brain Pathol* 2005;15:84–87. [PubMed: 15779241]
295. Klein WL, Krafft GA, Finch CE. Targeting small A β oligomers: the solution to an Alzheimer's disease conundrum? *Trends Neurosci* 2001;24:219–224. [PubMed: 11250006]
296. Schumacher M, Weill-Engerer S, Liere P, Robert F, Franklin RJ, Garcia-Segura LM, Lambert JJ, Mayo W, Melcangi RC, Parduc A, Suter U, Carelli C, Baulieu EE, Akwa Y. Steroid hormones and neurosteroids in normal and pathological aging of the nervous system. *Prog Neurobiol* 2003;71:3–29. [PubMed: 14611864]
297. Schonknecht P, Pantel J, Klinga K, Jensen M, Hartmann T, Salbach B, Schroder J. Reduced cerebrospinal fluid estradiol levels are associated with increased beta-amyloid levels in female patients with Alzheimer's disease. *Neurosci Lett* 2001;307:122–4. [PubMed: 11427315]
298. Chang D, Kwan J, Timiras PS. Estrogens influence growth, maturation, and amyloid beta-peptide production in neuroblastoma cells and in a beta-APP transfected kidney 293 cell line. *Advances in Experimental Medicine and Biology* 1997;429:261–71. [PubMed: 9413580]
299. Jaffe AB, Toran-Allerand CD, Greengard P, Gandy SE. Estrogen regulates metabolism of Alzheimer amyloid beta precursor protein. *J Biol Chem* 1994;269:13065–13068. [PubMed: 8175728]
300. Xu H, Gouras GK, Greenfield JP, Vincent B, Naslund J, Mazzarelli L, Fried G, Jovanovic JN, Seeger M, Relkin NR, Liao F, Checler F, Buxbaum JD, Chait BT, Thinakaran G, Sisodia SS, Wang R, Greengard P, Gandy S. Estrogen reduces neuronal generation of Alzheimer β -amyloid peptides. *Nature Med* 1998;4:447–451. [PubMed: 9546791]

