

# Sex Hormones and Adult Hippocampal Neurogenesis: Regulation, Implications, and Potential Mechanisms

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## **Abstract**

Neurogenesis within the adult hippocampus is modulated by endogenous and exogenous factors. Here, we review the role of sex hormones in the regulation of adult hippocampal neurogenesis in males and females. The review is framed around the potential functional implications of sex hormone regulation of adult hippocampal neurogenesis, with a focus on cognitive function and mood regulation, which may be related to sex differences in incidence and severity of dementia and depression. We present findings from preclinical studies of endogenous fluctuations in sex hormones relating to reproductive function and ageing, and from studies of exogenous hormone manipulations. In addition, we discuss the modulating roles of sex, age, and reproductive history on the relationship between sex hormones and neurogenesis. Because sex hormones have diverse targets in the central nervous system, we overview potential mechanisms through which sex hormones may influence hippocampal neurogenesis. Lastly, we advocate for a more systematic consideration of sex and sex hormones in studying the functional implications of adult hippocampal neurogenesis.

The hippocampus retains substantial plasticity throughout the lifespan, and this malleability is essential to hippocampal function (Jessberger et al., 2007; Scharfman and Myers, 2015; Surget et al., 2011; Wainwright et al., 2015). Considerable research suggests that the hippocampus is compromised in multiple neuropsychiatric and neurodegenerative disorders, including depression (McKinnon et al., 2009) and dementia (Henneman et al., 2009). This is perhaps not surprising given the importance of the hippocampus in cognition (Sweatt, 2004) and mood regulation (Campbell and Macqueen, 2004). Importantly, the hippocampus is a region in which neurogenesis persists throughout the lifespan in a wide variety of species including humans (Eriksson et al., 1998; Gould et al., 1999b; Gross, 2000; Spalding et al., 2013).

The majority of research efforts devoted to understanding the functional implications of adult hippocampal neurogenesis have been focused on learning and memory (Yau et al., 2015) and mood regulation (Sahay and Hen, 2007). Indeed, alterations in hippocampal neurogenesis are reported in post-mortem tissue from patients with depression (Boldrini et al., 2012) and Alzheimer's disease (Crews et al., 2010; K. Jin et al., 2004), and in animal models of these disorders (Bessa et al., 2009; Green and Galea, 2008; Mu and Gage, 2011; Wainwright et al., 2011). Although we first expand on the role of the hippocampus in the neurobiology of depression and cognitive function in this review, we direct the reader to other reviews with a primary focus on these topics (Broadbent et al., 2010; Campbell and Macqueen, 2004; MacQueen and Frodl, 2011; Sweatt, 2004). The current review focuses on the influence of sex and sex hormones on adult hippocampal neurogenesis, and explores ramifications to cognition and mood in health and disease.

Robust sex differences exist in the incidence rates of depression, where at least twice as many women are affected than men (Gutiérrez-Lobos et al., 2002; Angst et al., 2002). Similarly, Alzheimer's disease is more prevalent in women than men (Baum, 2005), in contrast to other forms of dementia (Gao et al., 1998). Sex differences in these neuropsychiatric and neurodegenerative disorders extend beyond incidence rates. The manifestation of a disorder can differ by sex, where in the case of depression, women are more likely to be diagnosed with atypical depression, and to present with co-morbid anxiety (Angst et al., 2002; Silverstein, 2002; Young et al., 1990). The severity of disease is also linked to sex, as Alzheimer's disease follows a more severe progression in women relative to men (Irvine et al., 2012). It is beyond the scope of this review to delve into why these sex differences in disease incidence and severity exist, but

the reader is directed to other reviews on this subject (Hammarström et al., 2009; Mielke et al., 2014; Vest and Pike, 2013). Beyond the context of disease, sex differences exist in certain domains of cognition, as reviewed in (Hamson et al., in press), and in neurogenesis, as reviewed in (Galea et al., 2013). In instances where sex differences are observed in a phenotype of interest, whether it is in the context of health or pathology, it is essential to examine whether sex hormones are involved. The role of sex hormones in any given phenotype can arise from effects early in development, during the pubertal transition, throughout adulthood, or across a combination of developmental windows in both males and females.

In light of the sex differences in cognitive function and mood regulation in health and disease, and the potential role of neurogenesis in these domains, the influence of sex hormones on adult hippocampal neurogenesis is essential to consider and will be the focus of our current review. After giving a brief overview of neurogenesis in the adult hippocampus, we expand on the role of the hippocampus, and links to hippocampal neurogenesis, in the neurobiology of depression and learning and memory. We then discuss evidence indicating that sex hormones are potent modulators of neurogenesis within the dentate gyrus. We highlight the complexities of sex hormone modulation of neurogenesis, in how it may be affected as a function of sex, age, and reproductive history. Further, we discuss potential implications of sex hormone regulation of hippocampal neurogenesis, with particular attention to affective and cognitive functions in health and disease. Lastly, we highlight potential mechanisms through which sex hormones may mediate their effects on neurogenesis within the dentate gyrus.

## **Adult Hippocampal Neurogenesis**

Altman first found neural stem cells in the brain of adult rodents more than fifty years ago (Altman, 1962) and neural stem cells have since been identified in a number of species, including humans (Eriksson et al., 1998). The production of new neurons in the adult brain is typically limited the subventricular zone (SVZ), which lines the lateral ventricles and sends newly generated cells along the rostral migratory stream to the olfactory bulb, and the subgranular zone of the hippocampus (Kaplan and Hinds, 1977). However, studies in humans indicate that adult neurogenesis may not occur in the SVZ, and is limited to the hippocampus (Sanai et al., 2011; Spalding et al., 2013). Multipotent neural stem cells are capable of producing multiple types of both neurons and glia and are located in the SVZ, while neural progenitor cells

located in the SGZ divide more frequently, in a finite manner, and into a limited number of cell types, as they may only produce daughter cells of either a defined glial or neuronal lineage (Gage, 2000; Seaberg and van der Kooy, 2003; 2002; van der Kooy and Weiss, 2000).

Newly generated neurons may be identified through the administration of DNA-markers such as  $^3\text{H}$ -thymidine, or a synthetic nucleoside such as 5-bromo-2-deoxyuridine (BrdU). Each marker is incorporated into the DNA of dividing cells during DNA synthesis, replacing thymidine nucleotides. The addition of BrdU into the DNA provides a unique epitope for antibody binding using immunocytochemistry. The controlled addition of the exogenous markers allows for a definitive timeline of neural development, as every cell undergoing DNA synthesis during the two hours after injection will be labelled. Labelled cells may be examined at time points ranging from hours, weeks, or even years later depending on the research question (Dayer et al., 2003; Eriksson et al., 1998; Kempermann et al., 2003).

There are limitations, however, in the use of exogenous markers, as they become diluted as cells undergo mitosis and are only distinguishable from background for 4-5 divisions following administration, thereby limiting the population of cells that may be labelled (Prickaerts et al., 2004; Stone et al., 1965); however, this limitation also provides the benefit of generating a “time stamp” for the occurrence of cell proliferation. Factors such as dose, toxicity, or the permeability of the blood-brain barrier may also disrupt the detection of true cell counts when using exogenous markers of DNA synthesis (Taupin, 2007). Exogenous markers must also be used in conjunction with endogenous markers, such as neuronal nuclei (NeuN) for mature neurons or glial fibrillary acidic protein (GFAP) for glial cells, in order to phenotype newly-labelled cells with BrdU or  $^3\text{H}$ -thymidine. It is therefore essential that BrdU or  $^3\text{H}$ -thymidine labelled cells be assessed with endogenous markers in order to demonstrate that neurogenesis has occurred (Ming and Song, 2005; Wojtowicz and Kee, 2006).

The measurement of neurogenesis may also be achieved through the assessment of endogenous protein expression. Measurement of the Ki67 protein, which is expressed throughout the active phases of the cell cycle but absent in quiescent cells, may be used as a marker of cell proliferation (Kee et al., 2002). The use of Ki67 in the measurement of cell proliferation also precludes issues with toxicity encountered with exogenous markers, however Ki67 is limited by the short timeline of expression - approximately 24 hours in rats, but is not limited to cells that

will become neurons (Cameron and McKay, 2001; Kee et al., 2002). Endogenous markers may also be used to identify and characterize both immature and mature neurons. Doublecortin (DCX) is a neuron-specific protein that functions in the stabilization of microtubules in early mitotic neurons which may be used to measure immature neurons and assess immature neuronal morphology (Bechstedt et al., 2014), and in the rat hippocampus is expressed from a few hours to 21 days after the production of a new neuron (Brown et al., 2003). The NeuN protein is expressed by most mature neurons and functions in RNA splicing within the nucleus, and may be used in the characterization of mature neurons (Kim et al., 2009; von Bohlen und Halbach, 2011). It is important to note that while NeuN is expressed in most neuronal cell types, it is not expressed in certain neuronal populations such as the cerebellar purkinje cells and retinal photoreceptor cells (Mullen et al., 1992). Importantly, expression patterns differ in the SVZ and in mice, with other species likely also differing from the rat (Ming and Song, 2005; Snyder et al., 2009).

It is important to note that neurogenesis, as defined here, requires the proliferation, migration, survival, and differentiation of newly generated cells into neurons (see Figure 1). Any number of internal and/or external factors may independently affect the proliferation of progenitor cells, migration, their differentiation into neurons, or their survival rates; including stress, gonadal hormones, or pharmaceuticals (Kempermann et al., 1998; van Praag et al., 1999; Malberg et al., 2000; Barker and Galea, 2008; Burgess et al., 2008; Pariante and Lightman, 2008; Anacker et al., 2011; Surget et al., 2011; Wainwright et al., 2011). It is therefore essential to understand which aspects of neurogenesis are being examined, and when they are being examined, in relation to experimental manipulation. For example, treatment prior to injections with a DNA synthesis marker may result in opposing effects on neurogenesis compared to treatments given just after injection of a DNA synthesis marker (Barker and Galea, 2008; Chan et al., 2014; Malberg et al., 2000; McClure et al., 2013; see figures 2 and 3). The differential effects depending on timing of DNA synthesis marker administration may relate to the manipulation itself (e.g. antidepressants do not independently alter the survival of new neurons (Malberg et al., 2000), and/or may be due to different populations of progenitor cells that respond at different times or in response to homeostatic disruptions (Hayes and Nowakowski, 2002)).

## The Hippocampus in the Neurobiology of Depression

The hippocampal formation is an area rich in mineralocorticoid (MR) and glucocorticoid receptors (GR) (McEwen, 1973). These receptors function in the maintenance of basal hypothalamic-pituitary-adrenal (HPA) axis tone, and in the regulation of negative feedback of glucocorticoid release during a stress response (Sapolsky et al., 1985). Briefly, as illustrated in figure 4, negative feedback refers to the inhibition of HPA axis activity by circulating glucocorticoids, acting on glucocorticoid and mineralocorticoid receptors in regions such as the hypothalamus, pituitary, and hippocampus (Smith and Vale, 2006). As some depressed patients show disrupted HPA negative feedback, diurnal rhythms and hypersecretion of cortisol (Pariante and Lightman, 2008), it is not surprising that the hippocampus is particularly vulnerable to the effects of stress and depression (Sapolsky, 1986; Sapolsky et al., 1988).

Imaging studies shown that depressed patients have smaller hippocampal volumes which co-vary with the number of episodes and duration of the illness (Sheline et al., 1996; Sheline et al., 2003; McKinnon et al., 2009). Similarly, post-mortem studies of depressed patients have shown alterations in gray matter density, reductions in neuropil, decreased neuron soma size, and decreased hippocampal neurogenesis; demonstrating significant changes in both the number and connectivity of hippocampal neurons, which may contribute to the observed reductions in hippocampal volume (Stockmeier et al., 2004; Boldrini et al., 2009; Hercher et al., 2009; Boldrini et al., 2012b; Boldrini et al., 2013a; Cobb et al., 2013). Alternations to both neurogenesis and neuroconnectivity have been associated with the etiology and amelioration of depression (see Wainwright and Galea, 2013; Wainwright et al., 2015).

Interestingly, the meta-analysis by McKinnon et al., (2009) showed that only patients with depression persisting longer than two years, or had greater than one episode of depression, showed significantly smaller hippocampal volume. The evidence for smaller hippocampal volume was, however, only found in children and middle-aged to older adults, where young adults showed no significant difference between depressed patients and control subjects. Thus the periods during life that the hippocampus is most vulnerable to the effects of chronic or recurrent depression are also the times when gonadal hormones are lower. Importantly, gonadal hormones function in then feedback regulation of the HPA axis in both males and females (see figure 4). Lastly, and importantly, men are more likely than women to present with a small hippocampal volume with depression (Frodl et al., 2002). It is important to note that reductions in hippocampal volume with depression are not solely due to neurogenesis, and may be due to a

number of factors such as reduced neuropil, glia, or neurons. An exhaustive review of factors that could contribute to the reduced volume is beyond our current scope, but the reviewer is directed to another review on the subject (Czéh and Lucassen, 2007).

## **Adult Hippocampal Neurogenesis and Depression**

The neurogenic hypothesis of depression is predicated not only on the findings, reviewed above, that chronic stress decreases hippocampal neurogenesis in animal models of depression (Gould et al., 1997; Santarelli et al., 2003; Bessa et al., 2009), but that antidepressant drugs can both prevent (Czeh et al., 2001; Alonso et al., 2004) and reverse (Malberg and Duman, 2003; Santarelli et al., 2003; Bessa et al., 2009) this effect (see Wainwright and Galea, 2013). The enhancement of adult hippocampal neurogenesis via antidepressant treatment is dependent on chronic exposure, and occurs in a manner that temporally coincides with the delayed time to substantial remission in depressed patients (Malberg et al., 2000; Santarelli et al., 2003; Mitchell, 2006). This delayed enhancement of neurogenesis is mediated through the increased proliferation of neural progenitor cells, while the proportion of cells that survive and differentiate into neurons remains constant (Malberg et al., 2000). Several classes of antidepressant drugs, including [selective serotonin reuptake inhibitors \(SSRI\)s](#), [tricyclic antidepressants \(TCA\)s](#), and [monoamine oxidase inhibitors \(MAOI\)s](#), increase neurogenesis in the hippocampus (Banasr et al., 2006; Dagyte et al., 2010). Moreover, a rodent model of the non-pharmacological antidepressant treatment of electroconvulsive therapy (ECT), which shows the greatest clinical efficacy against treatment-resistant depression (Pagnin et al., 2004), enhances neurogenesis to levels nearly twice that of pharmacological antidepressants in animal models (Madsen et al., 2000; Hellsten et al., 2002). Post-mortem studies have also shown antidepressant treatment enhances hippocampal neurogenesis in humans (Boldrini et al., 2009; Boldrini et al., 2012; Epp et al., 2013). Interestingly, one study shows treatment with TCAs increases cell proliferation levels beyond that of SSRIs (Boldrini et al., 2009), while another by the same research group shows a greater enhancement of proliferation by SSRIs over TCAs (Boldrini et al., 2012). There is a similar discrepancy in studies measuring hippocampal volume following antidepressant treatment, where SSRIs enhance hippocampal volume over TCA treatment (Boldrini et al., 2012; Boldrini et al., 2013; Boldrini et al., 2009). It is important to note that the majority of pre-clinical research described above has been conducted exclusively in male rodents (Bessa et al., 2009; Malberg et al., 2000; Santarelli et al., 2003), while post-mortem studies typically analyse both

men and women together (Boldrini et al., 2009; 2012; 2014). Thus, one factor that may contribute to the disparate findings above that is not always considered in the effects of antidepressants or classes of antidepressants is biological sex.

