



Published in final edited form as:

Front Neuroendocrinol. 2014 January ; 35(1): 42–57. doi:10.1016/j.yfrne.2013.09.001.

Sex Differences in Anxiety and Depression: Role of Testosterone

Jenna McHenry^{1,3,#}, Nicole Carrier^{2,#}, Elaine Hull^{1,3}, and Mohamed Kabbaj^{2,3,*}

¹Psychology Department, Florida State University

²Biomedical Sciences Department, College of Medicine, Florida State University

³Neuroscience Program, Florida State University

1. Introduction

Anxiety and depressive disorders are the most common of all psychiatric disorders; however, current human and animal research has yet to provide a clear understanding of the neural mechanisms underlying their etiology. Demographic analyses illustrate not only their widespread prevalence, but also pervasive sex differences in these affective disorders. In fact, approximately 18% of the adult American population suffers from an anxiety-related disorder and another 7% from major depressive disorder each year (Kessler et al., 2005b). Further, females are more than twice as likely as males to be afflicted by mood disorders (Kessler et al., 2005a, Bekker and van Mens-Verhulst, 2007). These sex differences are observed, not only in the U.S., but are also documented worldwide (Seedat et al., 2009). This sex disparity indicates a potential role for gonadal hormones in the etiology of anxiety and depressive disorders. In fact, studies have revealed that women are more likely to experience mood disturbances, anxiety, and depression during times of hormonal flux, such as puberty, menopause, perimenstrual and post-partum periods (Ahokas et al., 2001, Parker and Brotchie, 2004, Douma et al., 2005, Solomon and Herman, 2009). While hormonal flux in females appears to increase the likelihood of experiencing mood disturbances, clinical and preclinical studies in males suggest that testosterone yields protective benefits against anxiety and depression. These beneficial effects may stem from both organizational and activational effects of testosterone. The possible underlying neurobiological mechanisms that mediate such protective effects, including the brain sites, biochemical factors, and molecular pathways involved are discussed herein. Understanding the influence of hormones on neurobiological systems that regulate anxiety and depressive behavior will increase our capacity to develop new drug targets to treat various mental illnesses in both men and women.

© 2013 Elsevier Inc. All rights reserved.

*Corresponding author: Mohamed Kabbaj, PHD, Professor of Biomedical Sciences & Neurosciences, 1115 W. Call Street, College of Medicine-2330G, Florida State University, 32306-4300, Phone: 850-644-4930, Mohamed.Kabbaj@med.fsu.edu.

#Jenna McHenry and Nicole Carrier participated equally in this work.

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

2. The influence of testosterone on anxiety and depressive behaviors in men and women

The relationship between testosterone levels, anxiety disorders, and major depressive disorder in humans is evident in males with hypogonadism, a condition in which reduced functional activity of the gonads results in decreased levels of testosterone. Hypogonadal men exhibit a significantly higher prevalence of anxiety disorders and major depressive disorder, compared to those with normal physiological levels of androgens (Shores et al., 2004, Zarrouf et al., 2009). Similarly, men treated with androgen-depleting drugs for prostate cancer have a greater likelihood of developing an anxiety disorder or major depressive disorder (DiBlasio et al., 2008). Moreover, hypogonadal men with human immunodeficiency virus are more likely to experience depressive moods, an effect reversed by testosterone administration (Rabkin et al., 2000). Collectively, several reports suggest that testosterone-replacement therapy in hypogonadal men greatly improves mood, alleviates anxiety, and mitigates symptoms of depression (Wang et al., 1996, Pope et al., 2003, Kanayama et al., 2007, Zarrouf et al., 2009). However, this is not the case in all clinical studies. For example, one study reports that testosterone-replacement therapy in androgen-deficient men did not significantly alleviate symptoms of major depressive disorder, compared to placebo-treated controls (Seidman et al., 2001). Another study revealed that testosterone administration in elderly men with low levels of testosterone and mild cognitive impairments also did not improve symptoms of depression (Kenny et al., 2004). However, despite a few inconsistent reports, the majority of studies support the case that testosterone yields beneficial effects on mood in men, especially in those with lower than normal levels. Other important considerations in these discrepancies may include differences in age, degree of hypogonadism, and also the timing, dose, duration, and route of androgen replacement. In fact, studies indicate that route of administration may be an important factor, with transdermal application being more effective in improving mood than hormone injections (Zarrouf et al., 2009). Another major consideration is whether the individual tested had previously experienced a depressive episode or had never suffered from an affective disorder (Pope et al., 2003, Zarrouf et al., 2009). In general, testosterone appears to be most effective in alleviating symptoms of anxiety and/or depression in older hypogonadal men, but risk factors must also be assessed. For example, testosterone therapy also involves a potential increased risk of cardiovascular complications, sleep apnea, polycythemia, and prostate cancer (Surampudi et al., 2012). Therefore, additional studies are required in order to know more about when and how testosterone therapy may be effective in treating anxiety and mood disorders despite potential risks involved.

While the clinical studies of testosterone therapy in women are more limited, some evidence supports anxiolytic and antidepressant roles for testosterone. Administration of a low dose of testosterone in women with treatment-resistant major depressive disorder significantly improved ratings of depression, compared to placebo-treated subjects (Miller et al., 2009). In addition, surgical removal of the ovaries increased mood disturbances and depression, compared to placebo-treated controls, an effect reversed by testosterone (Shifren et al., 2000). Another study in women found that a single administration of testosterone reduced anxiety in the fear-potentiated startle response, compared to placebo-treated controls (Hermans et al., 2006). Furthermore, transdermal application of testosterone in women experiencing age-related declines in androgens resulted in substantially improved mood and psychological well-being, compared to placebo-treated individuals (Goldstat et al., 2003). However, some reports have noted that too much testosterone can also negatively impact mood in women and can even contribute to the onset of major depressive disorder (Rohr, 2002). Additional clinical studies in women are necessary in order to reveal whether, and

under what conditions, testosterone alleviates symptoms of anxiety and major depressive disorder.

Additional information can be obtained from examining mood and behavior in both men and women during times of testosterone flux, often due to age-related declines or circadian function. In adolescent males, but not females, decline in salivary testosterone throughout the day due to circadian flux, is correlated with an increase in anxiety- and depressive- like measures (Granger et al., 2003). Lower salivary levels of testosterone are also observed in individuals with anxiety disorders and major depressive disorder. In fact, women with major depressive disorder or a type of anxiety disorder, including generalized anxiety, social phobia, or agoraphobia express lower levels of salivary testosterone, compared to emotionally healthy women (Giltay, 2012). Socially anxious men also display a significant drop in testosterone levels after being defeated in a competition, an effect not observed in non-anxious men (Maner et al., 2008). In addition, both women and men taking a serotonin reuptake inhibitor (SSRI) for major depressive disorder have higher levels of salivary testosterone, compared to depressed individuals not taking SSRI medication (Giltay, 2012). Also, in more senior men and women, lower levels of testosterone are associated with an increased prevalence of major depressive disorder (Barrett-Connor et al., 1999, Morsink et al., 2007).

Clinical evidence suggests that testosterone has anxiolytic and antidepressant benefits, with the potential to promote improved mood and mental health in both women and men. However, the neurobiological mechanisms underlying the protective effects of testosterone in males and females remain poorly understood. In addition, given testosterone's disparate routes of action, it is not clear whether actions of androgens at androgen receptors or conversion to estrogen are responsible for these effects. Human imaging studies that examine possible sites in the brain are necessary to better understand how testosterone and its metabolites and receptors may mediate central effects on mood in both men and women. Animal studies are also required to corroborate findings in human studies, establish causal relationships, and elucidate possible neural and molecular mechanisms underlying testosterone's benefits. Below, we first provide a background summarizing the biosynthesis of testosterone and its metabolites. Then we present the genomic and nongenomic molecular actions of testosterone. Lastly, we provide a comprehensive review of the animal models and experiments that begin to elucidate the brain sites and neurobiological mechanisms by which testosterone exerts its generally beneficial effects on anxiety and depression.

3. Molecular mechanisms of testosterone

3.1 Steroidogenesis, biosynthesis, and metabolism

Testosterone is often referred to as a male hormone, in part because males have about ten times higher concentrations of testosterone compared to women, although women are actually more sensitive to testosterone (reviewed in Durdiakova et al., 2011). The gonads and adrenal cortex are the primary sources of testosterone in most vertebrate species of both sexes. Peripheral testosterone can cross the blood brain barrier and have a number of effects on the brain. In addition, small amounts of steroids, including testosterone, are synthesized *de novo* from cholesterol or steroidal precursors in the brain, and are referred to as neurosteroids (Baulieu et al., 2001, Melcangi et al., 2008) and are discussed in more detail below.

Cholesterol is the precursor of all steroid hormones, including testosterone (reviewed in Ghayee and Auchus, 2007). The rate-limiting step of steroid synthesis is the transport of cholesterol from the cytoplasm to the inner mitochondrial membrane, where steroidogenic enzymes reside (reviewed in, Sierralta et al., 2005, Abdulkarimi et al., 2012, Miller, 2013).

A protein complex called the transduceosome forms at the outer mitochondrial membrane of gonadal and adrenal cells. The transduceosome includes steroidogenic acute regulatory protein (StAR), as well as protein kinase A (PKA), and several other mitochondrial and cytosolic proteins. The process is initiated by the binding of luteinizing hormone (LH) or chorionic gonadotropin (CG; hCG in humans) to their G-protein-coupled receptors, which results in cAMP activating PKA, which in turn phosphorylates and thereby activates StAR. StAR passes through the outer mitochondrial membrane, carrying cholesterol in its hollow, hydrophobic C-terminus, and attaches to the inner mitochondrial membrane, where the cholesterol side-chain cleavage enzyme desmolase is located.

Figure 1 shows the series of reactions, beginning with the cleavage of a side-chain of carbons by the enzyme desmolase to form pregnenolone, an obligatory C21 steroid and prohormone to all other steroids. Pregnenolone can then be further processed either in the mitochondrion or the endoplasmic reticulum. Next, 17 α -hydroxylase converts pregnenolone to 17 α -hydroxypregnenolone, and, in the adrenal cortex 17,20 lyase catalyzes the conversion to dehydroepiandrosterone (DHEA), which circulates throughout the body, primarily in the more stable sulfated form (DHEA-S). However, small amounts of DHEA are also produced in the testes and ovaries (Traish et al., 2011). DHEA is also synthesized in the brain as a “neurosteroid” (Baulieu and Robel, 1998; see section 3.3, below regarding neurosteroids). From DHEA, 3 β -hydroxysteroid dehydrogenase (3 β -HSD) produces androstenedione, which is then converted to testosterone by 17 β -hydroxysteroid dehydrogenase (17 β -HSD). Testosterone has a number of biosynthetic pathways and disparate routes of metabolism that determine its precise molecular mechanism of action. For example, the cytochrome P450 enzyme, 5 α -reductase, can then reduce testosterone to a more potent androgen, dihydrotestosterone (DHT), which can be metabolized by aldo-keto reductase (AKR1C2) to 5 α -androstane-3 α ,17 β -diol (abbreviated 3 α -diol) or by AKR1C1 to 5 α -androstane-3 β , 17 β -diol (3 β -diol). 3 α -diol binds with relatively low affinity to the androgen receptor (Cunningham et al., 1979), but acts as a neurosteroid agonist at the GABAA receptor (Frye et al., 1996). Neurosteroids, including androstenediol, can act as allosteric modulators by increasing either the duration or the frequency of chloride channel opening in the GABAA receptor (Rupprecht, 2003, Reddy and Jian, 2010). Neuroactive steroids may initiate these effects by binding to discrete sites on the GABAA receptor (Rupprecht, 2003). 3 β -diol exerts most of its effects via the estrogen receptor β (ER β) (Pak et al., 2005). Conversely, the P450 enzyme aromatase can aromatize testosterone to estradiol. Given the many routes and disparate actions of testosterone and its metabolites, it is important to determine which biochemical factors, associated steroid receptors, and molecular pathways mediate the anxiolytic and antidepressant effects of testosterone.

3.2 Testosterone's effects on neurotransmitters implicated in mood disorders

Neuroactive steroids affect neurotransmitters, neuronal excitability, and have been implicated in mood disorders (Rupprecht et al., 2001, Dubrovsky, 2005, Eser et al., 2006). Neuroactive steroids exert modulatory effects on a number of neurotransmitters and/or their associated receptors, including γ -aminobutyric acid (GABA), dopamine, and serotonin (5-HT) and may underlie some of testosterone's protective benefits. These are briefly presented here and then mentioned when appropriate in subsequent sections.

Testosterone has been shown to affect a number of monoamines implicated in mental illness. In relation to depression, testosterone can enhance dopamine release in the mesolimbic system (Alderson and Baum, 1981), which may protect against depression-induced anhedonia and the associated decrease in dopamine activity in reward-related brain pathways. Additionally, intra-nasal administration of testosterone in intact male rodents increased dopamine and 5-HT release in the neostriatum and nucleus accumbens (de Souza Silva et al., 2009), while GNX reduced basal levels of dopamine in the nucleus accumbens

and septum (Alderson and Baum, 1981). The molecular mechanism underlying this relationship is unclear; however, there is evidence of hormonal control of dopamine release from studies examining hormone-neurotransmitter interactions in the MPOA in a model of male rat sexual behavior. Here, GNX was found to reduce extracellular dopamine but increase intracellular dopamine, suggesting that GNX impairs dopamine release, rather than synthesis (reviewed in Hull and Dominguez, 2006). While estradiol replacement in GNX rodents restored basal levels of dopamine within the MPOA, the addition of DHT was necessary to recover female-stimulated dopamine release (reviewed in Hull and Dominguez, 2006). Other studies found that estradiol acts in part through nitric oxide to increase extracellular dopamine levels. Specifically, estradiol up-regulates neuronal nitric oxide synthase (nNOS), resulting in increased production of nitric oxide, which in turn stimulates dopamine release (Hull and Dominguez, 2006, Sato et al., 2007). Testosterone may also affect serotonin function, which is clearly implicated in anti-depressant treatment. Electrophysiological recordings from the dorsal raphe nucleus serotonin-containing neurons showed that administration of testosterone or estradiol increased the firing rates of these neurons (Robichaud and Debonnel, 2005). Testosterone metabolites may also interact with serotonin receptors. For example, estradiol antagonizes the serotonin 5-HT₃ receptor, which is a ligand-gated ion channel (Rupprecht, 2003). Conversely, 5-HT reuptake inhibitors can increase the production of neuroactive steroids such as pregnenolone, which may contribute to antidepressant effects (reviewed in Schule et al., 2011). The biochemical mechanisms of serotonin/testosterone interactions remain poorly understood; however, such interactions may provide insights into the functions of SSRI antidepressants. Testosterone's influence on GABA may mediate some of its anxiolytic properties. Indeed, administration of a GABA_A receptor antagonist blocked the anxiolytic effects of testosterone (Gutierrez-Garcia et al., 2009). These actions may be similar to that of benzodiazepine drugs with anxiolytic properties; however, the precise mechanisms involved in testosterone-GABA interactions are still under investigation. Studies implicating testosterone-GABA interactions are discussed herein.

3.3 General intracellular genomic actions, non-genomic rapid effects, and specific molecular mechanisms implicated in affective disorders

Testosterone and its associated metabolites can exert actions through slower genomically mediated processes, in addition to rapid non-genomic actions. Both kinds of intracellular effects of steroid hormones in the brain may affect the manifestation of affective disorders and mediate the antidepressant and anxiolytic benefits of testosterone (Rupprecht et al., 2001). A brief overview of the general slow genomic effects and rapid actions of testosterone are reviewed below, along with some putative molecular candidates, which may mediate the protective benefits of testosterone.

