Published in final edited form as:

Neurosci Biobehav Rev. 2010 May ; 34(6): 791–807. doi:10.1016/j.neubiorev.2009.06.004.

PRENATAL ALCOHOL EXPOSURE: FETAL PROGRAMMING AND LATER LIFE VULNERABILITY TO STRESS, DEPRESSION AND ANXIETY DISORDERS

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Abstract

Children with Fetal Alcohol Spectrum Disorder (FASD) exhibit cognitive, neuropsychological and behavioral problems, and numerous secondary disabilities including depression and anxiety disorders. Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis is common in depression/anxiety, reflected primarily in increased HPA tone or activity. Prenatal alcohol exposure (PAE) increases HPA tone and results in HPA dysregulation throughout life, paralleling many of the HPA changes in depression/anxiety. We review data demonstrating altered HPA function and increased depression/anxiety in FASD. In the context of the stress-diathesis model, we discuss the hypothesis that fetal programming of the HPA axis by PAE alters neuroadaptive mechanisms that mediate the stress response, thus sensitizing the organism to stressors encountered later in life, and mediating, at least partly, the increased vulnerability to depression/anxiety disorders. Furthermore, we present evidence demonstrating sex-specific alterations in both hormonal and behavioral responsiveness to tasks measuring depressive- and anxiety-like behaviors in PAE offspring. Overall, the research suggests that the stress-diathesis model provides a powerful approach for elucidating mechanisms underlying the increased vulnerability to mental illness among individuals with FASD, and developing appropriate treatments for these individuals. Dr. Seymour Levine's seminal work on the long-term consequences of early life experiences formed a framework for the development of the research described in this review.

Keywords

HPA axis; prenatal ethanol; depression; behavior; animal models; rat; fetal programming; fetal alcohol spectrum disorder

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1. Fetal Alcohol Spectrum Disorder (FASD)

i. FASD: Description in humans and animal models

Fetal Alcohol Spectrum Disorder is an umbrella term that describes the range of adverse effects that can occur in children born to women who consume alcohol during pregnancy (Manning and Hoyme, 2007; Riley and McGee, 2005). At the most severe end of the spectrum is Fetal Alcohol Syndrome (FAS), which can occur with chronic consumption of high doses of alcohol (Jones and Smith, 1973; Jones, Smith, Ulleland, and Streissguth, 1973). The diagnostic criteria for FAS include pre- and post-natal growth retardation, characteristic facial dysmorphology, and central nervous system (CNS) alterations, including neurological abnormalities, developmental delays, and intellectual impairment (Stratton, Howe, and Battaglia, 1996, Table 1). Exposure to alcohol at levels that do not produce full FAS can result in either partial FAS, where only some of the diagnostic features occur, or in numerous alcohol-related effects that are either primarily physical (Alcohol-Related Birth Defects, ARBD) or primarily neurobehavioral (Alcohol-Related Neurodevelopmental Disorder, ARND) (Stratton et al., 1996). Interestingly, while level of alcohol exposure may impact the severity of effects observed, cognitive, neuropsychological, and behavioral deficits are consistently seen, including hyperactivity, poor attention span, impaired habituation, cognitive and perceptual problems, deficits in executive function, impulsivity, lack of inhibition, and poor sensitivity to social cues (Astley et al. 2009; Mattson, Schoenfeld, and Riley, 2001; Shaywitz, Cohen, and Shaywitz, 1980; Streissguth, 1986).

Features similar to those observed in human FASD are observed in rodent models of prenatal alcohol exposure (PAE). These include retarded pre- and post-natal growth and development, physical malformations, CNS abnormalities (Abel and Dintcheff, 1978; Chernoff, 1977; Druse, 1996; Leichter and Lee, 1979; Randall, Taylor, and Walker, 1977; Weinberg, 1993; Weinberg and Gallo, 1982), and a wide range of behavioral and cognitive deficits, including deficits in learning and memory, hyperactivity, hyper-responsivity to stressors, and deficits in both response inhibition and appropriate use of environmental cues (Abel, 1979; Anandam, Felegi, and Stern, 1980; Bond and Di Giusto, 1976; Bond and DiGiusto, 1977; Gallo and Weinberg, 1982; Kim et al., 1997; Lochry and Riley, 1980; Martin, Martin, Sigman, and Radow, 1978; Riley, Baron, and Hannigan, 1986; Riley, Lochry, and Shapiro, 1979; Weinberg, 1992b).

ii. Secondary disabilities associated with FASD

The extensive literature on neurodevelopmental deficits in children prenatally exposed to alcohol indicates that in addition to primary problems in cognition, communication, learning, memory and behavior, secondary problems in a number of life domains can occur (Olson, Morse, and Huffine, 1998; Streissguth, 1992; Streissguth et al., 2004). Of relevance to the present review, mental illness, and in particular, depression and anxiety disorders, are among the most commonly reported problems in children and adults with FASD (Famy et al., 1998; Famy, Streissguth, and Unis, 1998; Kodituwakku, 2007; O'Connor and Kasari, 2000; O'Connor et al., 2002; O'Connor and Paley, 2006; O'Connor, O'Halloran, and Shanahan, 2000; Roebuck, Mattson, and Riley, 1999; Streissguth et al., 2004), and a high proportion of patients and families seek help from mental health professionals (Streissguth

and O'Malley, 2000). Lemoine and colleagues, in a 30 year follow up of their original 1968 cohort, concluded that mental health problems constituted the most severe manifestation of FAS in adulthood (Lemoine, Harousseau, Borteyru, and Menuet, 2003). Similarly, 3–16 year old children with heavy prenatal alcohol exposure showed higher rates of depressive symptoms than control children (Roebuck et al., 1999), and 63% of a patient sample had at least one psychiatric abnormality, far exceeding findings from epidemiological studies in the general population (Steinhausen & Spohr, 1998). Importantly, this increased risk for depression is not related to mental retardation, as the link between prenatal alcohol and depression also occurs in children (O'Connor et al., 2002; O'Connor et al., 2000) and adults (Famy et al., 1998) with normal intelligence.

Environmental factors associated with prenatal alcohol exposure may play a role in the increased incidence of depression that has been observed. Among these factors are early maternal death, living with an alcoholic parent, maternal mood, child abuse and neglect, removal from the home by authorities, repetitive periods of foster care and other transient home placements, and being raised by adoptive or foster families (Streissguth and O'Malley, 2000; Streissguth et al., 2004; O'Connor & Kasari, 2000). In relation to maternal mood, for example, maternal depression was related to high levels of negative affect and the highest scores on the child measure of depressive symptoms in 4–6 year old girls with high levels of prenatal alcohol exposure (O'Connor & Kasari, 2000; O'Connor and Paley, 2006). Moreover, negative affect in the child was associated with lower levels of maternal emotional connectedness to their children, and in turn, those children had higher levels of depressive symptomatology (O'Connor and Paley, 2006). Importantly, however, a direct relation between prenatal alcohol exposure and child depressive symptoms was also reported. Thus, while environmental factors, including the mother-child interaction, might play a role in mediating the effects of prenatal alcohol exposure on child depressive symptoms, direct effects of alcohol also appear to mediate this relationship. Together, these findings indicate that problems such as anxiety and depression are not only a significant issue among adults and children with FASD, significantly impacting health and well being, but also may have a neurobiological basis and thus, at least in some instances, could be primary rather than secondary disabilities. To date, the mechanisms underlying the link between prenatal alcohol exposure and increased vulnerability to depressive and/or anxiety disorders are not known.

In this review, we will outline the neurobiological dysfunctions associated with depression and anxiety disorders, and link them to those observed following prenatal exposure to alcohol. We will review evidence from animal models demonstrating long-term alterations in hypothalamic-pituitary-adrenal (HPA) regulation and responsiveness under both basal and stress conditions in PAE males and females, reflecting increased HPA tone throughout life. We will then discuss work from our laboratory and others demonstrating that PAE animals exhibit altered behavioral and HPA responses to tasks measuring anxiety/depressive-like behaviors than their control counterparts. Further, we will show that exposure to chronic mild stress in adulthood differentially increases the incidence of depressive/anxiety-like symptomatology in PAE compared to control animals, and does so in a sexually dimorphic manner. These results will be discussed in the context of the stress-diathesis hypothesis of depression.

2. Depression: General Overview

i. Overview

Depression is a highly prevalent, chronic, recurring, and potentially life-threatening illness (Berton and Nestler, 2006; Nestler et al., 2002). Severe forms of depression affect at least 2–5% of the US population, and up to 20% of the population are affected by milder forms of depression (Nestler et al., 2002). A survey by the World Health Organization indicates that depression is one of the top ten causes of morbidity and mortality worldwide (Berton and Nestler, 2006). There is a strong heritable component, with approximately 40–50% of the risk for depression being genetic, and the remaining 50–60% due to non-genetic factors such as early childhood trauma, emotional stress, and physical illness (Berton and Nestler, 2006; Nestler et al., 2002). Remarkably, depression is almost twice as common in females as in males (Kessler, 1997), although the factors mediating this increased incidence are not well understood. This is an important issue clinically, and interestingly, work in animal models has demonstrated sexual dimorphism in the effects of stress on depressive- and anxiety-like behaviors, and in the effects of prenatal alcohol exposure on both hormonal and behavioral responsiveness. We will discuss these important issues further below.

ii. Categories and classification

It is generally agreed that depression cannot be viewed as a single disease, but rather a heterogeneous syndrome or spectrum of disorders, with highly variable symptoms and perhaps distinct pathophysiologies (Berton and Nestler, 2006; Hindmarch, 2002; Matthews, Christmas, Swan, and Sorrell, 2005; Nestler et al., 2002; Pryce et al., 2005). Indeed, there are no symptoms, nor other clinical features that are actually pathognomonic for depression, and even the core features of depression occur in several other medical and psychiatric disorders (Matthews et al., 2005).

There are three general categories of depression: *Major Depressive Disorder*, the most severe disorder; *Dysthymic Disorder*, a somewhat milder form (although often there is no clear clinical or symptomatic distinction between these two); and *Depressive Disorder Not Otherwise Specified*, when the depressive disorder does not meet the criteria for a specific disorder (American Psychiatric Association, 2000). Briefly, depression is characterized by the presence of two major symptoms: depressed mood (sadness, helplessness, irritability) and anhedonia (loss of interest/pleasure in daily activities). At least one of these must be present chronically, and the patient must show at least four additional symptoms including changes in body weight, changes in sleep, psychomotor agitation or retardation, fatigue or energy loss, feelings of worthlessness or guilt, diminished cognitive functioning, and recurrent thoughts of death (American Psychiatric Association, 2000).

iii. Co-morbidities

Co-occurrence of depression and anxiety is found in a large percentage of individuals diagnosed with either depression or an anxiety disorder (Cameron, Abelson, and Young, 2004; Gorman, 1996; Kalueff, Wheaton, and Murphy, 2007; Nemeroff, 2002; Young, Abelson, and Cameron, 2004), and co-morbidity of anxiety disorders and depression may actually be the rule rather than the exception (Lenze et al., 2001; Mineka, Watson, and

Clark, 1998; Nemeroff, 2002). The major diagnostic feature of *Generalized Anxiety Disorder* is excessive anxiety and worry that occurs more days than not for a period of at least 6 months, about a number of events or activities (American Psychiatric Association, 2000). However, many of the symptoms of anxiety and depression are similar, and mild anxiety can be difficult to distinguish from mild depression. In addition, the symptoms of depression and anxiety may respond to the same treatments, supporting the possibility of a common, or at least partially overlapping, neurobiological dysfunction (Kalueff et al., 2007). Given this overlap between depression and anxiety, it has been suggested (Kalueff et al., 2007) that animal models should be developed to assess common pathogenic mechanisms, risk factors and co-morbidity associated with these two disorders, in addition to utilizing “pure” anxiety and depression paradigms. The high co-morbidity of anxiety and depression has informed the development of the test battery and measures in our current work (see below).

