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Neurosteroid, GABAergic and Hypothalamic Pituitary Adrenal (HPA) Axis Regulation: What is the Current State of Knowledge in Humans?

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Abstract

Rationale—A robust epidemiological literature suggests an association between chronic stress and the development of affective disorders. However, the precise biological underpinnings of this relationship remain elusive. Central to the human response and adaptation to stress, activation and inhibition of the hypothalamic pituitary adrenal (HPA) axis involves a multi-level, multi-system, neurobiological stress response which is as comprehensive in its complexity as it is precarious. Dysregulation in this complex system has implications for human stress related illness.

Objectives—The pioneering research of Robert Purdy and colleagues has laid the groundwork for advancing our understanding of HPA-axis regulation by stress-derived steroid hormones and their neuroactive metabolites (termed neurosteroids), which are potent allosteric modulators of GABA_A receptor function in the central nervous system. This review will describe what is known about neurosteroid modulation of the HPA-axis in response to both acute and chronic stress, particularly with respect to the current state of our knowledge of this process in humans.

Results—Implications of this research to the development of human stress related illness are discussed in the context of two human stress-related psychiatric disorders - major depressive disorder and premenstrual dysphoric disorder.

Conclusions—Neurosteroid-mediated HPA-axis dysregulation is a potential pathophysiologic mechanism which may cross traditional psychiatric diagnostic classifications. Future research directions are identified.

Keywords

allopregnanolone; allotetrahydrodeoxycorticosterone; cortisol; depression; DHEA; GABA; HPA-axis; neurosteroids; premenstrual dysphoric disorder; stress

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Introduction

In industrialized societies, it is estimated that stress contributes to a \$150 billion dollar loss of revenue each year due to diminished productivity, absenteeism, stress-related mental illness, and substance abuse (Kalia 2002). However, the precise mechanisms by which stress “gets under the skin” to promote illness are still under investigation. Both experimental and epidemiological evidence suggest that dysregulation within integrated neuroendocrine, neurotransmitter and central networks governing the physiologic response to stress plays a role (Bifulco et al. 1998; Binder and Nemeroff 2010; Kendler et al. 1999).

Centrally important to the human response and adaptation to stress are the actions of the hypothalamic pituitary adrenal (HPA) axis (McEwen and Getz 2013). The pioneering research of Robert Purdy and colleagues has laid the groundwork for advancing our understanding of HPA-axis regulation by stress-derived steroid hormones and their neuroactive metabolites (termed neurosteroids). What is clear from animal studies is that the molecular underpinnings of neurosteroid regulation of the HPA-axis activity are complex and context-specific. Behavioral, hormonal, developmental, and environmental events play crucial and determinant roles in shaping physiological mechanisms underlying neurosteroid regulation of the HPA-axis.

The concept of allostatic load provides a framework by which to understand how regulation of the HPA-axis in response to stress may contribute to disease development. Allostasis is the process of achieving stability through change and, consequently, the process by which homeostasis in physiologic systems is maintained (Sterling and Eyer 1988). The body responds to stress by setting into motion a coordinated physiologic response involving hormonal and neurotransmitter mediators that determine physiological responses of cells and tissues in order to meet the organism’s metabolic needs (McEwen 2008). Glucocorticoids, the primary end products of the HPA axis, represent classic examples of allostatic mediators whose effects involve modulation of the HPA-axis to return the system to homeostasis following stress.

In the context of adaptation, activation of stress-responsive systems to an acute threat is appropriate. However, chronic activation of these systems, or failure to properly curtail activation following threat, while representing an ‘adaptation’ to environmental or historical context, may contribute to long-term physical or mental sequelae associated with stress. As stress responsive mediators are chronically mobilized to meet the homeostatic demands of stress (McEwen 2007), this causes some measure of wear on physiologic systems and this wear has been termed “allostatic load.” Allostatic load can manifest as: (1) repeated elevations of stress mediators (e.g., cortisol) over long periods; (2) a failure to adapt to a stressor; (3) a failure to shut off the normal stress response; or (4) an inadequate response to stress that may allow other systems that are normally counter-regulated to become overactive (McEwen and Seaman 1999).

We propose that the 3α , 5α – reduced metabolites of progesterone and deoxycorticosterone, allopregnanolone (ALLO) and allotetrahydrodeoxycorticosterone (ALLO-THDOC), respectively, represent allostatic mechanisms in the context of adaptation to stress by

limiting the extent and duration of reduction of Gamma-Aminobutyric Acid (GABA)ergic inhibitory transmission and activation of the HPA-axis. In addition, dehydroepiandrosterone (DHEA), an androgenic hormone and precursor to androstenedione, along with its sulfate ester DHEA-S, which together comprise the most abundant steroid hormone(s) in the human body (Maninger et al. 2009), are associated with GABA_A receptor (GABA_AR) interactions and also exert profound antiglucocorticoid effects (Labrie et al. 2005; Maninger et al. 2009). In this way, DHEA may represent additional neurosteroid mechanisms in humans that are of relevance to the regulation of the HPA-axis response to stress. An overarching conceptual model guiding this review, therefore, is that there are both short-term adaptive actions in neurosteroid regulation of the HPA-axis (allostasis) that are protective, and long-term effects that can be damaging (allostatic load; McEwen 2007).

This review will describe what is known about neurosteroid modulation of the HPA-axis in response to stress in humans, drawing from animal research when relevant. We will describe the available evidence for alterations in neurosteroids and the HPA-axis in two forms of human stress-related illness: major depressive disorder and premenstrual dysphoric disorder. We conclude by proposing that neurosteroid-HPA-axis regulation is a fundamental pathophysiologic mechanism cutting across psychiatric diagnoses, and we will suggest future avenues of research.

Neurosteroid and GABAergic Regulation of the HPA-Axis

Background

Activation of the HPA-axis in reaction to, or in anticipation of, a stressor requires a coordinated neuroendocrine response (Herman et al. 2003; Ulrich-Lai and Herman 2009), involving inputs from multiple brain regions, neurotransmitters, and feedback systems [for a review see (Herman 2013)]. In brief, stress induces the release of corticotrophin releasing hormone (CRH) from the paraventricular nucleus (PVN) of the hypothalamus. CRH subsequently stimulates the synthesis and release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary which, in turn, stimulates the synthesis and release of glucocorticoids from the adrenal cortex (Pariante and Lightman 2008). In the context of stress, the most important glucocorticoid in humans is cortisol (Groeneweg et al. 2011). Cortisol plays a critical role in regulating cardiovascular, metabolic, and immunologic changes during stress (Buckingham 2006; Dhabhar 2009), and homeostatic mechanisms exist to limit the extent and duration of cortisol's catabolic actions. The most obvious of these homeostatic mechanisms involves negative feedback regulation by glucocorticoids, via stimulating hippocampal, hypothalamic, and pituitary glucocorticoid receptors to inhibit further secretion of ACTH and CRH, thereby terminating the HPA-axis response to stress (Myers et al. 2012). Additionally, a role of GABA, the most prevalent inhibitory neurotransmitter in the mammalian central nervous system, in regulating the HPA-axis in response to stress is also well established (Cullinan et al. 2008; Decavel and Van den Pol 1990; Jones et al. 1984). Dysregulation in GABAergic transmission is emerging as a prominent theory underlying the etiology and perpetuation of stress-related psychiatric disorders (Luscher et al. 2011). While GABA exerts its central effects through activation of both GABA_A (ligand-gated ion channels) and GABA_B (G protein-coupled) receptors,

current evidence suggests that GABA_A receptors (GABA_AR's) are of greater relevance to stress-induced HPA-axis activation (Brickley and Mody 2012).