Steroid hormones can have long-lasting genomic effects through actions at intracellular steroid receptors, often referred to as the “classical” effects of steroids. Testosterone and its metabolites are lipid-soluble ligands that diffuse across the cell membrane and can interact with intracellular nuclear receptors (reviewed in Wierman, 2007). Upon reaching their target sites, androgens bind to the androgen receptor (AR), and estrogens bind to estrogen receptor alpha (ER α) or beta (ER β). These intracellular steroid receptor proteins have at least three functional domains: the ligand-binding domain located near the C-terminus, the DNA-binding domain located in the central region, and the variable transactivation domain located near the N-terminus, which mediates transcription. ARs and ERs are classified as homodimer receptor proteins, which are contained in the cytosol by chaperone proteins including heat-shock protein Hsp90 in the absence of ligand binding (Nestler et al., 2009). Ligand binding results in a conformational change, dissociation from chaperone proteins, and transport to the nucleus. Specifically, the hormone-receptor complex interacts with

hormone response element DNA sequences (e.g. androgen response elements and estrogen response elements) located within the promoter region of target genes. Co-activator proteins are also recruited and assemble into complexes that activate or repress gene expression through enzymatic actions (e.g. histone acetyl transferase, histone deacetylase, DNA methyltransferase) that modify chromatin structure (reviewed in Wierman, 2007, Vasudevan and Pfaff, 2008). Following transcription, the mRNA is transported out of the nucleus and into the rough endoplasmic reticulum, where it is translated into enzymes or other proteins, usually in the order of hours (Lodish, 2007). This is the general process by which testosterone mediates slower genomic effects that proceed over the course of hours to days. More detailed information regarding the molecular mechanisms of androgens and estrogens at their receptors can be found in Bennett et al. (2010).

In addition to the intracellular steroid receptors with slow, genomic effects, there are membrane-bound androgen and estrogen receptors that exert rapid, nongenomic effects (reviewed in Cato et al., 2002). These effects can occur within seconds or minutes of binding to the receptor (Wehling, 1997, Falkenstein et al., 2000). As early as the late 1960s, estrogen was reported to act within minutes to affect the firing of neurons in the hypothalamus, septum, and preoptic area of female rats (Lincoln, 1967) and testosterone similarly increased responsiveness of medial preoptic area (MPOA) neurons to the odor of a receptive female within minutes (Pfaff and Pfaffmann, 1969). Testosterone and estrogen may mediate these rapid effects by interacting with a number of membrane-associated receptors and/or non-receptor proteins. These rapid actions can influence a number of cellular actions, including changes in intracellular signaling pathways, neuronal membrane excitability, and plasticity (Yamada, 1979, Kubli-Garfias et al., 1982, Pluciennik et al., 1996). For example, neurons in the arcuate nucleus of the hypothalamus of ovariectomized guinea pigs showed increased excitability as a result of estradiol uncoupling μ -opioid receptors in pro-opiomelanocortin (POMC) neurons and uncoupling GABAB receptors in dopaminergic neurons (Kelly et al., 2002a). This effect was blocked by inhibitors of several intracellular effectors, including protein kinase A (PKA), protein kinase C (PKC), and phospholipase C (PLC) (Kelly et al., 2002a, Kelly et al., 2002b). Estradiol also induced Ca^{2+} influx via L-type calcium channels, leading to the activation of the Src/ERK/cyclic AMP response element binding protein pathway in hippocampal neurons (Wu et al., 2005). Rapid estrogen effects on extracellular signal regulated kinases (ERKs) were also found in rat cerebral cortex explants (Setalo et al., 2005). In vivo activation of ERK phosphorylation by estrogen administered into the lateral ventricle was also observed within 5 minutes in hippocampal neurons (Kuroki et al., 2000) and in many brain regions within 20 minutes after subcutaneous administration (Bryant et al., 2005). Other kinases that are rapidly activated by estrogen include the serine–threonine kinase Akt and cyclic AMP response element binding protein (CREB), which are activated in the same population of cortical neurons (Mannella and Brinton, 2006). Estrogen can also rapidly affect ion channels. As noted above, estrogen rapidly increased Ca^{2+} influx in hippocampal neurons (Wu et al., 2005). K^{+} channels can also be rapidly regulated in numerous types of neurons in the hypothalamus via G-coupled protein receptors and cAMP-induced phosphorylation (Kelly et al., 1999, Kelly et al., 2003).

In addition, these non-genomic actions can enhance the classical genomic effects of testosterone and estrogen (reviewed in Vasudevan and Pfaff, 2008). For example, PKA-mediated phosphorylation of ER α at its DNA binding domain may increase dimerization and, thereby, transcription (Chen et al., 1999). In addition, dendritic spine growth in hippocampal neurons may be increased via estrogen-induced PKA and pCREB actions (Murphy and Segal, 1997, Segal and Murphy, 1998). Finally, in caudomedial nidopallium (NCM), the songbird analog of the mammalian auditory association cortex, locally produced estradiol is both necessary and sufficient for song-stimulated MAPK-dependent gene

expression in awake birds (Tremere et al., 2009). Those authors observed that social interactions can increase estradiol levels in the NCM (Remage-Healey et al., 2008), and that the NCM is part of a “social behavioral network,” in which steroid hormones may directly influence sensory processing.

Both testosterone and estradiol can exert non-genomic actions in the cell by activating a number of intracellular signaling pathways, notably the mitogen-activated protein kinase (MAPK) pathway. Once activated, MAPK phosphorylates target substrates such as 3'-5'-cyclic adenosine monophosphate response element binding protein (CREB) (Song et al., 2002, Cheng et al., 2007). The MAPK-ERK(extracellular signal-regulated kinase)-CREB signal transduction pathway is of particular interest, given that it can be stimulated in response to androgens and has been implicated in the pathophysiology of depression (Liu et al., 2004, Shen et al., 2004). This may be one potential mechanism whereby androgens promote antidepressant actions; however, the activation of this pathway can result from a number of disparate molecular mechanisms. For instance, this pathway can be activated through membrane-associated receptors for androgen, estrogen, or progesterone, which interact with the SH2 and SH3 domains of the c-Src kinase, an important upstream component of the MAPK cascade (Migliaccio et al., 2000, Boonyaratankornkit et al., 2001, Kousteni et al., 2001). Specifically, Src kinase activates Shc, promoting the Shc/Grb2/SOS complex, facilitating activation of the monomeric GTPase, Ras, and finally activating the Raf/MEK/ERK pathway (Cheng et al., 2007). Src kinase can also stimulate membrane-bound epidermal growth factor (EGF), which activates a tyrosine kinase EGF receptor that can activate the MAP kinase pathway (Cheng et al., 2007). Alternatively, testosterone can associate with membrane-bound AR, which interacts with a Gq-protein, producing phospholipase C, which then cleaves the phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2) into inositol trisphosphate (IP3) and diacylglycerol (DAG) (as reviewed in, Rahman and Christian, 2007). This process initiates a cascade of events that leads to an increase in intracellular calcium, inhibition of inhibitory K⁺-ATP channels (leading to depolarization), and activation of ERK kinases (Rahman and Christian, 2007, Loss et al., 2011). Testosterone and its associated metabolites may activate one or more of these MAPK-ERK pathways in brain regions mediating mood and emotion, in turn influencing the onset, outcome, and/or treatment of affective disorders. Recently, our lab has implicated this pathway in the antidepressant actions of testosterone in the hippocampus of male rats and is presented below in further detail.

4. Origins of sex differences in anxiety and depression: organizational and activational effects

In both human and animal models, there are many factors, such as environment, age, and genetic sex, that contribute to sex differences in anxiety and depressive disorders. Sex differentiation of mammals begins with the fertilization of an oocyte with either an X or Y chromosome-bearing sperm. Y chromosomes usually carry the SRY (Sex-determining Region of the Y chromosome) gene, which activates the SOX1 gene, which in turn causes the primitive gonads to develop into testes. The testes secrete testosterone and anti-Müllerian hormone (AMH), which causes the Müllerian ducts to degenerate, rather than develop into oviducts and uterus. X-bearing sperm result in automatic development of the gonads as ovaries, due to genes on the X chromosome including DAX1, WNT4, and FOXL2 (Kousta et al., 2010). Occasionally, the SRY gene is translocated onto an X chromosome or an autosome during development of the sperm, resulting in a phenotypic male. Conversely, XY individuals lacking the SRY gene develop as phenotypic females. However, these individuals have some different characteristics compared to typical XY males and XX females (De Vries et al., 2002, Pfaff, 2010).

Organizational effects of hormones occur during critical periods of development when exposure to gonadal hormones can cause permanent sex differences. Activational effects are acute and transient effects that occur throughout life (Cooke et al., 1998). The organizational actions of gonadal hormones are highly evident when female rodents are exposed to neonatal testosterone (Arnold and Gorski, 1984, Simerly, 2002, Morris et al., 2004). A single testosterone treatment on the day of birth is sufficient to masculinize the brain and behavior of female mice and rats (Guillamon et al., 1988, Mong et al., 1999, Murray et al., 2009, Hisasue et al., 2010).

For most male rodents, masculinization of brain areas that control sexual behavior is caused primarily by estradiol, produced from testosterone by the enzyme aromatase. This is referred to as the aromatization hypothesis. Actions via estrogen receptor α (ER α) masculinize brain circuits, whereas actions via ER β defeminize circuits (Kudwa et al., 2006). Female rodents are protected from their mother's and their own estrogen by alpha-fetoprotein, which binds to estrogens and prevents them from entering neurons to masculinize them. However, androgens are the masculinizing and defeminizing hormones for primates and guinea pigs (Goy and McEwn, 1980). Although many studies concerning organizational sex differences have focused on sex behavior in rodents (Phoenix et al., 1959, Handa et al., 1985), there is recent support for a role of organizational effects of sex hormones partially underlying sex differences in emotionality in rodents (Goel and Bale, 2008, Seney et al., 2012).

Beginning in puberty, the predominant circulating gonadal hormones include estrogen and progesterone in females and testosterone in males. Puberty constitutes a second period of such organizational effects of steroid hormones (reviewed in Schulz et al., 2009). For example, castration shortly before puberty results in decreased sexual, aggressive, and territorial behaviors in male Syrian hamsters (Schulz et al., 2004, Schulz and Sisk, 2006, Schulz et al., 2009). Those males are also more sensitive to adult injections of estrogen and progesterone, showing shorter latencies to assume lordosis, compared to males castrated in adulthood. Pubertal hormones also promote the development of territorial scent marking in male tree shrews (Eichmann and Holst, 1999), as well as inter-male aggression in mice (Shrenker et al., 1985) and gerbils (Lumia et al., 1976). Prepubertal castration also increases the anxiogenic effect of a novel environment; such males show less ambulation in an open field (Brand and Slob, 1988) and also less male-male social interaction (Primus and Kellogg, 1990). Those behavioral effects may have resulted in part from changes in cell proliferation and/or survival in several brain areas. Prepubertal gonadectomy (GNX) abolished the sex differences in the acquisition of new neurons (bromodeoxyuridine, BrdU, immunoreactive) in the anteroventral periventricular nucleus (AVPV), which is larger in females, and in the sexually dimorphic nucleus of the preoptic area (SDN) and medial amygdala (MeA), both of which are larger in males (Ahmed et al., 2008). Ahmed et al. reported that only gonadally intact females acquired significantly more new neurons during puberty in the AVPV, and only intact males acquired more new neurons in the SDN and MeA. These new neurons increased the sex differences already present in those brain areas. The AVPV plays an important role in neural regulation of gonadotrophin secretion and luteinizing hormone surges (Sakuma, 2009, Semaan and Kauffman, 2010).; The functions of the SDN are not clear; lesions restricted to the SDN impaired copulation only in sexually naive male rats, not in experienced males (De Jonge et al., 1989). However, lesions of the SDN did increase the display of lordosis in male rats (Hennessey et al., 1986). Therefore, the SDN may be more important for defeminization, rather than masculinization, of behavior. The MeA is part of a social/sexual/emotional network. The neuropeptide substance P (SP) is important for pain perception and may promote depressive-like behavior in guinea pig pups separated from their mothers and also in humans (Kramer et al., 1998). SP is released in the MeA of male rats in response to restraint stress and placement on an elevated plus maze (Ebner et al., 2004). Therefore, the pubertal changes in hormones can enhance the sex differences in

neuroanatomy and behavior that were initially organized perinatally, and these changes may affect moods.

The influence of these gonadal hormones in adulthood, traditionally known as activational effects (Arnold and Breedlove, 1985, Williams, 1986) or hormonally modulated responses (McCarthy and Konkle, 2005), may also activate sex differences in many biological processes. Various animal models and methods can be utilized to distinguish between organizational and activational effects of hormones that may ameliorate the display of anxiety- and depressive-like behaviors. Common animal models utilize GNX in conjunction with hormone vs. vehicle replacement, performed either before or after critical periods of reproductive development. Testosterone may also be administered to gonadally intact subjects to determine if increasing testosterone above basal levels can improve measures of anxiety- and/or depressive-like behavior.

5. The influence of testosterone on anxiety- and depressive-like behaviors in animal models

5.1 The influence of testosterone on anxiety- and depressive-like measures in male animal models

Modeling affective disorders in animals is challenging, due to the subjective nature of many symptoms. Additionally, only a subset of symptoms (such as homeostatic changes, anhedonia, and psychomotor behaviors) can be objectively measured in rodents and most of the current behavioral tests used were initially developed for the rapid screening of antidepressant compounds. Two widely used tests for depression include the forced swim and the tail suspension tests (Cryan et al., 2002, Nestler et al., 2002a, Nestler et al., 2002b). The forced swim test, also known as the Porsolt test, involves placing a rat or mouse in a tank filled with water and measuring the amount of time the animal is immobile (Borsini and Meli, 1988, Lucki, 1997, Porsolt, 2000). In the tail suspension test, mice are suspended by their tails and the time it takes them to become immobile is measured. In both tests, acute antidepressant treatments decrease immobility. Another major class of tests for depression-related behaviors includes measures of anhedonia. The most frequent tests involve examining an animal's interest in pleasurable activities such as preference for a sucrose solution or engaging in social activity (reviewed in (Nestler and Hyman, 2010)). Behavioral tests commonly used to detect anxiety include the elevated plus maze, light dark box, and open field test. Anxiety-like behaviors are assessed based on the animal's willingness to explore a novel environment rather than avoid open exposure (reviewed in (Nestler and Hyman, 2010)).

Several activational effects of testosterone have been observed in measures of anxiety-like behavior in adult male rodents. Specifically, GNX in adult male rodents results in increased anxiety-like behaviors in a battery of behavioral tests, such as the elevated plus maze, open field test, and defensive probe-burying, compared to sham-operated controls (Slob et al., 1981, Adler et al., 1999, Frye and Seliga, 2001, Fernandez-Guasti and Martinez-Mota, 2003, 2005, Morsink et al., 2007), effects that were reversed by testosterone replacement (Slob et al., 1981, Adler et al., 1999, Frye and Seliga, 2001, Fernandez-Guasti and Martinez-Mota, 2005). In our recent work we have demonstrated that physiological levels of testosterone replacement in adult GNX male, but not female rats, have protective effects against the development of anxiety-like behaviors in a model of chronic social isolation (Carrier and Kabbaj, 2012d). Indeed, testosterone replacements were equally as effective as administration of the typical tricyclic antidepressant imipramine in alleviating anxiety-like behaviors induced by two-weeks of chronic social isolation. Testosterone administration in intact adult male rodents also reduces anxiety-like behavior in the elevated plus maze test,

compared to controls (Bitran et al., 1993). Similarly, in intact aged male rodents with lower levels of testosterone, administration of testosterone reduces anxiety-like behavior in the open field test and light-dark box test, compared to vehicle-treated controls (Frye et al., 2008). These data therefore support the hypothesis that the activational effects of testosterone can reduce behavioral measures of anxiety in male rodents.

Testosterone can also influence anxiety-like behaviors through organizational mechanisms. Perinatal and pubertal exposure to testosterone organizes adult behaviors in rodents by acting either upon ARs or on ERs following aromatization to estrogen. In contrast to the anxiolytic effects of androgens in adulthood, organizational effects of gonadal steroids may be anxiogenic, at least in some animal models. A study comparing wild-type and AR-deficient (Tfm, testicular feminization, due to a mutation of the gene for ARs) male rats that were GNX on the day of birth found that GNX decreased the incidence of anxiety-like behaviors in both groups, compared to sham-operated controls, with no differences between wild-type (WT) and AR-deficient rats (Zuloaga et al., 2011a). The lack of a difference between intact WT and AR-deficient males suggests that ARs do not mediate the anxiogenic effect, but rather that aromatization to estrogen confers the effect. Thus, testosterone or its estrogenic metabolites may act during development to increase adult anxiety-like behaviors in the open field, novel object exposure, light-dark box, and elevated plus maze. In line with these findings, an additional study found that GNX in neonatal male rats resulted in decreased measures of anxiety-like behavior in an elevated plus maze test during adulthood, compared to sham-operated controls (Lucion et al., 1996). Thus, in contrast to its anxiolytic effects in adulthood, testosterone during organizational periods may increase anxiety-like behavior. On the contrary, two additional studies from the same lab as above showed that Tfm males were more anxious in the light-dark and novel object tests, compared to wild type (WT) males (Zuloaga et al., 2008, Zuloaga et al., 2011b). Similarly, Tfm males and WT females showed greater increases in corticosterone in response to a novel object, compared to WT males (Zuloaga et al., 2008, Zuloaga et al., 2011b). Therefore, neonatal testosterone can have anxiolytic, as well as anxiogenic effects in adulthood. As noted above, prepubertal GNX also increased anxiety-like behavior in an open field and in male-male social interactions in hamsters (reviewed in Schulz et al., 2009). Future studies regarding the organizational effects of testosterone and its metabolites with respect to the manifestation of anxiety-like behaviors in adulthood will be needed to elucidate their contributing roles and the molecular mechanisms involved.