Depression and substance use disorders are also highly co-morbid (American Psychiatric Association, 2000; Bruijnzeel, Repetto, and Gold, 2004). Clinically, patients may receive a diagnosis of either primary (independent) or secondary (substance-induced) depression (Nunes and Rounsaville, 2006). In primary depression, symptoms can be distinguished as “temporally independent” in the lifetime history from addiction symptoms, whereas in secondary depression, symptoms are not temporally independent, exceed typical withdrawal effects and may require specific medical attention (Nunes and Rounsaville, 2006). It is interesting to note that women more often present with primary depression, while men more often present with primary addiction (Zilberman, Tavares, Blume, and el-Guebaly, 2003). Epidemiological studies suggest that women also experience worse symptoms and more depressive co-morbidity than men (Zilberman et al., 2003). For example, in the US, it has been estimated that of those with alcohol dependence, 19% of women but only 5% of men, experience a lifetime history of major depression (Pettinati, 2004). These data are particularly relevant in light of the large body of evidence indicating a high incidence of substance use disorders among FASD populations (Streissguth, 1992; Streissguth and O’Malley, 2000).

3. Depression: Neurobiology

i. The Monoamine Hypothesis

The monoamine hypothesis has been the dominant one in the depression field for many years. This hypothesis states that a deficiency in serotonergic (5-HT) and noradrenergic (NA) transmission underlies the symptoms of depression (Schildkraut, 1965). It is based primarily on the serendipitous finding that drugs that facilitate monoaminergic transmission can be effective antidepressants (Hindmarch, 2002; Nestler et al., 2002). However, over the years it has become apparent that one must go beyond monoamines to understand the neurobiology of depression. Deficits in monoamine activity are not always observed in clinically depressed patients, and may be related more to suicidal behaviour and/or impulsivity than depression (Placidi et al., 2001; van Praag and Kahn, 1988; van Praag and Plutchik, 1988). Moreover, facilitation of monoamine neurotransmission is only one component of antidepressant activity. While current antidepressants can be very effective, up

to 50% of patients still do not respond (Walsh, Seidman, Sysko, and Gould, 2002), and various side effects lead to discontinuation of treatment in many others. Furthermore, despite inducing an almost immediate elevation in synaptic monoamine levels, monoaminergic drugs show a long latency for clinical effect (Hindmarch, 2002), suggesting that the mechanism of action of these drugs may involve other neurobiological systems.

New approaches to drug development have come from our increasing knowledge of brain areas, neural circuits, and neurobiological substrates involved in depression and anxiety disorders. Among the most widely cited and accepted alternative hypotheses of depression is that which implicates a central role for the HPA axis (Barden, 2004; Berton and Nestler, 2006; Dinan, 2001; Dinan, 2005; Hindmarch, 2002; Holsboer, 2000; Nemeroff and Vale, 2005; Nestler et al., 2002; Young, 2004; Young, 2006). Depression is largely a disorder of the representation and regulation of mood and emotion (Davidson, Pizzagalli, Nitschke, and Putnam, 2002), and there is a large overlap of the neurobiological circuitry underlying emotion and depression. Importantly, this neural circuitry also overlaps to a large extent with that mediating both neuroendocrine and autonomic aspects of the stress response (Holsboer, 2001; Nestler et al., 2002). This circuitry includes, but is not limited to, the prefrontal and anterior cingulate cortices, nucleus accumbens, ventral tegmental area, hippocampus, amygdala, bed nucleus of the stria terminalis and hypothalamus (Davidson et al., 2002; Nestler et al., 2002). These areas are all interconnected and exhibit bidirectional feedback (Davidson et al., 2002). Abnormalities in morphometry and functioning of each of these have been reported in both depression and anxiety disorders (Abercrombie et al., 1998; Bertoglio, Joca, and Guimaraes, 2006; Berton and Nestler, 2006; Calfa, Bussolino, and Molina, 2007; Davidson et al., 2002; Jacobs, Praag, and Gage, 2000; Lloyd et al., 2004; Maren, 2005; Mayberg, 1997; Nestler et al., 2002; Nestler and Carlezon, 2006; Rainnie et al., 2004; Saxena et al., 2001; Sheline, Wang, Gado, Csernansky, and Vannier, 1996; Thayer and Lane, 2000).

ii. The Hypothalamic Pituitary Adrenal (HPA) Axis

One of the most consistently described biological abnormalities in depression is an alteration in HPA activity and regulation, which is typically normalized by successful antidepressant therapy (Dinan and Scott, 2005; Gold and Chrousos, 2002; Holsboer, 2001; Kaufman, Plotsky, Nemeroff, and Charney, 2000; Nestler et al., 2002; O'Connor and Kasari, 2000; Parker, Schatzberg, and Lyons, 2003; Swaab, Bao, and Lucassen, 2005; Watson, Gallagher, Ritchie, Ferrier, and Young, 2004). Inputs to the HPA axis are provided by stressors (stimuli that threaten homeostasis), and by the endogenous circadian rhythm (Dallman et al., 1987). These inputs act through central neural pathways to the medial parvocellular division of the paraventricular nucleus (PVN) of the hypothalamus, where corticotrophin-releasing hormone (CRH) is synthesized. CRH neurons in the PVN are the final common pathway in the stress response. CRH is released into the portal circulation and stimulates the release of proopiomelanocortin (POMC)-derived peptides (adrenocorticotropin [ACTH] and β -endorphin [β -EP]) from the anterior pituitary. CRH effects on the pituitary are potentiated by arginine vasopressin (AVP) (Jones and Gillham, 1988; Rivier and Plotsky, 1986), particularly during repeated or chronic stress (de Goeij et al., 1991). ACTH stimulates the synthesis and secretion of glucocorticoids from the adrenal cortex. Glucocorticoids regulate

energy substrate availability and utilization, and provide negative feedback to inhibit the HPA axis at the pituitary, and at brain sites including the PVN, hippocampus and prefrontal cortex (Aguilera, 1994; Dallman et al., 1987; Jones and Gillham, 1988; Rivier and Plotsky, 1986). In the short term, glucocorticoids, acting on their receptors (mineralocorticoid, MR or type I, and glucocorticoid, GR or type II), enable the organism to respond both behaviorally and physiologically to stressors, while in the long term they help to dampen the stress-activated defense reactions and prevent them from overshooting and themselves causing harm (de Kloet, Vreugdenhil, Oitzl, and Joels, 1998; Sapolsky, Romero, and Munck, 2000). Thus, successful adaptation to or recovery from stress requires not only the ability to respond initially, but also the ability to control or turn off the stress response appropriately (Aguilera, Kiss, Liu, and Kamitakahara, 2007; de Kloet et al., 1998; O'Connor et al., 2000; Sapolsky et al., 2000). Together, hypothalamic peptidergic drive and the efficacy of CORT mediated feedback largely determine the characteristics of basal and stress-related HPA activity (Patchev, Hayashi, Orikasa, and Almeida, 1995). Appropriate balance between drive and feedback, and maintenance of moderate or controlled levels of the glucocorticoid hormones are critical for optimal HPA function and health (de Kloet et al., 1998; McEwen, 2004; Sapolsky et al., 2000; Patchev et al., 1995). Furthermore, it is important to note that CRH in central neural structures that regulate HPA activity, independent of its HPA activating effects, has activating effects on behavior and coordinates behavioral responses to stressors (Heinrichs et al., 1995). Of particular relevance is the finding that activation of CRH receptors in the amygdala may provide a substrate for stress-induced alterations in affective behavior (Shekhar et al., 2005).

iii. HPA Axis dysregulation in depression

Depressed patients exhibit increased HPA drive and deficits in feedback regulation, manifest as increased basal cortisol levels (Board, Wadeson, and Persky, 1957; Gibbons, 1964); flattened diurnal cortisol rhythm, with morning peak levels only marginally raised, but significant elevations at the afternoon nadir (Deuschle et al., 1997; Wong et al., 2000); “break-through” in the dexamethasone (DEX) suppression test (DST); and/or increased responses in the combined DEX/CRH test. While baseline measures of HPA activity are routinely used in human studies of depression, data suggest that challenge tests of HPA function may be more sensitive, and provide more clues to the nature of the HPA dysfunction that is present (Holsboer, 2001; Watson, Gallagher, Smith, Ferrier, and Young, 2006). The DST, one of the most frequently used neuroendocrine tests to assess HPA function or hypercortisolism in depression (Carroll, 1982), measures negative feedback effects of DEX primarily at the pituitary level, as DEX is a GR agonist that does not readily cross the blood-brain barrier. Individuals receive a 1.5 mg oral dose of DEX at 2300 hr, and basal cortisol levels are measured the following day. Depressed patients frequently show higher basal cortisol levels than controls following DEX suppression. However, studies have suggested that the DST has relatively low sensitivity (cortisol suppression following DEX), averaging 25–50% (Carroll 1982; Heuser et al., 1994). For example, DST status was reported to have only a weak association with diurnal cortisol profiles or 24-hr plasma cortisol levels (Halbreich et al 1985a; 1985b). Other studies suggest that the DST lacks the sensitivity and specificity to differentiate mood disorders from other psychiatric conditions or from healthy controls, or to differentiate patients with different mood disorders (Arana et al 1985;

Braddock, 1986). To overcome these shortcomings, a combined DEX/CRH test was developed (Heuser et al, 1994). Subjects are given DEX at 2300 hr, as in the DST, and at 1500 hr on the next day are given 100 µg CRH i.v. Plasma levels of cortisol and ACTH are typically increased in depressed patients in this combined test, and sensitivity of the DEX/CRH test has been shown to exceed that of the DST (Deuschle et al, 1998; Heuser et al, 1994; Watson et al., 2006), averaging from ~62–80%. Further, sensitivity is increased to above 90% if subjects are clustered by age (Heuser et al, 1994). Importantly, HPA alterations in all of these challenge tests tend to normalize after successful antidepressant treatment (Ising et al., 2005). Moreover, favorable responses to antidepressant treatment can be predicted by determining the DEX/CRH response on admission, with optimal prediction of possible non-response to treatment requiring a second test. Thus, the combined DEX/CRH test has been proposed as a potential surrogate marker in depression (Ising et al., 2005).

Major depression is also associated with decreased MR activity, reflected as significantly increased cortisol secretion among depressed compared to control populations following administration of the MR antagonist spironolactone (Lopez, Chalmers, Little, and Watson, 1998). Such decreased sensitivity to MR antagonists suggests that depression may be associated with an imbalance in the MR/GR ratio, which could lead to downstream effects on serotonergic and other neurotransmitter and neuroendocrine systems, and ultimately may contribute to the etiology of depressive symptoms. HPA dysregulation is also observed in other measures. For example, post-mortem studies among suicide victims have revealed decreased CRH type 1 receptor binding in the frontal cortex (Nemeroff, Owens, Bissette, Andorn, and Stanley, 1988) and pituitary (Dinan and Scott, 2005), as well as decreased hippocampal volume (Lloyd et al., 2004) and hippocampal MR mRNA levels (Lopez et al., 1998), and adrenal hyperplasia (Barden, 2004; Berton and Nestler, 2006; Dinan, 2001; Hindmarch, 2002; Holsboer, 2000; Kaufman et al., 2000; Nemeroff and Vale, 2005; Nestler et al., 2002).

In addition to these findings, it is noteworthy that distinct HPA alterations have been characterized in co-morbid depressive-anxious patients (Cameron, 2006; Keck, 2006; Young et al., 2004). For example, subjects with pure mood or anxiety disorders show normal ACTH and cortisol responses to a social stressor, whereas patients with co-morbid depression and anxiety show exaggerated ACTH responses, suggesting that co-morbid anxiety disorders might play a role in the increased HPA activation observed in patients with major depression (Young et al., 2004).

It is not known whether the HPA abnormalities are a primary cause of depression/anxiety, represent an illness marker, or are secondary to another initiating cause. A recent study reported hypersecretion of cortisol (increased waking salivary cortisol levels) in asymptomatic individuals at genetic risk for depression, suggesting that it is a possible illness endophenotype (Mannie, Harmer, and Cowen, 2007). There is also strong evidence that HPA changes play a role in certain symptoms of depression (e.g., anxiety, insomnia, loss of appetite) and have an impact on the course of disease and its somatic sequelae (Gold and Chrousos, 2002; Nestler et al., 2002). In addition, normalization of HPA disturbances may be a prerequisite for successful treatment: the risk of relapse or resistance to treatment is

much higher in patients where a neuroendocrine abnormality persists (de Kloet, Joels, and Holsboer, 2005; Holsboer, 2000).