ALLO and ALLO-THDOC, the neuroactive steroid derivatives of progesterone, are stress responsive and among the most potent modulators of the GABA_AR's (Morrow et al. 1987). ALLO is synthesized not only in the ovaries and adrenals but also de novo in the brain (Paul and Purdy 1992), while ALLO-THDOC is derived primarily from adrenal sources (Reddy 2006). During stress, ACTH stimulates the conversion of cholesterol to pregnenolone in the adrenal cortex (Simpson and Waterman 1988), which is rapidly converted to progesterone and then to ALLO or ALLO-THDOC by 5 α -reductase and 3 α HSD reductase enzymes. Nonetheless, both ALLO and ALLO-THDOC readily cross into the brain, and the major proportion of CNS concentrations of these neuroactive steroids increased by stress comes from peripheral tissues (Purdy et al. 1991).

Both ALLO and ALLO-THDOC enhance GABAergic transmission by binding to "allosteric" sites (remote from the active site) on the GABA_AR. In so doing, these neuroactive steroids increase the likelihood that GABA will bind to the active site, and thereby potentiate chloride channel opening and membrane currents (Belelli and Lambert 2005; Chisari et al. 2010). It is through this allosteric action that ALLO and ALLO-THDOC exert profound anxiolytic, anti-conflict, and analgesic effects (Bitran et al. 1995; Engin and Treit 2007; Frye and Rhodes 2006; Kavaliers and Wiebe 1987; Pibiri et al. 2006; Pinna et al. 2003), all consistent with an integrated and adaptive response to stress (Purdy et al. 1991). It is also through their modulation of GABA_AR's and alterations in GABAergic neurotransmission that these neuroactive steroids regulate the HPA-axis (Belelli and Lambert 2005; Morrow et al. 1995).

DHEA is also stress responsive, and is co-released with cortisol (Wolf and Kirschbaum 1999) via ACTH stimulation of adrenal DHEA synthesis (Hornsby 1995; Parker and Odell 1980). DHEA is widely considered to have anti-glucocorticoid effects (Browne et al. 1992; Kalimi et al. 1994), though the precise mechanisms underlying these effects are still unclear. Current evidence suggests that the anti-glucocorticoid effects of DHEA, or its metabolites, on cortisol may involve inhibition of the intermediary enzymes involved in the conversion of the inactive cortisol metabolite, cortisone, to cortisol (Hennebert et al. 2007; Maninger et al. 2009).

Whether DHEA/DHEA-S in humans is produced exclusively from the periphery, or may also be synthesized in the brain is still under debate (Maninger et al. 2009). DHEA and DHEA-S are generally considered non-competitive negative allosteric modulators of the GABA_A receptor and positive modulators of NMDA and sigma-1 receptors (Amato et al. 2010; Baulieu 1998; Dubrovsky 2006; Imamura and Prasad 1998; Majewska 1992; Paul and Purdy 1992; Zheng 2009). However, the physiological significance, mechanism of action, and potential implications of DHEA and DHEA-S in human stress-related disease are, to date, not fully understood.

It has been established that complex relationships exist wherein both positive and negative modulators of the GABA_AR have the capacity to modulate network excitability via

GABAergic mechanisms (Majewska 1992; Park-Chung et al. 1999), thereby influencing the regulation of the HPA-axis. However, the relevance of DHEA and DHEA-S to human stress-related disease is complicated by the observed anxiolytic and antidepressant effects of exogenous DHEA administration (Bloch et al. 1999; Genazzani et al. 2003; Maayan et al. 2006; Schmidt et al. 2005; Strous et al. 2003; Wolf and Kirschbaum 1999; Wolkowitz et al. 1999), which appear counterintuitive to the hypothesized antagonistic effects of DHEA at the GABA_A receptor. This apparent paradox may be explained by (1) the capacity of DHEA to influence the release of other GABAergic neurosteroids which are positive modulators of the GABA_AR (Gartside et al. 2010; Genazzani et al. 2003; Majewska 1992); and/or (2) the anti-glucocorticoid properties of DHEA (Browne et al. 1992; Kalimi et al. 1994) which may serve to counteract the adverse effects of prolonged cortisol release (Wolkowitz et al. 2001).

Neurosteroid and GABAergic Regulation of the HPA-Axis: Acute Stress

Research in animal models has provided consistent evidence that stress-induced activation of the HPA-axis increases neurosteroid levels to concentrations which can functionally potentiate the effects of GABA on GABA_AR's (Akk et al. 2007; Purdy et al. 1991). Under the control of ACTH (Reddy 2006), DHEA (Maninger et al. 2010) and pregnenolone (Vallee et al. 2000), as well as their progesterone-derived and androstenedione-derived metabolites (Barbaccia et al. 1997; Paul and Purdy 1992), have been shown to increase in response to acute stress. Studies in rat models have shown that GABAergic transmission is rapidly decreased following acute stress (Barbaccia et al. 1996; Biggio et al. 2007; Sanna et al. 1992). Central nervous system increases in neurosteroids following stress are also rapid, though the time course of peripheral neurosteroid responses to acute stress in rats is delayed, peaking between 30 and 70 minutes as originally demonstrated by Purdy (Barbaccia et al. 1998; Paul and Purdy 1992; Purdy et al. 1991). Peripheral increases in neurosteroid concentrations have been correlated with restoration of GABAergic transmission in addition to anxiolytic actions (Barbaccia et al. 1998). Pretreatment of rats with ALLO, ALLO-THDOC, or progesterone attenuates stress-induced increases in plasma ACTH and cortisol (Owens et al. 1992; Patchev et al. 1996). These results implicate peripheral neurosteroids in curtailing the extent and duration of reduction in GABAergic inhibitory transmission and restoring HPA-axis homeostatic control following stress.