301. Yaffe K, Sawaya G, Lieberburg I, Grady D. Estrogen therapy in postmenopausal women: effects on cognitive function and dementia. *Jama* 1998;279:688–695. [PubMed: 9496988]
302. Vincent B, Smith JD. Effect of estradiol on neuronal Swedish-mutated beta-amyloid precursor protein metabolism: reversal by astrocytic cells. *Biochemical and Biophysical Research Communications* 2000;271:82–5. [PubMed: 10777685]
303. Greenfield JP, Leung LW, Cai D, Kaasik K, Gross RS, Rodriguez-Boulan E, Greengard P, Xu H. Estrogen lowers Alzheimer beta-amyloid generation by stimulating trans-Golgi network vesicle biogenesis. *J Biol Chem* 2002;277:12128–12136. [PubMed: 11823458]
304. Savage MJ, Trusko SP, Howland DS, Pinsker LR, Mistretta S, Reaume AG, Greenberg BD, Siman R, Scott RW. Turnover of amyloid beta-protein in mouse brain and acute reduction of its level by phorbol ester. *The Journal of Neuroscience : the Official Journal of the Society For Neuroscience* 1998;18:1743–52. [PubMed: 9464999]
305. Petanceska SS, Nagy V, Frail D, Gandy S. Ovariectomy and 17beta-estradiol modulate the levels of Alzheimer's amyloid beta peptides in brain. *Experimental Gerontology* 2000;35:1317–25. [PubMed: 11113610]
306. Levin-Allerhand JA, Lominska CE, Wang J, Smith JD. 17alpha-estradiol and 17beta-estradiol treatments are effective in lowering cerebral amyloid-beta levels in AbetaPPSWE transgenic mice. *J Alzheimers Dis* 2002;4:449–457. [PubMed: 12515896]
307. Zheng H, Xu H, Uljon SN, Gross R, Hardy K, Gaynor J, Lafrancois J, Simpkins J, Refolo LM, Petanceska S, Wang R, Duff K. Modulation of A(beta) peptides by estrogen in mouse models. *Journal of Neurochemistry* 2002;80:191–6. [PubMed: 11796757]
308. Green PS, Bales K, Paul S, Bu G. Estrogen therapy fails to alter amyloid deposition in the PDAPP model of Alzheimer's disease. *Endocrinology* 2005;146:2774–2781. [PubMed: 15731362]
309. Heikkinen T, Kalesnykas G, Rissanen A, Tapiola T, Iivonen S, Wang J, Chaudhuri J, Tanila H, Miettinen R, Puolivali J. Estrogen treatment improves spatial learning in APP + PS1 mice but does not affect beta amyloid accumulation and plaque formation. *Exp Neurol* 2004;187:105–17. [PubMed: 15081593]
310. Yue X, Lu M, Lancaster T, Cao P, Honda S, Staufenbiel M, Harada N, Zhong Z, Shen Y, Li R. Brain estrogen deficiency accelerates Abeta plaque formation in an Alzheimer's disease animal model. *Proc Natl Acad Sci U S A* 2005;102:19198–203. [PubMed: 16365303]
311. Mellon SH, Griffin LD. Neurosteroids: biochemistry and clinical significance. *Trends Endocrinol Metab* 2002;13:35–43. [PubMed: 11750861]
312. Mellon SH, Griffin LD. Synthesis, regulation, and function of neurosteroids. *Endocr Res* 2002;28:463. [PubMed: 12530649]
313. Compagnone NA, Mellon SH. Neurosteroids: biosynthesis and function of these novel neuromodulators. *Front Neuroendocrinol* 2000;21:1–56. [PubMed: 10662535]
314. Griffin LD, Mellon SH. Biosynthesis of the neurosteroid 3 alpha-hydroxy-4-pregnen-20-one (3 alpha hp), a specific inhibitor of FSH release. *Endocrinology* 2001;142:4617–22. [PubMed: 11606426]
315. Lauber ME, Lichtensteiger W. Ontogeny of 5 alpha-reductase (type 1) messenger ribonucleic acid expression in rat brain: early presence in germinal zones. *Endocrinology* 1996;137:2718–30. [PubMed: 8770891]
316. Noseworthy JH, Luchinetti C, Rodriguez M, Weinshenker BG. Multiple Sclerosis. *New England Journal of Medicine* 2000;342:938–952. [PubMed: 10738053]