4. Adverse early life events and later vulnerabilities

i. Fetal programming

Although the etiology of depression/anxiety disorders is at present unknown, it is generally accepted that the incidence of these mental illnesses occurs in greater proportion among vulnerable populations. One possible explanation for this could relate to early (pre- and early postnatal) adverse experience. The concept of fetal programming developed originally from a large body of data showing that low birth weight and other indices of poor fetal growth are associated with increased biological risk for coronary heart disease, hypertension, and type II diabetes/impaired glucose tolerance (i.e., metabolic syndrome) in adult life. Adult lifestyle factors such as smoking, alcohol consumption, and exercise appear to be additive to the early life influences, suggesting that early life effects have distinct roles and causes. These findings led to the hypothesis that common adult diseases might originate during fetal development, i.e., the “fetal origins of adult disease” hypothesis.

The underlying processes that link early growth restriction with long-term health consequences are not fully understood. It is generally accepted that low birth weight *per se* is unlikely to cause these increased risks for disease. Rather, birth weight likely serves as a marker for the effects of early life events, and common factors probably underlie both the intrauterine growth retardation and the altered physiological function (Welberg and Seckl, 2001). It has been suggested that the resetting of key hormonal systems by early environmental events may be one mechanism linking early life experiences with long term health consequences. Importantly, studies have shown that the HPA axis is highly susceptible to programming during development (Matthews, 2000; Matthews, Owen, Banjanin, and Andrews, 2002; Phillips et al., 1998; Phillips et al., 2000) and that there are strong correlations between birth weight, plasma cortisol concentrations, and the development of hypertension and Type II diabetes. Thus, intrauterine programming of the HPA axis may be a mechanism underlying the observed associations between low birth weight and increased risk for disease (Matthews, 2000; Symonds, Stephenson, Gardner, and Budge, 2007). Importantly, the hypothetical ‘function’ of programming is thought to be adaptive, preparing the offspring to face postnatal life under conditions similar to those present during gestation (for a review, see Gluckman and Hanson, 2004). However, a mismatch between prenatal and postnatal environments, or the presence of severe or prolonged adverse circumstances during early life, can result in deleterious outcomes.

Depression is often described as a stress-related disorder, and there is good evidence that episodes of depression often occur in the context of some form of stress (Nestler et al., 2002). Indeed, stressful life events are present in approximately 90% of first episodes of depression (Young and Altemus, 2004). However, stress *per se* is not sufficient to cause depression. Many people who experience significant life stressors never become depressed and conversely, many people who develop depression have not been exposed to significant life stressors. This suggests that depression, at least in some individuals, may be caused by

interactions between a genetic or biological predisposition and environmental factors (Agid, Kohn, and Lerer, 2000; Nestler et al., 2002), possibly related to fetal programming.

ii. Stress Diathesis Hypothesis

The Stress Diathesis hypothesis suggests that exposure to stressors over the life course can lead to a maladaptive cascade of events and an increased incidence of depression, but only in the context of an already sensitized HPA axis (Gutman and Nemeroff, 2003). In other words, exposure to stressors may not be a causative factor in itself for the onset of a depressive episode; rather, depressive symptoms are more likely to present following stress in those individuals who have experienced early life events or in whom there are other predisposing factors that have sensitized or programmed the stress system (Swaab et al., 2005).

Importantly, the Stress Diathesis hypothesis has support in the FASD literature. For example, Olson et al (Olson et al., 1998) suggest that problems emerge when individuals with FASD, who are constitutionally vulnerable, are exposed to stressors, which then mediate poor outcome. Thus, it is possible that cumulative stress exposure over time may play a role in triggering depressive episodes in individuals who have dysregulated HPA function.

In view of evidence (Phillips et al., 1998; Phillips et al., 2000) that fetal programming of the HPA axis, at least in part, underlies the connection between the early environment and adult stress-related and behavioral disorders in humans, we propose that prenatal alcohol exposure (PAE) be considered in the context of early life adversity or early adverse environment. Furthermore, as programming of the fetal HPA axis by alcohol can result in long-term alterations in the physiological and behavioural profiles of the offspring, we suggest that HPA programming ultimately confers increased susceptibility to develop depression/anxiety disorders if stressors are encountered later in life.

5. Prenatal alcohol exposure and HPA dysregulation

i. Modelling Prenatal Alcohol Exposure

In our well-established model of prenatal alcohol (ethanol) exposure (Weinberg, 1985; Weinberg, 1988; Weinberg, 1989), pregnant dams are assigned to one of three treatment groups. The ethanol (PAE) treatment group is offered liquid ethanol diet (36% ethanol-derived calories) *ad libitum*, formulated to provide adequate nutrition to pregnant rats regardless of ethanol intake (Weinberg, 1985). The pair-fed (PF) group is offered a liquid control diet with maltose-dextrin isocalorically substituted for ethanol, and intake is matched to the amount consumed by an ethanol-treated partner (g/kg body weight/gestation day). The PF group serves as a nutritional control for the reduced food intake in the ethanol group (Weinberg, 1984). PAE and PF animals are provided with fresh diet daily within 1.5 h prior to lights off to prevent a shift of the CORT circadian rhythms, which occurs in animals on a restricted feeding schedule, such as those in the PF group (Gallo and Weinberg, 1981). Finally, a control (C) group is offered standard lab chow or liquid control diet *ad libitum*. The diets are continued throughout gestation (i.e., until gestation day 21 or 22).

Although beyond the scope of this review, it is important to note briefly that pair-feeding is in many ways a treatment in itself. While pair-feeding is the accepted and indeed standard

procedure utilized to separate nutritional effects of ethanol from its direct effects, and to control for the reduced food intake that is typical of ethanol-consuming animals, pair-feeding is at best an imperfect control procedure. Pair-feeding can never control for ethanol's effects on absorption and utilization of nutrients. Furthermore, the pair-feeding procedure may in itself constitute a mild form of prenatal stress (Vieau et al., 2007). In contrast to ethanol-consuming dams, who consume their diet *ad libitum* throughout the 24 hr period, pair-fed dams get a reduced ration of food and thus typically consume their entire daily ration within a few hours of the food being presented. Despite the fact that experimental diets are generally formulated to provide optimal nutrition during pregnancy (Weinberg, 1985; Subcommittee on Laboratory Animal Nutrition, Committee on Animal Nutrition, Board on Agriculture, and National Research Council, 1995), these animals are hungry as they are without food for a large part of the 24 hr cycle. This introduces a stress component to the pair-feeding paradigm, above and beyond the nutritional aspect of receiving a reduced food ration, which itself can program offspring behavioral and physiological (including HPA) function.

ii. Prenatal alcohol exposure and HPA activity in human infants

Compared to the fairly large literature on the effects of ethanol consumption on the HPA axis of adults following acute or chronic ethanol alcohol intake, relatively few human studies have examined the effects of drinking during pregnancy on the HPA axis of the developing child. In case studies by Root and colleagues (Root, Reiter, Andriola, and Duckett, 1975), plasma cortisol concentrations were within the normal range in children with FAS. On the other hand, heavy drinking at conception and during pregnancy were associated with higher basal and post-stress (blood draw) cortisol concentrations in 13-month-old infants (Jacobson, Bihun, and Chiodo 1999), and two-month-old infants exposed *in utero* to ethanol or cigarettes were shown to have higher basal cortisol levels than control infants (Ramsay, Bendersky, and Lewis, 1996). More recently, Haley and colleagues (Haley, Handmaker, and Lowe, 2006) examined cortisol, heart rate and negative affect in 5–7 month old infants during a modified “still face” procedure, a standardised developmental paradigm used to study emotion and stress regulation. They found that greater prenatal alcohol exposure was associated with greater cortisol reactivity, negative affect, and elevated heart rate. Of relevance to the present review, these effects differed in boys and girls, with girls showing greater changes in heart rate and negative affect than boys, and boys showing greater changes in cortisol than girls. Despite these data and the clear effects observed among pre-clinical models, to our knowledge the effects of FASD on adult HPA reactivity has not been examined.

iii. PAE results in elevated basal HPA tone and increased stress reactivity in a sex specific manner in animal models of FASD

We and others have shown that PAE rats typically show normal basal CORT and ACTH levels, but are hyperresponsive to a variety of stressors and to challenges with drugs, such as ethanol or morphine (Kim, Osborn, and Weinberg, 1996; Taylor, Branch, van Zuylen, and Redei, 1988; Weinberg, 1995; Weinberg, 1993). Interestingly, while HPA hyperresponsiveness is a robust phenomenon, sex differences in response are often observed depending on the nature and intensity of the stressor, time course of testing, and hormonal

endpoint measured (Weinberg, 1985; Weinberg, 1992a; Weinberg, Taylor, and Gianoulakis, 1996; Weinberg et al., 2008). For example, both PAE males and females exhibit increased CORT, ACTH and/or β -EP (Weinberg, 1988; Weinberg, 1992a; Weinberg et al., 1996), as well as increased immediate early gene and CRH mRNA levels (Kim, Turnbull, Lee, and Rivier, 1999; Lee, Imaki, Vale, and Rivier, 1990; Lee, Schmidt, Tilders, and Rivier, 2000; Gabriel, Glavas, Ellis, and Weinberg 2005), in response to stressors such as repeated restraint, footshock, and immune challenges. PAE males and females also both show deficits in habituation to repeated restraint, although patterns of response may differ for the different HPA hormones measured (Weinberg et al., 1996). In contrast, in response to either prolonged or intense restraint stress, or cold stress, HPA hyperresponsiveness is observed primarily in PAE males (Kim, Giberson, Yu, and Weinberg, 1996; Kim, Giberson, Yu, Zoeller, and Weinberg, 1999; Weinberg, 1992a), whereas in response to acute restraint stress, or acute ethanol or morphine challenge, increased CORT and ACTH levels are observed primarily in PAE females (Taylor et al., 1988; Taylor, Branch, Liu, and Kokka, 1982; Taylor, Branch, Kokka, and Poland, 1983; Weinberg, Nelson, and Taylor, 1986; Weinberg and Gallo, 1982; Weinberg, 1985; Weinberg, 1992a). Interestingly, studies using rhesus monkeys as subjects have shown that the combination of moderate prenatal alcohol exposure and noise stress reduces birth weights in male but not female offspring, although both males and females showed increased HPA responses to the stress of maternal separation (Schneider, Moore, Kraemer, Roberts, and DeJesus, 2002). Together, these studies strongly suggest a role for the hypothalamic-pituitary-gonadal (HPG) axis in mediating at least some effects of ethanol on the offspring HPA axis (see further discussion of this issue in Weinberg, Sliwowska, Lan, and Hellemans, 2008).

iv. Changes in central HPA regulation are revealed following manipulation of the HPA axis, which may underlie the HPA hyperactivity in PAE animals

Despite the finding that basal hormone levels are typically normal in PAE animals, studies have shown that central regulation of HPA activity is altered under both basal and stress conditions. PAE weanlings (Lee et al., 1990; Lee and Rivier, 1996) and adults (Gabriel, Glavas, Ellis, and Weinberg, 2005; Redei, Clark, and McGivern, 1989) show increased hypothalamic steady state CRH mRNA levels compared to controls, and PAE males may also exhibit increased steady state levels of anterior pituitary POMC mRNA (Redei, Halasz, Li, Prystowsky, and Aird, 1993). Similarly, we have shown that following the challenge of adrenalectomy, basal ACTH levels are higher in PAE than in PF and C males (Glavas, Hofmann, Yu, and Weinberg, 2001), and PAE animals also show greater CRH mRNA increases, but lower pituitary CRH-R1 mRNA levels than controls (Glavas, Ellis, Yu, and Weinberg, 2007). Together, these data suggest increased central HPA drive in PAE offspring.