There is some evidence that men respond better to TCAs while women respond better to SSRIs (Kornstein) and this may be seen in terms of hippocampal volume and/or neurogenesis (Lorenzetti). Indeed women responders were more likely to show increased hippocampal volume over non-responders compared to men (Vakili et al., 2000). One recent study has shown that antidepressant treatment only enhances the expression of DCX/NeuN ratio in women, but not men, thereby demonstrating that pro-neurogenic effects of antidepressant treatment may not only depend on the mechanism of antidepressant action, but the sex of the person taking a particular antidepressant (Epp et al., 2013). Unfortunately, that study did not examine type of antidepressant treatment to determine whether the sex effects were also dependent on antidepressant mechanism of action. The age of the research subjects is also important, as two studies have shown that antidepressants fail to enhance neurogenesis in older patients (Lucassen et al., 2010; Epp et al., 2013). Thus, it is important to distinguish between findings in males versus females and with attention to age in order to fully understand the role of neurogenesis in depression.

The modulation of neurogenesis via antidepressant treatment is well defined in the preclinical literature, however the specific role, and thereby the potentially essential nature of neurogenesis, is less clear. The reduction of hippocampal neurogenesis via irradiation, disrupts antidepressant efficacy on novelty-induced hypophagia in a model of depression (Santarelli et al., 2003). However, reduction of neurogenesis via the cytostatic agent methylazoxymethanol (MAM) failed to disrupt the behavioural efficacy antidepressant treatment on sucrose preference, or in the forced swim test (Bessa et al., 2009). Furthermore, the reduction of neurogenesis via MAM did not eliminate the antidepressant treatment-induced increases in hippocampal volume and synaptic protein expression in the hippocampus (Bessa et al., 2009). Thus, there are thought to be neurogenesis-dependent and –independent effects of antidepressant treatment, where hippocampal neurogenesis is required for the alleviation of anxiety-like behaviours, at least in males (Holick et al., 2008; Surget et al., 2008; David et al., 2009). Indeed, hippocampal neurogenesis buffers the acute stress response and normalizes glucocorticoid release after acute stress in male mice (Snyder et al., 2011). Snyder and colleagues found that the reduction of

hippocampal neurogenesis, either transgenically or via irradiation, attenuated both the recovery of basal HPA tone following acute restraint stress and the normal suppression of glucocorticoid release during the dexamethasone suppression test (Snyder et al., 2011). Importantly, the ablation of neurogenesis does not alter the ability of the HPA axis to mount a response to stress (Santarelli et al., 2003). However, the ability of an antidepressant (fluoxetine) to improve negative feedback and normalize HPA tone in a chronic unpredictable stress (CUS) model of depression is abolished by the ablation of hippocampal neurogenesis in adult male mice (Surget et al., 2011). Further, the transgenic enhancement of hippocampal neurogenesis is sufficient to ameliorate some measures of anxiety- and depressive-like behaviour in a chronic CORT administration model of depression (Hill et al., 2015). As such, there is likely a role for hippocampal neurogenesis in re-establishing normal HPA tone and regulating a normal HPA response to stressors; possibly through GR-mediated feedback regulation, at least in males. The preponderance of depression research, including each of the aforementioned studies, is conducted in males and therefore, it is important to acknowledge that the functional significance of hippocampal neurogenesis may be different in females. Although previous work that directly examined the role of neurogenesis in depressive-like behaviour and antidepressant efficacy has been performed in males, there are a few studies that have examined depressive-like behaviour and antidepressant efficacy in females, and in some instances have examined alterations in neurogenesis (Lagunas et al., 2010; Mahmoud et al., in press; for review see: Dalla et al., 2010; Gobinath et al., 2015).

Experimental evidence has aligned to indicate a specific functional contribution of neurogenesis in stress reactivity and antidepressant action. However, there is also ample evidence demonstrating the importance of forms of neuroplasticity beyond neurogenesis in depression. The monoaminergic facets of antidepressant action, namely the modulation of serotonergic and dopaminergic neurotransmission, are unaffected by the ablation of neurogenesis (Bessa et al., 2009; David et al., 2009). The modification of receptor expression, synaptic weight, synaptic density, and neural connectivity, are tied to the antidepressant-induced modulation of neurotransmission and subsequently antidepressant efficacy (Shakesby et al., 2002; Duman and Aghajanian, 2012). In fact a study from our laboratory demonstrated that the enzymatic ablation of the polysialylated neural cell adhesion molecule (PSA-NCAM) disrupted antidepressant efficacy across neurogenesis-dependent and -independent behavioural measures (Wainwright et

al., 2015). The ablation of PSA-NCAM causes broad a disruption of neuroplasticity, including long-term potentiation (LTP), long-term depression (LTD), dendritic outgrowth, and synaptic density (Burgess et al., 2008; McCall et al., 2013; Muller et al., 1996; Rutishauser, 2008; for review see Wainwright and Galea, 2013). Taken together, these findings demonstrate the limited contribution of neurogenesis alone in antidepressant efficacy while demonstrating the essential contributions of all facets of neuroplasticity.

### **The Hippocampus in the Neurobiology of Learning and Memory**

There is little dispute that the hippocampus is involved in some aspects of learning and memory, however it is important to acknowledge that not all researchers agree to the degree of hippocampal involvement in learning and memory. The nuances and complexities are beyond the scope of this review and the reader is directed to many excellent reviews on the topic (Josselyn et al., 2015; Leal and Yassa, 2015; Rosenbaum et al., 2001; Winocur et al., 2013). Certainly a large amount of evidence, including the existence of place and grid cells, points to a role of the hippocampus in spatial memory (Moser et al., 2015). Indeed, the integrity of the hippocampus is important for pattern separation and completion (Clelland et al., 2009; Kesner and Rolls, 2015). However, it is also important to understand that the hippocampus, while vital in some forms of spatial cognition, is only part of a larger network of structures that are involved in various aspects of learning and memory, such as decision making and/or executive functions such as planning/shifting strategies (Orsini et al., 2015). The hippocampus does not act as a single, homogenous, structure; rather, there appears to be a functional dissociation along the dorsoventral axis (Moser and Moser, 1998). Anatomical, gene expression, and behavioural studies point to a primary role in cognition for the dorsal hippocampus, while the ventral hippocampus is primarily involved in affective functions (Fanselow and Dong, 2010), although whether or not this is a true double dissociation is up for debate (Strange et al., 2014). The dorsal region shows a greater density of place cells involved in the coding of spatial location and reciprocal connections with regions prominently involved in cognitive processing and locomotion, while the ventral region shares connections with the amygdala, medial prefrontal cortex (mPFC), and hypothalamus in a manner primarily associated with neuroendocrine regulation, stress, and affect (Herman and Cullinan, 1997; Fanselow and Dong, 2010). Few studies in neurogenesis research, however, have dichotomized the dentate gyrus, but those that do often find differences in the effects of treatment on neurogenesis in the dorsal

versus ventral dentate gyrus (O'Leary and Cryan, 2014; Surget et al., 2011; Tanti and Belzung, 2013; Yagi et al., 2015).

### **Adult Hippocampal Neurogenesis and Learning and Memory**

Neurogenesis in the hippocampus has been linked to cognition, and while the debate continues as to how much it is involved and in what types of learning and memory, it is certainly less disputed that neurogenesis is required for spatial pattern separation (Clelland et al., 2009). Indeed, new neurons, but not older neurons, in the dentate gyrus are needed for pattern separation (Nakashiba et al., 2012). However, ablation of neurogenesis does not lead to disruptions in acquisition in a spatial reference memory version of Morris Water Maze (Shors et al., 2002), but does lead to long-term disruption in spatial memory (Snyder et al., 2005). Ablation of neurogenesis has also lead to deficits in contextual fear and trace conditioning (Shors et al., 2002; Winocur et al., 2006). Other studies have indicated that an optimal level of neurogenesis is needed for optimal learning and memory (Akers et al., 2014; Jessberger et al., 2007; Scharfman and Myers, 2015). For example, computational models and empirical data suggest that large fluctuations in cell proliferation will be disruptive to the network and thus new neurons that have not fully integrated into the system disrupt new learning (Akers et al., 2014; Butz et al., 2006; Jessberger et al., 2007; Scharfman and Myers, 2015). The reader should be aware that the nature of the disruption of neurogenesis may have very different effects on cognition, as new neurons created under exercise versus seizure-like conditions have different properties (Jakubs et al., 2006), and depending on whether neurogenesis disruptions are affecting new or older memories (Epp et al., 2016).

Hippocampus-dependent learning (acquisition) influences neurogenesis and the activation of newly generated neurons in response to memory retrieval (Epp et al., 2013b). A study by Gould et al (1999) was the first to demonstrate that the number of BrdU-labeled cells was increased in male rats by training on the hippocampus-dependent Morris Water Maze (Gould et al., 1999a). Further work corroborated these findings (Epp et al., 2007), but also revealed that several factors including sex, strain, and methodological differences can affect the relationship between learning and neurogenesis (Epp et al., 2013 for review). An extensive review of the involvement of hippocampal neurogenesis in cognition is beyond the scope of this

review and the reader is directed to many other reviews on the subject (Abrous and Wojtowicz, 2015; Deng et al., 2010; Frankland and Josselyn, 2016; Johnston et al., 2015).

## Hippocampal Neurogenesis and Androgens

Accumulating evidence indicates a role for androgens, and androgenic signalling, in the modulation of adult hippocampal neurogenesis (Galea et al., 2013). In the following sections, we will review studies that have investigated the influence of endogenous fluctuations and exogenous manipulations of androgens on neurogenesis within the dentate gyrus (summarized in Figure 2). We will discuss potential implications for affective and cognitive function, and in turn depression and dementia.

### Androgens and Androgen Signalling: A Brief Overview

Androgens are the predominant gonadal hormones in men, derived from cholesterol via progestins, they include among others testosterone, androstenedione, and the testosterone metabolite produced via 5 $\alpha$ -reductase, 5 $\alpha$ -dihydrotestosterone (DHT). Testosterone and androstenedione may also be converted, via aromatase, to 17 $\beta$ -estradiol. 17 $\beta$ -estradiol is the most biologically active of the estrogens in men and women (Nelson, 2011). Androgens are produced primarily in the Leydig cells of the testes, and transported through the circulatory system via sex hormone-binding globulin produced in the liver (Waterman and Keeney, 1992). Androgens act via the androgen receptor (AR), which is classically expressed in the cytoplasm then, when bound by either testosterone or the higher-affinity DHT, translocates to the nucleus and functions as a transcription factor to directly regulate gene expression via AR binding domains (Heinlein and Chang, 2002; Bennett et al., 2010). ARs also have a non-genomic pathway to induce rapid signalling following the binding of a ligand (Heinlein and Chang, 2002). Though DHT does not bind or activate estrogen receptors (ERs), it can be further metabolised by cytochrome P450 enzymes to 5 $\alpha$ -androstane-3 $\alpha$ , 17 $\beta$ -diol (3 $\alpha$ -Diol) or 5 $\alpha$ -androstane-3 $\beta$ , 17 $\beta$ -diol (3 $\beta$ -Diol), both of which show affinity for ERs (Handa et al., 2008).

The androgen receptor protein contains an N-terminal domain, a hormone-binding domain, and a DNA-binding domain. The AR gene encoding the AR protein is located on the X chromosome and has a polymorphic CAG microsatellite that encodes variable-length glutamine repeats in the N-terminal domain (Lubahn et al., 1988). The CAG repeat length normally ranges

from 6 to 39 repeats, with a mean of 22, where CAG repeat length negatively correlates with the amount of AR transactivation (Seidman et al., 2001b). Androgen receptors are expressed throughout the rat brain, including the hippocampal formation in both males and females (Simerly et al., 1990). Sex differences are present however, as the density of AR expression in the hippocampus is greater in intact males than intact females, as may be expected (Xiao and Jordan, 2002). Within the hippocampal formation AR is expressed throughout Ammon's horn, with particularly high expression in the stratum pyramidale of CA1 and stratum lucidum of CA3 (Simerly et al., 1990; Kerr et al., 1995; Tabori et al., 2005). Interestingly, there is no apparent expression of AR in the dentate gyrus of adult male rats (Simerly et al., 1990; Kerr et al., 1995; Xiao and Jordan, 2002; Tabori et al., 2005), including findings from our laboratory indicating no expression of AR in the dentate gyrus or on adult-generated immature hippocampal neurons (Hamson et al., 2013; see figure 5).

### Hippocampal Neurogenesis and Androgens

Despite the fact that there is an absence of AR in the dentate, there is evidence showing that androgens influence neurogenesis in the adult male hippocampus (see Figure 2). In adult male rodents, long-term exposure to androgens reliably increase hippocampal neurogenesis via the modulation of the survival of new neurons (for review see Galea et al., 2013). Several studies have shown that the removal of testicular hormones, via castration, or a reduction of testicular hormone levels during the non-breeding season, is associated with a reduction in the number of newly generated neurons that survive to maturity in adult rodents (Ormerod and Galea, 2003; Spritzer and Galea, 2007; Spritzer et al., 2011a; Wainwright et al., 2011; Hamson et al., 2013). On the other hand, supplementation with either testosterone or dihydrotestosterone (DHT), but not estradiol, ameliorates the effect of castration on the survival of new neurons in males (Spritzer and Galea, 2007; Hamson et al., 2013). However, some studies have shown no effect of testosterone supplementation to increase the survival of new neurons after castration (Spritzer et al., 2011a; Carrier and Kabbaj, 2012a), or in intact males (Buwalda et al., 2010). The duration of androgen treatment appears to be critical in promoting the survival of new neurons. The studies that show enhanced survival of new neurons have used 30 or 90 days of androgen administration (Spritzer and Galea, 2007; Spritzer et al., 2011a; Wainwright et al., 2011; Hamson et al., 2013; Hannan and Ransome, 2012), while those failing to enhance the survival of new neurons use 15 or 21-day courses of androgen treatment in male rodents (Spritzer et al., 2011; Carrier and

Kabbaj, 2012b; Wainwright et al., 2016; see figure 2). Given the length of exposure required to elicit an increase in the survival of new neurons, the effect is likely mediated through a genomic mechanism. It is unclear why the effects on neurogenesis are not seen with 15 or 21 days of treatment, considering that these windows would also allow for the activation of genomic mechanisms. However, as we discuss later in the review, we speculate that androgens may act on the CA3 region to induce retrograde signalling of survival factors that ultimately influence the survival of new cells. This process, that likely requires the synthesis, release and action of survival factors emitted from the CA3, may therefore explain the length of exposure required to elicit an increase in neurogenesis.