To date, relatively few studies have examined stress-induced ALLO or ALLO-THDOC in humans, and even fewer have examined concurrent indicators of HPA-axis activation. Genazzani and colleagues (1998) were the first to demonstrate, in healthy men and women, that both gonadotropin-releasing hormone (GnRH) and corticotropin releasing factor (CRF) administration increased serum progesterone and ALLO concentrations, with an ALLO time course consistent with that seen in rats, peaking 60 min post-challenge. Subsequent work by this group has yielded a similar time course for both peak plasma ALLO and cortisol responses to CRF challenge in healthy controls, though not in women with hypothalamic amenorrhea who fail to show any increase in ALLO (Meczekalski et al. 2000), or in obese subjects who show an exaggerated ALLO response with an earlier peak (Menozzi et al. 2002). In healthy volunteers, an experimental panic induction procedure using cholecystokinin-tetrapeptide (cck-4) produced an ACTH peak at 10 min following administration, while cortisol and ALLO-THDOC peaked at 20 min (Eser et al. 2005). The

difference in time course of response for ACTH and ALLO-THDOC is consistent with the evidence that the formation of ALLO-THDOC requires availability of deoxycorticosterone from the adrenal cortex, the synthesis of which is under the control of ACTH (Simpson and Waterman 1988). Taken together, the results of these endocrine challenge studies suggest that neurosteroids increase in response to pharmacologically-induced stress in healthy humans with a time course similar to that seen in rodent models.

There have been only a handful of studies which have investigated progesterone-derived neurosteroid regulation of the HPA-axis following acute mental stress in humans, and they have focused almost exclusively on ALLO. Droogleever-Fortuyn and colleagues (2004), using a naturalistic stressor (PhD defense), observed, in a predominantly male sample, that both plasma ALLO and cortisol were significantly increased during the examination relative to concentrations four weeks earlier. In that study, stress-induced ALLO and cortisol concentrations were positively correlated, suggesting a coordinated HPA-axis and neurosteroid response to stress. However, in a study comparing postpartum women with healthy controls in the follicular phase of the menstrual cycle for cortisol and ALLO stress reactivity to the Trier Social Stress Test (TSST), a mental stressor battery that induces substantial HPA-axis activation (Kirschbaum et al. 1993), no effect of acute stress was seen for ALLO reactivity at a single 30 min post-stress time point in either group, though a significant cortisol response was observed (Altemus et al. 2001).

Childs and de Wit (2009) also used the TSST to examine progesterone and ALLO stress reactivity in healthy male smokers and non-smokers. The TSST induced significant increases in progesterone, but no changes in ALLO in either group when measured at 5, 10, 20 and 30 min post-stress. However, a negative association was observed between baseline concentrations of ALLO and the magnitude of the ALLO increase to the TSST, indicating that basal ALLO concentrations influence the degree of ALLO reactivity to acute psychosocial stress in humans. Subsequent work by this group (Childs et al. 2010) found that pretreatment with 50mg of progesterone 3hr prior to the TSST in healthy men attenuated the peak stress induced increase in cortisol, presumably reflecting the inhibitory action of progesterone-derived neurosteroids on the HPA-axis. This assumption is supported by their observations that progesterone also attenuated stress-induced increases in anger and frustration and promoted recovery from negative mood changes after stress. These data provide evidence in humans that progesterone (and possibly its neurosteroid metabolites) may play a role in recovery from stress (Childs et al. 2010).

While the studies summarized above are inconclusive regarding ALLO reactivity to acute psychological stress and HPA-axis modulation in humans, they relied on small samples, most were conducted in primarily or exclusively male samples (with substantially lower concentrations of progesterone-derived neurosteroids), and each used radioimmunoassay (RIA) techniques. While RIAs provide highly sensitive measurements of neuroactive steroids in brain, in serum samples neuroactive steroids are present at much lower concentrations. The use of gas chromatography and mass spectrometry (GC-MS), which allows the simultaneous detection of low amounts of neurosteroids in serum may advance our ability to study neurosteroid stress reactivity in humans as suggested by a prior study in

which GC-MS detected increases in both ALLO and ALLO-THDOC in response to progesterone in women (Porcu et al. 2009).

In addition, the possibility exists that the time course of the ALLO response to psychological stress in humans differs from that in rodent models. This was initially suggested to us by our observations for greater ALLO increases to psychological stress when ALLO was sampled 17 min after stress onset (Girdler et al. 2001) compared to the magnitude of reactivity when sampled 30 and 60 min post-stress onset (Klatzkin et al. 2006b) in healthy female subjects. We investigated this further, albeit in a post-hoc fashion, by analyzing ALLO in serum from 39 healthy premenopausal women tested in the luteal phase of their cycle after an extended rest and again during speech (12 minutes post-stress onset), mental arithmetic (17 minutes post-stress onset), and recovery following the TSST (30 min post-stress onset). As summarized in Figure 1 (unpublished data), although ALLO concentrations at both minute 12 ($F(1, 38) = 13.02, p < .001$) and minute 17 ($F(1, 38) = 5.50, p < .05$) were greater than baseline, ALLO concentrations at minute 12 were greater than those at minute 17 ($F(1, 38) = 4.43, p < .05$). While preliminary, these data suggest the possibility that ALLO reactivity to psychological stress may peak earlier than the time points selected for sampling in prior research summarized above, much of which failed to find significant increases in ALLO in response to the TSST in healthy samples (Altemus et al. 2001; Childs and de Wit 2009; Girdler et al. 2001; Klatzkin et al. 2006b). An alternative, though not mutually exclusive, explanation is that the nature of the stressor may influence the magnitude of neurosteroid responses in humans since speech stress elicits more robust physiologic responses than mental arithmetic (Dickerson and Kemeny 2004).

In addition to time course and stressor characteristics, inter-individual characteristics would be expected to influence the magnitude of neurosteroid reactivity to mental stress in humans. A prior study of ours illustrates this point (Girdler et al. 2006). In a sample of 85 healthy men and premenopausal women, half of each gender group was African American (AA) and the other half was non-Hispanic White (nHW), initial results revealed that in the entire sample, there was no significant change in ALLO in response to the TSST. However, about half the sample showed a significant stress-induced increase and half, a stress-induced decrease in ALLO. In women, the AA group had lower circulating ALLO concentrations, in general relative to nHW women, and were more likely to exhibit a stress-induced decrease in ALLO, while nHW women were more likely to exhibit a stress-induced increase in ALLO. Ethnicity did not predict ALLO reactivity in men. However, in the entire sample, both AA and nHW groups showed the expected negative correlation relating post-stress ALLO concentrations to post-stress cortisol.