Our data further demonstrate that PAE males and females exhibit deficits in feedback regulation of HPA activity in the intermediate (2–10 hr) (Osborn, Kim, Yu, Herbert, and Weinberg, 1996), but not the fast (sec to min) (Hofmann, Glavas, Yu, and Weinberg, 1999), feedback time domain. Furthermore, removal of the CORT feedback signal by adrenalectomy appears to unmask hippocampal MR- and GR-mediated effects on basal HPA regulation not observed in intact animals (Glavas, Ellis, Yu, and Weinberg, 2007). Whereas prenatal treatment had no effect on MR and GR upregulation following adrenalectomy,

CORT replacement was less effective in normalizing MR mRNA expression in PAE compared to PF and C males. This suggests an alteration in MR-mediated CORT signalling, and perhaps an altered MR/GR balance (Sliwowska et al., 2008). In addition, we found that MR and GR blockade resulted in greater ACTH release in PAE females but not males under basal or pre-stress conditions, compared to their PF and C counterparts (Glavas et al., 2006), which is consistent with what was observed by Young et al (2004) among depressed populations, as described above.

Finally, studies have shown that PAE animals exhibit altered HPA responsiveness in challenge tests similar to those used to assess depression in humans. Following DEX blockade, PAE males and females exhibit higher stress levels of CORT and ACTH, with the strongest effects at the peak of the circadian rhythm, when HPA feedback is less efficient (Osborn et al., 1996). Furthermore, in the DEX/CRH test (CRH infusion following DEX blockade), PAE, PF and C animals do not differ in adrenal sensitivity to ACTH, whereas both PAE males and females show an increased ACTH response to CRH compared to their respective controls (Osborn, Yu, Stelzl, and Weinberg, 2000). Interestingly, Lee et al (2000) found that if CRH is infused without prior DEX treatment, PAE animals do not differ from controls in their ACTH response to CRH, suggesting that blockade of endogenous HPA activity may increase sensitivity to CRH challenge. This finding is particularly noteworthy in view of data showing that the DEX/CRH test is sensitive in revealing HPA dysregulation in depressed individuals (Holsboer, 2000) and has good diagnostic utility for mood disorders (Watson et al., 2006).

In summary, changes in HPA activity and regulation in PAE animals present a marked parallel to what is observed in patients with major depression and are suggestive of increased HPA tone: PAE animals exhibit increased HPA activity and responsiveness, increases in central HPA drive and deficits in feedback regulation of HPA activity, a possible alteration in the MR/GR balance, ‘break-through’ of HPA activity following DEX blockade, and increased ACTH responses in the DEX/CRH test.

v. PAE animals exhibit altered serotonergic regulation of HPA activity

A wealth of evidence indicates that serotonergic function is altered in depression (e.g., see Nash et al., 2008; Nutt and Stein, 2006; Nutt, 2008; Trivedi, Hollander, Nutt, and Blier, 2008). Similarly, PAE results in marked changes in serotonergic function. For example, decreased concentrations of 5-HT or its metabolites have been reported during fetal life and in weanling animals (Druse, Kuo, and Tajuddin, 1991; Rathbun and Druse, 1985), and PAE also permanently alters 5-HT transmission in discrete brain regions (Zafar, Shelat, Redei, and Tejani-Butt, 2000). Importantly, prenatal administration of buspirone or ipsapirone, partial 5-HT_{1A} agonists, in conjunction with ethanol, ameliorates some of these deficits (Tajuddin and Druse, 1993). On a functional level, our data have shown that PAE animals exhibit physiological and behavioral abnormalities consistent with altered 5-HT function, including altered hypothermic responses to 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), a 5-HT_{1A} receptor agonist, and an increased rate of ‘wet dog shakes’ (stereotyped body movements that consist of shaking the body and head like a dog that has come out of the water) to 1-(2, 5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI), a 5-

HT_{2A/C} receptor agonist (Hofmann, Simms, Yu, and Weinberg, 2002). Furthermore, PAE females show blunted ACTH responses to 8-OH-DPAT, but increased ACTH responses to DOI compared to controls, suggesting an altered interaction between the HPA axis and the 5-HT system (Hofmann, Ellis, Yu, and Weinberg, 2007). In addition, 8-OH-DPAT increases, whereas DOI reduces, hippocampal 5-HT_{1A} receptor mRNA expression in PAE compared with PF and C females, overall. Moreover, PAE males show elevated hypothalamic CRH mRNA levels following DOI, whereas PAE and PF females show a trend toward lower levels compared to their C counterparts. These findings suggest that an important regulatory relationship between the 5-HT receptor and CRH may be disrupted by prenatal ethanol exposure (Hofmann et al., 2002; Hofmann, Patyk, and Weinberg, 2005; Hofmann et al., 2007). Recently, we have extended these studies and demonstrate, for the first time, that 5-HT_{1A} mRNA levels may be differentially altered in PAE animals depending on gonadal hormone status. We observed increased hippocampal 5-HT_{1A} mRNA levels in PAE compared to PF and C females but similar plasma estradiol and CORT levels during diestrus. Importantly, it appears that regulatory effects of estradiol on hippocampal 5-HT receptors may occur primarily through its effects on the HPA axis. As 5-HT_{1A} receptors are under tonic inhibition by CORT (Chalmers et al, 1993), our findings suggest that the inhibitory effects of CORT may be decreased by PAE and/or that increased expression of 5-HT_{1A} receptors may reflect decreased numbers of serotonergic neurons in these animals (Sliwowska et al., 2008). Together, these data indicate that PAE alters neuroendocrine responses to 5-HT receptor agonists in a sex-specific manner, suggesting that the HPA hyperresponsiveness observed in PAE animals may be mediated, at least partly, by an altered interaction between the serotonin system and the HPA axis.

In summary, the data presented above indicate that alterations in central HPA regulation are observed in PAE animals under both basal and stress conditions, and in many respects show parallels to the changes observed in depressed individuals. The marked sex differences in PAE effects on different components of the HPA axis demonstrate that the balance between HPA drive and feedback, and between MR and GR-mediated effects of CORT, are differentially altered in PAE males and females, and suggest a role for the gonadal hormones in mediating alcohol effects on HPA activity. Furthermore, altered HPA-serotonin interactions may play a role in the neuroendocrine dysregulations observed. Such alterations will impact the organism's ability to maintain homeostasis if challenged by adverse events, and could provide a basis for the enhanced vulnerability to behavioral and physiological disorders, such as the increased anxiety and depression observed in individuals with FASD.

6. Prenatal Alcohol Exposure and Depressive/Anxiety-like Symptoms

Although there are clear parallels between the neurobiological alterations observed in PAE animals and what is observed in human depression and anxiety disorders, our ability to model these disorders effectively is limited if we do not take into consideration the functional consequences of changes in central circuits. To explore this issue, we have utilized an animal model of chronic mild stress (CMS) to investigate the effects of prenatal alcohol exposure on vulnerability to depression/anxiety following exposure to later life stressors, as indexed by behavioral responses to tasks that measure depressive-/anxiety-like behaviors.

i. Animal models of depression

Animal models of disease are typically evaluated on four dimensions: 1) construct validity (manipulations of the model organism reflects the pathology and symptomatology of the disorder); 2) face validity (presentation of symptoms should be comparable to those seen in human patients); 3) predictive validity (alterations should be reversed by treatments which are also effective in humans; and 4) etiological validity (etiologic factors resulting in symptoms should be similar to those seen in human patients) (Geyer and Markou, 1995; McKinney and Bunney, 1969). An ideal animal model of depression would have identical causative factors, symptomatology, and response to currently effective treatments (Cryan and Slattery, 2007). However, there are a number of challenges that preclude this ideal. For example, animal models have inherent limitations in their ability to model certain psychiatric symptoms (e.g., feelings of guilt/worthlessness; suicidality), thereby compromising construct validity. Yet, animal models are unique in their ability to demonstrate neurobiological consequences of early environmental events, to control for factors such as sex and stress, and to reveal neurobiological changes underlying depressive- and anxiety-like behaviours (Altemus, 2006).

There is an important distinction between a model (to evoke pathology) and a test (to measure a symptom), and this distinction has not always been clear in the literature. Traditional tests of depressive-like behavior can be roughly categorized into those that measure: 1) Exploratory behavior (e.g., open field, light-dark box, elevated plus maze, novelty-induced suppression of feeding); 2) Social interaction (e.g., dominance/submissive interactions, distress vocalizations, social approach/avoidance); 3) Reward (e.g., sucrose intake/preference/contrast, sexual behavior, novelty-seeking); and 4) Behavioral ‘despair’ (e.g., forced swim, tail suspension, learned helplessness), (adapted from Berton and Nestler, 2006). While there is controversy as to whether despair-based tests produce depressive-like symptoms (i.e., are a model of depression), or rather, act as effective screens for detecting agents with antidepressant-like activity (McArthur and Borsini, 2006), or both, they are nevertheless widely used and effective in revealing effects of adverse or stressful events.

Importantly, and of relevance to our model, stress is known to play a key role in the pathogenesis of anxiety and depression, as discussed above (See Section 4), and as such, the majority of animal models of depression involve some form of stress exposure. Although many investigations have been conducted with acute stressors, such as restraint or footshock, these have been criticized for their low construct validity, as it is often the prolonged, unpredictable stress exposure (e.g. work pressure, marriage stress) that triggers a depressive episode in humans. To elucidate the impact of chronic stress in animals, the chronic mild stress (CMS) model, proposed by Katz *et al.* (1981) and further developed by Willner (1997), has been utilized in a variety of studies. This model involves exposing animals to chronic, unpredictable, mild stressors, typically over a 4–6 week period, which results in a variety of symptoms that are analogous to those observed in depression (Redei *et al.*, 2001; Willner, 2005). However, this model has been criticized for its impracticality and lack of reproducibility between laboratories (Nestler *et al.*, 2002; Redei *et al.*, 2001). Of relevance to this review, there also exist several, highly etiologically valid, models that involve manipulation of the early environment, including repetitive vs. varied prenatal stress, and

maternal deprivation/separation (Caldji, Diorio, and Meaney, 2000; Francis et al., 1996; Ladd et al., 2000; Meaney, 2001; Pryce and Feldon, 2003; Pryce et al., 2005). These models result in neurobehavioral alterations in rodents that resemble depression or anxiety in humans, persist into adulthood, and can be attenuated with antidepressants.

Studies that employ these tests and/or models are not without their limitations. Despite the fact that a diagnosis of depression is based on alterations on a number of behavioral dimensions, animal models often fail to reproduce this complex multi-syndromal disorder. Many studies measure only a limited number of behaviors, or neglect to measure some dimensions altogether (e.g., changes in activity, body weight, social interaction, or sexual behavior). As noted (Anisman and Matheson, 2005), an appropriate model of depression requires multiple behavioral tests that approximate the range of symptoms characterizing depressive illnesses. In addition, studies often utilize animals that are ‘normal’, rather than animals that have been sensitized by being subjected to an adverse manipulation (e.g., early life stress, PAE), the latter of which may more appropriately model the etiology of depression. This is a critical limitation, as antidepressant treatment administered to ‘normal’ or non-depressed humans almost certainly do not elicit the same neurobiological changes as when applied to someone with depression. Thus, in these instances, the biological basis of the animal symptoms may be very different from the biological basis of human symptoms (Nestler et al., 2002). Another important consideration is the necessity to account for the considerable symptom overlap between depression and anxiety disorders (Lenze et al., 2001; Mineka et al., 1998; Nemeroff, 2002; see 2.iii. above). Many animal studies do not discriminate between ‘anxious’ and ‘depressive’ behaviors, or neglect to include measures of anxiety altogether. Finally, few studies explore the influence of sex on the development of depressive and/or anxious behaviors, despite the known sex differences in humans, as well as evidence from animal studies that females are more anxious (Johnston and File, 1991; Palanza, 2001), more vulnerable following early life (Barr et al., 2004; Wigger and Neumann, 1999) and chronic mild (Dalla et al., 2005) stress, and respond differentially to antidepressants (Lifschytz, Shalom, Lerer, and Newman, 2006).