Androgenic regulation of the survival of new neurons within the dentate gyrus has been specifically attributed to the activation of the AR in rodents, as estrogenic metabolites of testosterone such as estradiol, produce no significant effect on survival of new neurons (Spritzer and Galea, 2007; Carrier and Kabbaj, 2012a). Indeed, testosterone exerts an effect on neurogenesis via an AR-dependent mechanism, as treatment with the testosterone metabolite DHT, which has a higher affinity for AR than testosterone, produces an increase in neurogenesis. Moreover, the enhancement of neurogenesis by DHT is blocked by the AR antagonist flutamide (Hamson et al., 2013). Similarly, testosterone treatment fails to enhance neurogenesis in Tfm (testicular feminization mutation) rats containing a mutation of the AR that renders it non-functional (Hamson et al., 2013). Given the absence of ARs in the dentate gyrus of most rodent strains, the site of androgen action to influence neurogenesis cannot be directly within the dentate gyrus. We have postulated that the binding of ARs in the CA3 region induces retrograde signalling of survival factors from CA3; however, a mechanism still needs to be delineated (Galea et al., 2013; Hamson et al., 2013).

While androgens, but not estrogens, modulate the survival of new neurons, estradiol has some effects on neurogenesis in males (Ormerod et al., 2004). Although 29 days of exposure to estradiol does not alter neurogenesis, 5 days of estradiol administered 6-10 days after BrdU does increase the survival of new neurons, one week after castration in male rodents (Ormerod et al., 2004). Furthermore, while 60 days of estradiol treatment did not affect the survival of new neurons, it did increase the density of immature doublecortin-expressing neurons (Saravia et al., 2007). This suggests that while long-term exposure of estradiol does not affect the survival of new neurons in the male rodent, short-term exposure to estradiol, limited to the axon extension

phase, does promote the survival of new neurons in adult male rodents. Collectively, these findings demonstrate that long-term androgen treatment has pro-neurogenic effects on cell survival via the AR in agonadal males, while short-term targeted (5 day; during axon extension), or very long term (60 day) estradiol treatment may promote increased survival of new neurons in adult males. It is also possible that the length of castration may influence these findings but as of yet this has not been examined in the literature.

Androgens have less of an influence on adult hippocampal cell proliferation as neither castration, nor repeated androgen supplementation (administration of testosterone or DHT) produced any significant change in cell proliferation using either exogenous or endogenous markers (Spritzer and Galea, 2007; Carrier and Kabbaj, 2012b; Hamson et al., 2013; Allen et al., 2015; Hannan and Ransome, 2012). Furthermore, neither the administration of the AR-antagonist flutamide, nor the expression of the insensitive androgen receptor in TfM rats given vehicle, altered levels of cell proliferation (Hamson et al., 2013). Though not a significant effect, there was a trend toward testosterone supplementation reducing cell proliferation in intact male rats (Buwalda et al., 2010). Interestingly, another study has found that administration of finasteride - a 5 $\alpha$ -reductase inhibitor that functions to inhibit the metabolism of testosterone to DHT- in intact male mice actually serves to reduce cell proliferation and the expression of DCX-immunoreactive neurons in the hippocampus (Romer et al., 2010). Collectively these findings demonstrate that while androgen treatment may have modest effects on cell proliferation, it may potentially have deleterious effects on cell proliferation in intact males. It is therefore possible that optimal androgen levels may be required to support hippocampal neurogenesis in rodents. On the other hand, estradiol treatment may have pro-proliferative effects in the hippocampus of male rodents, as 10 or 60 days of estradiol treatment (using continuous-release pellets) increased cell proliferation in middle aged (10-12 month old) male C57Bl/6 mice (Saravia et al., 2007).

A study in non-human primates showed gonadectomised males had increased survival of new neurons and enhanced differentiation of new cells to a neural phenotype (Allen et al., 2014). However, it is important to note that the Rhesus Macaques used in that study were gonadectomised *prior* to puberty, and neurogenesis was not measured until over two years after surgery. This is very different from the findings in rodents demonstrating that castration in adulthood can suppress the survival of new neurons (Spritzer and Galea, 2007). Given the pre-pubertal removal of gonadal hormones, and the long absence from testicular hormones, any

differences in hippocampal neurogenesis cannot be solely attributed to an AR-dependent neurogenic mechanism. Furthermore, new cells are produced during puberty in at least three sexually dimorphic regions in the rat brain; the medial amygdala, the anteroventral periventricular nucleus of the hypothalamus, and the sexually dimorphic nucleus of the preoptic area (Ahmed et al., 2008). Gonadectomy prior to puberty prevents the production of these new cells indicating that these effects are modulated by gonadal hormones during puberty (Ahmed et al., 2008). This indicates that pre-pubertal gonadectomy likely alters the numbers of neurons in many regions of the brain and manipulations in these animals, while informative to pubertal effects, are not likely to mirror effects seen in adults that were gonadectomised after puberty.

The modulation of androgens also alters the expression of neurotrophic factors and structural proteins associated with neuroplasticity in the hippocampus. Gonadectomised rats show reductions in genes associated with neurogenesis, neuroplasticity, and cell adhesion (Quintela et al., 2015). Conversely, 4-weeks of testosterone supplementation via silastic capsule in GDX male rats enhances the expression of the calcium-dependent cell adhesion molecule, N-cadherin, in the hippocampus (Monks et al., 2001). However, a study from our laboratory has shown that testosterone supplementation in GDX male rats for 3-weeks via daily injection failed to enhance the expression of the calcium-independent cell adhesion molecule PSA-NCAM (Wainwright et al., 2016). Given the differences in methodology and the proteins measured, conclusions are difficult to draw regarding the influence of androgens on cell adhesion molecule expression. These findings indicate, however, that androgens modulate hippocampal plasticity in the adult hippocampus beyond their influence on hippocampal neurogenesis.

### **Androgens and Neurogenesis: Potential Implications for Cognitive Function**

There is equivocal data to support that androgens significantly affect cognition in young adult males. Although studies show that testosterone or androgen manipulation does not influence acquisition of the Morris Water Maze (Sandstrom et al., 2006; Spritzer and Galea, 2007; Benice and Raber, 2010; Spritzer et al., 2011b), short-term testosterone supplementation can influence early (7 days) but not later (15 days) spatial memory retrieval in this task (Sandstrom et al., 2006; Spritzer and Galea, 2007; Spritzer et al., 2011b). **These findings are clearly uncoupled with the findings of testosterone-induced increases in hippocampal neurogenesis as it takes at least 30 day of testosterone treatment to see an upregulation in**

neurogenesis (Spritzer and Galea, 2007). However, shorter-term AR manipulations can modulate spatial acquisition and memory in the Morris water Maze, as the AR antagonist flutamide impaired MWM acquisition and memory retrieval when administered into the CA1 region (Naghdi et al., 2001; Edinger and Frye, 2007), and Tfm male rats showed impaired acquisition of the Morris water Maze (Jones and Watson, 2005). These studies collectively suggest that short-term exposure to testosterone may enhance spatial memory via an AR-dependent mechanism occurring within the CA1 region. Intriguingly, one study showed that short-term estradiol, given only during the axon-extension phase, not only increased the survival of new neurons in adult male meadow voles but also improved spatial memory in the Morris Water Maze (Ormerod et al., 2004). Together, this suggests that, at least in males, androgens and estradiol can improve spatial memory if given in limited duration.

There is also evidence that loss of androgens may contribute to memory decline in aging men and in men with Alzheimer's disease (Moffat et al., 2004). Lower brain and serum levels of testosterone are detected in men with AD (Moffat et al., 2004; Rosario et al., 2011), and these reductions are apparent 10 years prior to a diagnosis (Moffat et al., 2004). Interestingly, androgens may interact with APOE4 allele, a genetic risk factor for Alzheimer's disease, to enhance the risk for pathological cognitive ageing (Panizzon et al., 2010; 2014). For example, a smaller hippocampus is evidenced in middle-aged men with lower serum testosterone levels possessing an APOE4 allele (Panizzon et al., 2010). Further, better verbal memory in middle-aged men with APOE4 was found in those with higher levels of testosterone (Panizzon et al., 2014). Increased amyloid burden was associated with decreased testosterone levels in men with mild cognitive impairment (MCI) carrying at least one APOE4 allele (Verdile et al., 2014). Although some studies indicate that androgen supplementation in older men with MCI or AD can improve spatial memory (Cherrier et al., 2005), others find testosterone supplementation in men with MCI or AD did not have a significant effect on cognition (Cherrier et al., 2015; Lu et al., 2006). However, these studies did not account for APOE4 status, which may have contributed to the negative findings, and/or the duration or dose of testosterone may not have been adequate. Taken together, androgens may be neuroprotective against cognitive ageing in men, but may be less efficacious in aiding cognitive function after a diagnosis with Alzheimer's disease. Further, the interaction of testosterone with known genetic predispositions, such as APOE, should be considered in research on cognitive ageing in men.

## **Androgens and Neurogenesis: Potential Implications for Mood Regulation**

Numerous studies suggest an influence of androgens on mood and affect, both clinically and pre-clinically. There is evidence showing that androgen levels in men are associated with depression; for example, there is an increased incidence of depression in males coinciding with the age-related decline in testosterone levels (Shores et al., 2004; Shores et al., 2005; McIntyre et al., 2006; Shores et al., 2009). Similarly, young and middle-aged men in a hypogonadal state are far more susceptible to developing depression (Veras and Nardi, 2010; Westley et al., 2015), portending protective effects of testosterone against the development of depression. Testosterone has also shown antidepressant effects, as testosterone replacement therapies have some efficacy in alleviating depressive symptoms in hypogonadal men (Seidman and Rabkin, 1998; Shores et al., 2009; Zarrouf et al., 2009). A meta-analysis in men indicated that testosterone therapy improved overall depression scores (Hamilton depression rating scale; HAM-D) after examining 7 studies that fulfilled their criteria (Odds ratio 0.40). Testosterone therapy also showed improvement in depression scores in hypogonadal men, men with HIV/AIDS, and a mild improvement in eugonadal men (Zarrouf et al., 2009). Testosterone replacement has also shown efficacy as an adjunct treatment to clinical antidepressants in cases of treatment-resistant depression (Seidman and Rabkin, 1998; Pope et al., 2003). While other androgen supplementation is available, and new selective androgen receptors (SARMs) are being developed, to our knowledge no research has been published using SARMs to treat mood in men to date (Omwancha and Brown, 2006).

Androgen therapies are not always seen to be effective for men suffering depression (Seidman et al., 2001a; Pope et al., 2010). Studies differ widely on the administration of testosterone (topical gel appears more efficacious than intramuscular injections), time course, age of participants, depression scales, and what is considered an effective response rate (the meta-analysis used 50% effective change in HAM-D), which may all contribute to findings related to the efficacy of testosterone treatment (Zarrouf et al., 2009). Furthermore, individual differences, such as the glutamine (CAG) polymorphism of the AR, are also related to the effects of testosterone on depression incidence, dependent on age (Seidman et al., 2001b; Harkonen et al., 2003). For example, testosterone levels correlate negatively with depressed mood in men with short CAG repeat length but not in men with longer CAG repeats (Seidman et al., 2001b). However, greater CAG repeat length is positively correlated with depressed mood in older men

with no significant difference in serum testosterone levels (Harkonen et al., 2003). Thus, while there is controversy over the antidepressant-like action of testosterone supplementation in depressed patients, the majority of randomized control trials show some effectiveness of testosterone supplementation that may be limited to subpopulations and subtypes of depression (Rodgers et al., 2014).

The effects of testosterone supplementation, though less numerous, also indicate a positive effect on mood in women. Testosterone treatment in premenopausal women with low circulating levels of testosterone improved mood scores (Goldstat et al., 2003). Similarly, women that underwent oophorectomy showed decreased depressive mood scores when treated with a combination of conjugated equine estrogens and testosterone compared to treatment with estrogens alone (Shifren et al., 2000). Women presenting with treatment resistant depression showed improved mood scores or completely remitted in response to low-dose testosterone treatment as an adjunct therapy (Miller et al., 2009). Moreover, a meta-analysis of post-menopausal women shows testosterone treatment improves mood scores; both alone, and in combination with estrogens (Zweifel and O'Brien, 1997). These findings add credence to the supposition that androgens may function to improve mood in both men and women.

Animal studies support the clinical literature in demonstrating anxiolytic and antidepressant-like effects of androgens. Studies have shown that the removal of testicular hormones potentiates the stress response in males following acute stress (Viau et al., 1999; Viau et al., 2003), and testosterone replacement, or supplementation, is associated with a recovery of the stress response (Seale et al., 2004). Indeed, androgens function to directly inhibit HPA output at the level of the hypothalamus (Viau and Meaney, 1996) and the administration of DHT decreases the expression of GR in the CA1 region of GDX males (Kerr et al., 1996). In the NSF test, classically a test for anxiety-like behaviour and anxiolytic drugs, treatment with exogenous testosterone produces anxiolytic effects (Aikey et al., 2002). Similarly, research from our laboratory shows that testosterone treatment in gonadectomised males exposed to chronic stress ameliorates the anxiety/depressive-like behaviours in the NSF test (Wainwright et al., 2016). Gonadectomy has anxiogenic effects in rodents, which are ameliorated by testosterone treatment (Carrier and Kabbaj, 2012b; Khakpai, 2014). The anxiolytic effects of testosterone are blocked by the administration of the AR antagonist flutamide (Fernandez-Guasti and Martinez-Mota, 2005; Hodosy et al., 2012), suggesting these effects are mediated by the AR. However, several

metabolites of testosterone, including estradiol and  $3\alpha$ -diol, also produce anxiolytic effects independent of the AR (Frye and Edinger, 2004; Edinger and Frye, 2005; Carrier et al., 2015).

Androgens are also associated with depressive-like endophenotypes in rodent models. Research from our laboratory shows that gonadectomised male rats exposed to chronic unpredictable stress (CUS) show greater time spent immobile in the FST, a putative measure of depressive-like behaviour compared to intact males (Wainwright et al., 2011). Moreover, within this study, stress-exposed gonadectomised males had higher basal levels of corticosterone, indicative of disrupted HPA tone, and greater reductions in hippocampal neurogenesis and PSA-NCAM expression compared to intact males. Conversely, treatment with exogenous testosterone attenuated effects of CUS on gonadectomised male rats in the FST (Wainwright et al., 2016). In fact, androgen treatment serves to reduce immobility behaviour in both intact and GDX males, as well as female rats (Buddenberg et al., 2009; Frye and Walf, 2009; Carrier and Kabbaj, 2012b, a). Testosterone treatment also potentiates the effects of the tricyclic antidepressant drug imipramine in the FST when co-administered (Wainwright et al., 2016). Androgens also modulate the presentation of depressive-like behaviour in the sucrose preference test; gonadectomy serves to reduce sucrose preference, an effect reversed by testosterone supplementation (Carrier and Kabbaj, 2012a; Carrier et al., 2015), though not in male rats exposed to CUS (Wainwright et al., 2016). However, testosterone treatment facilitates the antidepressant effects of imipramine in CUS exposed male rats in the sucrose preference test (Wainwright et al., 2016).