Activational effects of testosterone contribute, not only to anxiolytic behaviors in rodents, but also to antidepressive-like behaviors. Similarly to hypogonadal men, rodents with low testosterone levels can exhibit increased depressive-like behaviors. In fact, depressive-like behaviors, including behavioral despair and anhedonia following GNX and vehicle, as opposed to testosterone, replacements in male rats, are well documented (Wainwright et al., 2011, Carrier and Kabbaj, 2012c, a, Herrera-Perez et al., 2012). Previously, we have demonstrated that GNX in adult male rats increased depressive-like measures in the forced swim test and in the sucrose preference test, a measure of anhedonia, compared to GNX males treated with low or high doses of testosterone. Interestingly, both the low, physiological levels, as well as the high, extraphysiological levels of testosterone replacements were equally effective in reducing depressive-like symptoms that were seen in GNX male rats receiving placebo replacements, and the behaviors of the testosterone-treated males were similar to those of intact sham controls. These effects were found to be mediated by estradiol, but not dihydrotestosterone, in the forced swim test (Carrier and Kabbaj, 2012b), suggesting that aromatization of testosterone to estradiol may be critical for the antidepressant effects of testosterone. In a related study, we investigated the protective effects of testosterone following chronic social isolation, a model used to induce a depressive-like state. Indeed, physiological levels of testosterone replacements had

antidepressant effects in both the sucrose preference and novelty-induced hypophagia tests (Carrier and Kabbaj, 2012b). Another study reported that GNX in male rats increased submissive/depressive behavior in response to social defeat, compared to sham-operated controls - an effect partially reversed by administration of testosterone or DHT (Solomon et al., 2009). Injections of testosterone, DHT, or 3 α -diol to intact adult male rodents dose-dependently decreased depressive-like behavior in the forced swim test, compared to vehicle-treated controls (Buddenberg et al., 2009). Similarly, administration of testosterone, DHT, or 3 α -diol to intact aged male rodents also decreased depressive-like behavior in the forced swim test, compared to vehicle-treated controls (Frye and Walf, 2009). Since similar antidepressant-like effects are observed following administration of testosterone, DHT, and 3 α -diol, it is possible that actions at androgen receptors and/or GABAA receptors are involved. As previously stated, 3 α -diol is a metabolite of DHT and binds with relatively low affinity to the androgen receptor (Cunningham et al., 1979), but acts as an agonist at the GABAA receptor (Frye et al., 1996). Testosterone, on the other hand, can act through a number of disparate actions that can stimulate estrogen receptors, androgen receptors, or GABAA receptors. Conversely, socially defeated males often display decreased levels of testosterone and increased depressive-like behavior following defeat (Schuurman, 1980). Together, these data support a role for the activational effects of testosterone administration in reducing depressive-like behaviors in male animal models.

Organizational effects of testosterone may also contribute to antidepressant-like behaviors later in life, as suggested from animal models. In fact, newborn rat pups treated with the AR antagonist, flutamide, displayed increased depressive-like behavior in the forced swim test and sucrose preference test later in life, relative to vehicle-treated controls (Zhang et al., 2010). Overall, a large body of accumulating evidence supports antidepressant and anxiolytic properties of testosterone in adult males, although there are contradictory findings regarding the anxiogenic vs. anxiolytic organizational effects of androgen (See table 1).

5.2 The influence of testosterone on anxiety- and depressive-like measures in female animal models

Preclinical research has generated inconsistent results with regard to an anxiolytic and antidepressant role of testosterone in female rodents. Many studies provide supporting evidence for a beneficial role of testosterone, while others report no effect. Further, it is unclear which of testosterone's disparate routes of action may be involved. As previously mentioned, effects resulting from testosterone, DHT, or 3 α -diol could be mediated by androgen or non-androgen mediated events. In intact adult female rodents, injections of testosterone, DHT, or 3 α -diol reduced anxiety-like behavior in the open field test and elevated plus maze, compared to vehicle-treated controls (Frye and Lacey, 2001). Similarly, testosterone administration in intact female rodents also decreased anxiety-like behavior in a defensive burying task through an androgen-mediated effect, compared to vehicle-treated controls (Gutierrez-Garcia et al., 2009). Interestingly, following two weeks of chronic social isolation to induce anxiety-like behaviors, we observed that the anxiolytic and antidepressant effects of testosterone administration were limited to male rats. We have shown anxiolytic and antidepressant effects of physiological levels of testosterone replacement in GNX male rats, but no effect of these same replacements in ovariectomized (OVX) female rats in the light-dark box, elevated plus maze, sucrose preference, and novelty-induced hypophagia tests (Carrier and Kabbaj, 2012d). These data suggest that physiological levels of testosterone in male rats have protective effects against the development of anxiety-like behaviors in an anxiety and depression model; however, female rats may not experience the same anxiolytic and antidepressant benefits of testosterone. Perhaps organizational sex differences prevent physiological levels of testosterone from producing behavioral outcomes in some OVX female rats. Higher doses may be needed in

females to observe an anti-anxiety affect. It is important to note that the majority of studies reporting beneficial effects of testosterone used intact females. Thus, the dose and reproductive status are important parameters to consider and likely contribute to the discrepancies reported for effects of testosterone administration in adult female rodents.

Similar to effects in males, testosterone also appears to decrease depressant-like behavior in female rodents, although the mechanism of action remains unclear. Indeed, administration of estradiol to intact female rodents decreased depressive-like effects in the forced swim test, an effect that was similar to the effect of fluoxetine in female rats (Estrada-Camarena et al., 2003). However, administration of testosterone, DHT, or 3 α -diol to intact aged female rodents also had antidepressant-like effects in the forced swim test, compared to oil vehicle-treated controls (Frye and Walf, 2009), suggesting that activation of androgen receptors or GABAA receptors may mediate some antidepressive effects of testosterone (see table 1). The potential brain sites where testosterone may be acting to exert its anxiolytic and antidepressant effects are discussed below.

6. Potential brain sites mediating testosterone's effects on anxiety and depression: parallels between human and animal studies

Several brain regions have been implicated in the pathophysiology of anxiety and depressive disorders. In humans, a number of anxiety disorders are associated with structural and functional abnormalities within the amygdala, prefrontal cortex, hippocampus, and hypothalamus (Shin and Liberzon, 2010). In animal models, more discrete sub-regions of these brain sites and others have also been implicated in anxiety and depressive-like behavior. Mice specifically bred for high anxiety behavior exhibit more expression of stress-induced c-Fos immunoreactivity within the central, medial, and basolateral regions of the amygdala, medial prefrontal cortex, anterior cingulate cortex, hippocampus, septum, and several hypothalamic nuclei including the medial preoptic area, lateral hypothalamus, and paraventricular nucleus, compared to wild type mice and those bred for low anxiety behavior (reviewed in Singewald, 2007). Also, anxiogenic stimuli induce neuronal Fos activity in the medial prefrontal cortex, lateral septum, bed nucleus of the stria terminalis (BNST), locus coeruleus, periaqueductal gray (PAG), and several nuclei within the amygdala and the hypothalamus in normal rodents (reviewed in Singewald, 2007). Depression is commonly associated with dysregulation of the mesolimbic system, the hypothalamic-pituitary-adrenal (HPA) axis, hypothalamic areas, hippocampus, and medial prefrontal cortex (reviewed in Nestler et al., 2002a, Krishnan and Nestler, 2008). In post-mortem tissue of depressed individuals, a decrease in volume of the hippocampus is observed, compared to healthy individuals (reviewed in Nestler et al., 2002a). Neuroimaging studies and post-mortem tissue analysis of depressed individuals also reveal both structural and functional abnormalities in the prefrontal cortex, cingulate cortex, hippocampus, striatum, amygdala, and thalamus (reviewed in Nestler et al., 2002a). Testosterone and its associated actions have been shown to have a number of neurobiological effects in several areas implicated in affective disorders, particularly the amygdala, BNST, and hippocampus as discussed below.

6.1 Amygdala

The amygdala plays a major role in anxiety and fear responses in humans and rodents alike (reviewed in Rauch et al., 2003, Rodrigues et al., 2009, Ressler, 2010). Hyperactivity of the amygdala has been reported in people with post-traumatic stress disorder and phobia disorders (Singewald, 2007, Shin and Liberzon, 2010) and in rodents displaying high levels of anxiety-like behavior (reviewed in Singewald, 2007). Although human imaging studies examining potential brain regions involved in the effects of testosterone on anxiety and/or

depression are extremely limited, a few have found that testosterone can influence the degree of amygdala activation in relation to fear, with a positive correlation observed between testosterone levels and amygdala activation in men and a negative correlation in women (Derntl et al., 2009, van Wingen et al., 2009). More research concerning the role of the amygdala in testosterone's influence on anxiety- and depressive-like behavior has been obtained from rodent models, although findings are complicated and somewhat contradictory.

Rodents display a high degree of natural variation in exploratory/novelty-seeking behavior (Kabbaj et al., 2000), which may be used to correlate biological processes with behavioral phenotypes. For example, male rodents with naturally high defensive burying express more ARs and vasopressin mRNA in the medial amygdala (MeA) and posterior BNST and less oxytocin mRNA in the PVN, compared to those with low defensive burying responses (Linfoot et al., 2009). The high-bury animals also display increased levels of adrenocorticotrophic hormone (ACTH) and corticosterone release in response to stress, compared to low-bury animals. While the higher expression of ARs in the high-bury animals appears contradictory to previous findings relating ARs to more anxiolytic behaviors, it has been suggested that burying behavior in rodents is complex and may reflect active versus passive stress-coping style (with high-burying behavior reflecting active stress-coping and vice versa). Thus, even though there is increased behavioral and physiological stress responsiveness in rodents with high numbers of ARs in the MeA and BNST, those males may respond more adaptively. Some of these individual differences may stem from testosterone's organizational effects on neural circuits mediating stress reactivity, which ultimately influence anxiety-like behavior. In fact, neonatal GNX results in fewer AR- and vasopressin-containing cells in the MeA and BNST in adulthood, compared to sham-operated controls (Bingham and Viau, 2008). Also, testosterone administered on postnatal days 1–5 reversed this effect, but testosterone administered in adulthood, did not.

The effects of androgens and estrogens on anxiety- and depressive-like behaviors have also been observed in female rodents. Injection of a 5 α -reductase inhibitor into the amygdala of intact and OVX hormone-primed female rodents increased anxiety-like behavior in the open field test, elevated plus maze, and defensive freezing tests, and depressive-like behavior in the forced swim test, compared to vehicle-treated controls (Walf et al., 2006). Therefore, metabolism of testosterone to DHT, or progesterone to 5 alpha progesterone, in the amygdala of female rodents may have anxiolytic and antidepressant effects. Aromatization of testosterone to estradiol may also have anxiolytic effects within the amygdala. Indeed, injection of estradiol into the amygdala of ovariectomized (OVX) female rats decreased anxiety-like behavior in the open field, elevated plus maze, and hot plate tasks, compared to sham-operated controls (Frye and Walf, 2004). In contrast, silencing ER α in the posterior medial amygdala of intact female rats decreased anxiety-like behavior in a light-dark box test, compared to controls, suggesting that estrogen in the amygdala may also have anxiogenic effects (Spiteri et al., 2010). It is not clear in which conditions estrogen in the amygdala may have anxiolytic vs. anxiogenic effects; perhaps different nuclei or different receptor subtypes mediate the opposite effects.

6.2 Bed Nucleus of the Stria Terminalis (BNST)

The BNST is implicated in central control of emotion associated with anxiety and stress responses (Walker et al., 2003) and is also highly sensitive to testosterone changes. In addition, the central nucleus of the BNST is considered a sexually dimorphic area that is twice as large in men as in women (Miller et al., 1989, Swaab, 2007). Its lateral and medial divisions are considered extensions of the central amygdala and medial amygdala, respectively (Dong et al., 2001a, Dong et al., 2001b). Further, lesions of the BNST reduced, and stimulation enhanced, anxiety- and stress-related behaviors (Walker et al., 2003,

Toufexis et al., 2006). The BNST also contains aromatase enzymes (Roselli, 1991), in addition to ER α , ER β , and AR (Zhou et al., 1994, Laflamme et al., 1998). GNX in adult rodents decreased vasopressin mRNA and increased ER α mRNA within the BNST, effects reversed by testosterone administration (Schulz et al., 2009, Auger et al., 2011). The effects of castration resulted from increased methylation at CpG sites in the vasopressin promoter and decreased methylation in the ER α promoter, establishing an epigenetic influence of testosterone. However, the specific role of testosterone in the BNST relating to anxiety and depression is understudied and requires additional focus.

6.3 Hippocampus

The hippocampus has been extensively documented as a critical site involved in anxiety disorders, major depressive disorder, and stress-induced alterations in the brain. Structural abnormalities and reduced activity in the hippocampus are observed in individuals with anxiety-related disorders and major depressive disorder, compared to healthy controls (Nestler et al., 2002a, Shin et al., 2006, Ferrari et al., 2008, Krishnan and Nestler, 2008, Hayes et al., 2011). The hippocampus also undergoes various morphological alterations during depression and anti-depressant treatment (McEwen, 2005, Rodrigues et al., 2009). Chronic stress causes atrophy of hippocampus cells, impairs neurogenesis (McEwen and Magarinos, 2001), and produces excitotoxic damage resulting from excess glucocorticoids (Magarinos and McEwen, 1995a, b). These effects can impair memory, impact HPA axis negative feedback, and contribute to the manifestation and maintenance of stress-related depression (Nestler et al., 2002a). Preclinical evidence has found that testosterone acting in the hippocampus has a number of anxiolytic, antidepressant, and protective cellular actions. Some of the protective effects of testosterone in the hippocampus may be due to its ability to lessen the aversive effects of stress and depression and facilitate molecular mechanisms that favor cell proliferation, growth and/or survival.

It is clear that testosterone has a number of protective effects on hippocampal cell growth and survival, during both organizational and activational periods. Perinatal androgen treatment increases neuronal soma size, dendritic length and branching, and also the volume of the CA3 pyramidal cell layer and the entire CA3 region of the hippocampus (Isgor and Sengelaub, 1998, 2003). Furthermore, neonatal GNX resulted in decreased hippocampal spine density in adulthood, an effect reversed with testosterone or DHT treatment (Dawson et al., 1975, Isgor and Sengelaub, 1998, 2003). These structural changes are associated with functional changes. In fact, neonatal androgens play an important role in protecting male rats from the development of depressive-like behaviors, and this protection is correlated with hippocampal neurogenesis as well as increased hippocampal spine density (Zhang et al., 2010). It has been suggested that the antidepressant effects of estradiol in females may be mediated in part by actions at serotonin (5-HT) receptors at the level of the hippocampus (Estrada-Camarena et al., 2006, Walf and Frye, 2007). In addition, testosterone administration in stressed tree shrews increased binding of serotonin 1A receptors in the hippocampus, which are usually down-regulated in response to stress and elevated corticosterone levels (Flugge et al., 1998). Additionally, mice with a knockout of ER β have significantly lower levels of serotonin in the BNST, preoptic area, and hippocampus, compared to wild type littermates. The organizational effects of testosterone within the hippocampus, also have important implications regarding depression. Administration of the AR antagonist flutamide to newborn male rat pups resulted in increased depressive-like behavior in the forced swim and sucrose preference tests prior to puberty; these behavioral effects were associated with decreased neurogenesis and dendritic spines in the hippocampus (Zhang et al., 2010). Overall, the influence of organizational effects of testosterone within the hippocampus appears to contribute significantly to its protective and antidepressant properties.