ii. PAE and chronic mild stress as a model of depression/anxiety

In order to account for the complex factors involved in the measurement of depressive and anxiety-like behaviors in animal models, we have developed a multidimensional model employing a battery of behavioral tests, sensitive to both depressive- and anxiety-like aspects of behavior. In alignment with both the fetal programming and stress diathesis hypotheses, we are testing animals that have had adverse early life experiences (i.e., PAE), and consequently, have a sensitized HPA axis. Extensive data from our laboratory and others have shown that this early exposure renders PAE animals hyperresponsive to subsequent life stressors, and we hypothesize that this will increase their vulnerability to stress-induced depressive symptomatology. Further, we hypothesize that the incidence of depressive/anxious symptomatology will be greater if PAE animals are exposed to stressors in adulthood, prior to behavioral analysis. To test these hypotheses, we have undertaken a series of studies in which PAE, PF, and C males and females are exposed to our modified CMS protocol, consisting of exposure to a series of psychological and physiological stressors over 10 consecutive days, twice daily, at random times of day. As noted, typical

CMS protocols tend to be long in duration, on the scale of several weeks. Based on the robust evidence that PAE animals are hyperresponsive to stressors, we opted for an attenuated version of this CMS protocol in order to avoid possible ceiling effects on HPA reactivity or behavior.

iii. Behavioral tests to assess depressive- and anxiety-like behaviors in rodents

Depressive and anxiety-like behaviors were assessed using the open field, elevated plus maze, Porsolt forced swim, sucrose contrast, and social interaction tests (Table 2). The **open field (OF)** is utilized as a test of exploratory locomotor behavior and emotionality (Archer, 1973; Prut and Belzung, 2003; Walsh and Cummins, 1976). Animals are placed in a novel arena for 5–15 min/day for one day or several consecutive days and time in the center/periphery, as well as overall locomotor activity, are recorded. Ambulation (squares crossed) is typically used as a measure of exploratory behavior, although locomotor hyperactivity is thought to reflect neophobia. By contrast, increased time in periphery or avoidance of the center of the field reflects anxiety/emotionality. Importantly, anxious or neophobic behavior is reversible with most anxiolytics that decrease anxiety in humans (Prut and Belzung, 2003). For our studies, animals were exposed to a small, dimly lit open field for 5 min over three consecutive days. The **elevated plus maze (EPM)** is an apparatus in the shape of a cross, with two open arms and two arms enclosed by walls. The EPM is a reliable and validated task used to measure anxiety/fear in rodents, as indicated by behavioral, physiological and pharmacological responses (Lister, 1987; Pellow, Chopin, File, and Briley, 1985; Pellow and File, 1986). Animals confined to the open arms of the EPM show elevated CORT levels (Pellow et al., 1985), and measures such as number of open-arm entries and time spent in the open arms are inversely related to anxiety (Cruz, Frei, and Graeff, 1994), as rats have an innate fear of open spaces. Locomotor activity can also be quantified by total distance traveled and total number of arm entries. This latter measure is independent of anxiety, as sedative treatments reduce total arm entries without changing time spent on the open arms (Pellow and File, 1986). The **Porsolt forced swim test (FST)** is utilized as a measure of ‘behavioral despair’, and is the most widely used preclinical test of antidepressant action (Cryan, Valentino, and Lucki, 2005; Porsolt, Anton, Blavet, and Jalfre, 1978; Porsolt, 1979). Animals are placed in a cylindrical tank of water (deep enough so the tail cannot touch the bottom) for 15 min on day 1 and re-exposed for 5 min on day 2, and time spent ‘immobile’ (minimal movements to keep the head above water) is analyzed. As antidepressants reverse immobility (Cryan et al., 2005), time immobile is interpreted as reflecting depressive-like behavior, or ‘behavioral despair’; i.e., animals “give up” and stop swimming or trying to escape. **Sucrose intake or preference tests** are used to measure anhedonia, or the inability to experience pleasure from rewarding stimuli, which is one of the core symptoms of depression. However, behavior in these tests can be confounded by changes in body weight, and can reflect a nonspecific consummatory disturbance unrelated to hedonic factors (Forbes, Stewart, Matthews, and Reid, 1996; Matthews, Forbes, and Reid, 1995). The **sucrose contrast test** overcomes some of these issues (Matthews et al., 2005). In the **positive contrast test**, animals are exposed for several consecutive days to a relatively low (e.g., 2.1%) concentration of a sucrose solution, and on the test day presented with a higher (e.g., 15%) concentration. Animals typically consume more of the higher concentration than they would if continued on the lower concentration (Flaherty and Geary,

1993; Flaherty, Turovsky, and Krauss, 1994). This shift reflects an increase in the incentive salience of the higher reward (Berridge and Robinson, 1998), and is considered a measure of 'hedonic behavior'. Anhedonia can be defined as no change in responding to the contrast, suggesting a deficit in sensitivity to alterations in reward value. Finally, the **social interaction (SI)** test is a validated measure of both social behavior and anxiety (File and Hyde, 1978). Animals are placed in a dimly lit, familiar open field along with a control animal of the same sex and weight range for 15 min. Affiliative (social sniffing, allogrooming, social rest, mutual circle/following) and non-affiliative (pouncing, wrestling/pinning) behaviors are recorded and coded by an independent observer blind to the prenatal treatment.

In our initial studies, to avoid the confounding effects of repeated testing, a unique cohort of animals from each prenatal group was tested on each task. Animals were exposed to CMS over 10 consecutive days, and behavioral testing began on day 11. A single cohort of male and female PAE, PF, and C animals remained undisturbed in their home cage as a non-stressed (Non-CMS) comparison group for the CMS animals tested on the EPM. As we were interested in sex differences among PAE, PF, and C rats exposed and not exposed to CMS, animals from both sexes were included in all behavioral, hormonal, and brain analyses.

iv. CMS exposure in adulthood induces greater depressive symptomatology in PAE than in control animals, and does so in a sexually dimorphic manner

In these studies, we found that, across prenatal groups, animals exposed to the 10 day CMS protocol showed attenuated weight gain compared to Non-CMS animals. Basal CORT levels did not change during CMS among males, whereas PAE females had lower CORT levels on the final (day 10), compared to the initial (day 1), day of CMS exposure. This latter is an important finding in the context of the literature on early life adversity and endocrine regulation. For example, Fisher and colleagues (Fisher, Gunnar, Dozier, Bruce, and Pears, 2006; Fisher, Stoolmiller, Gunnar, and Burraston, 2007) found that early adversity, particularly neglect, a younger age at first foster placement and higher number of placements, results in blunting of the daily cortisol rhythm, with atypically low morning cortisol levels. These children show the least resilience in the face of subsequent stressful events, and indeed, are often hyperresponsive to acute stressors or challenges despite the overall flattening of the circadian rhythm. It is possible that in our model, increased HPA tone and increased responsiveness to the CMS protocol may result in an overall blunting of basal hormone levels and/or the HPA circadian rhythm. In support of this possibility is our finding that basal MR mRNA levels are downregulated in PAE females (Sliwowska et al., 2008), suggesting an effect of increased HPA tone on basal HPA activity. This issue requires further investigation.

Elevated Plus Maze (EPM)—Figure 1 shows that CMS exposure led to a significant increase in anxiety-like behaviors overall on the EPM, which was significantly correlated with an increase in CORT 30 min post-test. Moreover, CMS exposure altered HPG function overall: CMS-exposed rats had higher testosterone and progesterone, but decreased estradiol, levels following exposure to the EPM.

Our previous studies demonstrated differential effects of PAE on EPM behavior, with greater deficits in PAE animals if they were exposed to stressors prior to EPM testing (Gabriel, Yu, Osborn, and Weinberg, 2006; Osborn, Kim, Steiger, and Weinberg, 1998; Osborn, Yu, Gabriel, and Weinberg, 1998). Similarly, in the present study, CMS revealed differences among prenatal groups depending on prior stress conditions: Whereas males from the three prenatal groups in the Non-CMS condition did not differ from each other, PAE males exposed to CMS spent significantly less time in the open arms compared to controls, and CMS significantly reduced the frequency of open-arm entries among PAE, but not PF or C females. Thus, CMS led to significant increases in anxiety in a sexually dimorphic manner among PAE animals. The effects of CMS and PAE on locomotor activity were also sexually dimorphic: PAE females but not males in the Non-CMS condition showed locomotor hyperactivity, and this hyperactivity was suppressed by CMS.

CMS exposure also differentially influenced HPA and HPG reactivity among prenatal groups following EPM testing. PAE females exposed to CMS showed significantly elevated CORT (Figure 2) and progesterone, and lower estradiol levels compared to PF and/or C females (Figure 3). By contrast, although CMS exposure elevated testosterone among all males, there were no effects of prenatal treatment (Figure 3). Together, these data suggest that CMS exposure may serve to activate an already sensitized HPA axis in PAE animals, with one consequence being an increase in anxiogenic behavior, suppressed locomotor activity, and elevated CORT following an acute stressor (EPM testing). Similarly, HPG activity, as well as HPA-HPG interactions, appear to be differentially impacted by CMS in PAE compared to control females.

Open field (OF)—Consistent with previous findings in the literature (Bond and Di Giusto, 1976; Mothes, Opitz, Werner, and Clausen, 1996; Riley et al., 1979) male PAE and PF rats exposed to CMS were hyperactive in the OF. As the dimensions of our open field were relatively small compared to those of traditional open fields, the increase in activity likely reflects locomotor hyperactivity rather than a change in emotionality. Importantly, our previous studies (Osborn et al., 1998) demonstrate that, depending upon the test conditions, PAE animals may also show hyperactivity on the EPM, an effect that may be mediated, in part, by the nutritional effects of ethanol. These findings are consistent with the literature demonstrating hyperactivity as a robust behavioral characteristic in children with FASD (e.g., Burd, Klug, Martsof, and Kerbeshian, 2003; Doig, McLennan, and Gibbard, 2008; Elgen, Bruaroy, and Laegreid, 2007; Kodituwakku et al., 2006; O'Malley and Nanson, 2002).

Forced swim test (FST)—Previous studies have shown that PAE increases immobility in the forced swim test in both weanling (Carneiro et al., 2005) and adult (Slone and Redei, 2002) rats. The increased immobility among adults is attenuated by adrenalectomy (Wilcoxon and Redei, 2007), suggesting that adrenal activity may, at least partly, underlie the expression of this behavior.

In the present study, we extend these findings, demonstrating sexually dimorphic effects of PAE on forced swim behavior (see Figure 4). While PAE males did not differ from their control counterparts on any behavioral measure, PAE females showed greater immobility in

the FST than C females during the final 5 min test period on Day 1, and both PAE and PF females were more immobile in the FST during retesting on Day 2. As noted, the FST is the most widely used preclinical test of antidepressant action (Cryan et al., 2005; Porsolt et al., 1978; Porsolt, 1979), and immobility is typically interpreted as a measure of 'behavioral despair'. Thus, our findings suggest that PAE selectively increases depressive-like behaviors in female offspring in the FST. However, we are well aware that test parameters can alter behavior, and that there are alternative interpretations of behavior in the FST (Abel and Hannigan, 1992; Abel and Hannigan, 1994; Abel, 1994), so further studies are needed to confirm our conclusions.

Positive sucrose contrast test—We found that PAE males, but not females, had elevated sucrose intake initially, but did not increase intake of sucrose at the higher concentration. That behavior did not change in response to the higher sucrose concentration suggests insensitivity to the change in reward value of the sucrose, and may be interpreted as anhedonia. However, in view of our previous finding that PAE animals are less able to respond appropriately to environmental cues (Weinberg, 1988; Weinberg, 1992b), it is possible that males could not discriminate between the low and high concentrations. It is not clear at present why PAE males drank more of the low sucrose concentration initially. One possible explanation is that sucrose consumption represents a compensatory response to modulate greater behavioral or HPA arousal (Dallman et al., 2003). Current investigations in our laboratory are underway to explore whether using additional control groups that do not receive the contrast exposure on day 5 can elucidate possible mechanisms underlying the differences observed in PAE males and why PAE females did not show these same alterations.

Social interaction (SI)—Figure 5 illustrates that PAE males showed a significant decrease in the amount of time spent in affiliative and non-affiliative behaviours in the SI test, particularly in the first 5 min of testing. Moreover, whereas PF and C females showed increased frequency of non-affiliative behaviors in the last 5 min of the SI test, PAE females did not alter their behavior accordingly. These data suggest dysfunctional social interaction in both male and female PAE offspring. Depressed social behavior is a key characteristic of depression, and the present data provide strong evidence that CMS can reveal deficits in social interactions in PAE animals.