There is ample evidence to support anxiolytic- and antidepressant-like effects of androgen treatment, alone and as an adjunct therapy, in both clinical and preclinical studies. Androgen-mediated modulation of hippocampal neurogenesis and neuroplasticity likely plays a role conferring mood-altering actions resulting from the disruption or supplementation of endogenous androgens; both through the alteration of HPA reactivity and monoameric neurotransmission. However, these effects may ultimately be mediated via the metabolization of testosterone into other active steroid hormones, which then exert their effects via estrogenic mechanisms or the direct modulation of neurotransmission.

## Hippocampal Neurogenesis and Estrogens

There is convincing evidence that estrogens are important players in the regulation of adult hippocampal neurogenesis (Galea et al., 2013; Pawluski et al., 2009). Below, we will review studies that have investigated the influence of endogenous fluctuations and exogenous manipulations of estrogens on neurogenesis within the dentate gyrus (summarized in Figure 3). We will discuss potential implications for affective and cognitive function, and in turn depression and dementia. Finally, we highlight potential cellular and molecular mechanisms that may underlie the effects of estrogens on neurogenesis and identify areas that necessitate further investigation.

### **Estrogen classes and estrogen signalling in the CNS: a brief overview**

Estradiol, estrone, and estriol are the major naturally occurring estrogens. Estradiol exists in two optical isomers,  $17\beta$ -estradiol and  $17\alpha$ -estradiol, with  $17\beta$ -estradiol being the more potent of the two (Shappell et al., 2010). In premenopausal women, estradiol, the most potent of the three major estrogens, is also the most abundant (Rannevik et al., 1986). After the menopausal transition, and while all circulating estrogens decline, the ratio of estrone to estradiol is gradually reversed and estrone becomes the more abundant estrogen (Rannevik et al., 1986). Estrogen signalling in the CNS is highly complex (Toran-Allerand, 2004), however, the actions of estrogens can be roughly categorized into genomic and non-genomic mechanisms. Estrogens can bind with estrogen receptors (ERs), ER $\alpha$  or ER $\beta$ , which are typically located in the cytoplasm or nucleus. The bound receptors act as transcription factors to regulate gene expression; they form homo or heterodimers that translocate to the nucleus and bind to estrogen response elements (EREs) located in the promoter regions of target genes. However, approximately one third of genes regulated by ERs do not include estrogen response element sequences (O'Lone et al., 2004), therefore it is becoming increasingly clear that bound ERs can regulate gene expression through other mechanisms, such as via protein-protein interactions with other transcription factors and their respective response elements (Björnström and Sjöberg, 2005). On the other hand, rapid, non-genomic effects are mediated through membrane associated ERs, such as the G-protein coupled estrogen receptor (GPER) (Olde and Leeb-Lundberg, 2009; Revankar et al., 2005; Thomas et al., 2005). While the terms genomic and non-genomic are used it is also important to note that even non-genomic signalling pathways can result in gene transcription indirectly through downstream cascades, and these genes may differ from those directly activated through EREs (Marino et al., 2006). The hippocampus contains ER- $\alpha$ , ER- $\beta$  and GPER

receptors (Shima et al., 2003; Shughue et al., 1997). Intracellular ERs are located in the CA1, CA3, and the dentate gyrus in males and females, while GPER is most dense in the CA3 region in female rats (Duarte-Guterman et al., 2015a; Loy et al., 1988; Maggi et al., 1989). ERs are regulated by age, hormone exposure and reproductive experience (M. Jin et al., 2005; Pawluski et al., 2010), but while there is less work done on GPER, GPER expression is reduced by estradiol in the GCL, but not in the CA1 and CA3 regions in females (Duarte-Guterman et al., 2015a).

### **Endogenous fluctuations in ovarian hormones influence hippocampal neurogenesis**

Females exhibit natural fluctuations in ovarian hormones across the lifespan; this is evident across the menstrual and estrous cycles, in women and rodents respectively, but also in relation to reproductive events, and in the transition to reproductive senescence (Butcher et al., 1974; Rannevik et al., 1986; Rosenblatt et al., 1988; Sherman and Korenman, 1975; Yoshinaga et al., 1969). It is also important to note that menstrual cycle and estrous cycle fluctuations in hormones can differ dramatically with age (Klein et al., 1996) and parity (Barrett et al., 2014).

### ***Estrous cycle and breeding seasons***

The rodent estrous cycle follows a 4-5 day periodicity and is divided into four phases; metestrus, diestrus, proestrus, and estrus. The cycle is characterized by distinct fluctuations in ovarian hormones, in which estradiol and progesterone peak during proestrus then fall sharply following ovulation and remain low during other phases of the estrous cycle (Butcher et al., 1974). These fluctuations in ovarian hormone milieu are transient and are accompanied by changes in the levels of cell proliferation within the subgranular zone (Rummel et al., 2010; Tanapat et al., 1999; Tzeng et al., 2014). In both rats and mice, highest levels of cell proliferation occur in proestrous females relative to estrous and diestrous controls (Lagace et al., 2007; Rummel et al., 2010; Tanapat et al., 1999; Tzeng et al., 2014), reaching levels 50% higher than during diestrus (Tanapat et al., 1999). In comparison to the estrous phase, the survival of new neurons generated during proestrus is increased at 14 but not 22 days (Tanapat et al., 1999), suggesting that the upregulation of cell proliferation during proestrus results in a transient increase in the survival of these new neurons. Some studies have failed to find a significant effect of estrous cycle phase on cell proliferation in rodents (Lagace et al., 2007), but it should be noted that estradiol levels during the estrous cycle can change rapidly over the course of a few hours, and thus some studies

may miss the peak of estradiol levels. Thus, it appears that the effects of endogenous fluctuations of ovarian hormones are primarily on cell proliferation rather than an independent effect on the survival of these new neurons.

Female meadow voles, on the other hand, are seasonal breeders and induced ovulators, in which circulating estradiol levels are high during the breeding season and low during the non-breeding season (Sawrey and Dewsbury, 1985). As such, they exhibit a different profile of endogenous hormone fluctuations than mice and rats. Seasonal differences are also evidenced in cell proliferation levels within the dentate gyrus, in which female meadow voles captured during the non-breeding season had higher levels of cell proliferation relative to those captured during the breeding season (Galea and McEwan, 1999). In the same study, plasma levels of estradiol were negatively correlated with cell proliferation levels (Galea and McEwan, 1999). Although seemingly contradictory to the studies discussed above indicating increased cell proliferation during periods of higher estradiol in rats and mice, additional work clarified that the effects of estradiol on cell proliferation are time-dependent (Ormerod et al., 2003), and this will be discussed below.

Although beyond the emphasis of the current review, it is important to note that besides neurogenesis, substantial plasticity has been detected in the hippocampus driven by estrous cyclicity. For example, an approximately 30% difference in the density of hippocampal synapses is observed between the estrous and proestrous phases in rats, with estradiol levels being positively correlated to synaptic density (Woolley and McEwen, 1992). In addition, there is increased excitability in the CA1 and CA3 regions of the hippocampus during phases of the estrous cycle that are characterized by highest estradiol levels (Scharfman et al., 2003). Estrous cycle phase also influences long-term potentiation (LTP) in the CA1 region of the hippocampus, as evidenced by significantly greater potentiation during proestrus (Warren et al., 1995), although this is dependent on time of day (Sabaliauskas et al., 2015). Contrary to the idea that greater LTP would be correlated with better learning, proestrus is correlated with impaired spatial learning (Tada et al., 2015; Warren et al., 1995), again dependent on time of day (Sabaliauskas et al., 2015). Furthermore, inhibitory avoidance task conditioning failed to drive LTP during proestrus in rats (Tada et al., 2015). Taken together, the evidence suggests that proestrus is associated with structural and functional adaptations in the hippocampus of female

rodents, including enhanced excitability and potentiation, and increased synaptic density and cell proliferation.

### ***Ageing and the effects of exogenous estrogen manipulations***

In ageing female rats, the estrous cycle becomes irregular and prolonged, with most rats eventually reaching an acyclic stage of persistent-diestrus (LeFevre and McClintock, 1988). Although adult neurogenesis persists throughout the lifespan, ageing is accompanied by a decrease in cell proliferation levels (Driscoll et al., 2006; Kuhn et al., 1996; Nacher et al., 2003). In female rats, this decline begins at middle age but plateaus thereafter, as levels of cell proliferation do not significantly differ between middle aged and old female rats (Driscoll et al., 2006; Kuhn et al., 1996; Nacher et al., 2003). Likewise, the survival of newly generated neurons also declines in ageing female rats (Kuhn et al., 1996; Merrill et al., 2003). This age-related decline in neurogenesis may be in part related to the natural reductions in ovarian hormone levels.

Most studies have used ovariectomized rats to model menopause in women, and to study the effects of hormone treatment on neurogenesis in older age. In a study using 12-month-old nulliparous (i.e. never pregnant) ovariectomized rats, a single dose of estradiol, given one week after ovariectomy, was not effective in increasing cell proliferation (Barha and Galea, 2011). On the other hand, cell proliferation was increased in 22-month-old nulliparous rats that were ovariectomized at 10 months of age, following 10 weeks of estradiol treatment or of supplementation with the phytoestrogen-rich soybean extract (Perez-Martin et al., 2005). Together, these findings indicate that progenitor cells in the ageing female hippocampus may become less responsive to the acute effects of estradiol, but retain a level of responsiveness to chronic estradiol treatment. Thus, it appears that the ability of estradiol to influence neurogenesis is altered across the lifespan. The mechanism behind this altered plasticity potential is yet to be elucidated, but it may relate to changes in receptor levels, or alterations that are downstream of receptor activation.

### **Ovarian hormone depletion and replacement influence hippocampal neurogenesis**

#### ***Hormone depletion and acute replacement***

Additional evidence for the role of estrogens in neurogenesis is derived from studies of exogenous hormone manipulation in adult ovariectomized animals. Following the removal of the ovaries, serum levels of estradiol plummet within twenty-four hours (Woolley and McEwen, 1993). Short-term ovariectomy (6-7 days) reduces cell proliferation (Tanapat et al., 1999), but long-term ovariectomy (3-4 weeks) does not influence cell proliferation in rats or mice (Green and Galea, 2008; Lagace et al., 2007; Tanapat et al., 2005). This points to the existence of some unknown mechanism that restores cell proliferation levels after longer periods of ovariectomy. *De novo*, or local, synthesis of estrogens may be one mechanism, but we feel that it is an unlikely mechanism given that plasma and hippocampal estradiol levels correlate during the estrous cycle in female rodents (Kato et al., 2013). Furthermore, because steroid hormones are lipophilic and hydrophobic, the better comparison to demonstrate significant *de novo* synthesis of estradiol in the hippocampus would be to show significant levels of estradiol post-ovariectomy in the hippocampus. Indeed, studies indicated that estradiol within the hippocampus was undetectable or very low when examined 2-3 weeks post-ovariectomy in adult rat and mice (Barker and Galea, 2009; Fester et al., 2012; Kato et al., 2013). However, because estrogens have stimulatory effects on the HPA axis (Goel et al., 2014), and corticosterone can reduce cell proliferation (Brummelte and Galea, 2010), it is possible that reduced circulating levels of corticosterone following long-term ovariectomy may play a role in the return of cell proliferation to pre-ovariectomy levels. Further, a transient decrease in serotonin turn-over is observed in the hippocampus following ovariectomy (J. Zhang et al., 1999), coinciding with the transient decrease in neurogenesis. As will be discussed below, this indicates that the normalization of neurogenesis after longer periods of ovariectomy may be related to a normalization of serotonin turnover.

In young adult female rats, acute estradiol replacement after short-term ovariectomy (1 week) restores cell proliferation to levels of sham controls, and this effect is dose dependent and non-linear (Barha et al., 2009; Tanapat et al., 2005). For example, a single injection of 0.3 µg (Barha et al., 2009) or 10 µg (Barha et al., 2009; Tanapat et al., 2005; 1999) of estradiol restores the ovariectomy-induced reduction in cell proliferation, while doses of 1 µg (Barha et al., 2009; Tanapat et al., 2005) or 50 µg (Tanapat et al., 2005) are ineffective at restoring cell proliferation levels. Below we will provide evidence indicating that the duration of hormone exposure is an important determinant of estradiol's effects on cell proliferation. In these studies, Tanapat and

colleagues administered 17 $\beta$ -estradiol two hours prior to BrdU, and perfused the animals two hours after BrdU. Barha and colleagues, on the other hand, administered 17 $\beta$ -estradiol treatment either 30 minutes or 4 hours before BrdU, and perfused the animals 24 hours later. Importantly, the 0.3  $\mu$ g and 10  $\mu$ g doses of estradiol are physiologically relevant as they result in circulating levels comparable to diestrus and proestrus, respectively (Sohrabji et al., 1994; Viau and Meaney, 1991). The same acute dose of 10  $\mu$ g estradiol fails to rescue cell proliferation levels 4 weeks post-ovariectomy (Tanapat et al., 2005), indicating that progenitor cells in the hippocampus may become less sensitive to the effects of estradiol after a longer duration of ovarian hormone deprivation.

The duration of hormone exposure is also critical as it affects the outcome of estrogen exposure on cell proliferation in the dentate gyrus of female rodents (Ormerod et al., 2003). Indeed, acute administration of estradiol benzoate in female meadow voles results in an initial enhancement of cell proliferation at 4 hours post-administration, followed by a suppression in cell proliferation at 48 hours post-administration (Ormerod et al., 2003). This estradiol-induced suppression of cell proliferation was mediated by the stimulatory effects of estradiol on adrenal steroids as it was shown to be reversed by adrenalectomy (Ormerod et al., 2003).

Relatively less is known about the effects of other estrogens on adult hippocampal neurogenesis. However, in female rats, cell proliferation levels were increased 30 minutes following the administration of 17 $\beta$ -estradiol, estrone, and 17 $\alpha$ -estradiol, but not estradiol benzoate, all administered one week post-ovariectomy (Barha et al., 2009), and this relates to the faster metabolism of estradiol in comparison to the slower acting estradiol benzoate. These effects were dose-dependent, all with a similar pattern in which low and high, but not moderate doses, resulted in an upregulation in cell proliferation (Barha et al., 2009). In the same study, the hormone treatments had no significant effect of cell proliferation at 4 hours post-administration (Barha et al., 2009), thus further emphasizing importance of exposure duration and the injection of a DNA synthesis marker relative to treatment see (Taupin, 2007).