Activational effects of testosterone in hippocampal cells also produce favorable cellular outcomes. In GNX adult male rodents, testosterone administration reduced cellular oxidative damage and morphological alterations in the hippocampus, compared to vehicle-treated controls (Meydan et al., 2010). In intact female rodents, testosterone, DHT, or 3 α -diol administration following adrenalectomy decreased the number of pyknotic cells undergoing cell death in the hippocampus, compared to control-treated females (Frye and McCormick, 2000).

In addition, activational effects of testosterone in the hippocampus produce anxiolytic effects. For instance, GNX males with replacement of testosterone, DHT, or 3 α -diol in the hippocampus display decreased anxiety-like behavior in the elevated plus maze, open field test, and defensive freezing tests, compared to GNX without replacement controls (Edinger and Frye, 2004). Further, in GNX+DHT replaced male rodents, intra-hippocampal administration of indomethacin, which blocks conversion of DHT to 3 α -diol, decreased DHT's anxiolytic effects in the elevated plus maze and open field test and increased freezing behavior, compared to intact males and GNX+DHT replaced males treated with vehicle (Frye and Edinger, 2004). This suggests that 3 α -diol in the hippocampus contributes to DHT's anxiolytic effects. As previously stated, 3 α -diol binds with relatively low affinity to the androgen receptor (Cunningham et al., 1979), but acts as a neurosteroid agonist at the GABAA receptor (Frye et al., 1996). Several studies found that testosterone or its metabolites enhance the actions of GABA at its ionotropic GABAA receptor in cortical and hippocampal regions (Bitran et al., 1993, Frye et al., 2008). In addition to neurotransmitter interactions, androgen receptors may also be involved, since intra-hippocampal administration of the AR antagonist, flutamide, increased anxiety-like behavior, compared to intact and DHT-replaced controls (Edinger and Frye, 2006). Thus, it appears that testosterone within the hippocampus may have protective actions in males through a number of mechanisms at different points across the lifespan.

While it is clear that testosterone exerts a number of protective effects on cell growth and survival in the hippocampus, it remains unclear whether testosterone can also stimulate neurogenesis. Adult neurogenesis involves a population of stem cells that proliferate, migrate, and differentiate into new neurons within the brain. In mammals, the subgranular zone in the dentate gyrus sub-region of the hippocampal formation is a critical site for neurogenesis (Christie and Cameron, 2006) that is highly sensitive to multiple endogenous and environmental factors, particularly stress and antidepressant treatments (Galea et al., 2006). Two major components of hippocampal neurogenesis are typically studied: 1) the number of newly proliferating cells that are produced, and 2) the number of these new cells that survive to specific time points. Given that adult hippocampal neurogenesis is sexually dimorphic, such that females exhibit higher cell proliferation than males (Galea and McEwen, 1999), and the survival of newly proliferated cells is higher in males than in females (Westenbroek et al., 2004), it is quite predictable that sex hormones influence these processes. The extent of hormonal regulation of hippocampal neurogenesis on depressive-like behaviors is still not fully understood.

Studies investigating the effects of GNX on cell proliferation have been inconsistent. Several studies have found no effect of GNX on cell proliferation in male rats (Spritzer and Galea, 2007, Buwalda et al., 2010, Carrier and Kabbaj, 2012b); however, a decrease in both proliferation and survival following GNX has been reported (Wainwright et al., 2011). Previously, we have shown that three weeks of testosterone supplementation, in the form of slow release pellet implants, had no effect on hippocampal cell proliferation or survival in GNX male rats (Carrier and Kabbaj, 2012b). In agreement with our findings, two similar studies using testosterone implants also found no effect of testosterone on cell proliferation; however, they did report that GNX decreased cell survival (Benice and Raber, 2010), an

effect that was reversed with DHT and testosterone, but not estradiol (Spritzer & Galea, 2007). Indirect evidence associates seasonal decreases in testosterone levels with decreased hippocampal cell survival in male meadow voles during the non-breeding season (Galea and McEwen, 1999). Overall, there may be an optimal dose of circulating testosterone for increasing neurogenesis, and supraphysiological surges may even have a negative impact (Spritzer et al., 2011). However, the effects of testosterone on neurogenesis in the dentate gyrus of the hippocampus are complicated by a report that the majority of its neuroprotective effects may be mediated by aromatization to estradiol (Azcoitia et al., 2001). In contrast, another study provides evidence that testosterone may provide its beneficial effects via reduction to DHT (Spritzer and Galea, 2007). Specifically, in GNX rats 30 days of replacement with testosterone or DHT, but not estradiol, enhanced cell survival without affecting the number of newly proliferating cells. Therefore, it is not clear whether androgenic or estrogenic metabolites mediate most of the effects of testosterone on hippocampal cell survival.

Testosterone may act as a neuromodulator or in conjunction with other biochemical factors to enhance cell proliferation. We investigated the potential protective effects of concomitant treatment of testosterone and imipramine in rats that were exposed to chronic social isolation as a stress model. Although we found that testosterone alone did not influence cell proliferation in socially isolated GNX male rats, when compared to placebo-treated controls, we found an enhanced neurogenic effect with testosterone and imipramine co-administration on the number of proliferating cells in the dentate gyrus of the hippocampus, compared to testosterone or imipramine administration alone or to vehicle controls (Carrier and Kabbaj, 2012d). These data suggest an interesting potential influence of testosterone on antidepressant activity in male rats (Carrier and Kabbaj, 2012d). Although testosterone treatment in males has antidepressant activity that is similar to traditional drug treatments, few studies have investigated the potential interaction between testosterone and antidepressants. The physiological mechanism of these interactions remains unknown; however, imipramine, a tricyclic antidepressant that inhibits both 5-hydroxytryptamine and norepinephrine reuptake, likely interacts with testosterone through the noradrenergic system (Carrier and Kabbaj, 2012d). Indeed, Martinez-Mota and Fernandez-Guasti (2004) reported that GNX rats did not respond to the noradrenaline reuptake inhibitor desipramine, and testosterone supplementation restored its antidepressant activity. Furthermore, in that same study, testosterone did not influence the antidepressant efficacy of serotonin reuptake inhibitors such as fluoxetine and clomipramine. While the physiological mechanisms of testosterone's actions within the hippocampus remain unknown, the timing, dose, and route of testosterone administration appear to substantially impact observations regarding the effects of testosterone on hippocampal neurogenesis and interactions with typical antidepressants.

Elucidating underlying molecular signaling pathways stimulated by testosterone is crucial in understanding how testosterone is affecting intra-cellular processes. One potential candidate is the MAPK/ERK pathway, a major convergence point for signaling pathways activated by testosterone and its metabolites (Cheng et al., 2007) related to cell growth, differentiation, and neuronal plasticity (Schafe et al., 2000, Chen et al., 2001, Sweatt, 2001). Recently proposed theories implicate signaling pathways related to synaptic plasticity as critical to the molecular mechanisms of antidepressants, with particular focus on the ERK pathway regarding emotional responses (Malberg et al., 2000, Manji et al., 2001, Einat et al., 2003, Qi et al., 2008). Chronic stress increases depressive-like behaviors and decreases ERK2 in the hippocampus, an effect alleviated in part by treatment with fluoxetine (Qi et al., 2008). Furthermore, chronic administration of lithium or valproate, mood stabilizers used in the treatment of manic depression, stimulates the MAPK pathway in the rat hippocampus (Einat et al., 2003). In our previous work, we have shown that mRNA and protein expression of

ERK2, specifically within the hippocampus, is testosterone dependent (Carrier and Kabbaj, 2012d). GNX adult male rats had reduced ERK2 expression throughout the hippocampal formation, compared to sham-operated controls. Both physiological and extraphysiological testosterone replacements in GNX male rats increased ERK2 expression to the level seen in sham-operated controls. Furthermore, ERK2 expression within the hippocampus mediated the antidepressant effects of testosterone. In fact, GNX placebo-treated male rats exhibited depressive-like symptoms in the forced swim test and sucrose preference test, compared to sham-operated and GNX testosterone-replaced male rats (Carrier and Kabbaj, 2012b). Furthermore, we induced depressive-like behaviors by inhibiting downstream signaling of ERK2 activity in the dentate gyrus of testosterone-replaced GNX male rats, using a dominant negative herpes simplex viral vector construct, whereas over-expression of ERK2 in GNX placebo-treated male rats reversed this effect (Carrier and Kabbaj, 2012b). Therefore, the testosterone-dependent regulation of ERK2 signaling within the dentate gyrus area of the hippocampus appears to be an important link between circulating levels of peripheral testosterone, MAPK expression, and depressive-like behaviors. Overall, studies agree that testosterone has activational effects through signaling pathways in the hippocampus/dentate gyrus.

7. Testosterone's attenuating effects on stress response systems

In humans, excess stress has deleterious effects on mental health and often contributes to the manifestation and maintenance of anxiety-related disorders (Pego et al., 2010) and major depressive disorder (Holzel et al., 2011). Similarly in animal models, chronic stress increases anxiety- and depressive-like behaviors (Gronli et al., 2005, Becker et al., 2008, Kompagne et al., 2008). Moreover, long-term stress has been shown to induce functional alterations in brain regions implicated in anxiety and/or depressive disorders, including the hippocampus, amygdala, and prefrontal cortex (reviewed in McEwen, 2005, Rodrigues et al., 2009). The aversive effects of stress most likely result from over-activation of biological stress response systems. Stress acutely stimulates the sympathetic adrenomedullary (SAM) division of the autonomic nervous system, resulting in increased adrenal epinephrine release, followed by elevated heart rate, blood pressure, and vasoconstriction (Henry, 1992, Pacak, 2004). Stress also activates the hypothalamic-pituitary-adrenal (HPA) axis via the PVN, which contains the stimulatory peptides corticotrophin releasing hormone (CRH) (Henry, 1992, Pacak, 2004, Papadimitriou and Priftis, 2009). CRH stimulates the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary, ultimately promoting the secretion of adrenal glucocorticoids such as corticosterone/cortisol (cort.) into the bloodstream (Henry, 1992, Pacak, 2004, Papadimitriou and Priftis, 2009). Over-activation of the HPA axis with chronic stress, which results in excess glucocorticoids and impaired negative feedback, has been associated with a number of stress-related mental illnesses, including anxiety disorders and major depressive disorder (Sapolsky, 2000, Pariante and Miller, 2001, Varghese and Brown, 2001, Barden, 2004, Parker and Brotchie, 2004, Gillespie and Nemeroff, 2005). Testosterone may act during development and/or adulthood to dampen stress responsiveness. For example, women treated with testosterone display a suppressed stress response compared to controls (Hermans et al., 2007). In addition, testosterone can counteract the effects of early-life stress, which would otherwise result in increased stress and anxiety-like responses (Kapoor and Matthews, 2011).

Testosterone can suppress activity of the HPA axis, and there are opposing interactions between the HPA and hypothalamic-pituitary-gonadal (HPG) axes (reviewed in Viau, 2002). For example, stress inhibits gonadotrophins, which in turn leads to suppression of testosterone (Sapolsky, 2004). Conversely, testosterone also can attenuate levels of glucocorticoids and other stress hormones (Viau and Meaney, 1996). The precise mechanisms whereby testosterone inhibits the HPA axis remain unclear. GNX in adult male

rodents increases basal and stress-induced activity of the HPA axis, compared to controls, an effect reversed by testosterone or DHT replacement (Viau and Meaney, 1991, Handa et al., 1994, Viau et al., 2003, Viau and Meaney, 2004). However, while T suppresses activation of the HPA axis, it probably does not do so directly, since CRH neurosecretory cells in the PVN contain few to no gonadal steroid receptors (Simerly et al., 1990, Schuchard et al., 1993, Zhou et al., 1994, Shughrue et al., 1997, Laflamme et al., 1998, Viau, 2002). Thus, the influence of T on stress responses and HPA function is likely to be controlled above the level of the PVN. Indeed testosterone can act in brain areas up-stream from the PVN, such as the medial preoptic area (MPOA), to prevent stress responses of the HPA axis (Viau and Meaney, 1996, Williamson et al., 2010). Levels of testosterone and CRH also appear to be inversely related. In humans, CRH is often dysregulated in those with an anxiety disorder or major depression (Heuser et al., 1998, Arborelius et al., 1999, Reul and Holsboer, 2002b). In animals, males also have lower levels of CRH, compared to females, an effect reversed by GNX, rescued by DHT replacement, and exacerbated by estradiol treatment (Haas and George, 1988, Bingaman et al., 1994, Lund et al., 2004). Interestingly, the CRH promoter contains hormone response elements for both estrogens and androgens (Vamvakopoulos and Chrousos, 1993, Bao et al., 2006), suggesting a potential way for gonadal steroids to regulate CRH gene expression. Furthermore, DHT can significantly increase mRNA expression of CRH receptor 2 (CRH2) in the hippocampus, compared to vehicle-treated controls (Weiser et al., 2008). CRH2 may promote stress coping (Reul and Holsboer, 2002a) and knockout or deletion of CRH2 results in increased anxiety and hyperactive responses to stress (Bale et al., 2000, Kishimoto et al., 2000). Collectively, sex steroids can influence stress response systems and either enhance or lower stress responsiveness, depending on the brain region and specific hormone, metabolite, and/or receptor involved. In this way, sex steroids may influence stress-related anxiety and/or depressive disorders.

8. Conclusions

Innate sex differences develop under the influence of differential hormonal milieus, resulting in dimorphisms in brain structure, circuitry, and function. These differences are of extreme importance to the understanding of anxiety and depression, complex behaviors that exhibit sex-specific phenotypes. Overall, the studies presented here underscore the importance of testosterone and its metabolites in mediating patterns of gene expression within areas of the brain that are fundamental to the etiology of anxiety and depression. While our work has implicated the MAPK pathway in the hippocampus as an important mediator of testosterone effects, future studies will elucidate more molecular mechanisms in the hippocampus and other brain areas underlying the effects of testosterone on these complex psychological disorders.

References

- Abdulkarimi R, Aghazadeh AM, Daneshyar M. Effect of mentha extract (*Mentha piperita*) supplementation in drinking water on performance, plasma lipoproteins, carcass characteristic and liver color index or weight in broiler chickens. *Indian J Anim Sci.* 2012; 82:1070–1074.
- Adler A, Vescovo P, Robinson JK, Kritzer MF. Gonadectomy in adult life increases tyrosine hydroxylase immunoreactivity in the prefrontal cortex and decreases open field activity in male rats. *Neuroscience.* 1999; 89:939–954. [PubMed: 10199626]
- Ahmed EI, Zehr JL, Schulz KM, Lorenz BH, DonCarlos LL, Sisk CL. Pubertal hormones modulate the addition of new cells to sexually dimorphic brain regions. *Nat Neurosci.* 2008; 11:995–997. [PubMed: 19160494]
- Ahokas A, Kaukoranta J, Wahlbeck K, Aito M. Estrogen deficiency in severe postpartum depression: successful treatment with sublingual physiologic 17beta-estradiol: a preliminary study. *J Clin Psychiatry.* 2001; 62:332–336. [PubMed: 11411813]