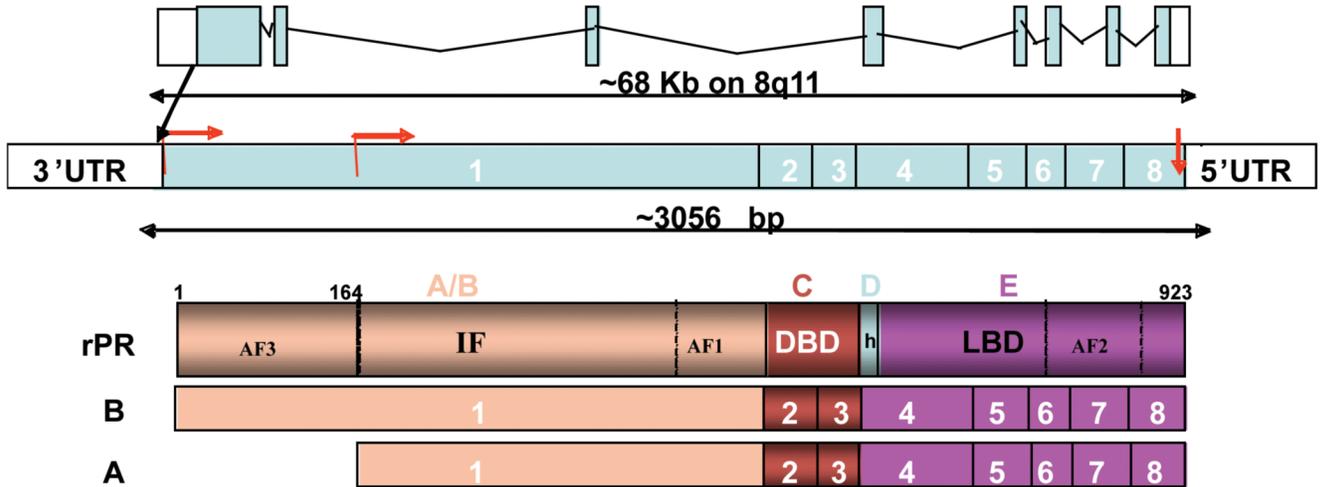


Fig. 1. Gene structure and functional domains of rat cPRA and cPRB. In rat, the classical progesterone receptor is composed of 8 exons with a 3100-bp coding region and 5'- and 3'-untranslated region. Both cPRB and cPRA are transcribed from this gene, but use alternative initiation codons (red horizontal arrows) driven by different promoters. The cPRs have a highly conserved DNA binding domain (DBD), an activation function1 (AF1) domain immediately upstream of the DBD, a hinge region downstream of the DBD, as well as a ligand binding domain (LBD) and a C-terminal AF2 domain. An inhibition factor (IF) is present upstream of AF1. The N-terminus of cPRB contains an AF3 domain, which acts in synergy with AF1 and AF2.

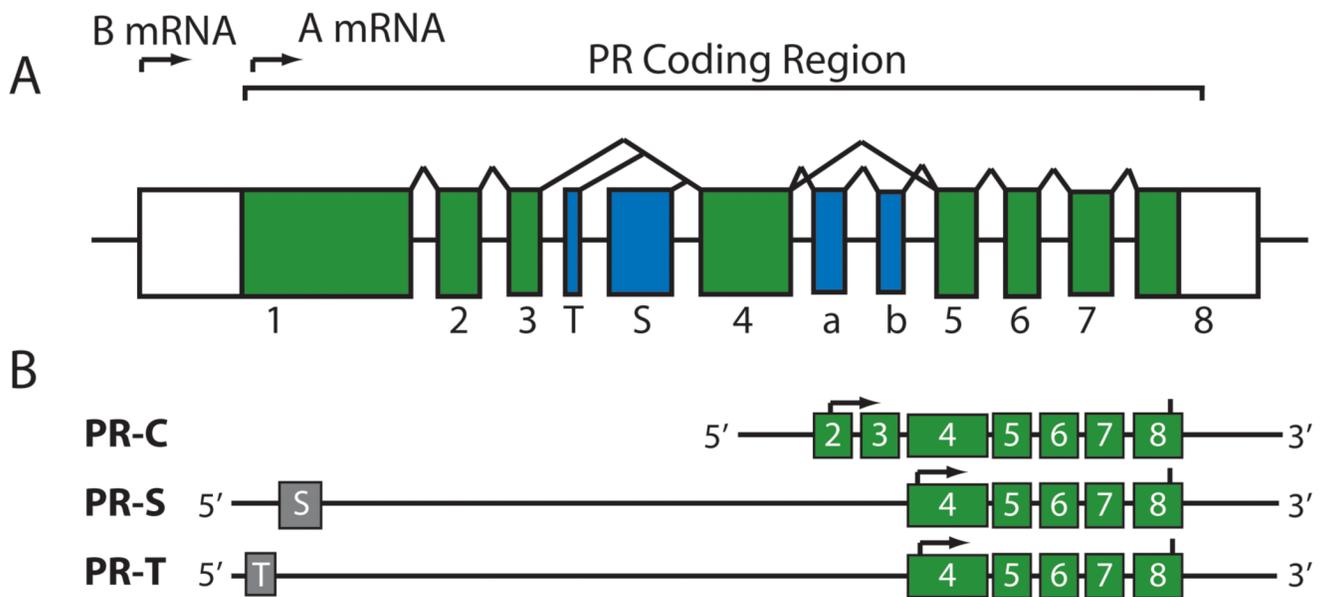


Fig. 2. Splice variants of cPR. A) Variants can be generated through insertion of T or S between exon 3 and 4 as well as through insertion of a or b between exon 4 and 5. B) Alternatively, variants can be generated through exon skipping. In PR-c, exon 1 is omitted. In PR-S and PR-T, exons 1-3 are omitted, but the 5'-untranslated exons S and T are retained.