To the extent that lower ALLO concentrations, especially following stress, are associated with diminished capacity to negatively modulate the HPA-axis and facilitate its recovery following stress exposure (Guo et al. 1995; Patchev and Almeida 1996), then the blunted ALLO stress response that was observed in AA women may be one mechanism increasing their vulnerability to stress and stress-related illness (Geronimus et al. 2006; Williams et al. 1997). Although the origin of the ALLO profile observed in AA women is unknown, it may reflect the greater chronic psychosocial stress experienced by AA women (Perry et al. 2012; Perry et al. 2013). While speculative at present, this hypothesis is consistent with an

allostatic load model of blunted stress reactivity resulting from chronic stress, and data from animal models of chronic stress and diminished neurosteroid availability/synthesis (see below).

Acute psychosocial stress has also been shown to substantially increase DHEA and DHEA-S concentrations in humans (by as much as 60 – 75% above baseline concentrations) (Izawa et al. 2008; Lennartsson et al. 2012; Marceau et al. 2012), and most studies suggest a coordinated response with that of cortisol. For example, in 36 female university students, both DHEA and cortisol were significantly elevated and positively correlated 10 minutes after the cessation of a speech and mental arithmetic stressor (Pico-Alfonso et al. 2007). In studies using the standardized TSST, salivary DHEA has been shown to peak about 10 minutes before that of cortisol (Izawa et al. 2008; Lennartsson et al. 2012; Shirotaki et al. 2009), and to correlate positively with the magnitude of the cortisol and ACTH response (Izawa et al. 2008; Lennartsson et al. 2012). DHEA-S reactivity to the TSST, on the other hand, may peak later than DHEA, and does not appear to correlate with HPA-axis reactivity (Lennartsson et al. 2012). Few studies have examined gender differences in DHEA or DHEA-S reactivity to acute stress. In a study of adolescents exposed to venipuncture stress, no gender differences existed in serum DHEA or DHEA-S reactivity, per se, but only for boys did DHEA stress reactivity correlate with cortisol reactivity (Marceau et al. 2012). In a study of adults (Lennartsson et al. 2012), no gender differences existed in the concentrations of DHEA or in DHEA stress reactivity to the TSST, but DHEA-S concentrations were significantly higher in men than women, though the comparable response pattern to the TSST was observed in both genders. The coordinated release of DHEA with cortisol in response to acute stress is thought to protect against the potentially damaging effects of excessive cortisol activity (Maninger et al. 2009), and also predicts less negative affect in response to stress (Izawa et al. 2008).

Neurosteroid and GABAergic Regulation of the HPA-Axis: Chronic Stress

Importantly, historical context plays a determinant role in the response of neuroendocrine stress mediators to challenge. Chronic social isolation in rats is associated with lower brain and plasma neuroactive steroid concentrations including ALLO and ALLO-THDOC (Dong et al. 2001; Guidotti et al. 2001; Serra et al. 2000; Serra et al. 2004). Findings that the expression of 5 α -reductase, the rate-limiting enzyme in the conversion of progesterone to ALLO, is downregulated following chronic social isolation (Agis-Balboa et al. 2007; Reddy 2006) indicates the synthesis of ALLO and ALLO-THDOC may be diminished in the context of chronic stress. The observed reductions in endogenous brain and plasma ALLO concentrations in socially isolated rats has been associated with reduced feedback sensitivity of the HPA-axis to dexamethasone (DEX) challenge (Evans et al. 2012; Serra et al. 2005), suggesting that diminished neurosteroid availability may impair the normal negative feedback regulation of the HPA-axis and, thus, contribute to elevated HPA-axis activation. Since stress-induced increases in ACTH are involved in the biosynthesis of neurosteroids of adrenal origin, chronic HPA-axis activation could contribute to reduced ALLO and ALLO-THDOC in socially-isolated animals via a chronic “wear and tear” on adrenal functioning, eventually leading to a state of adrenal “exhaustion” and reduced neurosteroid synthesis – a prototypical expression of allostatic load.

Studies measuring both ALLO and HPA-axis activity in chronically stressed human samples are currently nonexistent. However, one recent study investigated chronic work-related stress and burnout and sensitivity of saccadic eye velocity (SEV) to exogenously administered ALLO and flumazenil, a GABA_AR antagonist (Bäckström et al. 2013). Reductions in SEV are a measure of sedation strictly controlled by GABAergic transmission. Those with work related burn-out displayed an increased sensitivity in response to ALLO, as evidenced by larger decreases in the SEV/ALLO ratio, compared to controls. A greater reduction in SEV in response to flumazenil was also seen in the chronically stressed subjects. In animals, flumazenil changes its actions from a GABA_AR antagonist (or inert compound) to a GABA_AR agonist, but only in the presence of a conformational change in the GABA_AR (Gangisetty and Reddy 2010; Smith et al. 1998). Although neurosteroid concentrations were not measured, the results suggest that in humans, chronic stress is associated with an increased GABA_AR sensitivity, which may reflect compensatory changes in the GABA_AR in response to low ALLO concentrations resulting from chronic stress, as shown in animals (Dong et al. 2001; Matsumoto et al. 2005; Serra et al. 2000).

Results from human studies which have investigated associations between chronic stress and peripheral DHEA or DHEA-S concentrations have been mixed. However, the majority of studies suggest reduced basal levels of DHEA or DHEA-S in association with chronic psychosocial stress (Bellingrath et al. 2009; Izawa et al. 2012; Jeckel et al. 2010; Lennartsson et al. 2013; Vedhara et al. 2002), though elevated levels have also been found (Lac et al. 2012; Mommersteeg et al. 2007). Because DHEA is a multifunctional steroid capable of a broad range of biological effects (Lac et al. 2012; Maninger et al. 2009; Oberbeck and Kobbe 2010), methodological differences within and across studies likely contribute to the heterogeneity of findings. For example, DHEA and DHEA-S concentrations in human studies have been shown to be highly influenced by age (Guazzo et al. 1996; Kraemer et al. 2001; Kushnir et al. 2010; Šulcová et al. 1997) and sex (Kushnir et al. 2010; Šulcová et al. 1997). Moreover, in light of the proposed antagonistic effects of DHEA and DHEA-S on cortisol, the cortisol/DHEA or cortisol/DHEA-S ratios may be more informative measures of allostatic load (and therefore risk for stress-related disease), than individual concentrations (McEwen 1998; Sapolsky et al. 1986; Wolkowitz et al. 2001).