- Alderson LM, Baum MJ. Differential effects of gonadal steroids on dopamine metabolism in mesolimbic and nigro-striatal pathways of male rat brain. *Brain research*. 1981; 218:189–206. [PubMed: 7272735]
- Arborelius L, Owens MJ, Plotsky PM, Nemeroff CB. The role of corticotropin-releasing factor in depression and anxiety disorders. *J Endocrinol*. 1999; 160:1–12. [PubMed: 9854171]
- Arnold AP, Breedlove SM. Organizational and activational effects of sex steroids on brain and behavior: a reanalysis. *Horm Behav*. 1985; 19:469–498. [PubMed: 3910535]
- Arnold AP, Gorski RA. Gonadal steroid induction of structural sex differences in the central nervous system. *Annual review of neuroscience*. 1984; 7:413–442.
- Auger CJ, Coss D, Auger AP, Forbes-Lorman RM. Epigenetic control of vasopressin expression is maintained by steroid hormones in the adult male rat brain. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108:4242–4247. [PubMed: 21368111]
- Aydogan U, Aydogdu A, Akbulut H, Sonmez A, Yuksel S, Basaran Y, Uzun O, Bolu E, Saglam K. Increased frequency of anxiety, depression, quality of life and sexual life in young hypogonadotropic hypogonadal males and impacts of testosterone replacement therapy on these conditions. *Endocr J*. 2012; 59:1099–105. [PubMed: 22972022]
- Azcoitia I, Sierra A, Veiga S, Honda S, Harada N, Garcia-Segura LM. Brain aromatase is neuroprotective. *Journal of neurobiology*. 2001; 47:318–329. [PubMed: 11351342]
- Bale TL, Contarino A, Smith GW, Chan R, Gold LH, Sawchenko PE, Koob GF, Vale WW, Lee KF. Mice deficient for corticotropin-releasing hormone receptor-2 display anxiety-like behaviour and are hypersensitive to stress. *Nat Genet*. 2000; 24:410–414. [PubMed: 10742108]
- Bao AM, Fischer DF, Wu YH, Hol EM, Balesar R, Unmehopa UA, Zhou JN, Swaab DF. A direct androgenic involvement in the expression of human corticotropin-releasing hormone. *Mol Psychiatry*. 2006; 11:567–576. [PubMed: 16446741]
- Barden N. Implication of the hypothalamic-pituitary-adrenal axis in the physiopathology of depression. *J Psychiatry Neurosci*. 2004; 29:185–193. [PubMed: 15173895]
- Barrett-Connor E, Von Muhlen DG, Kritz-Silverstein D. Bioavailable testosterone and depressed mood in older men: the Rancho Bernardo Study. *The Journal of clinical endocrinology and metabolism*. 1999; 84:573–577. [PubMed: 10022418]
- Baulieu EE, Robel P. Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) as neuroactive neurosteroids. *Proc Natl Acad Sci U S A*. 1998; 95:4089–4091. [PubMed: 9539693]
- Baulieu EE, Robel P, Schumacher M. Neurosteroids: beginning of the story. *Int Rev Neurobiol*. 2001; 46:1–32. [PubMed: 11599297]
- Becker C, Zeau B, Rivat C, Blugeot A, Hamon M, Benoliel JJ. Repeated social defeat-induced depression-like behavioral and biological alterations in rats: involvement of cholecystokinin. *Mol Psychiatry*. 2008; 13:1079–1092. [PubMed: 17893702]
- Bekker MH, van Mens-Verhulst J. Anxiety disorders: sex differences in prevalence, degree, and background, but gender-neutral treatment. *Gend Med*. 2007; 4(Suppl B):S178–193. [PubMed: 18156102]
- Benice TS, Raber J. Castration and training in a spatial task alter the number of immature neurons in the hippocampus of male mice. *Brain research*. 2010; 1329:21–29. [PubMed: 20233585]
- Bennett NC, Gardiner RA, Hooper JD, Johnson DW, Gobe GC. Molecular cell biology of androgen receptor signalling. *Int J Biochem Cell Biol*. 2010; 42:813–827. [PubMed: 19931639]
- Bingaman EW, Magnuson DJ, Gray TS, Handa RJ. Androgen inhibits the increases in hypothalamic corticotropin-releasing hormone (CRH) and CRH-immunoreactivity following gonadectomy. *Neuroendocrinology*. 1994; 59:228–234. [PubMed: 8159272]
- Bingham B, Viau V. Neonatal gonadectomy and adult testosterone replacement suggest an involvement of limbic arginine vasopressin and androgen receptors in the organization of the hypothalamic-pituitary-adrenal axis. *Endocrinology*. 2008; 149:3581–3591. [PubMed: 18403478]
- Bitran D, Kellogg CK, Hilvers RJ. Treatment with an anabolic-androgenic steroid affects anxiety-related behavior and alters the sensitivity of cortical GABAA receptors in the rat. *Hormones and behavior*. 1993; 27:568–583. [PubMed: 8294123]

- Boonyaratanakornkit V, Scott MP, Ribon V, Sherman L, Anderson SM, Maller JL, Miller WT, Edwards DP. Progesterone receptor contains a proline-rich motif that directly interacts with SH3 domains and activates c-Src family tyrosine kinases. *Mol Cell*. 2001; 8:269–280. [PubMed: 11545730]
- Borsini F, Meli A. Is the forced swimming test a suitable model for revealing antidepressant activity? *Psychopharmacology*. 1988; 94:147–160. [PubMed: 3127840]
- Bryant DN, Bosch MA, Ronnekleiv OK, Dorsa DM. 17-Beta estradiol rapidly enhances extracellular signal-regulated kinase 2 phosphorylation in the rat brain. *Neuroscience*. 2005; 133:343–352. [PubMed: 15893655]
- Buddenberg TE, Komorowski M, Ruocco LA, Silva MA, Topic B. Attenuating effects of testosterone on depressive-like behavior in the forced swim test in healthy male rats. *Brain Res Bull*. 2009; 79:182–186. [PubMed: 19429189]
- Buwalda B, van der Borght K, Koolhaas JM, McEwen BS. Testosterone decrease does not play a major role in the suppression of hippocampal cell proliferation following social defeat stress in rats. *Physiology & behavior*. 2010; 101:719–725. [PubMed: 20732337]
- Carbone S, Pnzo OJ, Gobetto N, Samaniego YA, Reynoso R, Scacchi P, Moguevsky JA, Cutrera R. Antiandrogenic effect of perinatal exposure to the endocrine disruptor di-(2-ethylhexyl) phthalate increases anxiety-like behavior in male rats during sexual maturation. *Horm Behav*. 2013; 63:692–9. [PubMed: 23399322]
- Carrier N, Kabbaj M. Extracellular Signal-Regulated Kinase 2 Signaling in the Hippocampal Dentate Gyrus Mediates the Antidepressant Effects of Testosterone. *Biol Psychiat*. 2012a; 71:642–651. [PubMed: 22265242]
- Carrier N, Kabbaj M. Extracellular Signal-Regulated Kinase 2 Signaling in the Hippocampal Dentate Gyrus Mediates the Antidepressant Effects of Testosterone. *Biol Psychiatry*. 2012b
- Carrier N, Kabbaj M. Testosterone and imipramine have antidepressant effects in socially isolated male but not female rats. *Hormones and behavior*. 2012c; 61:678–685. [PubMed: 22426002]
- Carrier N, Kabbaj M. Testosterone and imipramine have antidepressant effects in socially isolated male but not female rats. *Hormones and behavior*. 2012d; 61:678–685. [PubMed: 22426002]
- Cato AC, Nestl A, Mink S. Rapid actions of steroid receptors in cellular signaling pathways. *Sci STKE*. 2002; 2002:re9. [PubMed: 12084906]
- Chen D, Pace PE, Coombes RC, Ali S. Phosphorylation of human estrogen receptor alpha by protein kinase A regulates dimerization. *Mol Cell Biol*. 1999; 19:1002–1015. [PubMed: 9891036]
- Chen Z, Gibson TB, Robinson F, Silvestro L, Pearson G, Xu B, Wright A, Vanderbilt C, Cobb MH. MAP kinases. *Chem Rev*. 2001; 101:2449–2476. [PubMed: 11749383]
- Cheng J, Watkins SC, Walker WH. Testosterone activates mitogen-activated protein kinase via Src kinase and the epidermal growth factor receptor in sertoli cells. *Endocrinology*. 2007; 148:2066–2074. [PubMed: 17272394]
- Christie BR, Cameron HA. Neurogenesis in the adult hippocampus. *Hippocampus*. 2006; 16:199–207. [PubMed: 16411231]
- Cooke B, Hegstrom CD, Villeneuve LS, Breedlove SM. Sexual differentiation of the vertebrate brain: principles and mechanisms. *Frontiers in neuroendocrinology*. 1998; 19:323–362. [PubMed: 9799588]
- Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: recent developments and future needs. *Trends in pharmacological sciences*. 2002; 23:238–245. [PubMed: 12008002]
- Cunningham GR, Huckins C. Persistence of complete spermatogenesis in the presence of low intratesticular concentrations of testosterone. *Endocrinology*. 1979; 105:177–186. [PubMed: 446406]
- Dawson JL, Cheung YM, Lau RT. Developmental effects of neonatal sex hormones on spatial and activity skills in the white rat. *Biol Psychol*. 1975; 3:213–229. [PubMed: 1203405]
- De Jonge FH, Louwse AL, Ooms MP, Evers P, Ender E, van de Poll NE. Lesions of the SDN-POA inhibit sexual behavior of male Wistar rats. *Brain Res Bull*. 1989; 23:483–492. [PubMed: 2611691]

- de Souza Silva MA, Mattern C, Topic B, Buddenberg TE, Huston JP. Dopaminergic and serotonergic activity in neostriatum and nucleus accumbens enhanced by intranasal administration of testosterone. *Eur Neuropsychopharmacol.* 2009; 19:53–63. [PubMed: 18818056]
- De Vries GJ, Rissman EF, Simerly RB, Yang LY, Scordalakes EM, Auger CJ, Swain A, Lovell-Badge R, Burgoyne PS, Arnold AP. A model system for study of sex chromosome effects on sexually dimorphic neural and behavioral traits. *The Journal of neuroscience: the official journal of the Society for Neuroscience.* 2002; 22:9005–9014. [PubMed: 12388607]
- Derntl B, Windischberger C, Robinson S, Kryspin-Exner I, Gur RC, Moser E, Habel U. Amygdala activity to fear and anger in healthy young males is associated with testosterone. *Psychoneuroendocrinology.* 2009; 34:687–693. [PubMed: 19136216]
- DiBlasio CJ, Hammett J, Malcolm JB, Judge BA, Womack JH, Kincade MC, Ogles ML, Mancini JG, Patterson AL, Wake RW, Derweesh IH. Prevalence and predictive factors for the development of de novo psychiatric illness in patients receiving androgen deprivation therapy for prostate cancer. *Can J Urol.* 2008; 15:4249–4256. discussion 4256. [PubMed: 18814813]
- Dong HW, Petrovich GD, Swanson LW. Topography of projections from amygdala to bed nuclei of the stria terminalis. *Brain Res Brain Res Rev.* 2001a; 38:192–246. [PubMed: 11750933]
- Dong HW, Petrovich GD, Watts AG, Swanson LW. Basic organization of projections from the oval and fusiform nuclei of the bed nuclei of the stria terminalis in adult rat brain. *J Comp Neurol.* 2001b; 436:430–455. [PubMed: 11447588]
- Douma SL, Husband C, O'Donnell ME, Barwin BN, Woodend AK. Estrogen-related mood disorders: reproductive life cycle factors. *ANS Adv Nurs Sci.* 2005; 28:364–375. [PubMed: 16292022]
- Dubrovsky BO. Steroids, neuroactive steroids and neurosteroids in psychopathology. *Prog Neuropsychopharmacol Biol Psychiatry.* 2005; 29:169–192. [PubMed: 15694225]
- Durdiakova J, Ostatnikova D, Celec P. Testosterone and its metabolites - modulators of brain functions. *Acta Neurobiol Exp (Wars).* 2011; 71:434–454. [PubMed: 22237492]
- Ebner K, Rupniak NM, Saria A, Singewald N. Substance P in the medial amygdala: emotional stress-sensitive release and modulation of anxiety-related behavior in rats. *Proceedings of the National Academy of Sciences of the United States of America.* 2004; 101:4280–4285. [PubMed: 15024126]
- Edinger KL, Frye CA. Testosterone's analgesic, anxiolytic, and cognitive-enhancing effects may be due in part to actions of its 5alpha-reduced metabolites in the hippocampus. *Behav Neurosci.* 2004; 118:1352–1364. [PubMed: 15598144]
- Edinger KL, Frye CA. Intrahippocampal administration of an androgen receptor antagonist, flutamide, can increase anxiety-like behavior in intact and DHT-replaced male rats. *Hormones and behavior.* 2006; 50:216–222. [PubMed: 16631174]
- Eichmann F, Holst DV. Organization of territorial marking behavior by testosterone during puberty in male tree shrews. *Physiol Behav.* 1999; 65:785–791. [PubMed: 10073481]
- Einat H, Yuan P, Gould TD, Li J, Du J, Zhang L, Manji HK, Chen G. The role of the extracellular signal-regulated kinase signaling pathway in mood modulation. *J Neurosci.* 2003; 23:7311–7316. [PubMed: 12917364]
- Eser D, Schule C, Romeo E, Baghai TC, di Michele F, Pasini A, Zwanzger P, Padberg F, Rupprecht R. Neuropsychopharmacological properties of neuroactive steroids in depression and anxiety disorders. *Psychopharmacology.* 2006; 186:373–387. [PubMed: 16247651]
- Estrada-Camarena E, Fernandez-Guasti A, Lopez-Rubalcava C. Antidepressant-like effect of different estrogenic compounds in the forced swimming test. *Neuropsychopharmacology.* 2003; 28:830–838. [PubMed: 12637949]
- Estrada-Camarena E, Lopez-Rubalcava C, Fernandez-Guasti A. Facilitating antidepressant-like actions of estrogens are mediated by 5-HT1A and estrogen receptors in the rat forced swimming test. *Psychoneuroendocrinology.* 2006; 31:905–914. [PubMed: 16843610]
- Falkenstein E, Tillmann HC, Christ M, Feuring M, Wehling M. Multiple actions of steroid hormones--a focus on rapid, nongenomic effects. *Pharmacol Rev.* 2000; 52:513–556. [PubMed: 11121509]
- Fernandez-Guasti A, Martinez-Mota L. Orchidectomy sensitizes male rats to the action of diazepam on burying behavior latency: role of testosterone. *Pharmacology, biochemistry, and behavior.* 2003; 75:473–479.

- Fernandez-Guasti A, Martinez-Mota L. Anxiolytic-like actions of testosterone in the burying behavior test: role of androgen and GABA-benzodiazepine receptors. *Psychoneuroendocrinology*. 2005; 30:762–770. [PubMed: 15919582]
- Ferrari MC, Busatto GF, McGuire PK, Crippa JA. Structural magnetic resonance imaging in anxiety disorders: an update of research findings. *Rev Bras Psiquiatr*. 2008; 30:251–264. [PubMed: 18833427]
- Flugge G, Kramer M, Rensing S, Fuchs E. 5HT1A-receptors and behaviour under chronic stress: selective counteraction by testosterone. *The European journal of neuroscience*. 1998; 10:2685–2693. [PubMed: 9767398]
- Frye CA, Edinger K, Sumida K. Androgen administration to aged male mice increases anti-anxiety behavior and enhances cognitive performance. *Neuropsychopharmacology*. 2008; 33:1049–1061. [PubMed: 17625503]
- Frye CA, Edinger KL. Testosterone's metabolism in the hippocampus may mediate its anti-anxiety effects in male rats. *Pharmacology, biochemistry, and behavior*. 2004; 78:473–481.
- Frye CA, Lacey EH. Posttraining androgens' enhancement of cognitive performance is temporally distinct from androgens' increases in affective behavior. *Cogn Affect Behav Neurosci*. 2001; 1:172–182. [PubMed: 12467112]
- Frye CA, McCormick CM. Androgens are neuroprotective in the dentate gyrus of adrenalectomized female rats. *Stress*. 2000; 3:185–194. [PubMed: 10938579]
- Frye CA, Seliga AM. Testosterone increases analgesia, anxiolysis, and cognitive performance of male rats. *Cogn Affect Behav Neurosci*. 2001; 1:371–381. [PubMed: 12467088]
- Frye CA, Van Keuren KR, Erskine MS. Behavioral effects of 3 alpha-androstane diol. I: Modulation of sexual receptivity and promotion of GABA-stimulated chloride flux. *Behav Brain Res*. 1996; 79:109–118. [PubMed: 8883822]
- Frye CA, Walf AA. Estrogen and/or progesterone administered systemically or to the amygdala can have anxiety-, fear-, and pain-reducing effects in ovariectomized rats. *Behav Neurosci*. 2004; 118:306–313. [PubMed: 15113256]
- Frye CA, Walf AA. Depression-like behavior of aged male and female mice is ameliorated with administration of testosterone or its metabolites. *Physiology & behavior*. 2009; 97:266–269. [PubMed: 19254734]
- Galea LA, McEwen BS. Sex and seasonal differences in the rate of cell proliferation in the dentate gyrus of adult wild meadow voles. *Neuroscience*. 1999; 89:955–964. [PubMed: 10199627]
- Galea LA, Spritzer MD, Barker JM, Pawluski JL. Gonadal hormone modulation of hippocampal neurogenesis in the adult. *Hippocampus*. 2006; 16:225–232. [PubMed: 16411182]
- Ghayee HK, Auchus RJ. Basic concepts and recent developments in human steroid hormone biosynthesis. *Rev Endocr Metab Disord*. 2007; 8:289–300. [PubMed: 17926129]
- Gillespie CF, Nemeroff CB. Hypercortisolemia and depression. *Psychosom Med*. 2005; 67(Suppl 1):S26–28. [PubMed: 15953796]
- Giltay EJ, Enter D, Zitman FG, Penninx B, Van Pelt J, Spinhoven P, Roelofs K. Salivary testosterone: Associations with depression, anxiety disorders, and antidepressant use in a large cohort study. *Journal of Psychosomatic Research*. 2012 in press.
- Goel N, Bale TL. Organizational and activational effects of testosterone on masculinization of female physiological and behavioral stress responses. *Endocrinology*. 2008; 149:6399–6405. [PubMed: 18687782]
- Goldstat R, Briganti E, Tran J, Wolfe R, Davis SR. Transdermal testosterone therapy improves well-being, mood, and sexual function in premenopausal women. *Menopause*. 2003; 10:390–398. [PubMed: 14501599]
- Goy, RW.; McEwen, BS. *Sexual Differentiation of the Brain*. Oxford University Press; 1980.
- Granger DA, Shirtcliff EA, Zahn-Waxler C, Usher B, Klimes-Dougan B, Hastings P. Salivary testosterone diurnal variation and psychopathology in adolescent males and females: individual differences and developmental effects. *Dev Psychopathol*. 2003; 15:431–449. [PubMed: 12931836]