PR A and B Isoforms in Rat Brain

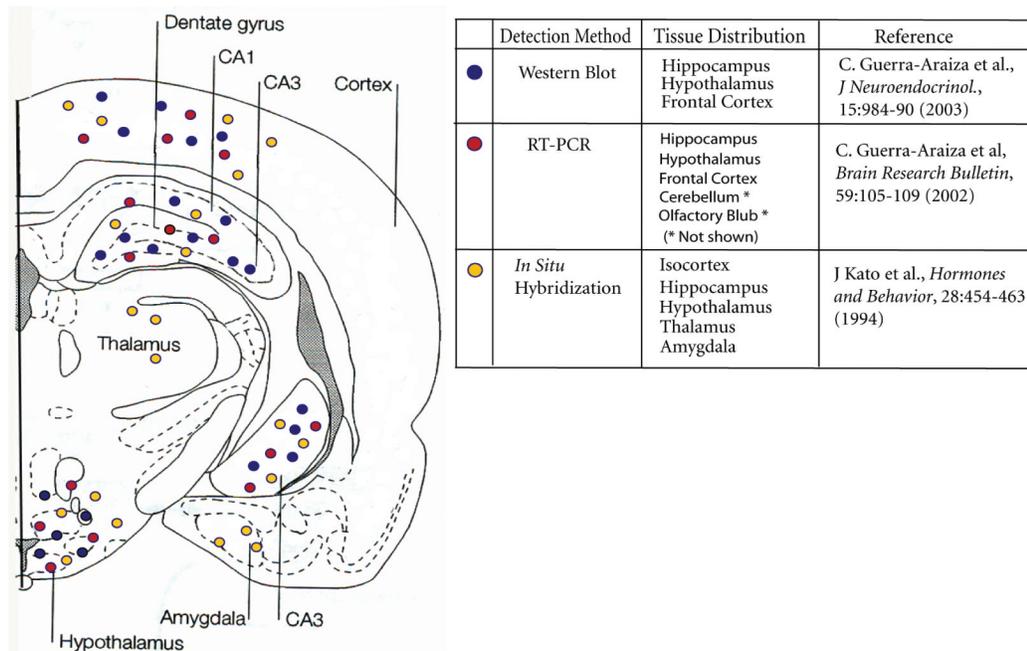


Fig. 3. Distribution of the 25 Dx PR transmembrane domain in the rat brain. The classical progesterone receptors PRA and PRB have been localized to regions throughout the brain using the indicated methods.