Together, these data provide compelling evidence that the combination of PAE and CMS in adulthood increases the expression of depressive/anxiety-like behaviors, and that many of these occur in a sexually dimorphic manner. These findings suggest PAE effects on HPA-HPG interactions, and provide support for the Stress Diathesis hypothesis of depression.

7. Prenatal Alcohol Exposure as Early Adverse Experience

Taken together, our recent studies demonstrate that 1) our modified CMS procedure is successful in altering both behavioral and endocrine measures of activity in a manner parallel in many respects to that observed in depressive-/anxiety-like disorders; 2) exposure of PAE animals to stressors (CMS) in adulthood increases depressive- and anxiety-like behaviors relative to those in C animals, and does so in a sexually dimorphic manner. PAE

males exposed to CMS showed greater anxiety (EPM), impaired hedonic responsivity (sucrose contrast test), locomotor hyperactivity (open field), and alterations in affiliative and non-affiliative social behaviors (social interaction) compared to control males. By contrast, while PAE females were similar to males in showing greater anxiety (EPM) and altered social interactions, they also showed greater levels of behavioral despair (FST) compared to their control counterparts. Importantly, even on tasks where both PAE males and females showed deficits, they differed in how these were manifest. For example, CMS caused a decrease in percent time on open arms for PAE males, but reduced total open arm entries for PAE females, and also eliminated the locomotor hyperactivity shown in PAE females who had not experienced CMS. In addition, both affiliative and non-affiliative social behaviors were reduced in PAE males during the first 5 min of testing, whereas PAE females did not show the increase in non-affiliative behaviors shown by PF and C females during the final 5 min of testing.

These data highlight the value of employing a multi-dimensional test battery rather than single behavioral tests in order to explore and characterize fully the range of deficits that may occur following PAE, or in any studies that aim to examine depressive- and anxiety-like behaviors in rodent models. Moreover, these data demonstrate the importance of assessing sex differences in the effects of prenatal ethanol exposure or any adverse early life experience on behavioral or physiological outcome. Finally, in conjunction with our studies showing that CMS leads to significant increases in CORT and decreases in estradiol in PAE compared to control females following acute stress (Hellemans et al., 2008), and data demonstrating HPA hyperresponsiveness to prolonged or repeated exposure to stressors in PAE males (reviewed in Weinberg et al., 2008), these results provide support for the Stress Diathesis hypothesis. Our findings suggest that fetal programming by PAE permanently alters sensitivity to stressors, and thus may be a predisposing factor for the prevalence of mood disorders in FASD populations.

The stress-diathesis hypothesis has been criticized as oversimplifying the pathophysiology of depression (Matthews et al., 2005). While a wealth of evidence links HPA dysregulation to depression, and particularly, major depression, there is, in fact, great heterogeneity of neuroendocrine changes in depression, with only a subset of individuals demonstrating HPA abnormalities (e.g., Strickland and Deakin, 2002). Furthermore, HPA alterations may be observed in patients with other psychiatric diagnoses, e.g., substance abuse. On the other hand, as noted above, studies suggest that pre-existing HPA abnormalities may be a major contributory factor in the genesis of some forms of depression (Matthews et al., 2005). In particular, a large literature points to a relationship between depression in adulthood and adverse early life events (Gutman and Nemeroff, 2003; Nemeroff and Vale, 2005). For example, a history of physical or sexual abuse increases the likelihood of lifetime psychopathology, including depression (Bernet and Stein, 1999; Harkness and Monroe, 2002), with a stronger association for women than men (MacMillan et al., 2001). Women with a history of childhood physical or sexual abuse or neglect also exhibit increased HPA and autonomic responses to a standard laboratory stressor compared to non-abused controls (Heim et al., 2000), with the largest effect in women with current symptoms of depression and anxiety. A link between early life events and depression in adulthood appears to hold even with events that are much less severe than the physical and sexual abuse described

above. For example, social dependence, family overcrowding, poor physical care, poor mothering, and marital or family instability are associated with a greater incidence of depression (Sadowski, Ugarte, Kolvin, Kaplan, and Barnes, 1999). Similarly, people from lower socioeconomic backgrounds have nearly a twofold increase in risk for major depression compared to those from the highest socioeconomic background, independent of childhood sociodemographic factors, family history of mental illness, or adult socioeconomic status (Gilman, Kawachi, Fitzmaurice, and Buka, 2002; Gilman, Kawachi, Fitzmaurice, and Buka, 2003). Thus, early experience is clearly one of the most potent influences on the vulnerability to depression, and may explain inconsistencies of reports from clinical and preclinical studies. We submit that prenatal exposure to alcohol can be viewed as an adverse early life experience that programs neurobiological and neuroadaptive mechanisms such that vulnerability to subsequent life stressors is increased, and, in turn, the vulnerability to depressive and anxiety disorders also increases.

Importantly, early programming events may also be involved in the co-morbidity between addiction and depression, and the sexual dimorphism in their presentation. Early adverse experiences, such as sexual abuse and trauma, appear to act as a specific risk factor for the presentation of co-morbid depression and anxiety symptoms, when compared to purely depressed and anxious populations (Leviton, Rector, Sheldon, and Goering, 2003), and put women at greater risk than men for developing addictive disorders (Goeders, 2003). In a study of 50 cocaine addicts, experience of poor parental relationship in childhood was a risk factor for both elevated HPA tone and co-morbid depression (Gerra et al., 2007). The risk for co-morbid addiction and depressive disorders does not appear to be specific to a substance type. However there is a strong role for HPA programming as a mediator for this co-morbid disease presentation.

In view of the increased rates of mental health problems, with high levels of depression/anxiety and substance use disorders in children and adults with FASD, it is clinically important to identify vulnerabilities (whether biological or psychological) as well as environmental stressors and protective factors among affected individuals, and to understand the mechanisms by which stressors bring on problems in those who are vulnerable. These data highlight the importance of developing animal models such as ours that examine how early life stress or other early adverse experiences (e.g., PAE) modify behavioral and physiological phenotypes to confer vulnerability to depression, anxiety and other mental health problems later in life. Moreover, studies of this nature are important in identifying therapeutic targets for depression as well as the dual diagnosis of FASD and anxiety/depressive disorders.

References

- Abel EL. Prenatal effects of alcohol on adult learning in rats. *Pharmacol Biochem Behav.* 1979; 10(2): 239–243. [PubMed: 450933]
- Abel EL. A further analysis of physiological changes in rats in the forced swim test. *Physiol Behav.* 1994; 56(4):795–800. [PubMed: 7800751]
- Abel EL, Dintcheff BA. Effects of prenatal alcohol exposure on growth and development in rats. *J Pharmacol Exp Ther.* 1978; 207(3):916–921. [PubMed: 731439]

- Abel EL, Hannigan JH. Effects of chronic forced swimming and exposure to alarm substance: Physiological and behavioral consequences. *Physiol Behav.* 1992; 52(4):781–785. [PubMed: 1409953]
- Abel EL, Hannigan JH. The immobility response in the forced swim test: Paradoxical effect of imipramine. *Eur J Pharmacol.* 1994; 258(3):261–264. [PubMed: 8088358]
- Abercrombie HC, Schaefer SM, Larson CL, Oakes TR, Lindgren KA, Holden JE, et al. Metabolic rate in the right amygdala predicts negative affect in depressed patients. *Neuroreport.* 1998; 9(14):3301–3307. [PubMed: 9831467]
- Agid O, Kohn Y, Lerer B. Environmental stress and psychiatric illness. *Biomed Pharmacother.* 2000; 54(3):135–141. [PubMed: 10840590]
- Aguilera G. Regulation of pituitary ACTH secretion during chronic stress. *Front Neuroendocrinol.* 1994; 15(4):321–350. [PubMed: 7895891]
- Aguilera G, Kiss A, Liu Y, Kamitakahara A. Negative regulation of corticotropin releasing factor expression and limitation of stress response. *Stress.* 2007; 10(2):153–161. [PubMed: 17514584]
- Altman M. Sex differences in depression and anxiety disorders: Potential biological determinants. *Horm Behav.* 2006; 50(4):534–538. [PubMed: 16920114]
- American Psychiatric Association. Diagnostic and statistical manual of mental disorders DSM-IV-TR. 4. Washington, DC: American Psychiatric Press, Inc; 2000. text revision ed
- Anandam N, Felegi W, Stern JM. In utero alcohol heightens juvenile reactivity. *Pharmacol Biochem Behav.* 1980; 13(4):531–535. [PubMed: 7433484]
- Anisman H, Matheson K. Stress, depression, and anhedonia: Caveats concerning animal models. *Neurosci Biobehav Rev.* 2005; 29(4–5):525–546. [PubMed: 15925696]
- Arana GW, Baldessarini RJ, Ornstein M. The dexamethasone suppression test for diagnosis and prognosis in psychiatry. Commentary and review. *Arch Gen Psychiatry.* 1985; 42(12):1193–204. [PubMed: 3000317]
- Archer J. Tests for emotionality in rats and mice: A review. *Anim Behav.* 1973; 21(2):205–235. [PubMed: 4578750]
- Astley SJ, Olson HC, Kerns K, Brooks A, Aylward EH, Coggins TE, Davies J, Dorn S, Gendler B, Jirikowic T, Kraegel P, Maravilla K, Richards T. Neuropsychological and behavioral outcomes from a comprehensive magnetic resonance study of children. *Can J Clin Pharmacol.* 2009; 16(1):178–201.
- Barden N. Implication of the hypothalamic-pituitary-adrenal axis in the pathophysiology of depression. *J Psychiatry Neurosci.* 2004; 29(3):185–193. [PubMed: 15173895]
- Barr CS, Newman TK, Lindell S, Shannon C, Champoux M, Lesch KP, et al. Interaction between serotonin transporter gene variation and rearing condition in alcohol preference and consumption in female primates. *Arch Gen Psychiatry.* 2004; 61(11):1146–1152. [PubMed: 15520362]
- Bernet CZ, Stein MB. Relationship of childhood maltreatment to the onset and course of major depression in adulthood. *Depress Anxiety.* 1999; 9(4):169–174. [PubMed: 10431682]
- Berridge KC, Robinson TE. What is the role of dopamine in reward: Hedonic impact, reward learning, or incentive salience? *Brain Res. Brain Res Rev.* 1998; 28(3):309–369. [PubMed: 9858756]
- Bertoglio LJ, Joca SR, Guimaraes FS. Further evidence that anxiety and memory are regionally dissociated within the hippocampus. *Behav Brain Res.* 2006; 175(1):183–188. [PubMed: 16996146]
- Berton O, Nestler EJ. New approaches to antidepressant drug discovery: Beyond monoamines. *Nat Rev Neurosci.* 2006; 7(2):137–151. [PubMed: 16429123]
- Board F, Wadeson R, Persky H. Depressive affect and endocrine functions; blood levels of adrenal cortex and thyroid hormones in patients suffering from depressive reactions. *AMA Arch Neurol Psychiatry.* 1957; 78(6):612–620. [PubMed: 13478217]
- Bond NW, Di Giusto EL. Effects of prenatal alcohol consumption on open-field behaviour and alcohol preference in rats. *Psychopharmacologia.* 1976; 46(2):163–165. [PubMed: 951453]
- Bond NW, DiGiusto EL. Effects of prenatal alcohol consumption on shock avoidance learning in rats. *Psychol Rep.* 1977; 41(3 pt 2):1269–1270. [PubMed: 601158]