- Gronli J, Murison R, Fiske E, Bjorvatn B, Sorensen E, Portas CM, Ursin R. Effects of chronic mild stress on sexual behavior, locomotor activity and consumption of sucrose and saccharine solutions. *Physiology & behavior*. 2005; 84:571–577. [PubMed: 15811392]
- Guillamon A, de Blas MR, Segovia S. Effects of sex steroids on the development of the locus coeruleus in the rat. *Brain research*. 1988; 468:306–310. [PubMed: 3382961]
- Gutierrez-Garcia AG, Contreras CM, Vasquez-Hernandez DI, Molina-Jimenez T, Jacome-Jacome E. Testosterone reduces cumulative burying in female Wistar rats with minimal participation of estradiol. *Pharmacology, biochemistry, and behavior*. 2009; 93:406–412.
- Haas DA, George SR. Gonadal regulation of corticotropin-releasing factor immunoreactivity in hypothalamus. *Brain Res Bull*. 1988; 20:361–367. [PubMed: 3284608]
- Handa RJ, Corbier P, Shryne JE, Schoonmaker JN, Gorski RA. Differential effects of the perinatal steroid environment on three sexually dimorphic parameters of the rat brain. *Biology of reproduction*. 1985; 32:855–864. [PubMed: 3924134]
- Handa RJ, Nunley KM, Lorens SA, Louie JP, McGivern RF, Bollnow MR. Androgen regulation of adrenocorticotropin and corticosterone secretion in the male rat following novelty and foot shock stressors. *Physiology & behavior*. 1994; 55:117–124. [PubMed: 8140154]
- Hayes JP, LaBar KS, McCarthy G, Selgrade E, Nasser J, Dolcos F, Morey RA. workgroup VM-AM. Reduced hippocampal and amygdala activity predicts memory distortions for trauma reminders in combat-related PTSD. *J Psychiatr Res*. 2011; 45:660–669. [PubMed: 21047644]
- Hennessey AC, Wallen K, Edwards DA. Preoptic lesions increase the display of lordosis by male rats. *Brain Res*. 1986; 370:21–28. [PubMed: 3708321]
- Henry JP. Biological basis of the stress response. *Integr Physiol Behav Sci*. 1992; 27:66–83. [PubMed: 1576090]
- Hermans EJ, Putman P, Baas JM, Gecks NM, Kenemans JL, van Honk J. Exogenous testosterone attenuates the integrated central stress response in healthy young women. *Psychoneuroendocrinology*. 2007; 32:1052–1061. [PubMed: 17904297]
- Hermans EJ, Putman P, Baas JM, Koppeschaar HP, van Honk J. A single administration of testosterone reduces fear-potentiated startle in humans. *Biol Psychiatry*. 2006; 59:872–874. [PubMed: 16458259]
- Herrera-Perez JJ, Martinez-Mota L, Chavira R, Fernandez-Guasti A. Testosterone prevents but not reverses anhedonia in middle-aged males and lacks an effect on stress vulnerability in young adults. *Hormones and behavior*. 2012; 61:623–630. [PubMed: 22373497]
- Heuser I, Bissette G, Dettling M, Schweiger U, Gotthardt U, Schmider J, Lammers CH, Nemeroff CB, Holsboer F. Cerebrospinal fluid concentrations of corticotropin-releasing hormone, vasopressin, and somatostatin in depressed patients and healthy controls: response to amitriptyline treatment. *Depress Anxiety*. 1998; 8:71–79. [PubMed: 9784981]
- Hisasue S, Seney ML, Immerman E, Forger NG. Control of cell number in the bed nucleus of the stria terminalis of mice: role of testosterone metabolites and estrogen receptor subtypes. *The journal of sexual medicine*. 2010; 7:1401–1409. [PubMed: 20102443]
- Dodosy J, Zelmanova D, Majzunova M, Filova B, Malinova M, Ostatnikova D, Celec P. The anxiolytic effect of testosterone in the rat is mediated via the androgen receptor. *Pharmacol Biochem Behav*. 2012; 102:191–5. [PubMed: 22546276]
- Holzel L, Harter M, Reese C, Kriston L. Risk factors for chronic depression--a systematic review. *J Affect Disord*. 2011; 129:1–13. [PubMed: 20488546]
- Hull EM, Dominguez JM. Getting his act together: roles of glutamate, nitric oxide, and dopamine in the medial preoptic area. *Brain research*. 2006; 1126:66–75. [PubMed: 16963001]
- Isgor C, Sengelaub DR. Prenatal gonadal steroids affect adult spatial behavior, CA1 and CA3 pyramidal cell morphology in rats. *Hormones and behavior*. 1998; 34:183–198. [PubMed: 9799628]
- Isgor C, Sengelaub DR. Effects of neonatal gonadal steroids on adult CA3 pyramidal neuron dendritic morphology and spatial memory in rats. *Journal of neurobiology*. 2003; 55:179–190. [PubMed: 12672016]
- Kabbaj M, Devine DP, Savage VR, Akil H. Neurobiological correlates of individual differences in novelty-seeking behavior in the rat: differential expression of stress-related molecules. *The*

- Journal of neuroscience: the official journal of the Society for Neuroscience. 2000; 20:6983–6988. [PubMed: 10995843]
- Kanayama G, Amiaz R, Seidman S, Pope HG Jr. Testosterone supplementation for depressed men: current research and suggested treatment guidelines. *Experimental and clinical psychopharmacology*. 2007; 15:529–538. [PubMed: 18179305]
- Kapoor A, Matthews SG. Testosterone is involved in mediating the effects of prenatal stress in male guinea pig offspring. *J Physiol*. 2011; 589:755–766. [PubMed: 21173081]
- Kelly MJ, Lagrange AH, Wagner EJ, Ronnekleiv OK. Rapid effects of estrogen to modulate G protein-coupled receptors via activation of protein kinase A and protein kinase C pathways. *Steroids*. 1999; 64:64–75. [PubMed: 10323674]
- Kelly MJ, Qiu J, Ronnekleiv OK. Estrogen modulation of G-protein-coupled receptor activation of potassium channels in the central nervous system. *Ann N Y Acad Sci*. 2003; 1007:6–16. [PubMed: 14993035]
- Kelly MJ, Qiu J, Wagner EJ, Ronnekleiv OK. Rapid effects of estrogen on G protein-coupled receptor activation of potassium channels in the central nervous system (CNS). *J Steroid Biochem Mol Biol*. 2002a; 83:187–193. [PubMed: 12650715]
- Kelly MJ, Ronnekleiv OK, Ibrahim N, Lagrange AH, Wagner EJ. Estrogen modulation of K(+) channel activity in hypothalamic neurons involved in the control of the reproductive axis. *Steroids*. 2002b; 67:447–456. [PubMed: 11960620]
- Kenny AM, Fabregas G, Song C, Biskup B, Bellantonio S. Effects of testosterone on behavior, depression, and cognitive function in older men with mild cognitive loss. *J Gerontol A Biol Sci Med Sci*. 2004; 59:75–78. [PubMed: 14718489]
- Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry*. 2005a; 62:593–602. [PubMed: 15939837]
- Kessler RC, Chiu WT, Demler O, Merikangas KR, Walters EE. Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry*. 2005b; 62:617–627. [PubMed: 15939839]
- Kishimoto T, Radulovic J, Radulovic M, Lin CR, Schrick C, Hooshmand F, Hermanson O, Rosenfeld MG, Spiess J. Deletion of *crhr2* reveals an anxiolytic role for corticotropin-releasing hormone receptor-2. *Nat Genet*. 2000; 24:415–419. [PubMed: 10742109]
- Kompagne H, Bardos G, Szenasi G, Gacsalyi I, Harsing LG, Levay G. Chronic mild stress generates clear depressive but ambiguous anxiety-like behaviour in rats. *Behav Brain Res*. 2008; 193:311–314. [PubMed: 18590771]
- Kousteni S, Bellido T, Plotkin LI, O'Brien CA, Bodenner DL, Han L, Han K, DiGregorio GB, Katzenellenbogen JA, Katzenellenbogen BS, Roberson PK, Weinstein RS, Jilka RL, Manolagas SC. Nongenotropic, sex-nonspecific signaling through the estrogen or androgen receptors: dissociation from transcriptional activity. *Cell*. 2001; 104:719–730. [PubMed: 11257226]
- Krishnan V, Nestler EJ. The molecular neurobiology of depression. *Nature*. 2008; 455:894–902. [PubMed: 18923511]
- Kubli-Garfias C, Canchola E, Arauz-Contreras J, Feria-Velasco A. Depressant effect of androgens on the cat brain electrical activity and its antagonism by ruthenium red. *Neuroscience*. 1982; 7:2777–2782. [PubMed: 6185879]
- Kudwa AE, Michopoulos V, Gatewood JD, Rissman EF. Roles of estrogen receptors alpha and beta in differentiation of mouse sexual behavior. *Neuroscience*. 2006; 138:921–928. [PubMed: 16338079]
- Kuroki Y, Fukushima K, Kanda Y, Mizuno K, Watanabe Y. Putative membrane-bound estrogen receptors possibly stimulate mitogen-activated protein kinase in the rat hippocampus. *Eur J Pharmacol*. 2000; 400:205–209. [PubMed: 10988335]
- Laflamme N, Nappi RE, Drolet G, Labrie C, Rivest S. Expression and neuropeptidergic characterization of estrogen receptors (ERalpha and ERbeta) throughout the rat brain: anatomical evidence of distinct roles of each subtype. *Journal of neurobiology*. 1998; 36:357–378. [PubMed: 9733072]

- Lincoln DW. Unit activity in the hypothalamus, septum and preoptic area of the rat: characteristics of spontaneous activity and the effect of oestrogen. *J Endocrinol.* 1967; 37:177–189. [PubMed: 6017305]
- Linfoot I, Gray M, Bingham B, Williamson M, Pinel JP, Viau V. Naturally occurring variations in defensive burying behavior are associated with differences in vasopressin, oxytocin, and androgen receptors in the male rat. *Prog Neuropsychopharmacol Biol Psychiatry.* 2009; 33:1129–1140. [PubMed: 19540895]
- Liu YF, Bertram K, Perides G, McEwen BS, Wang D. Stress induces activation of stress-activated kinases in the mouse brain. *Journal of neurochemistry.* 2004; 89:1034–1043. [PubMed: 15140201]
- Lodish, H.; Darnell, J.; Baltimore, D.; Wolfgang Montes, J. *Molecular Cell Biology.* New York: W.H. Freeman and company; 2007. Regulation of transcription-factor activity.
- Loss ES, Jacobus AP, Wassermann GF. Rapid signaling responses in Sertoli cell membranes induced by follicle stimulating hormone and testosterone: calcium inflow and electrophysiological changes. *Life sciences.* 2011; 89:577–583. [PubMed: 21703282]
- Lucion AB, Charchat H, Pereira GA, Rasia-Filho AA. Influence of early postnatal gonadal hormones on anxiety in adult male rats. *Physiology & behavior.* 1996; 60:1419–1423. [PubMed: 8946485]
- Lucki I. The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. *Behavioural pharmacology.* 1997; 8:523–532. [PubMed: 9832966]
- Lumia AR, Canino G, Drozdowski B. Effects of androgen on scent marking and aggressive behavior of neonatally bulbectomized female Mongolian gerbils (*Meriones unguiculatus*). *Hormones and behavior.* 1976; 7:461–471. [PubMed: 1035203]
- Lund TD, Munson DJ, Haldy ME, Handa RJ. Androgen inhibits, while oestrogen enhances, restraint-induced activation of neuropeptide neurones in the paraventricular nucleus of the hypothalamus. *J Neuroendocrinol.* 2004; 16:272–278. [PubMed: 15049858]
- Magarinos AM, McEwen BS. Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: comparison of stressors. *Neuroscience.* 1995a; 69:83–88. [PubMed: 8637635]
- Magarinos AM, McEwen BS. Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: involvement of glucocorticoid secretion and excitatory amino acid receptors. *Neuroscience.* 1995b; 69:89–98. [PubMed: 8637636]
- Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *The Journal of neuroscience: the official journal of the Society for Neuroscience.* 2000; 20:9104–9110. [PubMed: 11124987]
- Maner JK, Miller SL, Schmidt NB, Eckel LA. Submitting to defeat: social anxiety, dominance threat, and decrements in testosterone. *Psychol Sci.* 2008; 19:764–768. [PubMed: 18816282]
- Manji HK, Drevets WC, Charney DS. The cellular neurobiology of depression. *Nat Med.* 2001; 7:541–547. [PubMed: 11329053]
- Mannella P, Brinton RD. Estrogen receptor protein interaction with phosphatidylinositol 3-kinase leads to activation of phosphorylated Akt and extracellular signal-regulated kinase 1/2 in the same population of cortical neurons: a unified mechanism of estrogen action. *J Neurosci.* 2006; 26:9439–9447. [PubMed: 16971528]
- Martinez-Mota L, Fernandez-Guasti A. Testosterone-dependent antidepressant-like effect of noradrenergic but not of serotonergic drugs. *Pharmacology, biochemistry, and behavior.* 2004; 78:711–718.
- McCarthy MM, Konkle AT. When is a sex difference not a sex difference? *Frontiers in neuroendocrinology.* 2005; 26:85–102. [PubMed: 16083951]
- McEwen BS. Glucocorticoids, depression, and mood disorders: structural remodeling in the brain. *Metabolism.* 2005; 54:20–23. [PubMed: 15877308]
- McEwen BS, Magarinos AM. Stress and hippocampal plasticity: implications for the pathophysiology of affective disorders. *Hum Psychopharmacol.* 2001; 16:S7–S19. [PubMed: 12404531]
- Melcangi RC, Garcia-Segura LM, Mensah-Nyagan AG. Neuroactive steroids: state of the art and new perspectives. *Cell Mol Life Sci.* 2008; 65:777–797. [PubMed: 18038216]