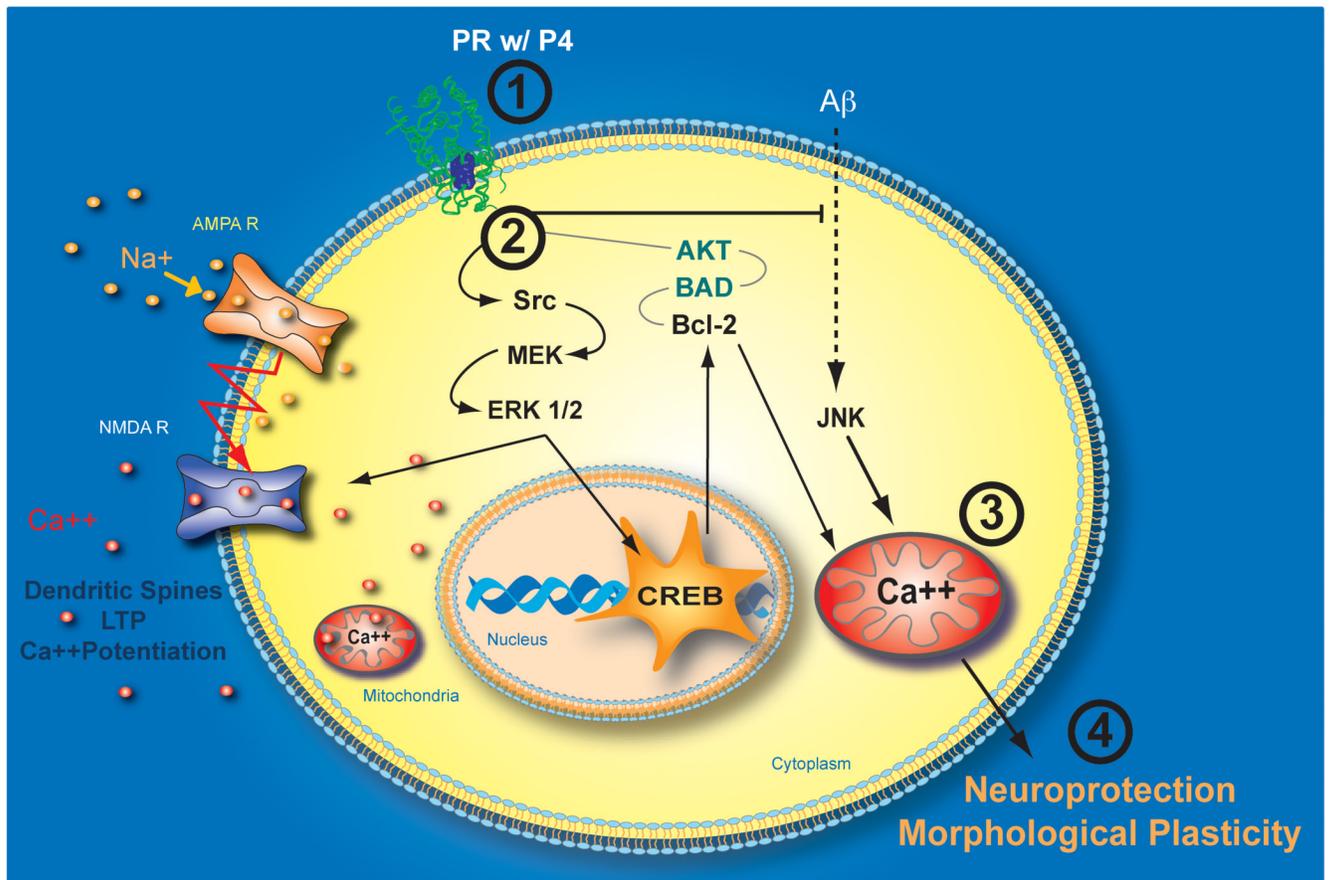


Fig. 4. Model of progesterone-induced neuroprotective signaling. Progesterone prevents synaptic dysfunction associated with aging and neurodegeneration through (1) ligand activation of a progesterone receptor, (2) which initiates second messenger signaling cascades (3) to promote neuronal survival. These signaling cascades converge on mitochondrial function to protect against against toxic insults and include the passive prevention signaling pathway and the active protection signaling pathway. In the passive prevention signaling pathway, both ERK/CREB/Bcl-2 and Akt pathways are simultaneously activated, which (4) enhances mitochondrial function and enables neurons to better withstand neurodegenerative insults. The active protection pathway acts to block Aβ-induced JNK activation and mitochondrial dysfunction.

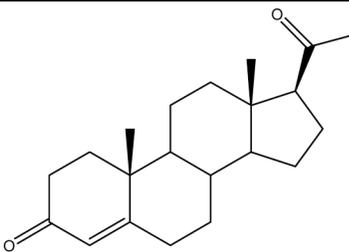
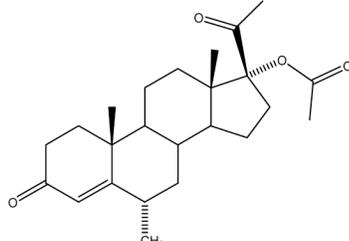
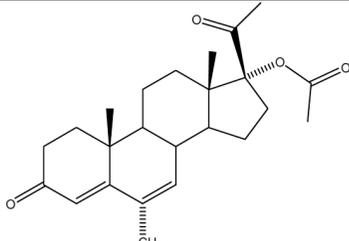
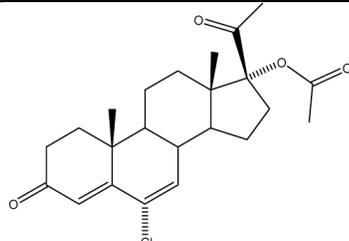
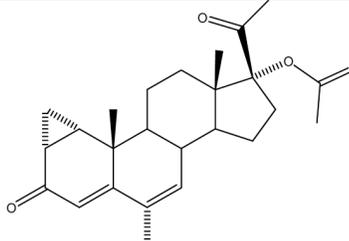
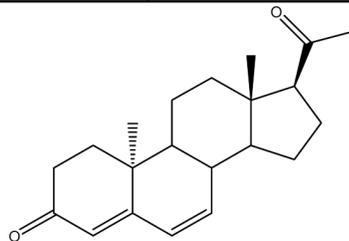
Table 1