- Braddock L. The dexamethasone suppression test. Fact and artefact. *Br J Psychiatry*. 1986; 148:363–74. [PubMed: 3524723]
- Bruijnzeel AW, Repetto M, Gold MS. Neurobiological mechanisms in addictive and psychiatric disorders. *Psychiatr Clin North Am*. 2004; 27(4):661–674. [PubMed: 15550286]
- Burd L, Klug MG, Martsolf JT, Kerbeshian J. Fetal alcohol syndrome: Neuropsychiatric phenomics. *Neurotoxicol Teratol*. 2003; 25(6):697–705. [PubMed: 14624969]
- Caldji C, Diorio J, Meaney MJ. Variations in maternal care in infancy regulate the development of stress reactivity. *Biol Psychiatry*. 2000; 48(12):1164–1174. [PubMed: 11137058]
- Calfa G, Bussolino D, Molina VA. Involvement of the lateral septum and the ventral hippocampus in the emotional sequelae induced by social defeat: Role of glucocorticoid receptors. *Behav Brain Res*. 2007; 181(1):23–34. [PubMed: 17445915]
- Cameron OG. Anxious-depressive comorbidity: Effects on HPA axis and CNS noradrenergic functions. *Essent Psychopharmacol*. 2006; 7(1):24–34. [PubMed: 16989290]
- Cameron OG, Abelson JL, Young EA. Anxious and depressive disorders and their comorbidity: Effect on central nervous system noradrenergic function. *Biol Psychiatry*. 2004; 56(11):875–883. [PubMed: 15576065]
- Carneiro LM, Diogenes JP, Vasconcelos SM, Aragao GF, Noronha EC, Gomes PB, et al. Behavioral and neurochemical effects on rat offspring after prenatal exposure to ethanol. *Neurotoxicol Teratol*. 2005; 27(4):585–592. [PubMed: 16039829]
- Carroll BJ. The dexamethasone suppression test for melancholia. *Br J Psychiatry*. 1982; 140:292–304. [PubMed: 7093598]
- Chalmers DT, Kwak SP, Mansour A, Akil H, Watson SJ. Corticosteroids regulate brain hippocampal 5-HT_{1A} receptor mRNA expression. *J Neurosci*. 1993; 13:914. [PubMed: 8441016]
- Chernoff GF. The fetal alcohol syndrome in mice: An animal model. *Teratology*. 1977; 15(3):223–229. [PubMed: 560725]
- Cruz AP, Frei F, Graeff FG. Ethopharmacological analysis of rat behavior on the elevated plus-maze. *Pharmacol Biochem Behav*. 1994; 49(1):171–176. [PubMed: 7816869]
- Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: Recent developments and future needs. *Trends Pharmacol Sci*. 2002; 23:238–245.
- Cryan JF, Valentino RJ, Lucki I. Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. *Neurosci Biobehav Rev*. 2005; 29(4–5):547–569. [PubMed: 15893822]
- Cryan JF, Slattery DA. Animal models of mood disorders: Recent developments. *Curr Opin Psychiatry*. 2007; 20(1):1–7. [PubMed: 17143074]
- Dalla C, Antoniou K, Drossopoulou G, Xagoraris M, Kokras N, Sfikakis A, et al. Chronic mild stress impact: Are females more vulnerable? *Neuroscience*. 2005; 135(3):703–714. [PubMed: 16125862]
- Dallman MF, Akana SF, Cascio CS, Darlington DN, Jacobson L, Levin N. Regulation of ACTH secretion: Variations on a theme of B. *Recent Prog Horm Res*. 1987; 43:113–173. [PubMed: 2819993]
- Dallman MF, Pecoraro N, Akana SF, La Fleur SE, Gomez F, Houshyar H, et al. Chronic stress and obesity: A new view of “comfort food”. *Proc Natl Acad Sci USA*. 2003; 100(20):11696–11701. [PubMed: 12975524]
- Davidson RJ, Pizzagalli D, Nitschke JB, Putnam K. Depression: Perspectives from affective neuroscience. *Annu Rev Psychol*. 2002; 53:545–574. [PubMed: 11752496]
- de Goeij DC, Kvetnansky R, Whitnall MH, Jezova D, Berkenbosch F, Tilders FJ. Repeated stress-induced activation of corticotropin-releasing factor neurons enhances vasopressin stores and colocalization with corticotropin-releasing factor in the median eminence of rats. *Neuroendocrinology*. 1991; 53(2):150–159. [PubMed: 1849619]
- de Kloet ER, Joels M, Holsboer F. Stress and the brain: From adaptation to disease. *Nat Rev Neurosci*. 2005; 6(6):463–475. [PubMed: 15891777]
- de Kloet ER, Vreugdenhil E, Oitzl MS, Joels M. Brain corticosteroid receptor balance in health and disease. *Endocr Rev*. 1998; 19(3):269–301. [PubMed: 9626555]

- Deuschle M, Schweiger U, Weber B, Gotthardt U, Korner A, Schmider J, et al. Diurnal activity and pulsatility of the hypothalamus-pituitary-adrenal system in male depressed patients and healthy controls. *J Clin Endocrinol Metab.* 1997; 82(1):234–238. [PubMed: 8989265]
- Deuschle M, Schweiger U, Gotthardt U, Weber B, Körner A, Schmider J, Standhardt H, Lammers CH, Krumm B, Heuser I. The combined dexamethasone/corticotropin-releasing hormone stimulation test is more closely associated with features of diurnal activity of the hypothalamo-pituitary-adrenocortical system than the dexamethasone suppression test. *Biol Psychiatry.* 1998; 43(10): 762–6. [PubMed: 9606531]
- Dinan T. Novel approaches to the treatment of depression by modulating the hypothalamic -pituitary -adrenal axis. *Hum Psychopharmacol.* 2001; 16(1):89–93. [PubMed: 12404603]
- Dinan TG. Stress: The shared common component in major mental illnesses. *Eur Psychiatry.* 2005; 20(Suppl 3):S326–8. [PubMed: 16459244]
- Dinan TG, Scott LV. Anatomy of melancholia: Focus on hypothalamic-pituitary-adrenal axis overactivity and the role of vasopressin. *J Anat.* 2005; 207(3):259–264. [PubMed: 16185250]
- Doig J, McLennan JD, Gibbard WB. Medication effects on symptoms of attention-deficit/hyperactivity disorder in children with fetal alcohol spectrum disorder. *J Child Adolesc Psychopharmacol.* 2008; 18(4):365–371. [PubMed: 18759646]
- Druse, MJ. Neurotransmitter function: Changes associated with in utero alcohol exposure. In: Abel, EL., editor. *Fetal alcohol syndrome: From mechanisms to prevention.* CRC Press; 1996. p. 171-189.
- Druse MJ, Kuo A, Tajuddin N. Effects of in utero ethanol exposure on the developing serotonergic system. *Alcohol Clin Exp Res.* 1991; 15(4):678–684. [PubMed: 1928643]
- Elgen I, Bruaroy S, Laegreid LM. Complexity of foetal alcohol or drug neuroimpairments. *Acta Paediatr.* 2007; 96(12):1730–1733. [PubMed: 17971190]
- Famy C, Streissguth AP, Unis AS. Mental illness in adults with fetal alcohol syndrome or fetal alcohol effects. *Am J Psychiatry.* 1998; 155(4):552–554. [PubMed: 9546004]
- File SE, Hyde JR. Can social interaction be used to measure anxiety? *Br. J Pharmacol.* 1978; 62(1):19–24.
- Fisher PA, Gunnar MR, Dozier M, Bruce J, Pears KC. Effects of therapeutic interventions for foster children on behavioral problems, caregiver attachment, and stress regulatory neural systems. *Ann NY Acad Sci.* 2006; 1094:215–225. [PubMed: 17347353]
- Fisher PA, Stoolmiller M, Gunnar MR, Burraston BO. Effects of a therapeutic intervention for foster preschoolers on diurnal cortisol activity. *Psychoneuroendocrinology.* 2007; 32(8–10):892–905. [PubMed: 17656028]
- Flaherty C, Geary N. Contrast in consummatory behavior. *Appetite.* 1993; 21(1):81. [PubMed: 8239637]
- Flaherty CF, Turovsky J, Krauss KL. Relative hedonic value modulates anticipatory contrast. *Physiol Behav.* 1994; 55(6):1047–1054. [PubMed: 8047570]
- Forbes NF, Stewart CA, Matthews K, Reid IC. Chronic mild stress and sucrose consumption: Validity as a model of depression. *Physiol Behav.* 1996; 60(6):1481–1484. [PubMed: 8946494]
- Francis D, Diorio J, LaPlante P, Weaver S, Seckl JR, Meaney MJ. The role of early environmental events in regulating neuroendocrine development. moms, pups, stress, and glucocorticoid receptors. *Ann NY Acad Sci.* 1996; 794:136–152. [PubMed: 8853600]
- Gabriel KI, Glavas MM, Ellis LI, Weinberg J. Postnatal handling does not normalize corticotropin releasing factor mRNA levels in animals prenatally exposed to ethanol. *Devel Brain Res.* 2005; 157:74–82. [PubMed: 15939087]
- Gabriel KI, Yu CL, Osborn JA, Weinberg J. Prenatal ethanol exposure alters sensitivity to the effects of corticotropin-releasing factor (CRF) on behavior in the elevated plus-maze. *Psychoneuroendocrinology.* 2006; 31(9):1046–1056. [PubMed: 16934947]
- Gallo PV, Weinberg J. Corticosterone rhythmicity in the rat: Interactive effects of dietary restriction and schedule of feeding. *J Nutr.* 1981; 111(2):208–218. [PubMed: 7463165]
- Gallo PV, Weinberg J. Neuromotor development and response inhibition following prenatal ethanol exposure. *Neurobehav Toxicol Teratol.* 1982; 4(5):505–513. [PubMed: 7177300]

- Gerra G, Leonardi C, Cortese E, Zaimovic A, Dell'agnello G, Manfredini M, et al. Childhood neglect and parental care perception in cocaine addicts: Relation with psychiatric symptoms and biological correlates. *Neurosci Biobehav Rev*. 2007
- Geyer, MA., Markou, A. Animal models of psychiatric disorders. In: Bloom, FE., Kupfer, DJ., editors. *Psychopharmacology - the 4th generation of progress*. New York: Raven; 1995. p. 787-798.
- Gibbons JL. Cortisol secretion rate in depressive illness. *Arch Gen Psychiatry*. 1964; 10:572–575. [PubMed: 14159257]
- Gilman SE, Kawachi I, Fitzmaurice GM, Buka SL. Socioeconomic status in childhood and the lifetime risk of major depression. *Int J Epidemiol*. 2002; 31(2):359–367. [PubMed: 11980797]
- Gilman SE, Kawachi I, Fitzmaurice GM, Buka SL. Family disruption in childhood and risk of adult depression. *Am J Psychiatry*. 2003; 160(5):939–946. [PubMed: 12727699]
- Glavas MM, Yu WK, Weinberg J. Effects of mineralocorticoid and glucocorticoid receptor blockade on hypothalamic-pituitary-adrenal function in female rats prenatally exposed to ethanol. *Alcohol Clin Exp Res*. 2006; 30(11):1916–24. [PubMed: 17067357]
- Glavas MM, Ellis L, Yu WK, Weinberg J. Effects of prenatal ethanol exposure on basal limbic-hypothalamic-pituitary-adrenal regulation: Role of corticosterone. *Alcohol Clin Exp Res*. 2007; 31:598–610.
- Glavas MM, Hofmann CE, Yu WK, Weinberg J. Effects of prenatal ethanol exposure on hypothalamic-pituitary-adrenal regulation after adrenalectomy and corticosterone replacement. *Alcohol Clin Exp Res*. 2001; 25(6):890–897. [PubMed: 11410726]
- Gluckman PD, Hanson MA. The developmental origins of the metabolic syndrome. *Trends Endocrinol Metab*. 2004; 15(4):183–187. [PubMed: 15109618]
- Goeders NE. The impact of stress on addiction. *Eur Neuropsychopharmacol*. 2003; 13(6):435–441. [PubMed: 14636959]
- Gold PW, Chrousos GP. Organization of the stress system and its dysregulation in melancholic and atypical depression: High vs low CRH/NE states. *Mol Psychiatry*. 2002; 7(3):254–275. [PubMed: 11920153]
- Gorman JM. Comorbid depression and anxiety spectrum disorders. *Depress Anxiety*. 1996; 4(4):160–168. [PubMed: 9166648]
- Gutman DA, Nemeroff CB. Persistent central nervous system effects of an adverse early environment: Clinical and preclinical studies. *Physiol Behav*. 2003; 79(3):471–478. [PubMed: 12954441]
- Halbreich U, Asnis GM, Shindledecker R, Zumoff B, Nathan RS. Cortisol secretion in endogenous depression. I Basal plasma levels. *Arch Gen Psychiatry*. 1985; 42(9):904–8. [PubMed: 4037990]
- Halbreich U, Asnis GM, Shindledecker R, Zumoff B, Nathan RS. Cortisol secretion in endogenous depression. II Time-related functions. *Arch Gen Psychiatry*. 1985; 42(9):909–14. [PubMed: 4037991]
- Haley DW, Handmaker NS, Lowe J. Infant stress reactivity and prenatal alcohol exposure. *Alcohol Clin Exp Res*. 2006; 30(12):2055–2064. [PubMed: 17117971]
- Harkness KL, Monroe SM. Childhood adversity and the endogenous versus nonendogenous distinction in women with major depression. *Am J Psychiatry*. 2002; 159(3):387–393. [PubMed: 11870001]
- Heim C, Newport DJ, Heit S, Graham YP, Wilcox M, Bonsall R, et al. Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. *JAMA*. 2000; 284(5):592–597. [PubMed: 10918705]
- Heinrichs SC, Menzaghi F, Merlo Pich E, Britton KT, Koob GF. The role of CRF in behavioral aspects of stress. *Ann N Y Acad Sci*. 1995; 771:92–104. [PubMed: 8597448]
- Hellemans K, Verma P, Yoon E, Yu W, Weinberg J. Prenatal alcohol exposure increases vulnerability to stress and anxiety-like disorders in adulthood. *Ann N Y Acad Sci*. 2008; 1144:154–75. [PubMed: 19076375]
- Heuser I, Yassouridis A, Holsboer FJ. The combined dexamethasone/CRH test: a refined laboratory test for psychiatric disorders. *Psychiatr Res*. 1994; 28(4):341–56.
- Hindmarch I. Beyond the monoamine hypothesis: Mechanisms, molecules and methods. *Eur Psychiatry*. 2002; 17(Suppl 3):294–299.