- Meydan S, Kus I, Tas U, Ogeturk M, Sancakdar E, Dabak DO, Zararsiz I, Sarsilmaz M. Effects of testosterone on orchietomy-induced oxidative damage in the rat hippocampus. *J Chem Neuroanat.* 2010; 40:281–285. [PubMed: 20696235]
- Migliaccio A, Castoria G, Di Domenico M, de Falco A, Bilancio A, Lombardi M, Barone MV, Ametrano D, Zannini MS, Abbondanza C, Auricchio F. Steroid-induced androgen receptor-oestradiol receptor beta-*Src* complex triggers prostate cancer cell proliferation. *Embo J.* 2000; 19:5406–5417. [PubMed: 11032808]
- Miller KK, Perlis RH, Papakostas GI, Mischoulon D, Losifescu DV, Brick DJ, Fava M. Low-dose transdermal testosterone augmentation therapy improves depression severity in women. *CNS Spectr.* 2009; 14:688–694. [PubMed: 20394176]
- Miller MA, Vician L, Clifton DK, Dorsa DM. Sex differences in vasopressin neurons in the bed nucleus of the stria terminalis by in situ hybridization. *Peptides.* 1989; 10:615–619. [PubMed: 2780420]
- Miller WL. Steroid hormone synthesis in mitochondria. *Mol Cell Endocrinol.* 2013
- Miner MM, Bhattacharya RK, Blick G, Kushner H, Khara M. 12-Month observation of testosterone replacement effectiveness in a general population of men. *Postgrad Med.* 2013; 125:8–18. [PubMed: 23816767]
- Mong JA, Glaser E, McCarthy MM. Gonadal steroids promote glial differentiation and alter neuronal morphology in the developing hypothalamus in a regionally specific manner. *The Journal of neuroscience: the official journal of the Society for Neuroscience.* 1999; 19:1464–1472. [PubMed: 9952422]
- Morris JA, Jordan CL, Breedlove SM. Sexual differentiation of the vertebrate nervous system. *Nature neuroscience.* 2004; 7:1034–1039.
- Morsink LF, Vogelzangs N, Nicklas BJ, Beekman AT, Satterfield S, Rubin SM, Yaffe K, Simonsick E, Newman AB, Kritchevsky SB, Penninx BW. Health ABCs. Associations between sex steroid hormone levels and depressive symptoms in elderly men and women: results from the Health ABC study. *Psychoneuroendocrinology.* 2007; 32:874–883. [PubMed: 17651906]
- Murphy DD, Segal M. Morphological plasticity of dendritic spines in central neurons is mediated by activation of cAMP response element binding protein. *Proceedings of the National Academy of Sciences of the United States of America.* 1997; 94:1482–1487. [PubMed: 9037079]
- Murray EK, Hien A, de Vries GJ, Forger NG. Epigenetic control of sexual differentiation of the bed nucleus of the stria terminalis. *Endocrinology.* 2009; 150:4241–4247. [PubMed: 19497973]
- Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. *Neuron.* 2002a; 34:13–25. [PubMed: 11931738]
- Nestler EJ, Gould E, Manji H, Buncan M, Duman RS, Greshenfeld HK, Hen R, Koester S, Lederhendler I, Meaney M, Robbins T, Winsky L, Zalcman S. Preclinical models: status of basic research in depression. *Biol Psychiatry.* 2002b; 52:503–528. [PubMed: 12361666]
- Nestler EJ, Hyman SE. Animal models of neuropsychiatric disorders. *Nature neuroscience.* 2010; 13:1161–1169.
- Nestler, EJ.; Hyman, SE.; Malenka, RC. *Molecular neuropharmacology: a foundation for clinical neuroscience.* New York: McGraw-Hill Medical; 2009.
- Pacak, K. *Stress: current neuroendocrine and genetic approaches.* New York: New York Academy of Sciences; 2004.
- Pak TR, Chung WC, Lund TD, Hinds LR, Clay CM, Handa RJ. The androgen metabolite, 5 α -androstane-3 β , 17 β -diol, is a potent modulator of estrogen receptor- β 1-mediated gene transcription in neuronal cells. *Endocrinology.* 2005; 146:147–155. [PubMed: 15471969]
- Papadimitriou A, Priftis KN. Regulation of the hypothalamic-pituitary-adrenal axis. *Neuroimmunomodulation.* 2009; 16:265–271. [PubMed: 19571587]
- Pariante CM, Miller AH. Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. *Biol Psychiatry.* 2001; 49:391–404. [PubMed: 11274650]
- Parker GB, Brotchie HL. From diathesis to dimorphism: the biology of gender differences in depression. *J Nerv Ment Dis.* 2004; 192:210–216. [PubMed: 15091302]
- Pego JM, Sousa JC, Almeida OF, Sousa N. Stress and the neuroendocrinology of anxiety disorders. *Curr Top Behav Neurosci.* 2010; 2:97–117. [PubMed: 21309108]

- Pfaff, DW. *Man and Woman: An inside story*. Oxford University Press; 2010.
- Pfaff DW, Pfaffmann C. Olfactory and hormonal influences on the basal forebrain of the male rat. *Brain Res*. 1969; 15:137–156. [PubMed: 5807760]
- Phoenix CH, Goy RW, Gerall AA, Young WC. Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. *Endocrinology*. 1959; 65:369–382. [PubMed: 14432658]
- Pluciennik F, Verrecchia F, Bastide B, Herve JC, Joffre M, Deleze J. Reversible interruption of gap junctional communication by testosterone propionate in cultured Sertoli cells and cardiac myocytes. *J Membr Biol*. 1996; 149:169–177. [PubMed: 8801349]
- Pope HG Jr, Cohane GH, Kanayama G, Siegel AJ, Hudson JI. Testosterone gel supplementation for men with refractory depression: a randomized, placebo-controlled trial. *The American journal of psychiatry*. 2003; 160:105–111. [PubMed: 12505808]
- Porsolt RD. Animal models of depression: utility for transgenic research. *Reviews in the neurosciences*. 2000; 11:53–58. [PubMed: 10716655]
- Primus RJ, Kellogg CK. Developmental influence of gonadal function on the anxiolytic effect of diazepam on environment-related social interaction in the male rat. *Behav Pharmacol*. 1990; 1:437–446. [PubMed: 11175428]
- Qi X, Lin W, Li J, Li H, Wang W, Wang D, Sun M. Fluoxetine increases the activity of the ERK-CREB signal system and alleviates the depressive-like behavior in rats exposed to chronic forced swim stress. *Neurobiology of disease*. 2008; 31:278–285. [PubMed: 18586506]
- Rabkin JG, Wagner GJ, Rabkin R. A double-blind, placebo-controlled trial of testosterone therapy for HIV-positive men with hypogonadal symptoms. *Arch Gen Psychiatry*. 2000; 57:141–147. discussion 155–146. [PubMed: 10665616]
- Rahman F, Christian HC. Non-classical actions of testosterone: an update. *Trends Endocrinol Metab*. 2007; 18:371–378. [PubMed: 17997105]
- Rauch SL, Shin LM, Wright CI. Neuroimaging studies of amygdala function in anxiety disorders. *Annals of the New York Academy of Sciences*. 2003; 985:389–410. [PubMed: 12724173]
- Reddy DS, Jian K. The testosterone-derived neurosteroid androstane diol is a positive allosteric modulator of GABAA receptors. *The Journal of pharmacology and experimental therapeutics*. 2010; 334:1031–1041. [PubMed: 20551294]
- Remage-Healey L, Maidment NT, Schlinger BA. Forebrain steroid levels fluctuate rapidly during social interactions. *Nat Neurosci*. 2008; 11:1327–1334. [PubMed: 18820691]
- Ressler KJ. Amygdala activity, fear, and anxiety: modulation by stress. *Biol Psychiatry*. 2010; 67:1117–1119. [PubMed: 20525501]
- Reul JM, Holsboer F. Corticotropin-releasing factor receptors 1 and 2 in anxiety and depression. *Curr Opin Pharmacol*. 2002a; 2:23–33. [PubMed: 11786305]
- Reul JM, Holsboer F. On the role of corticotropin-releasing hormone receptors in anxiety and depression. *Dialogues Clin Neurosci*. 2002b; 4:31–46. [PubMed: 22033745]
- Robichaud M, Debonnel G. Oestrogen and testosterone modulate the firing activity of dorsal raphe nucleus serotonergic neurones in both male and female rats. *J Neuroendocrinol*. 2005; 17:179–185. [PubMed: 15796770]
- Rodrigues SM, LeDoux JE, Sapolsky RM. The influence of stress hormones on fear circuitry. *Annual review of neuroscience*. 2009; 32:289–313.
- Rohr UD. The impact of testosterone imbalance on depression and women's health. *Maturitas*. 2002; 41(Suppl 1):S25–46. [PubMed: 11955793]
- Roselli CE. Sex differences in androgen receptors and aromatase activity in microdissected regions of the rat brain. *Endocrinology*. 1991; 128:1310–1316. [PubMed: 1999152]
- Rupprecht R. Neuroactive steroids: mechanisms of action and neuropsychopharmacological properties. *Psychoneuroendocrinology*. 2003; 28:139–168. [PubMed: 12510009]
- Rupprecht R, di Michele F, Hermann B, Strohle A, Lancel M, Romeo E, Holsboer F. Neuroactive steroids: molecular mechanisms of action and implications for neuropsychopharmacology. *Brain Res Brain Res Rev*. 2001; 37:59–67. [PubMed: 11744074]

- Sakuma Y. Gonadal Steroid Action and Brain Sex Differentiation in the Rat. *J Neuroendocrinol.* 2009; 21:410–414. [PubMed: 19226349]
- Sapolsky RM. Stress hormones: good and bad. *Neurobiology of disease.* 2000; 7:540–542. [PubMed: 11042072]
- Sapolsky, RM. *Why zebras don't get ulcers*/Robert M. Sapolsky. New York: Times Books; 2004.
- Sato SM, Wersinger SR, Hull EM. The effects of nitric oxide-cGMP pathway stimulation on dopamine in the medial preoptic area and copulation in DHT-treated castrated male rats. *Hormones and behavior.* 2007; 52:177–182. [PubMed: 17467707]
- Schafe GE, Atkins CM, Swank MW, Bauer EP, Sweatt JD, LeDoux JE. Activation of ERK/MAP kinase in the amygdala is required for memory consolidation of pavlovian fear conditioning. *J Neurosci.* 2000; 20:8177–8187. [PubMed: 11050141]
- Schuchard M, Landers JP, Sandhu NP, Spelsberg TC. Steroid hormone regulation of nuclear proto-oncogenes. *Endocr Rev.* 1993; 14:659–669. [PubMed: 8119231]
- Schule C, Eser D, Baghai TC, Nothdurfter C, Kessler JS, Rupprecht R. Neuroactive steroids in affective disorders: target for novel antidepressant or anxiolytic drugs? *Neuroscience.* 2011; 191:55–77. [PubMed: 21439354]
- Schulz KM, Molenda-Figueira HA, Sisk CL. Back to the future: The organizational-activational hypothesis adapted to puberty and adolescence. *Hormones and behavior.* 2009; 55:597–604. [PubMed: 19446076]
- Schulz KM, Richardson HN, Zehr JL, Osetek AJ, Menard TA, Sisk CL. Gonadal hormones masculinize and defeminize reproductive behaviors during puberty in the male Syrian hamster. *Horm Behav.* 2004; 45:242–249. [PubMed: 15053940]
- Schulz KM, Sisk CL. Pubertal hormones, the adolescent brain, and the maturation of social behaviors: Lessons from the Syrian hamster. *Mol Cell Endocrinol.* 2006; 254–255:120–126.
- Schuurman T. Hormonal correlates of agonistic behavior in adult male rats. *Progress in brain research.* 1980; 53:415–420. [PubMed: 7192876]
- Seedat S, Scott KM, Angermeyer MC, Berglund P, Bromet EJ, Brugha TS, Demyttenaere K, de Girolamo G, Haro JM, Jin R, Karam EG, Kovess-Masfety V, Levinson D, Medina Mora ME, Ono Y, Ormel J, Pennell BE, Posada-Villa J, Sampson NA, Williams D, Kessler RC. Cross-national associations between gender and mental disorders in the World Health Organization World Mental Health Surveys. *Arch Gen Psychiatry.* 2009; 66:785–795. [PubMed: 19581570]
- Segal M, Murphy DD. CREB activation mediates plasticity in cultured hippocampal neurons. *Neural plasticity.* 1998; 6:1–7. [PubMed: 9920677]
- Seidman SN, Spatz E, Rizzo C, Roose SP. Testosterone replacement therapy for hypogonadal men with major depressive disorder: a randomized, placebo-controlled clinical trial. *J Clin Psychiatry.* 2001; 62:406–412. [PubMed: 11465516]
- Semaan SJ, Kauffman AS. Sexual differentiation and development of forebrain reproductive circuits. *Curr Opin Neurobiol.* 2010; 20:424–431. [PubMed: 20471241]
- Seney ML, Walsh C, Stolakis R, Sibille E. Neonatal testosterone partially organizes sex differences in stress-induced emotionality in mice. *Neurobiology of disease.* 2012; 46:486–496. [PubMed: 22394611]
- Setalo G Jr, Singh M, Nethrapalli IS, Toran-Allerand CD. Protein kinase C activity is necessary for estrogen-induced Erk phosphorylation in neocortical explants. *Neurochem Res.* 2005; 30:779–790. [PubMed: 16187213]
- Shen CP, Tsimberg Y, Salvatore C, Meller E. Activation of Erk and JNK MAPK pathways by acute swim stress in rat brain regions. *BMC Neurosci.* 2004; 5:36. [PubMed: 15380027]
- Shifren JL, Braunstein GD, Simon JA, Casson PR, Buster JE, Redmond GP, Burki RE, Ginsburg ES, Rosen RC, Leiblum SR, Caramelli KE, Mazer NA. Transdermal testosterone treatment in women with impaired sexual function after oophorectomy. *N Engl J Med.* 2000; 343:682–688. [PubMed: 10974131]
- Shin LM, Liberzon I. The neurocircuitry of fear, stress, and anxiety disorders. *Neuropsychopharmacology.* 2010; 35:169–191. [PubMed: 19625997]
- Shin LM, Rauch SL, Pitman RK. Amygdala, medial prefrontal cortex, and hippocampal function in PTSD. *Annals of the New York Academy of Sciences.* 2006; 1071:67–79. [PubMed: 16891563]

- Shores MM, Sloan KL, Matsumoto AM, Mocerri VM, Felker B, Kivlahan DR. Increased incidence of diagnosed depressive illness in hypogonadal older men. *Arch Gen Psychiatry*. 2004; 61:162–167. [PubMed: 14757592]
- Shrenker P, Maxson SC, Ginsburg BE. The role of postnatal testosterone in the development of sexually dimorphic behaviors in DBA/1B mice. *Physiol Behav*. 1985; 35:757–762. [PubMed: 4080838]
- Shughrue PJ, Lane MV, Merchenthaler I. Comparative distribution of estrogen receptor- α and - β mRNA in the rat central nervous system. *J Comp Neurol*. 1997; 388:507–525. [PubMed: 9388012]
- Sierralta WD, Kohen P, Castro O, Munoz A, Strauss JF 3rd, Devoto L. Ultrastructural and biochemical evidence for the presence of mature steroidogenic acute regulatory protein (StAR) in the cytoplasm of human luteal cells. *Mol Cell Endocrinol*. 2005; 242:103–110. [PubMed: 16162390]
- Simerly RB. Wired for reproduction: organization and development of sexually dimorphic circuits in the mammalian forebrain. *Annual review of neuroscience*. 2002; 25:507–536.
- Simerly RB, Chang C, Muramatsu M, Swanson LW. Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. *J Comp Neurol*. 1990; 294:76–95. [PubMed: 2324335]
- Singewald N. Altered brain activity processing in high-anxiety rodents revealed by challenge paradigms and functional mapping. *Neurosci Biobehav Rev*. 2007; 31:18–40. [PubMed: 16620984]
- Slob AK, Bogers H, van Stolk MA. Effects of gonadectomy and exogenous gonadal steroids on sex differences in open field behaviour of adult rats. *Behav Brain Res*. 1981; 2:347–362. [PubMed: 7225223]
- Solomon MB, Herman JP. Sex differences in psychopathology: of gonads, adrenals and mental illness. *Physiology & behavior*. 2009; 97:250–258. [PubMed: 19275906]
- Solomon MB, Karom MC, Norvelle A, Markham CA, Erwin WD, Huhman KL. Gonadal hormones modulate the display of conditioned defeat in male Syrian hamsters. *Hormones and behavior*. 2009; 56:423–428. [PubMed: 19651128]
- Song RX, McPherson RA, Adam L, Bao Y, Shupnik M, Kumar R, Santen RJ. Linkage of rapid estrogen action to MAPK activation by ER α -Shc association and Shc pathway activation. *Mol Endocrinol*. 2002; 16:116–127. [PubMed: 11773443]
- Spiteri T, Musatov S, Ogawa S, Ribeiro A, Pfaff DW, Agmo A. The role of the estrogen receptor α in the medial amygdala and ventromedial nucleus of the hypothalamus in social recognition, anxiety and aggression. *Behav Brain Res*. 2010; 210:211–220. [PubMed: 20184922]
- Spritzer MD, Galea LA. Testosterone and dihydrotestosterone, but not estradiol, enhance survival of new hippocampal neurons in adult male rats. *Dev Neurobiol*. 2007; 67:1321–1333. [PubMed: 17638384]
- Spritzer MD, Ibler E, Inglis W, Curtis MG. Testosterone and social isolation influence adult neurogenesis in the dentate gyrus of male rats. *Neuroscience*. 2011; 195:180–190. [PubMed: 21875652]
- Surampudi PN, Wang C, Swerdloff R. Hypogonadism in the aging male diagnosis, potential benefits, and risks of testosterone replacement therapy. *Int J Endocrinol*. 2012; 2012:625434. [PubMed: 22505891]
- Swaab DF. Sexual differentiation of the brain and behavior. *Best Pract Res Clin Endocrinol Metab*. 2007; 21:431–444. [PubMed: 17875490]
- Sweatt JD. The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. *J Neurochem*. 2001; 76:1–10. [PubMed: 11145972]
- Toufexis DJ, Myers KM, Davis M. The effect of gonadal hormones and gender on anxiety and emotional learning. *Hormones and behavior*. 2006; 50:539–549. [PubMed: 16904674]
- Traish AM, Kang HP, Saad F, Guay AT. Dehydroepiandrosterone (DHEA)—a precursor steroid or an active hormone in human physiology. *J Sex Med*. 2011; 8:2960–2982. quiz 2983. [PubMed: 22032408]