In a recent prospective study of the effects of chronic stress (in the form of a two-week teaching practice) on salivary measures of the cortisol/DHEA ratio in 33 young adult women (Izawa et al. 2012), higher perceived stress following the chronic stressor was associated with an elevated cortisol/DHEA ratio at bedtime, and higher levels of negative mood were associated with elevated cortisol/DHEA ratio after awakening, though neither reached statistical significance. However, compared with pre-stressor concentrations, basal cortisol averaged over awakening, 30 min after awakening, and at bedtime significantly increased during the first and second week of the teaching practice, while DHEA concentrations significantly decreased. These findings are generally in agreement with other human studies of chronic stress, which have shown that DHEA and DHEA-S concentrations tend to decline with chronic or repeated stress, while cortisol levels tend to rise or remain unchanged, resulting in elevated cortisol to DHEA and/or DHEA-S ratios (Jeckel et al. 2010; Lennartsson et al. 2013; Wolkowitz et al. 2001).

In summary, studies using acute and chronic stress paradigms indicate that age, sex, and race/ethnicity are among the factors that may modify the neurosteroid response to psychological stress in humans. Indeed, data from both animal and human studies suggest that repeated and/or prolonged exposure to elevated levels of neurosteroids in response to chronic stress has the potential to result in long-term alterations in neurosteroid concentrations, and possible alterations in the plasticity and functioning of GABA_AR's, resulting in altered GABAergic transmission and potential dysregulation of the HPA-axis. If this is the case, impairment in neurosteroid-HPA-axis regulation could well play an etiopathological role in stress-related illness.

Implications of Neurosteroid – HPA-axis Regulation for Human Stress Related Illness

Major Depressive Disorder

Major depressive disorder (MDD) is one of the most extensively studied stress-related disorders in human psychopathology, and an apt model for investigation of neurosteroid-mediated HPA-axis dysregulation in the context of allostatic load. Epidemiological evidence suggests that depression is associated with increased daily stress (D'Angelo and Wierzbicki 2003; Hutchinson and Williams 2007; Wichers et al. 2009), and both new onset depression and risk of depression recurrence is increased by stressful life events (Bifulco et al. 1998; Brilman and Ormel 2001; Kendler et al. 2004). The olfactory bulbectomy (OB) model of depression (created by bilateral lesioning of the olfactory bulbs in animals) is considered one of the most valid animal models of human depression since the model produces behavioral and neuroendocrine alterations similar to those observed in depressed patients (Harkin et al. 2003; Kelly et al. 1997; Leonard and Tuite 1981; Leonard 1984; Song and Leonard 2005). In rats, OB significantly increases peripherally measured corticosterone (Harkin et al. 2003; Marcilhac et al. 1999) and significantly decreases centrally measured levels of ALLO (Uzunova et al. 2003). This decrease in ALLO has been hypothesized to reflect a distinct pathophysiological mechanism underlying the observed depressive-like behaviors associated with induced bulbectomy syndrome in rats (Uzunova et al. 2003), suggesting an etiopathological link among reduced neurosteroid function, dysregulation in HPA-axis activity, and depressive disorders.

In humans, both plasma and CSF ALLO concentrations are consistently decreased in patients with MDD (Eser et al. 2006; Nappi et al. 2001; Romeo et al. 1998; Strohle et al. 1999; Strohle et al. 2000; Uzunova et al. 1998; Uzunova et al. 2006), while basal cortisol levels are elevated (Young et al. 2004a; Young et al. 2004b; Young and Veldhuis 2006). In contrast, results from studies of DHEA and DHEA-S concentrations in individuals with depression, have shown them to be elevated (Hansen et al. 1982; Heuser et al. 1998; Takebayashi et al. 1998), decreased (Barrett-Connor et al. 1999; Michael et al. 2000; Morsink et al. 2007; Wong et al. 2011) or unchanged (Erdincler et al. 2004; Fabian et al. 2001), compared to controls. However, higher cortisol to DHEA ratios have been more consistently observed in individuals with depression vs. healthy controls (Markopoulou et al. 2009; Michael et al. 2000; Young et al. 2002). Higher cortisol/DHEA ratios are associated

with longer duration of depressive illness (Young et al. 2002) and predictive of persistent MDD in first episode patients (Goodyer et al. 2003).

Individual differences in age, sex, or race may exert profound effects on the relationship of DHEA or DHEA-S concentration to depressive symptomatology (van Broekhoven and Verkes 2003). This is particularly well-illustrated in a cross-sectional study conducted by Morrison and colleagues (2001) which investigated the association between DHEA-S concentrations and depressive symptoms in adult women (ages 35–47y). In this study, the authors found that DHEA-S concentrations were inversely associated with depressive symptoms in women in the older half of their cohort, while DHEA-S concentrations were positively associated with depressive symptoms in the younger women. Interestingly, in AA women, the association between DHEA-S and depressive symptoms transitioned from a positive association to an inverse association at a younger age, compared to nHW women. Thus, in line with our prior work on ALLO reactivity to acute stress in AA women (described above), race may influence associations among neurosteroids, the HPA-axis, and risk for stress-related psychopathology.

The clinical relevance of neurosteroid concentrations to MDD is derived from studies showing inverse relationships between ALLO concentrations and the severity of depressive illness (Nappi et al. 2001; Uzunova et al. 1998), and from studies showing that clinically efficacious treatment for MDD is associated with increases in ALLO (Romeo et al. 1998; Strohle et al. 1999; Strohle et al. 2000; Uzunova et al. 1998). Hypercortisolemia resulting from chronic activation of the HPA-axis, including greater CRH concentrations and gene expression (Gillespie and Nemeroff 2005; Nemeroff 1998; Plotsky et al. 1998; Raadsheer et al. 1994), is implicated in the pathophysiology of depressive disorders (Nemeroff 1998). Given the role of neurosteroids as endogenous negative modulators of the HPA-axis, HPA-axis hyperactivity in many individuals with MDD may be reflective of an eventual depletion in neurosteroid synthesis resulting from chronically heightened HPA activation, as seen in animal models of neurosteroid depletion associated with chronic stress (Serra et al. 2000). Therefore, the ability of ALLO (or other neurosteroids) to limit the extent and duration of reduction in GABAergic inhibitory transmission is reduced, allowing the HPA-axis to become overactive.

It should be noted, however, that not all studies report hypercortisolemia in MDD (Ahrens et al. 2008; Appelhof et al. 2006; Barden 2004; Bockting et al. 2012; Gillespie and Nemeroff 2005; Zobel et al. 2001). Heterogeneity due to potential methodological differences in study design and/or cortisol sampling is beyond the scope of this review; however, viewed from an allostatic load perspective, differences in HPA-axis activation may also be representative of different disease (allostatic) states of the individual. This is supported by evidence that, in humans, initial HPA-axis hyperactivity associated with stress is reduced in response to prolonged chronic stress exposure, and may even manifest as blunted HPA-axis activation over time (Miller et al. 2007). Additionally, higher tonic activation of the HPA-axis following chronic stress may lead to blunted cortisol reactions to acute stress, due to a “ceiling effect,” and thereby inhibit the potential for appropriate HPA-axis stress reactivity (Richards et al. 2011). This phenomenon has been demonstrated in patients with MDD (Burke et al. 2005). Therefore, context of disease state (e.g., first episode depression vs.

recurrent depression, or history of chronic life stress) may play an influential role in neurosteroid regulation of the HPA-axis, thereby influencing whether patients with depression exhibit hyper- or hypo-cortisolemia relative to controls.