#### ***Hormone depletion and chronic replacement:***

The literature on the effects of chronic estrogen replacement on hippocampal neurogenesis is much less extensive and perhaps more complex than that of acute estrogen replacement. Most studies show that chronic estradiol exposure in female rats does not affect cell

proliferation, as seen with 21 days of treatment using continuous release pellets, injections or a cyclical regimen of injections every 4 days for 20 days, all of which were initiated approximately one week post-ovariectomy (Chan et al., 2014; Tanapat et al., 2005). On the other hand, 15 days of continuous estradiol benzoate injections, administered starting one week after ovariectomy, increased cell proliferation in female rats (Barker and Galea, 2008). Given the critical role of hormone exposure duration discussed above, the dissimilar findings may be attributed to the timeline of estradiol exposure relative to the examination of cell proliferation, or to methodological differences relating to the length of estradiol exposure (continuous vs. cyclic). It is also possible that these differences may have arisen from differential ER (including GPER) adaptation from cyclical versus continuous exposure to estrogens (Brown et al., 1996).

The effect of chronic estradiol replacement on the survival of new neurons in ovariectomized female rats depends on the timing of estradiol exposure relative to that of cell proliferation. Specifically, the survival of new neurons is enhanced in cell populations that are produced after the initiation of estradiol treatment (McClure et al., 2013), but is suppressed in cell populations that are produced prior to the initiation of estradiol treatment (Barker and Galea, 2008; Chan et al., 2014). In other words, the hormone-related environment during which the cells are generated determines the outcome of chronic estradiol treatment on cell survival. Given that the effects of estradiol to enhance cell survival are only seen when the labeled cells are produced in an estradiol-enriched environment, this effect is probably attributed to the estradiol-induced upregulation of cell proliferation. However, this pattern does not necessarily apply to all forms of estrogens. For example, the survival of new neurons generated in an estrone-enriched environment is decreased, thus, a hormone-enriched environment is not necessarily conducive to the survival of newly generated neurons. For example, in female rats, although a single dose of 10 µg of estrone increased cell proliferation (Barha et al., 2009), 20 days of treatment with the same dose reduced the survival of new neurons generated after the initiation of estrone replacement (McClure et al., 2013). Intriguingly, experience affects the ability of estrogens to alter neurogenesis and spine density in the CA1 region. The estrone-based hormone replacement therapy Premarin increased the survival of new neurons in spatially trained female rats but not in cage controls (Barha and Galea, 2013), although the authors acknowledge that this effect may have been due to food deprivation. An additional example in which spatial training can interact with hippocampal plasticity comes from a study in which spatial training eliminated the effects

of estradiol to increase CA1 spine density that is observed in behaviorally-naïve female rats (Frick et al., 2004).

### **Progestins influence adult hippocampal neurogenesis**

Estrogens and progesterone naturally fluctuate across the estrous and menstrual cycles, and have a dynamic relationship in the CNS and the periphery (Mani and Blaustein, 2012). Thus, it is important to study the influence of progesterone on neurogenesis, both in isolation and in unison with estrogens. Although limited, the existing literature suggests that progesterone can modulate the effects of estradiol to influence neurogenesis in the hippocampus of adult female rats (Tanapat et al., 2005). Indeed, a single dose of progesterone given 24 hours after estradiol administration decreased the estradiol-induced enhancement of cell proliferation (Tanapat et al., 2005). Therefore, progesterone may antagonize the effects of estradiol on cell proliferation. It remains unknown, however, if this relationship is time- or sex-dependent. In adult ovariectomized female rats, chronic progesterone and estradiol benzoate, starting one week post-ovariectomy, had no significant effect on cell proliferation when given alone or on combination, but decreased the survival of new neurons that were administered BrdU prior to hormone treatment (Chan et al. 2014). Thus, the interaction of progesterone and estradiol to affect neurogenesis may differ from acute (Tanapat et al., 2005) to chronic (Chan et al., 2014) administration. Progesterone treatment for 7 days increased cell proliferation in adult male rats (Barha et al., 2011), and *in vitro*, progesterone dose-dependently enhanced cell proliferation in neural progenitor cell cultures derived from adult rat dentate gyrus (Liu et al., 2009), although the sex of the rats was not disclosed in the latter study. Furthermore in two models of CNS injury – cerebral ischemia and traumatic brain injury (TBI), progesterone suppressed the injury-induced enhancement of cell proliferation (Barha et al., 2011; Zhang et al., 2010), enhanced the ischemia-suppression of the survival of new neurons (Zhang et al., 2010), and reduced the injury-induced cell death in adult males (Barha et al., 2011). Collectively, these findings indicate that progesterone normalizes the injury-induced alterations in neurogenesis in male rodents. However, it is important to note that these studies were conducted in male subjects, thus it is not certain that the same findings will be observed in females. Sex differences in the effects of progesterone are important to investigate, especially given that the efficacy of progesterone for acute traumatic brain injury may differ by sex, where men may have more favorable outcomes than women (Wright et al., 2014).

## **Reproductive experiences influence hippocampal neurogenesis**

### ***Pregnancy and the postpartum period***

Pregnancy and the postpartum period are accompanied by dramatic changes in steroid and peptide hormones, and with behavioural changes that are necessary for offspring survival (Kinsley and Lambert, 2008). In rodents, early pregnancy is characterized by low levels of estradiol and high levels of progesterone, and this pattern is reversed towards the end of pregnancy wherein progesterone levels decline and estradiol levels rapidly increase (Garland et al., 1987; Rosenblatt et al., 1988). At parturition, estradiol levels drop abruptly and remain low during the postpartum period (Bloch et al., 2003a). Not surprisingly, the maternal brain undergoes numerous structural and functional changes, including gross neuroplastic changes such as reduced hippocampal volume in rats (Galea et al., 2000), and a transient reduction in overall brain volume in women (Oatridge et al., 2002). In addition, changes in dendritic morphology and spine densities in the CA1 and CA3 regions are detected with primiparous (pregnant once) rats exhibiting reductions compared to nulliparous and multiparous (pregnant more than once) rats (Pawluski and Galea, 2006).

Cell proliferation within the dentate gyrus is not significantly affected during gestation, as examined in early gestation, on gestation day 1 (GD1) (Pawluski et al., 2010), GD7 (Shingo et al., 2003) or late gestation (GD21) (Furuta and Bridges, 2005). However, expression of polysialylated neuronal cell adhesion molecule (PSA-NCAM), which is expressed on newly generated and migrating neurons, is increased in mid-late gestation (gestation day 18), suggesting a potential enhancement of neurogenesis in mid-late gestation (Banasr et al., 2001). Other reports suggest that the survival of new neurons produced in early gestation (GD1) is not significantly affected across gestation in rats (Pawluski et al., 2010). On the other hand, neurogenesis was suppressed in pregnant mice during mid- and late-gestation, but this suppression was transient as it normalized by postpartum day 21 (Rolls et al., 2008). Taken together, these findings suggest that while cell proliferation is not affected, the survival of new neurons may be altered during mid pregnancy (after GD7), but is normalized by late pregnancy (GD21).

More robust alterations in hippocampal neurogenesis are evidenced during the postpartum period. In rats, several studies demonstrate reduced cell proliferation in early

postpartum in primiparous and biparous dams (Darnaudéry et al., 2007; Leuner et al., 2007a; Pawluski and Galea, 2007). Leuner and colleagues found that in comparison to nulliparous females in diestrus, cell proliferation is suppressed in primiparous females during the early postpartum period (PPD 2 and 8), but not following weaning (PPD 28; see reviews by Leuner and Sabihi (2016) and Slattery and Hillerer (2016) in this issue). This postpartum-related reduction in cell proliferation was prevented by the removal of pups, or by adrenalectomy with low-dose corticosterone replacement, suggesting that this suppression may be attributed to elevations in adrenal steroids related to maternal experience and lactation (Leuner et al., 2007a). This is in partial agreement with a study from our group, in which cell proliferation was decreased in primiparous and multiparous rats in the early postpartum (postpartum day; PD 2), compared to nulliparous controls (Pawluski and Galea, 2007). Interestingly, primiparous rats had decreased 21-day survival of BrdU-labeled cell the postpartum period (PD 22), regardless of pup-exposure, in comparison with nulliparous and multiparous groups (Pawluski and Galea, 2007). Further, in nulliparous rats exposed to pups, cell proliferation, cell survival, and cell death were all increased in comparison to parous rats and nulliparous rats not exposed to pups, indicating that pup exposure differentially influenced neurogenesis with parity (Pawluski and Galea, 2007). Another study reported decreased expression of doublecortin in the dentate gyrus of primiparous rats on PPD 21, relative to nulliparous controls (Workman et al., 2015), also indicating a reduction in neurogenesis in the later postpartum period. The contrasting effects of pup exposure between these studies may be attributed to methodological differences relating to the timing of pup removal, the timeline of BrdU administration and perfusion, or a combination of these factors. Specifically, Leuner and colleagues removed pups shortly after birth on PD0, administered BrdU on PD3, and perfused the animals 2 hours later (Leuner et al., 2007b). On the other hand, Pawluski and colleagues administered BrdU on PD1, removed pups 24 hours later, and perfused the animals on PD22 (Pawluski and Galea, 2007). Thus, in the Leuner et al study, new cells produced after pup removal would have been labelled with BrdU, while in the Pawluski and Galea study, new cells produced prior to pup removal would have been labelled with BrdU. With that design, the Pawluski and Galea experiment tested the effects of pup removal (pregnancy only) on the survival of new cells independent of effects on proliferation, while the Leuner and colleagues examined the effect of pup removal on cell proliferation. In addition, Pawluski and Galea examined the influence of pup removal on the survival of new neurons later in the postpartum period (PPD22), while the Leuner et al study examined cell

proliferation three days after pup removal in early postpartum (PPD3). It is therefore not surprising that the effects of pup removal vary in the two studies, considering that different aspects of neurogenesis were examined (proliferation versus survival), and during different times in the postpartum period (earlier versus later).

In addition, estradiol withdrawal after a hormone simulated pregnancy in rats resulted in suppressed cell proliferation (Green and Galea, 2008). This mirrors what is seen naturally in the postpartum period, and suggests that the drop in estradiol following parturition may play a key role in the postpartum-related changes in cell proliferation. Indeed, treatment with a selective ER $\beta$  agonist during the simulated postpartum period rescued the suppression in cell proliferation (Green and Galea, 2008). Collectively, these studies provide incontrovertible evidence for the influence of pregnancy-related fluctuations in ovarian hormones on hippocampal neurogenesis.

### ***Partiy, Aging, and Neurogenesis***

Previous pregnancy and motherhood also affect the trajectory by which additional pregnancies influence hippocampal neurogenesis (Barha et al., 2015; Pawluski and Galea, 2007). While both primiparous and multiparous females show reduced cell proliferation in the early postpartum (PPD2) in comparison to nulliparous females, the survival of new neurons during the postpartum period was modulated by previous reproductive experience (Pawluski and Galea, 2007). Specifically, cell survival was suppressed in primiparous but not multiparous females in the postpartum, relative to nulliparous controls, and this effect was independent of exposure to pups (Pawluski and Galea, 2007). Interestingly, multiparous middle-aged rats had a greater density of immature neurons in the dentate gyrus than age-matched nulliparous rats (Barha et al., 2015). Thus, previous reproductive experiences have long lasting effects on hippocampal plasticity, and these studies underscore the need for considering the role of previous reproductive experience on the subsequent effects of sex hormones to influence hippocampal plasticity.

### **Estrogens and Neurogenesis: Potential Implications for Cognitive Function**

Male rats outperform female rats in spatial acquisition, and coupled with this, male, but not female, rats show increased survival of new neurons after spatial training (Chow et al., 2013). Interestingly, the activation of newly generated neurons in the dorsal dentate gyrus in response to spatial memory retrieval was positively correlated with performance in females but not males

(Chow et al., 2013). Similar findings were obtained from a study using a spatial pattern separation task previously shown to require hippocampal neurogenesis (Clelland et al., 2009), in which sex differences favouring males were found in spatial strategy users. Male spatial strategy users outperformed female spatial strategy users in separating similar (adjacent) patterns, and had greater neurogenesis levels compared to female spatial strategy users (Yagi et al., 2015). Interestingly, the survival of new neurons was positively correlated with the ability to separate similar patterns in female but not male rats (Yagi et al., 2015). Not all tasks show male superiority in performance; in a study using the hippocampus- and cerebellum-dependent trace eyeblink conditioning task, sex differences in conditioning favouring females was found, and this superior performance in females was accompanied by increased survival of new neurons relative to males (Dalla et al., 2009). These sex differences in the relationship between learning and neurogenesis point to a highly probable role of gonadal hormones, but to our knowledge a direct relationship is yet to be investigated. Collectively, it is clear that sex can modulate the relationship between learning and neurogenesis, dependent on the type of task.

Estrogens modulate both neurogenesis and learning and memory. An extensive review of the literature on estrogens modulation of learning is beyond the scope of this review, but the readers are directed to other reviews (Duarte-Guterman et al., 2015b; Frick et al., 2015; Korol and Pisani, 2015; Tuscher et al., 2015; Ervin et al., 2015). However, the estrone-based Premarin increased survival of new neurons but impaired both spatial working and reference memory in the spatial radial arm maze (Barha and Galea, 2013). The activation of new neurons in response to spatial memory retrieval was reduced in the lower dose of Premarin (1 ug) but not in the higher dose (2ug) used (Barha and Galea, 2013). Further, activation of new neurons was associated with poorer acquisition in the radial arm maze in Premarin-treated rats indicating that new neurons surviving under Premarin-rich conditions did not contribute to spatial memory and were associated with decrements in performance (Barha and Galea, 2013), perhaps indicating that these new neurons did not make appropriate connections within the CA3. Another study showed similar findings, in which activation of new neurons produced and surviving under estrone-rich conditions were not associated with spatial memory retrieval, while activation of new neurons produced under estradiol-rich conditions tended to be associated with enhanced spatial memory retrieval (McClure et al., 2013). Together, these studies indicate that while estrogens influence neurogenesis and cognition, there is not a direct association with number of

new neurons or number of activated new neurons. Importantly, new neurons may not always make appropriate connections, and thus studies in the future should be directed towards investigating the targets of new neurons surviving and created under estrogens.

### **Estrogens and Neurogenesis: Potential implications for mood regulation**

There is an abundance of literature, preclinical and clinical, indicating an intimate link between ovarian hormones and mood regulation (Steiner et al., 2003). Besides the robust sex difference in the incidence of depression favouring women, windows of heightened vulnerability to depression in women are characterized by dramatic fluctuations or sustained reductions in ovarian steroids (Cohen et al., 2006; Hendrick et al., 1998). Namely, in women, times of greatest vulnerability to develop depression are in the postpartum period, in which women are hypogonadal after experiencing sustained extreme levels of estrogens and progesterone (Hendrick et al., 1998), and in the transition to menopause (Freeman et al., 2006), which is characterised by more dramatic fluctuations in ovarian hormones preceding a persistent hypogonadal state. Rodent studies of ovariectomy demonstrate increased depressive like behaviour that is reversed by estradiol replacement (Bekku and Yoshimura, 2005). In addition, withdrawal from a hormone-simulated pregnancy resulted in increased depressive-like behaviour (Craft et al., 2010; Galea et al., 2001; Suda et al., 2008), which is reversed by estradiol treatment (Galea et al., 2001; Green and Galea, 2008). These behavioural phenotypes in animal models are also accompanied by reductions in neurogenesis (Green and Galea, 2008). Importantly, the implications of altered neurogenesis on mood regulation in these models are uncertain.