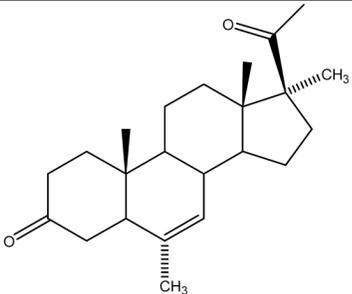
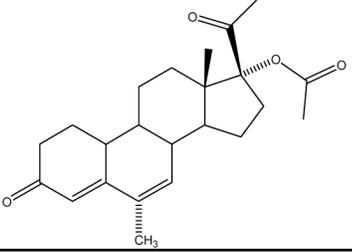
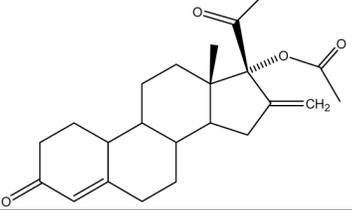
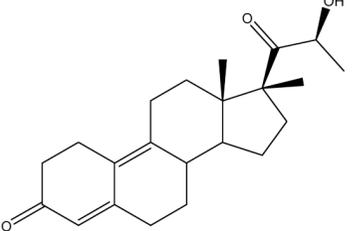
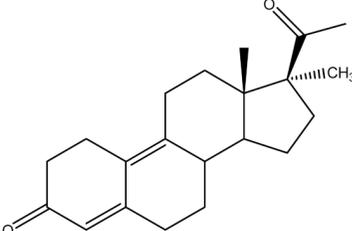
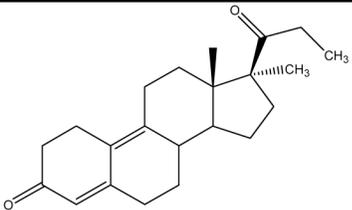
Classification of Progestogens

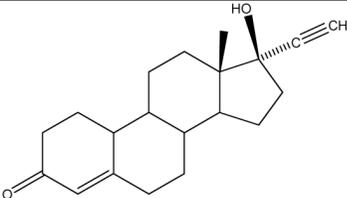
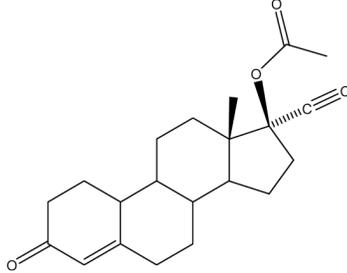
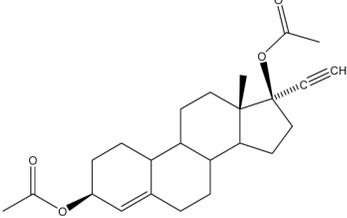
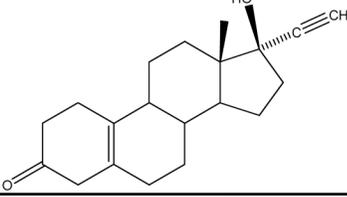
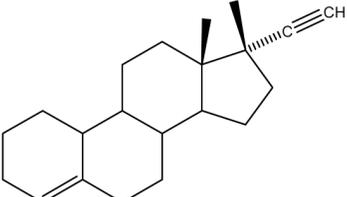
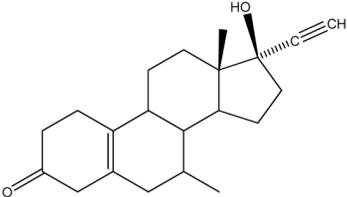
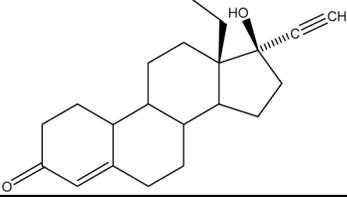
I. Natural	II. Synthetic Progestins	
Progesterone	A. Structurally Related to Progesterone	
	<p data-bbox="391 283 878 304">1 Pregnane Derivatives</p> <p data-bbox="483 304 878 367"> a. <u>Acetylated</u>: medroxyprogesterone acetate, megestrol acetate, cyproterone acetate, chlormadinone acetate, medrogestone</p> <p data-bbox="483 378 878 409"> b. <u>Non-acetylated</u>: dydrogesterone</p> <p data-bbox="391 420 878 451">2 19-Norpregnane derivatives</p> <p data-bbox="483 451 878 483"> a. <u>Acetylated</u>: nomegestrol acetate</p> <p data-bbox="483 493 878 546"> b. <u>Non-acetylated</u>: demegestone, trimesgestone, promgeestone, nesterone</p>	<p data-bbox="938 262 1383 283">Structurally Related to Androgen</p> <p data-bbox="987 283 1383 304">1 Ethinylated</p> <p data-bbox="1079 304 1383 399"> a. <u>Estranes</u>: norethindrone, norethynodrel, lynestrenol, norethindrone acetate, ethynodiol diacetate, tibolone</p> <p data-bbox="1079 409 1383 472"> b. <u>Gonanes</u>: levonorgestrel, desogestrel, norgestimate, gestodene</p> <p data-bbox="987 483 1383 514">2 Non-ethinylated: dienogest, drospirenone</p>