Our laboratory has assessed neurosteroid stress reactivity in euthymic women with or without a history of depression, and some of that research has included HPA-axis assessment. In our first study (Klatzkin et al. 2006b), though there were no diagnosis-related differences in resting ALLO concentrations, women with a history of depression had a blunted ALLO response to mental stress relative to never depressed women. We also observed that, in contrast to never depressed controls, there was a lack of positive correlation between ALLO and progesterone concentrations in women with depression histories. This finding is consistent with a report in women with current postpartum depression who also failed to show the expected relationship between ALLO and progesterone (Nappi et al. 2001). We concluded at the time that these findings may reflect depression-related alterations in the conversion of progesterone to neuroactive steroids (Strohle et al. 1999; Strohle et al. 2000). Consequently, our subsequent study tested women in the early follicular phase of their menstrual cycle (low endogenous neurosteroids) following oral micronized progesterone (Klatzkin et al. 2006a). Histories of depression predicted lower plasma concentrations of both ALLO and progesterone over 255 minutes. However, contrary to expectations, our analyses of the ALLO/progesterone ratio as an index of metabolism revealed no evidence for depression-related differences and, consequently, did not suggest alterations in the conversion of progesterone to ALLO in women with histories of depression.

While we were unable to elucidate mechanisms contributing to the lower plasma ALLO following oral progesterone in that study, our subsequent report (Girdler et al. 2012), based on secondary analyses of the progesterone challenge study described above, assessed a broader array of GABAergic neuroactive steroids using GC-MS methods. The primary finding of those analyses was a generalized reduction in GABAergic neuroactive steroid concentrations (both before and after the oral progesterone challenge) in women with histories of depression relative to never depressed women. This was true not only for ALLO and ALLO-THDOC, but for other progesterone derived neuroactive steroids and their precursor, pregnenolone; as well as for androstenedione-derived neuroactive steroids and their precursor, DHEA (see Figure 2). We also observed that women with a depression history had lower cortisol at rest and in response to stress than never depressed controls. The most parsimonious explanation for the findings was one of adrenal suppression since the adrenals are the main secretory source of progesterone during the early follicular phase (the time of testing in this study). Adrenal suppression is also supported, in part, by the finding for lower rest and stress cortisol concentrations in those with a history of depression, and is consistent with other research in euthymic women with histories of depression who show lower resting cortisol and a blunted cortisol and ACTH response to mental stress relative to never depressed women (Ahrens et al. 2008). We hypothesized that the pattern of diminished GABAergic neuroactive steroid and cortisol concentrations at rest and in response to challenge reflects a physiologic adaptation resulting from depression and/or chronic stress, though the absence of measures of psychosocial stress limit that interpretation.

Regardless of mechanism, a remarkable feature of these findings is for persistent disturbance in neurosteroid and HPA-axis function in women who were in remission for, on average, five years. If a history of depressive illness contributes to long-term alterations in neuroactive steroid concentrations, and potentially to neuroactive steroid regulation of the HPA-axis, this may represent one mechanism contributing to the increased risk of recurrent depression in those with a history of depression (Lewinsohn et al. 1989).

Premenstrual Dysphoric Disorder

Evidence from both animal and human studies suggest that periods of neurosteroid fluctuation (such as during the ovarian cycle) may be linked to alterations in the sensitivity of GABA_AR's to neurosteroids (Turkmen et al. 2011), resulting in mood instability in vulnerable women, such as those with premenstrual dysphoric disorder (PMDD). PMDD is a depressive disorder, characterized by the cyclic recurrence of emotional and physical symptoms during the luteal phase of the menstrual cycle that are of sufficient severity to interfere with function, but remit with the onset of menses. PMDD afflicts 5 – 10% of women in their reproductive years (Cohen et al. 2002). Since plasma levels of ALLO follow closely those of progesterone during the luteal phase, substantial interest has existed in investigating neurosteroid-related pathophysiology in PMDD. Overall, findings from studies comparing differences in basal concentrations of neurosteroids between women with PMDD and controls have been mixed (Girdler et al. 2001; Lombardi et al. 2004; Monteleone et al. 2000; Rapkin et al. 1997; Schmidt et al. 1994; Segebladh et al. 2013; Wang et al. 1996), and research which has included investigation of DHEA/DHEA-S in PMDD has been limited to two small studies (Eriksson et al. 1992; Lombardi et al. 2004). However, evidence does suggest that women with PMDD exhibit differences in GABA_AR sensitivity to neurosteroids (Kask et al. 2008). In women with PMDD, greater symptom severity predicts reduced sensitivity to positive allosteric modulators of the GABA_AR including pregnanolone, benzodiazepines, and alcohol (Sundstrom et al. 1997a; Sundstrom et al. 1997b; Sundstrom et al. 1998).

The research of Sundstrom and colleagues has demonstrated that women with PMDD show less of a reduction in SEV (a sensitive measure of benzodiazepine/GABA_AR sensitivity) and report less sedation in response to benzodiazepines relative to healthy controls (Sundstrom et al. 1997a; Sundstrom et al. 1997b; Sundstrom et al. 1998). Since there is no evidence that PMDD women differ in the density or affinity of peripheral benzodiazepine receptors, at least as measured on lymphocytes (Daly et al. 2001), these results suggest a diminished functional sensitivity of GABA_ARs in PMDD. Moreover, the work of Le Melledo et al. (2000) has shown that exposure to flumazenil, a benzodiazepine receptor antagonist, in the luteal phase elicits marked increases in panic symptoms in PMDD women but not in non-PMDD controls. This effect is consistent with a shift in benzodiazepine sensitivity toward inverse agonism, as is seen with panic patients exposed to flumazenil (Nutt et al. 1990). Although speculative at present, the rodent animal model work of Smith and colleagues suggests that alterations in GABA_AR function may reverse the anxiolytic effects of luteal phase increases in ALLO and trigger dysphoric mood states (Smith et al. 2006).