As we discuss earlier, the reduction in hippocampal volume in individuals with depression is more profound in men than women (Frodl et al., 2002; Vakili et al., 2000), and is detected only in children and middle aged to older individuals (McKinnon et al., 2009). Together these findings indicate that ovarian hormones, particularly during the reproductive years, may protect the female hippocampus from depression-related reductions in volume. Interestingly, chronic antidepressant treatment was associated with an increased hippocampal volume in women experiencing an alleviation of depression symptoms relative to non-responders, but the same effect was not seen in men (Vakili et al., 2000). Although studies investigating hippocampal volume changes in depression do not provide direct evidence for alterations in neurogenesis, there is evidence from post-mortem studies that age and sex, and thus likely sex

hormones, are implicated in antidepressant effects on hippocampal neurogenesis (Epp et al., 2013a; Lucassen et al., 2010). Specifically, chronic antidepressant treatment increased the ratio of immature to mature neurons in depressed women but not men (Epp et al., 2013a), and the pro-neurogenic effects of antidepressants were not detected in older depressed individuals (Epp et al., 2013a; Lucassen et al., 2010). Together, these data indicate that sex hormones, and particularly ovarian hormones in women, may facilitate the pro-neurogenic effects of antidepressants. The specific role of sex hormones in this respect, and the implications for antidepressant efficacy, are two avenues that necessitate future investigation.

### **Potential mechanisms for the influence of estrogens on adult hippocampal neurogenesis**

Estrogens have extremely diverse effects in the central nervous system (McEwen et al., 2012; McEwen and Alves, 1999), and the downstream signalling cascades that drive the effects of estrogens on neurogenesis in the hippocampus are largely unknown. In the following section we discuss potential mechanism that may underlie the effects of estrogens on neurogenesis in the hippocampus, and consider functional implications when appropriate. Importantly, we acknowledge that direct evidence for a mediating role of these mechanisms is scarce, and thus this section largely serves to highlight potentially mechanisms and to identify areas for future investigation.

### **Receptor mechanisms underlying the influence of estrogens on neurogenesis**

Given the complexity of estrogen signalling, the mechanisms by which estrogens influence adult hippocampal neurogenesis have not been fully deciphered. However, the effects of estradiol on cell proliferation in the dentate gyrus appear to be at least partially mediated through ER $\alpha$  and ER $\beta$  (Mazzucco et al., 2006). In adult ovariectomized female rats, treatment with the ER $\alpha$ -selective agonist propylpyrazole-triol (PPT), or the ER $\beta$ -selective agonist diarylpropionitrile (DPN) increased cell proliferation, suggesting that both nuclear estrogen receptors participate in the pro-proliferative effects of estradiol (Mazzucco et al., 2006). To further support the role of estrogen receptors, the estrogen receptor antagonist ICI 182,780 was shown to reverse the estradiol-induced upregulation of cell proliferation (Nagy et al., 2006). In addition, because both intracellular ERs colocalize with progenitor cells in subgranular zone of the dentate gyrus, albeit at low levels (Mazzucco et al., 2006), it is likely that estrogens can upregulate cell proliferation through direct interactions with intracellular ERs in progenitor cells (see figure 5). Interestingly,

treatment with the GPER agonist (G1) decreased cell proliferating in adult ovariectomized rats (Duarte-Guterman et al., 2015a), indicating an estradiol-independent role of GPER in hippocampal neurogenesis, or alternatively an antagonistic effect of intracellular and membrane bound ER activation to maintain levels of neurogenesis. Lastly, unlike intracellular ERs, GPER did not co-localize with progenitor cells in the subgranular zone of the dentate gyrus (Duarte-Guterman et al., 2015a), indicating that the effects of GPER activation on neurogenesis may be indirect.

### ***Immunoregulation: a potential role of microglia***

There is a growing awareness that neuroinflammatory processes are involved in the regulation of adult hippocampal neurogenesis (Ekdahl et al., 2009). Neuroinflammation is a complex response, however microglia, the resident immune cells of the brain, are the primary mediators of neuroinflammation. The first studies investigating the effects of inflammation on neurogenesis utilized the bacterial endotoxin lipopolysaccharide (LPS), which activates microglia and induces an inflammatory response (Kloss et al., 2001). Intracortical or systemic administration of LPS suppressed the survival of BrdU-labeled cells, but had no significant effect on cell proliferation in male rats (Ekdahl et al., 2003). Further, treatment with the microglial inhibitor minocycline prevented the suppression in the survival of new neurons (Ekdahl et al., 2003), supporting a direct role for microglia in the effects of neuroinflammation on neurogenesis. Importantly, the role of microglia in neurogenesis is not limited to pathological contexts. Indeed, ramified microglia support basal neurogenesis in the healthy hippocampus of mice (Sierra et al., 2010). A large proportion of newly generated cells in the hippocampus do not survive to maturity (Cameron and McKay, 2001), where the majority of newly born cells undergo apoptosis within the first 1-4 days, and are rapidly phagocytosed by ramified microglia mice (Sierra et al., 2010).

The anti-inflammatory effects of estrogens in the brain are well documented in a variety of CNS disorders, including stroke and neurodegenerative diseases (Pozzi et al., 2006; Spence and Voskuhl, 2012; Vegezo et al., 2008). This is corroborated by *in vitro* studies demonstrating that estrogens are capable of significantly altering microglial function in primary rat microglial cultures or N9 microglia lines (Bruce-Keller et al., 2000; Vegezo et al., 2001). Specifically, estradiol is capable of suppressing microglial activation induced by LPS treatment, and this effect was blocked by ER antagonists, suggesting an ER-mediated mechanism for the anti-

inflammatory effects of estradiol on microglia, however unfortunately the sex of the subjects was not specified (Vegeto et al., 2001). This literature is corroborated by *in vivo* studies showing that selective estrogen receptor modulators are capable of reducing LPS-induced microglial activation in male and female rats (Arevalo et al., 2011; DonCarlos et al., 2009).

Given the role of inflammation and microglia in neurogenesis, and the anti-inflammatory effects of estrogens, it is conceivable that the effects of estrogens on neurogenesis are in part afforded through the regulation of microglia-related inflammatory processes. That is to say, it is possible that an estrogen-deficient microenvironment may contribute to a failure of proper immunoregulation and in turn alterations in neurogenesis. To our knowledge, a direct link between estrogens and inflammation on neurogenesis has not been investigated.

Importantly, peripheral and central inflammation and microglial activation are evidenced in disorders of affective and cognitive function, in which sex hormones (Bloch et al., 2003b; Paganini-Hill and Henderson, 1994; Schmidt, 2005) and alterations in neurogenesis (Crews et al., 2010; Jin et al., 2004; Sahay and Hen, 2007), are also implicated. For example, neuroinflammation and microglial alterations are found in Alzheimer's Disease and other dementias (Dickson, 2001; Mackenzie, 2000; Wyss-Coray and Rogers, 2012). In addition, depression is accompanied by central and peripheral inflammation and increased microglial activation (Kaestner et al., 2005; Miller et al., 2009; Setiawan et al., 2015). Thus, the effects of estrogens on microglia-mediated inflammatory processes, and potentially neurogenesis, may have important implications for cognitive and affective functioning.

### ***Mediation of the serotonergic system***

There is strong evidence indicating a role for the serotonergic system in the regulation of adult hippocampal neurogenesis in males and females (Djavadian, 2004). The well-established pro-neurogenic effects of SSRI antidepressants represent one strong line of evidence for the role of serotonin (5-HT) (Malberg et al., 2000). Further, the depletion of serotonin via the inhibition of serotonin synthesis, or the ablation of serotonin neurons, decreased cell proliferation and the expression of PSA-NCAM in the dentate gyrus of female rats (Brezun and Daszuta, 1999a).

Estrogens have substantial effects on the serotonergic system, and those effects are most often stimulatory in females (Amin et al., 2005). For example, acute estradiol administration to

ovariectomized rats increased serotonin turnover in several brain regions, as seen by increased 5-HT and its metabolite 5HIAA (Johnson and Crowley, 1983; Morissette et al., 1990). Given this evidence, it is not surprising that effects of estrogens on serotonin may mediate estrogens' influence on neurogenesis. In fact, although the inhibition of serotonin synthesis decreased cell proliferation (Brezun and Daszuta, 1999a; 1999b), the ovariectomy-induced reductions in cell proliferation were not further affected by serotonin synthesis inhibition (Banasr et al., 2001), suggesting that the effects of ovariectomy on cell proliferation may be mediated, at least in part, through effects on serotonergic transmission. Indeed, stimulation of serotonin synthesis in ovariectomized rats restored levels of cell proliferation, but estradiol did not reverse cell proliferation reductions induced by 5-HT synthesis inhibition (Banasr et al., 2001). Thus, alterations in serotonergic transmission downstream of estradiol signalling may be involved in the regulation of hippocampal neurogenesis. Interestingly, the transient decrease in neurogenesis after ovariectomy coincides with a transient decrease in 5-HT turn-over in the hippocampus (Zhang et al., 1999), suggesting that the normalization of neurogenesis levels following longer periods of ovariectomy may be related to a normalization of 5-HT turnover in the hippocampus.

### ***Regulation of Neurotropic factors: a focus on BDNF***

Brain-derived neurotrophic factor (BDNF), is a member of the neurotrophin family; a group of small proteins involved in both the survival and function of neurons. Interestingly, estrogens and BDNF show remarkable interactions and parallel effects in the CNS, and this is particularly well described in the hippocampus (see Scharfman and MacLusky, 2006 for review). There is substantial evidence for a direct modulation of BDNF by estrogens, including the identification of an estrogen receptor element on the BDNF gene (Sohrabji et al., 1995). In addition, studies using estradiol replacement in ovariectomized rodents show that ovariectomy reduced BDNF mRNA and this is reversed by estradiol treatment (Cavus and Duman, 2003; Singh et al., 1995; Sohrabji et al., 1995). Because BDNF facilitates neurogenesis in the adult dentate gyrus in males (Lee et al., 2002; Scharfman et al., 2005; Waterhouse et al., 2012), it is conceivable that the effects of estrogens on adult hippocampal neurogenesis may be in part mediated through the modulation of BDNF. Finally, alterations in BDNF levels are implicated in men and women with Alzheimer's disease (O'Bryant et al., 2009; Phillips et al., 1991) and in depression (Karege et al., 2005; B.-H. Lee and Kim, 2010; Shimizu et al., 2003); pathologies that, as we discussed earlier, are linked to reductions/fluctuations in ovarian hormones (Bloch et al., 2003b; Paganini-Hill and

Henderson, 1994; Schmidt, 2005) and compromised neurogenesis (Crews et al., 2010; Jin et al., 2004; Sahay and Hen, 2007). However, there are also reported sex differences in the interactions between sex hormones and BDNF in the hippocampus (Harte-Hargrove et al., 2013), and as such we urge the research community to be aware of this possible difference in the relationship between sex hormones and BDNF.

## Conclusions

The evidence we review here strongly indicates that sex hormones are powerful modulators of neurogenesis within the adult hippocampus of both males and females. We highlight that several factors are important to consider when assessing the influence of sex hormones on neurogenesis, and these including the timing of DNA synthesis marker and/or hormone manipulation, in addition to sex, age, and reproductive history. Sex as a factor is rarely studied in the literature, and to address this issue, funding agencies in Europe and North America are placing a focus on the inclusion of female subjects in all studies. These calls for inclusion of females in research have been met with enthusiasm (Klein et al., 2015; McCullough et al., 2014), scepticism, and calls for restraint (McCarthy et al., 2012; Richardson et al., 2015). However, the literature we review here strongly suggests that in the pursuit of enhancing our understanding of the functional significance of adult hippocampal neurogenesis in health and disease, it is vitally important to consider sex differences and the role of sex hormones. It is critical to understand, however, that the mere inclusion of both sexes in neurogenesis research is not sufficient. A careful understanding of variables that can modulate the effects of sex hormones on neurogenesis, such as age and reproductive history, is essential. Lastly, the mechanisms by which sex hormones may influence hippocampal neurogenesis are largely unknown, and we discuss potential targets for future investigation.

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## Figure Captions

Figure 1 Diagram depicting the stages of adult neurogenesis in the dentate gyrus and approximate timelines are given according to studies in adult male rats. It is important to note that timelines are different in adult mice (Synder et al., 2009) and likely other species. A non-exhaustive list of endogenous markers (Ki67, DCX, PSA-NCAM and NeuN) that are often used in conjunction with neurogenesis are given for each stage to indicate the timeline of cell maturity. Briefly once a progenitor cell (blue) divides it is capable of incorporate a thymidine analogue during the cell synthesis stage of mitosis. The cell proliferation stage lasts 24 hours (Cameron and McKay, 2001). Once two daughter cells (blue) are produced at least one of these cells (green) will progress through differentiation and migration to become a mature neuron. Daughter cells will express DCX within hours which lasts for approximately 21 days (Brown et al., 2003). Immature neurons will express the mature neuronal marker (NeuN) approximately 14-21 days after production (Brown et al., 2003). PSA-NCAM expression occurs in daughter cells 1-2 days post-division, and will be expressed predominantly by immature neurons on the cell body and growing neurites for between 2-4 weeks (Bonfanti, 2006). The timelines for each differentiation marker are given relative to the production of a newly produced cell at time 0 in the subgranular zone. A new cell, labelled with a DNA synthesis marker, such as BrdU, at time 0 would show co-expression with these endogenous markers at these approximate timelines in a young adult male rat. DCX- a Doublecortin; GFAP- glial fibrillary acidic protein; NeuN - Neuronal nuclei; PSA-NCAM- Polysialylated neural cell adhesion molecule; BrdU – Bromoxyuridine.

Figure 2. An illustration depicting different timelines of testicular hormone manipulations in adult male rodents examining different components of adult neurogenesis across various studies. It is important to note that seemingly slight changes to protocols and timing of hormones relative to BrdU can influence the effects of hormones on various stages of neurogenesis. Grey bars depict castration /GDX (gonadectomy); Green bars depict testosterone propionate (TP) administration; Blue bars depict dihydrotestosterone (DHT) administration; Orange bars depict estradiol benzoate (EB) administration. BrdU- bromodeoxyuridine; E<sub>2</sub>- 17 $\beta$ -estradiol; CUS- chronic unpredictable stress; SI- social isolation.