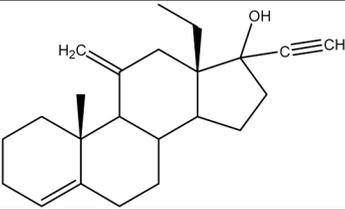
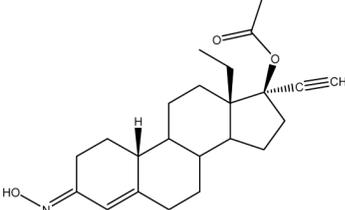
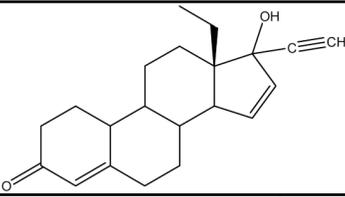
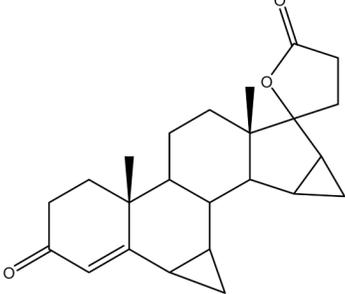
Table 2

Structures of selected progestogens and list of their active metabolites

Category	Progestogens	Structure	Active Compound of Prodrug
Natural Progestogens	Progesterone		
Pregnane (Acetylated)	Medroxyprogesterone Acetate		
	Megestrol Acetate		
	Chlormadionone Acetate		
	Cyproterone Acetate		
Pregnane (Non-acetylated)	Dydrogesterone		

Category	Progestogens	Structure	Active Compound of Prodrug
	Medrogestone		
19-Norpregnane (Acetylated)	Nomegestrol Acetate		
	Nesterone		
Norpregnane (Non-acetylated)	Trimegestone		
	Demegestone		
	Promegestone		

Category	Progestogens	Structure	Active Compound of Prodrug
Tesosterone Derivative (Estranes)	Norethindrone		
	Noethindrone acetate		Norethindrone
	Ethynodiol diacetate		Norethindrone
	Norethynodrel		Norethindrone
	Lynestrenol		Norethindrone
	Tibolone		Δ^4 -Tibolone 3 α -OH-Tibolone 3 β -OH-Tibolone
Testosterone Derivatives (Gonanes)	Levonorgestrel		

Category	Progestogens	Structure	Active Compound of Prodrug
	Desogestrel		3-Ketodesogestrel
	Norgestimate		Levonorgestrel-3-oxime Levonorgestrel
	Gestodene		
Testosterone Derivatives (Non-ethinylated)	Drospirenone		
	Dienogest	