Since ALLO and ALLO-THDOC are stress responsive, and since women with PMDD report more stressful life events (Girdler et al. 1993; Woods et al. 1997) and have substantially higher rates of sexual and physical abuse histories than non-PMDD women (Girdler et al. 1998; Girdler et al. 2003; Girdler et al. 2007; Golding et al. 2000; Paddison et al. 1990), it became of interest to us to examine whether neurosteroid reactivity to stress is altered in PMDD. We assessed luteal phase ALLO following an extended rest and 17 minutes after the onset of a mental stress and found that PMDD women displayed significantly higher plasma ALLO and lower plasma cortisol concentrations at rest than controls (Girdler et al. 2001), the latter being consistent with the majority of the literature suggesting blunted HPA-axis function in PMDD (Girdler et al. 1998; Klatzkin et al. 2010; Rabin et al. 1990; Redei and Freeman 1993). Consistent with a negative modulatory role of ALLO on the HPA-axis, ALLO concentrations were negatively correlated with plasma cortisol concentrations both at rest and in response to stress in the entire sample. These results are consistent with a recent report on diurnal ALLO and cortisol concentrations in PMDD women tested in the late luteal phase (Segebladh et al. 2013) since PMDD patients with higher morning ALLO had blunted nocturnal cortisol concentrations relative to controls with low morning ALLO concentrations.

Regarding ALLO stress reactivity, we found that 83% of controls showed the expected stress-induced increase in ALLO, while only 42% of PMDD women showed an increase. Moreover, lack of ALLO responsiveness to stress in PMDD women was related to their greater baseline concentrations since ALLO reactivity and baseline concentrations were negatively correlated in the PMDD group but not in the controls. Our finding for blunted ALLO stress reactivity in PMDD is consistent with the work of Monteleone et al. (2000) and Lombardi et al. (2004). Despite the fact that those investigators reported lower, and not higher, baseline ALLO in PMDD, in response to an ACTH stimulation test following dexamethasone suppression, PMDD women had a blunted adrenal ALLO response (Lombardi et al. 2004); and blunted ovarian ALLO response to a GnRH challenge relative to controls (Monteleone et al. 2000).

Taken together, the results of the studies assessing both ALLO and HPA-axis measures in PMDD are consistent with a negative modulatory role of ALLO on the HPA-axis (Girdler et al. 2001; Segebladh et al. 2013). However, to the extent that ALLO reactivity to stress is blunted in women with PMDD, these results also suggest an impaired GABA_AR mediated response to acute stress in PMDD. Subsequent work from our laboratory indicated that the pathophysiological role of altered ALLO reactivity to stress is especially apparent in PMDD women who also have a history of MDD (Klatzkin et al. 2006b). Only in PMDD women with a MDD history did ALLO stress reactivity predict the severity of premenstrual symptoms. However, since 45 – 60% of PMDD women have a history of MDD (Cohen et al. 2002; Pearlstein et al. 1990), MDD-related alterations in ALLO reactivity has special relevance to this population and is consistent with a heterogeneous etiology to the disorder (Halbreich 2003).

Conclusions and Future Directions

It is evident that neurosteroids play important roles in GABAergic, antigluco-corticoid, and other homeostatic mechanisms involved in the regulation of HPA-axis activity following stress. Indeed, the influence of neurosteroids on human stress-related illness comes from evidence that neurosteroids function as allostatic mediators in the homeostatic control of the HPA-axis, and that both neurosteroids and GABA_AR's are susceptible to long-term alterations in the context of stressful conditions, particularly if chronic.

The focus of this review centered on ALLO, ALLO-THDOC, DHEA and DHEA-S and their roles in the regulation of the HPA-axis response to stress because the human literature to date has almost exclusively focused on these neurosteroids with regards to HPA axis modulation. However, basic science research has demonstrated that a variety of other neuroactive steroids can exert both positive and negative modulation of GABAergic transmission. Therefore, these other neuroactive steroids are additional physiologic regulators of central nervous system excitability (Morrow 2007). GC – MS methods now allow for the simultaneous detection of multiple progesterone-derived neurosteroids and androstenedione-derived neuroactive steroids using small quantities of human serum (Porcu et al. 2009). Given the stress-responsiveness of neuroactive steroids (Purdy et al. 1991), their role in HPA-axis regulation (Morrow et al. 1995), and their effects on behavior (van Broekhoven and Verkes 2003), the field is poised to investigate the inter-relationships among other neuroactive steroids, and their relationship to HPA-axis function and the expression of dysphoric symptomatology.

Based on the evidence that impairment in neurosteroid regulation of the HPA-axis may play a role in symptoms of anxiety, depression, as well as irritability, all common features to many psychiatric diagnostic categories, neurosteroid-HPA-axis regulation may represent a fundamental pathophysiological mechanism cutting across multiple psychopathologies. However, prior to informing our understanding of how neurosteroid-HPA-axis regulation contributes to dimensional aspects of psychopathology, more fundamental research must be done on this regulation in healthy humans. This would include establishing a reliable neurosteroid acute stress response profile, as has been done for the HPA-axis response to social stress (Kirschbaum et al. 1993; Kirschbaum et al. 1995a; Kirschbaum et al. 1995b; Young et al. 2000), and including concurrent measures of HPA-axis function as well as mood and behavior. Finally, prospective studies are needed which assess neurosteroid regulation of HPA-axis activity in the context of adaptation, in order to begin to untangle the complex relationships underlying the transition from adaptive stress mechanisms to stress response dysregulation in the etiology of human psychopathology.