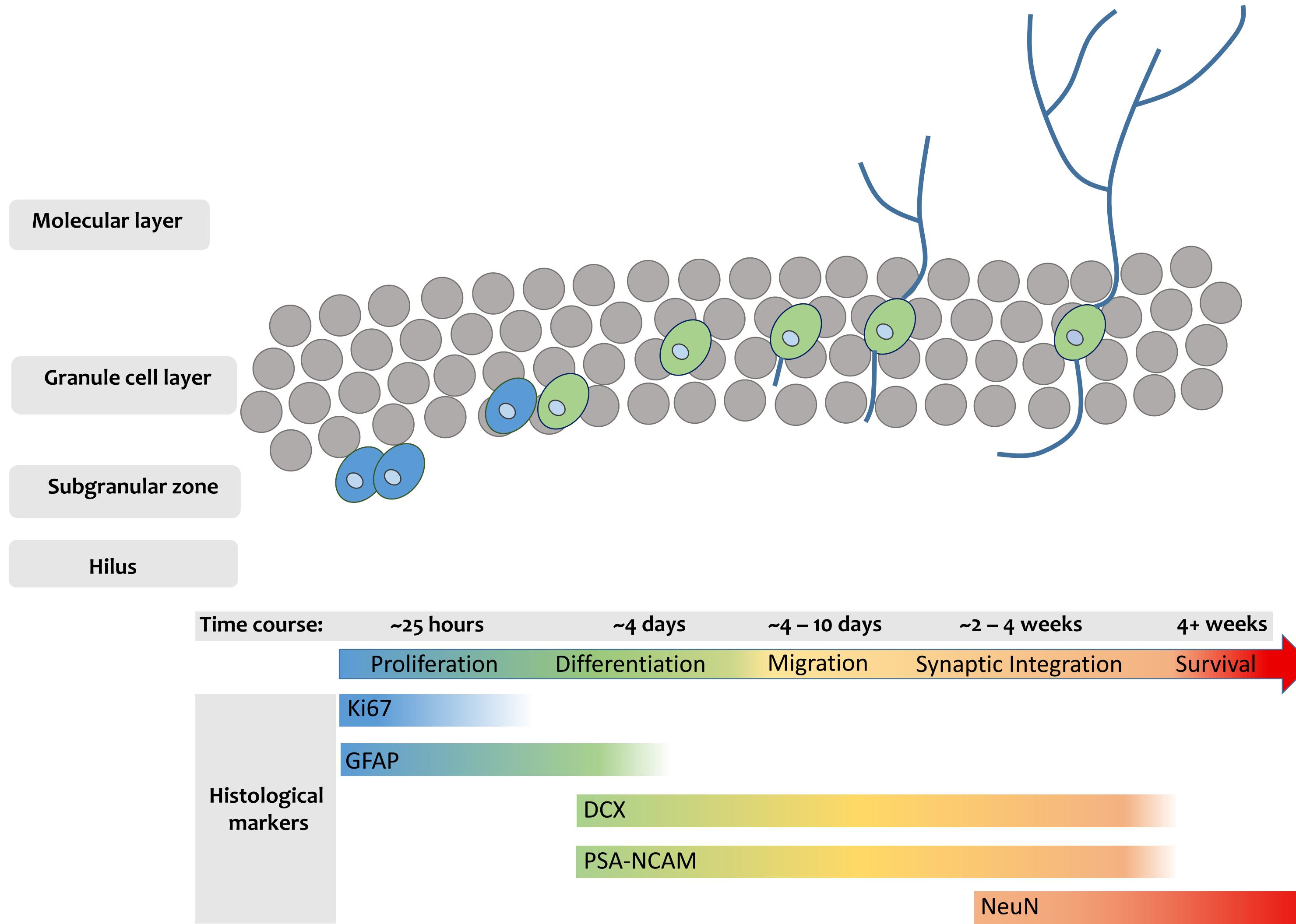
Figure 3 An illustration depicting different timelines of estrogens and progesterone manipulations in adult female rodents examining different components of adult neurogenesis. It is important to note that seemingly slight changes to protocols and timing (and duration) of hormones relative to BrdU can influence the effects of hormones on various stages of neurogenesis. Orange bars depict estradiol benzoate (EB) or 17 $\beta$ -estradiol (E<sub>2</sub>) administration; Purple bars depict progesterone (P) administration; Red bars depict estrone (E<sub>1</sub>) administration; yellow bars depict G<sub>1</sub>, a GPER agonist, administration. BrdU- bromodeoxyuridine; MWM- Morris Water Maze.

Figure 4: A simplified schematic diagram of the functional interactions and feedback mechanisms of the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary gonadal (HPG) axes in males and females. HPA axis: In response to a stressor, the activation of the hypothalamus results in the release of corticotropin releasing hormone and arginine vasopressin, which stimulate the anterior pituitary to release adrenocorticotropic hormone (Smith and Vale, 2006) Adrenocorticotropic hormone acts on the adrenal glands to release glucocorticoids into the

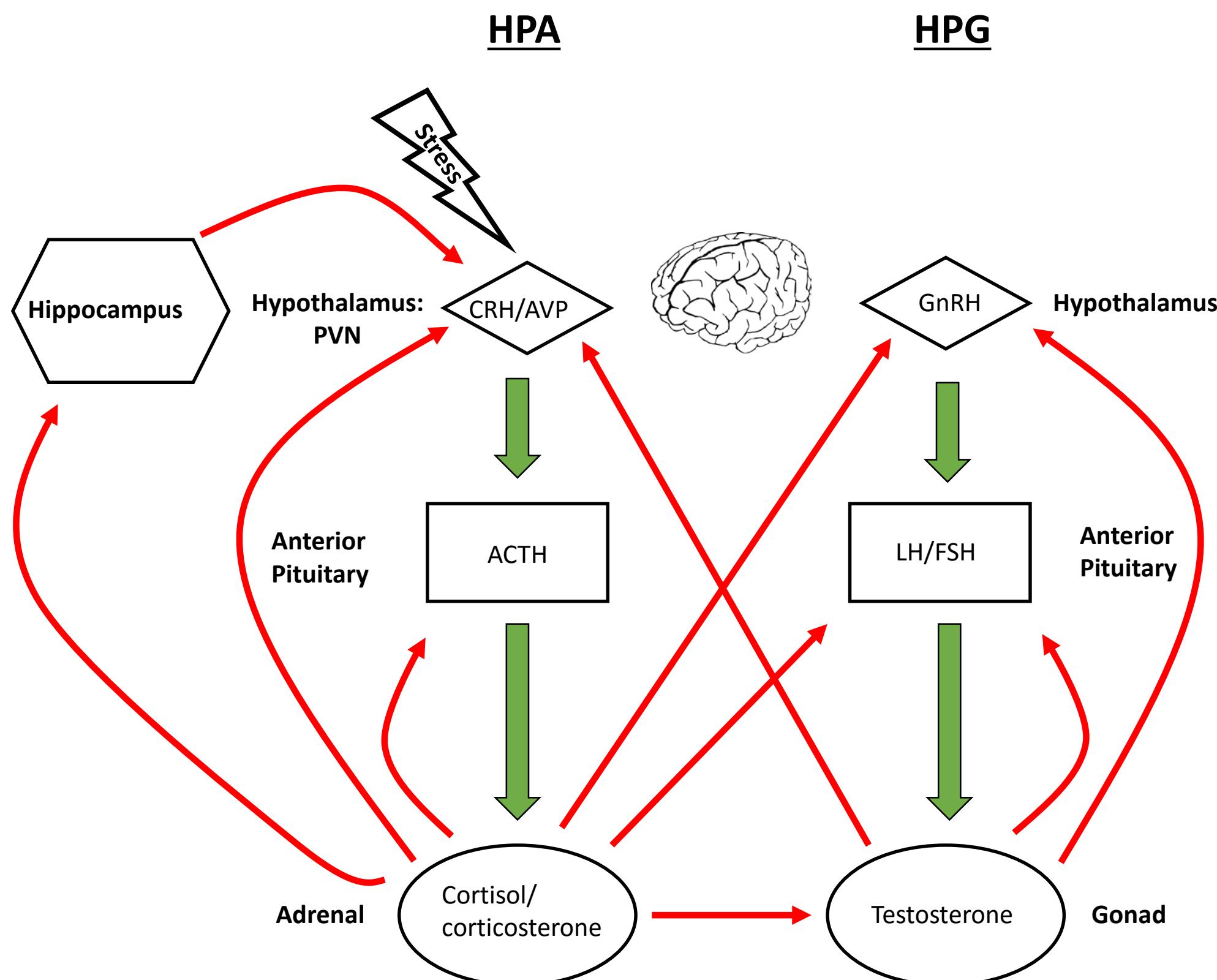
blood stream (Smith and Vale, 2006). Cortisol is the primary glucocorticoid in humans, while corticosterone is the primary glucocorticoid in rodents. Circulating glucocorticoids exert inhibitory actions on HPA axis activity via glucocorticoid and mineralocorticoid receptors in regions such as the paraventricular nucleus of the hypothalamus, the anterior pituitary, and the hippocampus (Smith and Vale, 2006). HPG axis: activation of the hypothalamus results in the release of gonadotropin releasing hormone, which acts on the anterior pituitary to prompt the release of luteinizing hormone and follicle-stimulating hormone (Vadakkadath Meethal and Atwood, 2005). These hormones, in turn, act on the gonads (ovaries in females and testes in males) to release sex hormones (Vadakkadath Meethal and Atwood, 2005). HPA - HPG interactions: glucocorticoids exert inhibitory effects on all levels of the HPG axis in both males and females. Androgens and estrogens have opposing effects on the paraventricular nucleus, where androgens exert inhibitory, and estrogens exert stimulatory, effects to influence the release of corticotropin releasing hormone (Goel et al., 2014; Viau, 2002). Green arrows = stimulatory actions or positive feedback; red arrows = inhibitory actions or negative feedback; HPG = hypothalamic-pituitary gonadal axis; HPA = hypothalamic-pituitary-adrenal axis; PVN = paraventricular nucleus; CRH = corticotropin releasing hormone; AVP = arginine vasopressin; GnRH = gonadotropin releasing hormone; LH = luteinizing hormone; FSH = follicle-stimulating hormone

Figure 5: A simplified schematic diagram showing the pattern and density of androgen receptors (AR)s and estrogen receptors (ER)s expression in the hippocampal formation along with what is known of whether these receptors are co-localized on progenitor or immature neurons in the dentate gyrus. Where there is no expression a dark grey cell is indicated when it is not yet known about co-expression a lighter grey cell is shown and question marks are shown below. It is important to note that AR and ER expression patterns change with age, sex, species, and, in

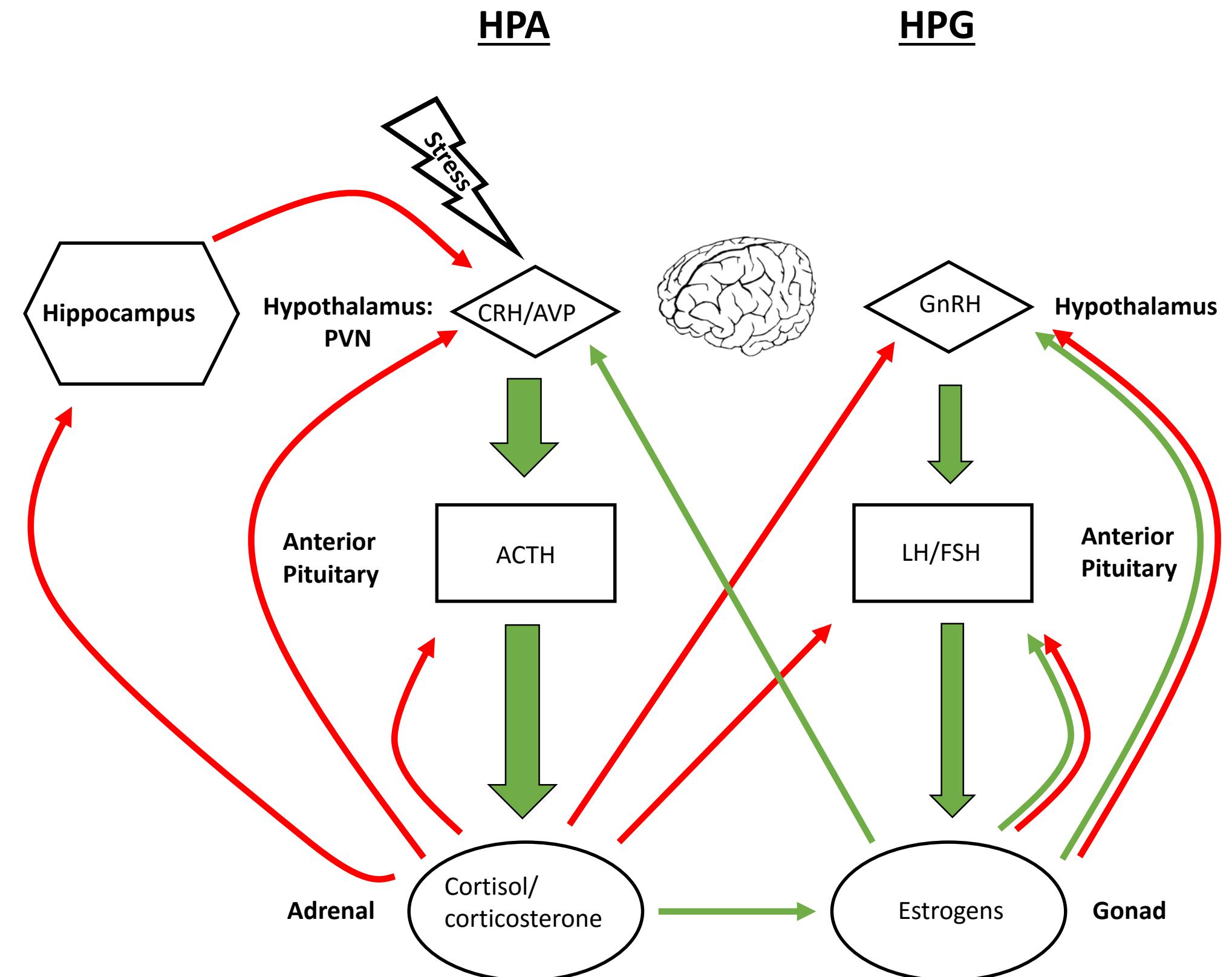
females, with estrous cycle phase and parity and it is possible that AR or ER colocalization with new neurons at different stages change with age (Brännvall et al., 2002). Depicted are the CA1 and CA3 subdivisions of the cornu ammonis (CA), and the dentate gyrus (DG). **(A)** ARs are classically expressed in the cytoplasm, but translocate to the nucleus when bound. In the male hippocampus, ARs are highly expressed in the CA1 and CA3 regions, but there is no evidence for AR expression in the dentate gyrus in most strains of male rodents (with the exception of Wistar rats). Concurrent with that lack of AR expression in the DG, neither progenitor nor immature neurons colocalized with AR in the DG of male rats. **(B)** ER $\alpha$  and ER $\beta$  (**C**) are classically located in the cytoplasm, but translocate to the nucleus when bound. ER $\alpha$  and ER $\beta$  are expressed in the CA1, CA3, and the dentate gyrus of female rodents. Both ERs are co-localized with Ki67-expressing cells at low percentage rates in adult female rats (Mazzucco et al., 2006) and ER $\beta$  are co-localized with a subset of DCX-expressing cells, including dendrites (depicted in red) in adult female rats (Herrick et al., 2006) and in adult male rats (Isgor and Watson, 2005). It is not currently known whether ER $\alpha$  is expressed on DCX-expressing cells in female rodents (and the blue ? depicts that this is unknown in females). However, a greater percentage of co-expression of both ERs with Ki67 and DCX-expression is seen in adult male Sprague-Dawley rats (Isgor and Watson, 2005). **(D)** G-protein-coupled estrogen receptor (GPER) is an extranuclear receptor. GPER is most densely expressed in the CA3 region, but is also expressed in the CA1 and hilus of the DG of adult female rodents, however expression is barely detectable in the granule cell layer of the DG. GPER were not colocalized with Ki67-expressing cells (Duarte-Guterman et al., 2015). The purple ? depicts that it is not known if GPER is co-expressed with DCX in females or males. CA1 = subdivision 1 of the cornu ammonis; CA3 = and subdivision 3 of the cornu ammonis; DG = dentate gyrus; AR = androgen receptor; ER $\alpha$  = estrogen receptors  $\alpha$ ; ER $\beta$  = estrogen receptor  $\beta$ ; GPER = G-protein-coupled estrogen receptor.