- Tremere LA, Jeong JK, Pinaud R. Estradiol shapes auditory processing in the adult brain by regulating inhibitory transmission and plasticity-associated gene expression. *J Neurosci*. 2009; 29:5949–5963. [PubMed: 19420261]
- Vamvakopoulos NC, Chrousos GP. Evidence of direct estrogenic regulation of human corticotropin-releasing hormone gene expression. Potential implications for the sexual dimorphism of the stress response and immune/inflammatory reaction. *J Clin Invest*. 1993; 92:1896–1902. [PubMed: 8408641]
- van Wingen GA, Zylicz SA, Pieters S, Mattern C, Verkes RJ, Buitelaar JK, Fernandez G. Testosterone increases amygdala reactivity in middle-aged women to a young adulthood level. *Neuropsychopharmacology*. 2009; 34:539–547. [PubMed: 18235425]
- Varghese FP, Brown ES. The Hypothalamic-Pituitary-Adrenal Axis in Major Depressive Disorder: A Brief Primer for Primary Care Physicians. *Prim Care Companion J Clin Psychiatry*. 2001; 3:151–155. [PubMed: 15014598]
- Vasudevan N, Pfaff DW. Non-genomic actions of estrogens and their interaction with genomic actions in the brain. *Frontiers in neuroendocrinology*. 2008; 29:238–257. [PubMed: 18083219]
- Viau V. Functional cross-talk between the hypothalamic-pituitary-gonadal and -adrenal axes. *J Neuroendocrinol*. 2002; 14:506–513. [PubMed: 12047726]
- Viau V, Lee P, Sampson J, Wu J. A testicular influence on restraint-induced activation of medial parvocellular neurons in the paraventricular nucleus in the male rat. *Endocrinology*. 2003; 144:3067–3075. [PubMed: 12810563]
- Viau V, Meaney MJ. Variations in the hypothalamic-pituitary-adrenal response to stress during the estrous cycle in the rat. *Endocrinology*. 1991; 129:2503–2511. [PubMed: 1657578]
- Viau V, Meaney MJ. The inhibitory effect of testosterone on hypothalamic-pituitary-adrenal responses to stress is mediated by the medial preoptic area. *The Journal of neuroscience: the official journal of the Society for Neuroscience*. 1996; 16:1866–1876. [PubMed: 8774455]
- Viau V, Meaney MJ. Testosterone-dependent variations in plasma and intrapituitary corticosteroid binding globulin and stress hypothalamic-pituitary-adrenal activity in the male rat. *J Endocrinol*. 2004; 181:223–231. [PubMed: 15128271]
- Wainwright SR, Lieblich SE, Galea LAM. Hypogonadism predisposes males to the development of behavioural and neuroplastic depressive phenotypes. *Psychoneuroendocrinology*. 2011; 36:1327–1341. [PubMed: 21481538]
- Walf AA, Frye CA. Gestational or acute restraint in adulthood reduces levels of 5alpha-reduced testosterone metabolites in the hippocampus and produces behavioral inhibition of adult male rats. *Front Cell Neurosci*. 2012; 6 Epub Date 12/25/2012.
- Walf AA, Frye CA. Administration of estrogen receptor beta-specific selective estrogen receptor modulators to the hippocampus decrease anxiety and depressive behavior of ovariectomized rats. *Pharmacology, biochemistry, and behavior*. 2007; 86:407–414.
- Walf AA, Sumida K, Frye CA. Inhibiting 5alpha-reductase in the amygdala attenuates antianxiety and antidepressive behavior of naturally receptive and hormone-primed ovariectomized rats. *Psychopharmacology*. 2006; 186:302–311. [PubMed: 16220340]
- Walker DL, Toufexis DJ, Davis M. Role of the bed nucleus of the stria terminalis versus the amygdala in fear, stress, and anxiety. *European journal of pharmacology*. 2003; 463:199–216. [PubMed: 12600711]
- Wang C, Alexander G, Berman N, Salehian B, Davidson T, McDonald V, Steiner B, Hull L, Callegari C, Swerdloff RS. Testosterone replacement therapy improves mood in hypogonadal men—a clinical research center study. *The Journal of clinical endocrinology and metabolism*. 1996; 81:3578–3583. [PubMed: 8855804]
- Wehling M. Specific, nongenomic actions of steroid hormones. *Annu Rev Physiol*. 1997; 59:365–393. [PubMed: 9074769]
- Weiser MJ, Goel N, Sandau US, Bale TL, Handa RJ. Androgen regulation of corticotropin-releasing hormone receptor 2 (CRHR2) mRNA expression and receptor binding in the rat brain. *Experimental neurology*. 2008; 214:62–68. [PubMed: 18706413]

- Westenbroek C, Den Boer JA, Veenhuis M, Ter Horst GJ. Chronic stress and social housing differentially affect neurogenesis in male and female rats. *Brain Res Bull.* 2004; 64:303–308. [PubMed: 15561464]
- Wierman ME. Sex steroid effects at target tissues: mechanisms of action. *Adv Physiol Educ.* 2007; 31:26–33. [PubMed: 17327579]
- Williams CL. A reevaluation of the concept of separable periods of organizational and activational actions of estrogens in development of brain and behavior. *Annals of the New York Academy of Sciences.* 1986; 474:282–292. [PubMed: 3555228]
- Williamson M, Bingham B, Gray M, Innala L, Viau V. The medial preoptic nucleus integrates the central influences of testosterone on the paraventricular nucleus of the hypothalamus and its extended circuitries. *The Journal of neuroscience: the official journal of the Society for Neuroscience.* 2010; 30:11762–11770. [PubMed: 20810896]
- Wu TW, Wang JM, Chen S, Brinton RD. 17Beta-estradiol induced Ca²⁺ influx via L-type calcium channels activates the Src/ERK/cyclic-AMP response element binding protein signal pathway and BCL-2 expression in rat hippocampal neurons: a potential initiation mechanism for estrogen-induced neuroprotection. *Neuroscience.* 2005; 135:59–72. [PubMed: 16084662]
- Yamada Y. Effects of testosterone on unit activity in rat hypothalamus and septum. *Brain Res.* 1979; 172:165–168. [PubMed: 466460]
- Zarrouf FA, Artz S, Griffith J, Sirbu C, Kommor M. Testosterone and depression: systematic review and meta-analysis. *J Psychiatr Pract.* 2009; 15:289–305. [PubMed: 19625884]
- Zhang JM, Tonelli L, Regenold WT, McCarthy MM. Effects of neonatal flutamide treatment on hippocampal neurogenesis and synaptogenesis correlate with depression-like behaviors in preadolescent male rats. *Neuroscience.* 2010; 169:544–554. [PubMed: 20399256]
- Zhou L, Blaustein JD, De Vries GJ. Distribution of androgen receptor immunoreactivity in vasopressin- and oxytocin-immunoreactive neurons in the male rat brain. *Endocrinology.* 1994; 134:2622–2627. [PubMed: 8194487]
- Zuloaga DG, Jordan CL, Breedlove SM. The organizational role of testicular hormones and the androgen receptor in anxiety-related behaviors and sensorimotor gating in rats. *Endocrinology.* 2011a; 152:1572–1581. [PubMed: 21325044]
- Zuloaga DG, Morris JA, Jordan CL, Breedlove SM. Mice with the testicular feminization mutation demonstrate a role for androgen receptors in the regulation of anxiety-related behaviors and the hypothalamic-pituitary-adrenal axis. *Hormones and behavior.* 2008; 54:758–766. [PubMed: 18775430]
- Zuloaga DG, Poort JE, Jordan CL, Breedlove SM. Male rats with the testicular feminization mutation of the androgen receptor display elevated anxiety-related behavior and corticosterone response to mild stress. *Hormones and behavior.* 2011b; 60:380–388. [PubMed: 21801726]

Highlights

- Women are twice as likely as men to suffer from anxiety and depressive disorders.
- Women's mood disturbances are more frequent during times of hormonal flux.
- Testosterone has anxiolytic and antidepressant effects in women, men, and animals.
- Its organizational and/or activational effects may mediate such benefits.
- The hippocampal MAPK pathway may mediate Testosterone's protective effects.

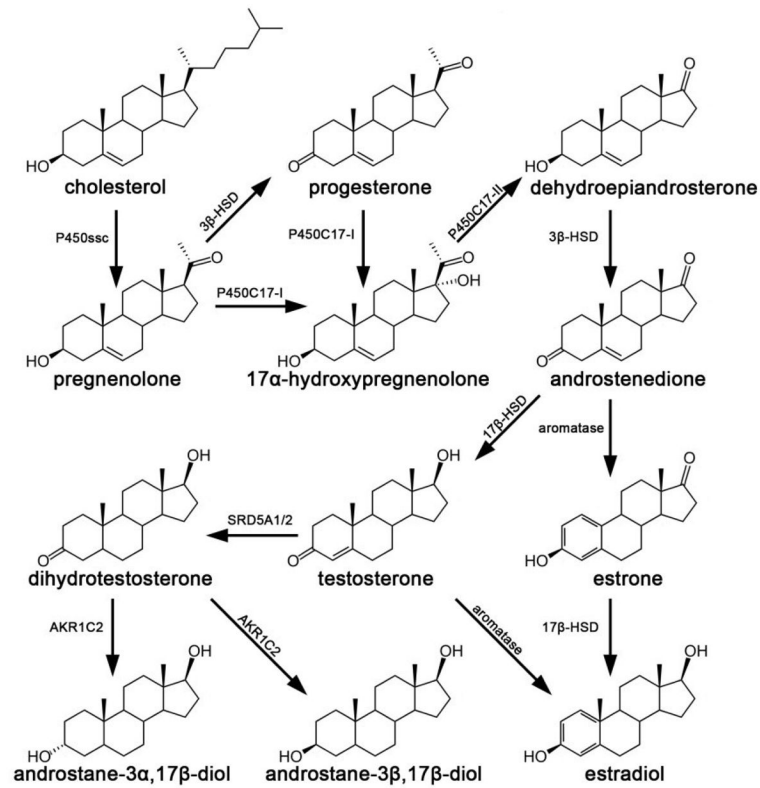


Figure 1. Steroidogenesis

Cholesterol is the precursor of all steroid hormones. The synthesis of testosterone involves a series of enzymatic steps and can occur through a number of disparate routes. Testosterone can then be aromatized to estradiol or reduced to dihydrotestosterone by the aromatase 5 α -reductase.

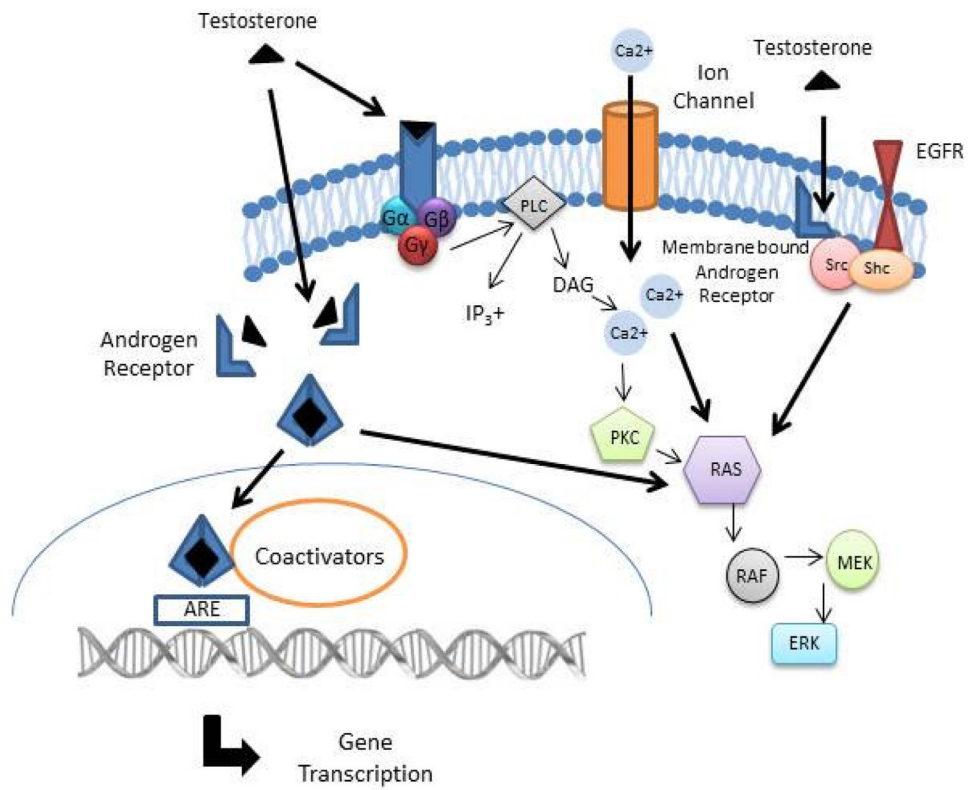


Figure 2. Testosterone's genomic and nongenomic effects

Testosterone exerts slower genomic actions by diffusing through the plasma membrane and binding with intracellular androgen receptors to form complexes. These complexes can then homodimerize and translocate to the nucleus and act as transcription factors at androgen response element (ARE) DNA sequences to enhance or repress transcription. Testosterone can also exert a number of rapid nongenomic effects through actions at membrane bound receptors (shown as examples, G protein coupled receptor and epidermal growth factor receptor). Rapid effects of androgens can induce a number of intracellular events such as stimulation of the MAPK-ERK pathway.

Table 1

Summary of some studies that examined anxiety and depression in human and animal models.

Study	Sex/Species	Age	Depression	Anxiety
Miner et al, Postgraduate Med, 2013	Hypogonadal Men	52.1 ± 12.3 yrs	Symptoms improved with 3 moths of T therapy	Not investigated
Giltay et al, Journal of Psychosomatic Research, 2012	Men	44.9 ± 12.6 yrs	No change in salivary T levels	No change in salivary T levels
	Women	42.8 ± 13.2 yrs	Decreased salivary T levels	Decreased salivary T levels
Aydoğan et al, Endocrine Journal, 2012	Men CHH	21 ± 2.04 yrs	Symptoms improved with T therapy	No improvement with T therapy
	Men controls	23 ± 2.47 yrs	N/A	N/A
Granger et al, Development and Psychopathology, 2003	Men	adolescent	Low salivary T associated with increased symptoms	Low salivary T associated with increased symptoms
	Women	adolescent	Low salivary T associated with increased symptoms	Low salivary T associated with increased symptoms
Carbone et al, Hormones and Behavior, 2013	Male rats	perinatal/adult	Not investigated	DEHP increased in adults; reversed by T
	Female rats	perinatal/adult	Not investigated	DEHP had no effect
Carrier and Kabbaj, Biological Psychiatry, 2012	Male rats	adult	removing T has depressive-like effects	Not investigated
Hodosy et al, Pharmacology, Biochemistry, and Behavior, 2012	Male rats	12 weeks	N/A	Flutamide blocked T anxiolytic effects
Walf and Frye, Front. Cell Neurosci, 2012	Male rats	Adult	Gestational stress increased symptoms	Gestational stress increased symptoms
Seney et al, Neurobiology of Disease, 2012	Female mice	neonatal/adult	neonatal T treatment partially decreased vulnerability to stress in adults	neonatal T treatment partially decreased vulnerability to stress in adults
Carrier and Kabbaj, Hormones and Behavior, 2012	Male rats	adult	T replacement in castrated animals decreased symptoms	T replacement in castrated animals decreased symptoms
	Female rats	adult	T replacement in castrated animals had no effect	T replacement in castrated animals had no effect