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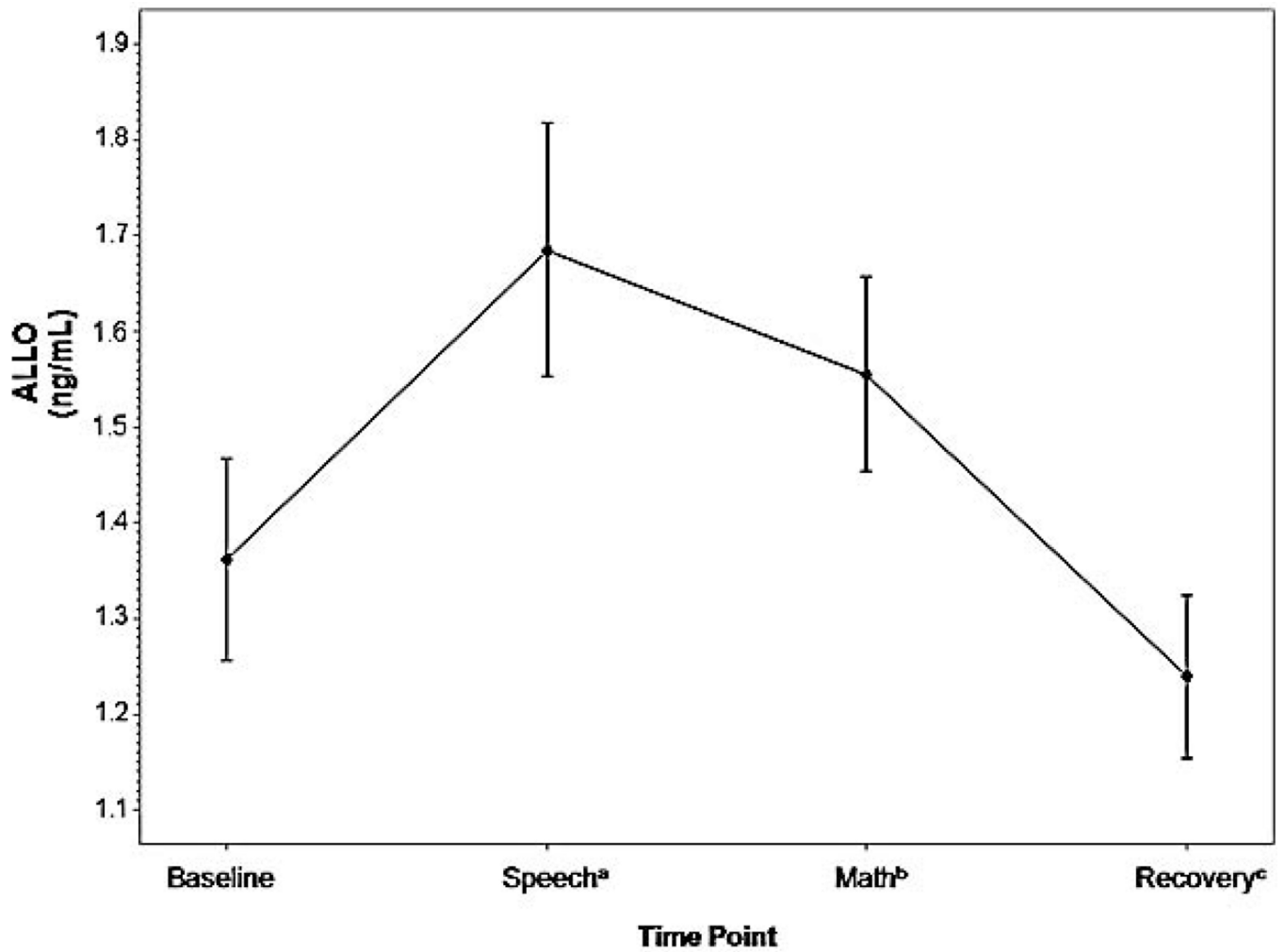


Fig. 1.
Time course of ALLO stress response in women, measured via radioimmunoassay. Data represent mean \pm SEM as a function of time across the task
^aSample taken 12 minutes post-task onset
^bSample taken 17 minutes post-task onset
^cSample taken 30 minutes post-task onset

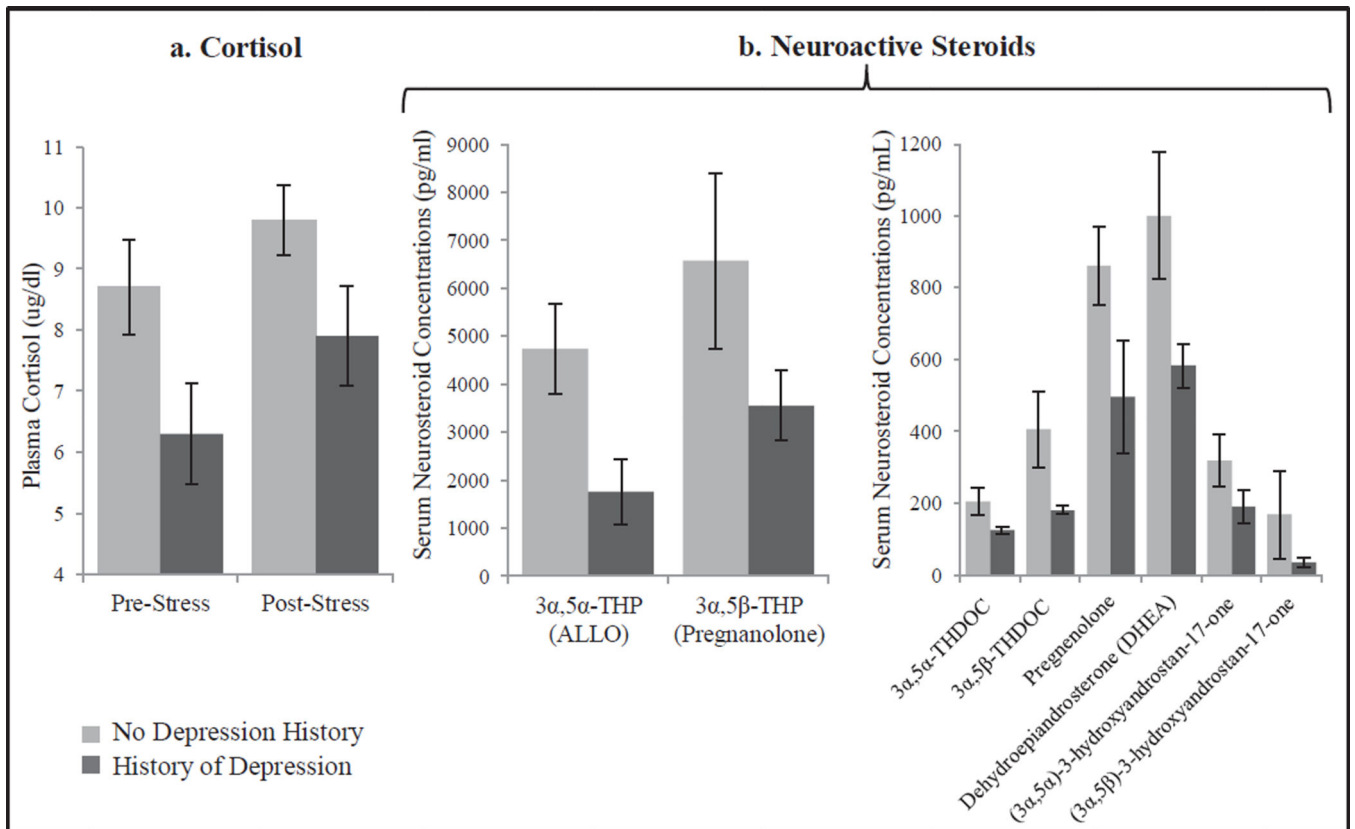


Fig. 2. Mean (\pm SEM) plasma cortisol pre and post-laboratory mental stress challenge (a) and serum GABAergic neurosteroids following progesterone challenge (b) in women with (n=11) or without (n=17) a history of depression