## Males



## Females

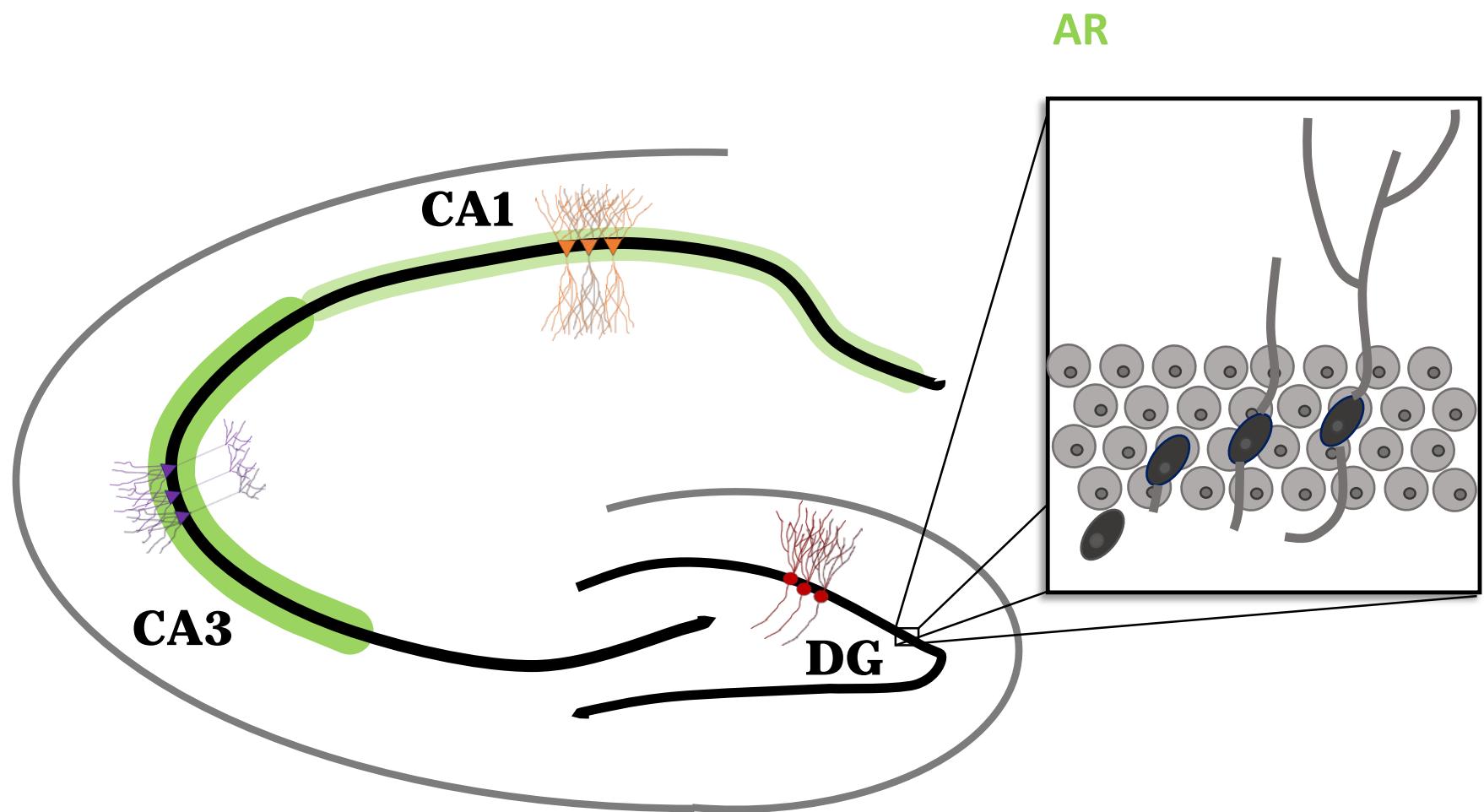
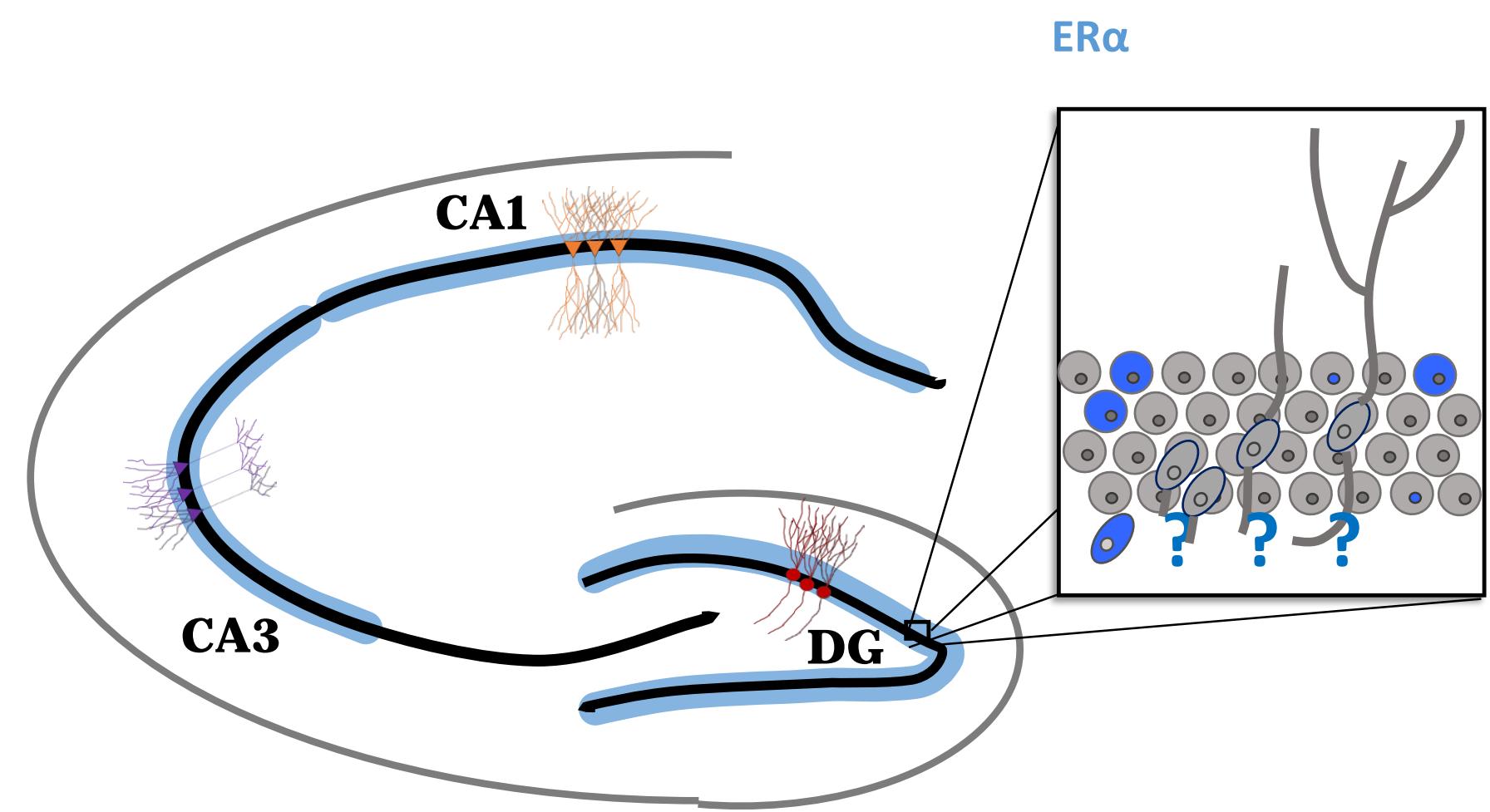
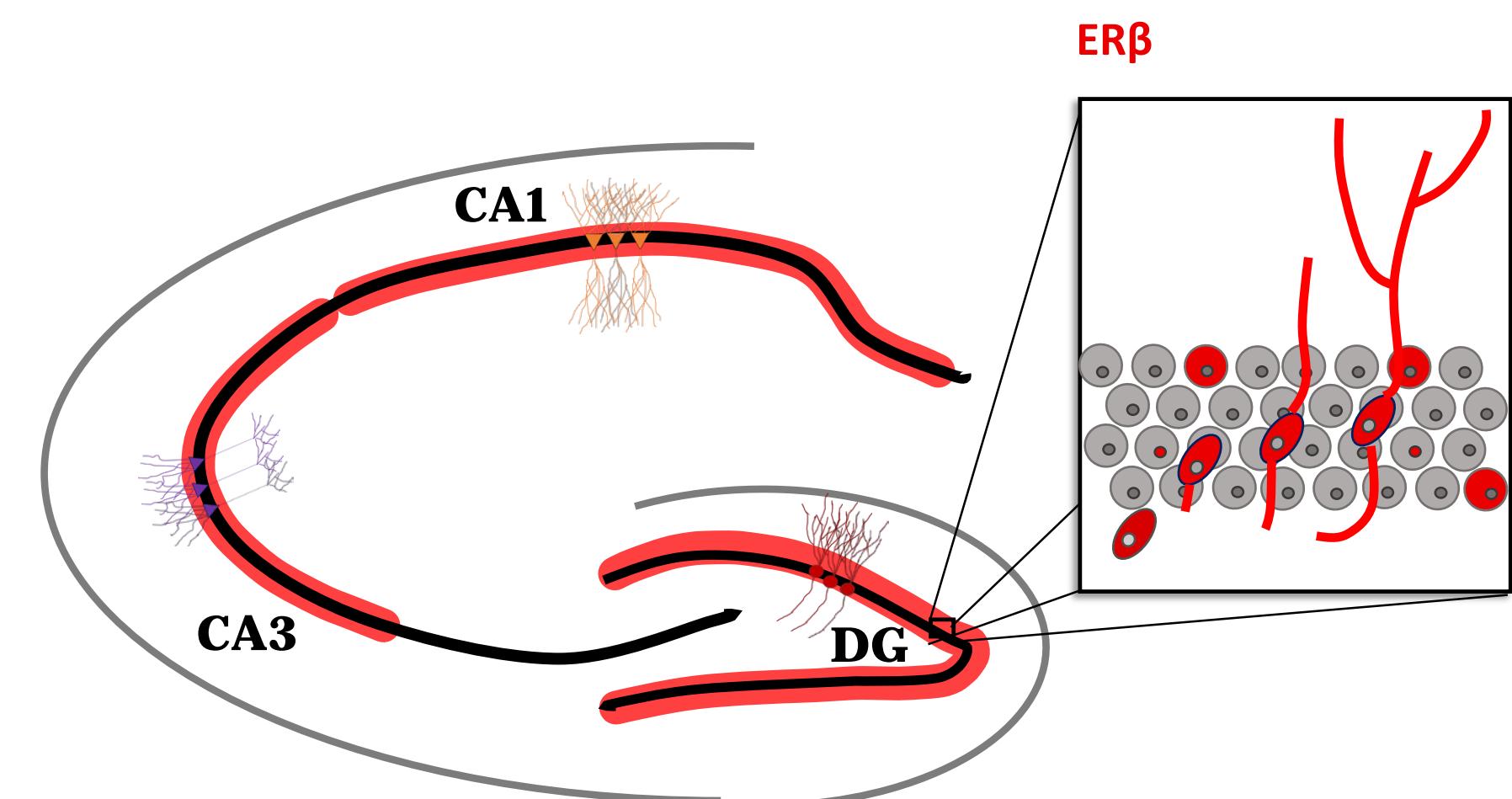


# Males

Species	Stress	Proliferation	Survival	Differentiation	Study
GDX ↓ 7d BrdU Perfusion		—			
GDX ↓ 7d BrdU Perfusion			↓		
GDX ↓ 7d BrdU TP 24h 29d Perfusion		↑		—	Spritzer et al., 2007
GDX ↓ 7d BrdU DHT 24h 29d Perfusion		↑		—	
GDX ↓ 7d BrdU EB 24h 29d Perfusion		—		—	
GDX ↓ 7d BrdU EB 24h 16d Perfusion		—	—	—	Barker and Galea, 2008
BrdU BrdU E2 ↓ 24h 2d 25d Perfusion		—			Zhang et al., 2010
GDX ↓ 7d BrdU 21d Perfusion	SD Rats	—	↓	—	
GDX ↓ 7d BrdU 21d Perfusion	SD Rats	—	↓	—	Wainwright et al., 2011
GDX ↓ 9-10d BrdU 16d Perfusion	SD Rats	SI	↓		Spritzer et al., 2011
GDX ↓ 9-10d BrdU 16d Perfusion	SD Rats	SI	↓	↑	
GDX TP ↓ 21d BrdU 24h Perfusion	SD Rats	SI	—		Carrier and Kabbaj, 2012
GDX TP ↓ 8d BrdU 30d Perfusion	SD Rats TFM SD Rats		↑		Hamson et al., 2013
GDX ↓ 8d BrdU DHT 24h 30d 24h Perfusion	SD Rats	—	↑	—	
GDX ↓ 7d BrdU 21d Perfusion	SD Rats	CUS	—	—	Wainwright et al., 2016
GDX TP ↓ 25d BrdU BrdU BrdU 24h 24h 24h Perfusion	SD Rats		↓		Shin et al., 2016

## Females

<u>Species</u>	<u>Behavior</u>	<u>Proliferation</u>	<u>Survival</u>	<u>Study</u>
SD Rats		↑		Tanapat et al. (1999)
SD Rats		↑		Ormerod et al. (2003) Barha et al. (2009) Mazzucco et al. (2006)
SD Rats		↓		Ormerod et al. (2003)
SD Rats		↑		Tanapat et al. (2005)
SD Rats		—		
SD Rats		—		
SD Rats		↑		Barha et al. (2009) Duarte-Guterman et al. (2015)
SD Rats		↑		Barha et al. (2009)
SD Rats		↓		Duarte-Guterman et al. (2015)
SD Rats		↑	↓	Barker and Galea (2008)
SD Rats	MWM	—	↑	McClure et al. (2013)
SD Rats	MWM	—	↓	McClure et al. (2013)
SD Rats		—	↓	Chan et al. (2014)
Wistar Rats	FST	—	—	Vega-Rivera et al., 2015

**A****B****C****D**