- Hofmann C, Glavas M, Yu W, Weinberg J. Glucocorticoid fast feedback is not altered in rats prenatally exposed to ethanol. *Alcohol Clin Exp Res*. 1999; 23(5):891–900. [PubMed: 10371411]
- Hofmann CE, Ellis L, Yu WK, Weinberg J. Hypothalamic-pituitary-adrenal responses to 5-HT1A and 5-HT2A/C agonists are differentially altered in female and male rats prenatally exposed to ethanol. *Alcohol Clin Exp Res*. 2007; 31(2):345–355. [PubMed: 17250628]
- Hofmann CE, Patyk IA, Weinberg J. Prenatal ethanol exposure: Sex differences in anxiety and anxiolytic response to a 5-HT1A agonist. *Pharmacol Biochem Behav*. 2005; 82(3):549–558. [PubMed: 16359722]
- Hofmann CE, Simms W, Yu WK, Weinberg J. Prenatal ethanol exposure in rats alters serotonergic-mediated behavioral and physiological function. *Psychopharmacology (Berl)*. 2002; 161(4):379–386. [PubMed: 12073165]
- Holsboer F. The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology*. 2000; 23(5):477–501. [PubMed: 11027914]
- Holsboer F. Stress, hypercortisolism and corticosteroid receptors in depression: Implications for therapy. *J Affect Disord*. 2001; 62(1–2):77–91. [PubMed: 11172875]
- Ising M, Kunzel HE, Binder EB, Nickel T, Modell S, Holsboer F. The combined dexamethasone/CRH test as a potential surrogate marker in depression. *Prog Neuropsychopharmacol Biol Psychiatry*. 2005; 29(6):1085–1093. [PubMed: 15950349]
- Jacobs BL, Praag H, Gage FH. Adult brain neurogenesis and psychiatry: A novel theory of depression. *Mol Psychiatry*. 2000; 5(3):262–269. [PubMed: 10889528]
- Jacobson SW, Bihun JT, Chiodo LM. Effects of prenatal alcohol and cocaine exposure on infant cortisol levels. *Dev Psychopathol*. 1999; 11:195–208. [PubMed: 16506530]
- Johnston AL, File SE. Sex differences in animal tests of anxiety. *Physiol Behav*. 1991; 49(2):245–250. [PubMed: 2062894]
- Jones KL, Smith DW. Recognition of the fetal alcohol syndrome in early infancy. *Lancet*. 1973; 2(7836):999–1001.
- Jones KL, Smith DW, Ulleland CN, Streissguth P. Pattern of malformation in offspring of chronic alcoholic mothers. *Lancet*. 1973; 1(7815):1267–1271. [PubMed: 4126070]
- Jones MT, Gillham B. Factors involved in the regulation of adrenocorticotrophic hormone/beta-lipotrophic hormone. *Physiol Rev*. 1988; 68(3):743–818. [PubMed: 2839858]
- Kalueff AV, Wheaton M, Murphy DL. What's wrong with my mouse model? advances and strategies in animal modeling of anxiety and depression. *Behav Brain Res*. 2007; 179(1):1–18. [PubMed: 17306892]
- Katz MM, Shulman R, Robins E. Behavioral aspects and relationships to outcome [proceedings. *Psychopharmacol Bull*. 1981; 17(1):69–70. [PubMed: 7232661]
- Kaufman J, Plotsky PM, Nemeroff CB, Charney DS. Effects of early adverse experiences on brain structure and function: Clinical implications. *Biol Psychiatry*. 2000; 48(8):778–790. [PubMed: 11063974]
- Keck ME. Corticotropin-releasing factor, vasopressin and receptor systems in depression and anxiety. *Amino Acids*. 2006; 31(3):241–250. [PubMed: 16733617]
- Kessler RC. The effects of stressful life events on depression. *Annu Rev Psychol*. 1997; 48:191–214. [PubMed: 9046559]
- Kim CK, Giberson PK, Yu W, Weinberg J. Response to cold stress following prenatal ethanol exposure. [Abstract]. *Soc Neurosci Abstr*. 1996; 22:1342.
- Kim, CK., Osborn, JA., Weinberg, J. Stress reactivity in fetal alcohol syndrome. In: Abel, E., editor. *Fetal alcohol syndrome: From mechanism to behavior*. CRC Press; 1996. p. 215–236.
- Kim CK, Giberson PK, Yu W, Zoeller RT, Weinberg J. Effects of prenatal ethanol exposure on hypothalamic-pituitary-adrenal responses to chronic cold stress in rats. *Alcohol Clin Exp Res*. 1999; 23(2):301–310. [PubMed: 10069560]
- Kim CK, Kalynchuk LE, Kornecook TJ, Mumby DG, Dadgar NA, Pinel JP, et al. Object-recognition and spatial learning and memory in rats prenatally exposed to ethanol. *Behav Neurosci*. 1997; 111(5):985–995. [PubMed: 9383519]

- Kim CK, Turnbull AV, Lee SY, Rivier CL. Effects of prenatal exposure to alcohol on the release of adrenocorticotrophic hormone, corticosterone, and proinflammatory cytokines. *Alcohol Clin Exp Res.* 1999; 23(1):52–59. [PubMed: 10029203]
- Kodituwakku P, Coriale G, Fiorentino D, Aragon AS, Kalberg WO, Buckley D, et al. Neurobehavioral characteristics of children with fetal alcohol spectrum disorders in communities from Italy: Preliminary results. *Alcohol Clin Exp Res.* 2006; 30(9):1551–1561. [PubMed: 16930218]
- Kodituwakku PW. Defining the behavioral phenotype in children with fetal alcohol spectrum disorders: A review. *Neurosci Biobehav Rev.* 2007; 31(2):192–201. [PubMed: 16930704]
- Ladd CO, Huot RL, Thirivikraman KV, Nemeroff CB, Meaney MJ, Plotsky PM. Long-term behavioral and neuroendocrine adaptations to adverse early experience. *Prog Brain Res.* 2000; 122:81–103. [PubMed: 10737052]
- Lee S, Imaki T, Vale W, Rivier C. Effect of prenatal exposure to ethanol on the activity of the hypothalamic-pituitary-adrenal axis of the offspring: Importance of the time of exposure to ethanol and possible modulating mechanisms. *Molec Cell Neurosci.* 1990; 1:168–177. [PubMed: 19912768]
- Lee S, Rivier C. Gender differences in the effect of prenatal alcohol exposure on the hypothalamic-pituitary-adrenal axis response to immune signals. *Psychoneuroendocrinology.* 1996; 21(2):145–155. [PubMed: 8774059]
- Lee S, Schmidt D, Tilders F, Rivier C. Increased activity of the hypothalamic-pituitary-adrenal axis of rats exposed to alcohol in utero: Role of altered pituitary and hypothalamic function. *Mol Cell Neurosci.* 2000; 16(4):515–528. [PubMed: 11085885]
- Leichter J, Lee M. Effect of maternal ethanol administration on physical growth of the offspring in rats. *Growth.* 1979; 43(4):288–293. [PubMed: 546691]
- Lemoine P, Harousseau H, Borteyru JP, Menuet JC. Children of alcoholic parents--observed anomalies: Discussion of 127 cases. *Ther Drug Monit.* 2003; 25(2):132–136. [PubMed: 12657907]
- Lenze EJ, Mulsant BH, Shear MK, Alexopoulos GS, Frank E, Reynolds CF 3rd. Comorbidity of depression and anxiety disorders in later life. *Depress Anxiety.* 2001; 14(2):86–93. [PubMed: 11668661]
- Leviton RD, Rector NA, Sheldon T, Goering P. Childhood adversities associated with major depression and/or anxiety disorders in a community sample of ontario: Issues of co-morbidity and specificity. *Depress Anxiety.* 2003; 17(1):34–42. [PubMed: 12577276]
- Lifshytz T, Shalom G, Lerer B, Newman ME. Sex-dependent effects of fluoxetine and triiodothyronine in the forced swim test in rats. *Eur Neuropsychopharmacol.* 2006; 16(2):115–121. [PubMed: 16112557]
- Lister RG. The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology (Berl).* 1987; 92(2):180–185. [PubMed: 3110839]
- Lloyd AJ, Ferrier IN, Barber R, Gholkar A, Young AH, O'Brien JT. Hippocampal volume change in depression: Late- and early-onset illness compared. *Br J Psychiatry.* 2004; 184:488–495. [PubMed: 15172942]
- Lochry EA, Riley EP. Retention of passive avoidance and T-maze escape in rats exposed to alcohol prenatally. *Neurobehav Toxicol.* 1980; 2:107–115. [PubMed: 7290306]
- Lopez JF, Chalmers DT, Little KY, Watson SJ. A.E. bennett research award. regulation of serotonin1A, glucocorticoid, and mineralocorticoid receptor in rat and human hippocampus: Implications for the neurobiology of depression. *Biol Psychiatry.* 1998; 43(8):547–573. [PubMed: 9564441]
- MacMillan HL, Fleming JE, Streiner DL, Lin E, Boyle MH, Jamieson E, et al. Childhood abuse and lifetime psychopathology in a community sample. *Am J Psychiatry.* 2001; 158(11):1878–1883. [PubMed: 11691695]
- Mannie ZN, Harmer CJ, Cowen PJ. Increased waking salivary cortisol levels in young people at familial risk of depression. *Am J Psychiatry.* 2007; 164(4):617–621. [PubMed: 17403975]
- Manning MA, Hoyme HE. Fetal alcohol spectrum disorders: A practical clinical approach to diagnosis. *Neurosci Biobehav Rev.* 2007; 31(2):230–238. [PubMed: 16962173]
- Maren S. Building and burying fear memories in the brain. *Neuroscientist.* 2005; 11(1):89–99. [PubMed: 15632281]

- Martin JC, Martin DC, Sigman G, Radow B. Maternal ethanol consumption and hyperactivity in cross-fostered offspring. *Physiol Psychol*. 1978; 6:362–365.
- Matthews K, Christmas D, Swan J, Sorrell E. Animal models of depression: Navigating through the clinical fog. *Neurosci Biobehav Rev*. 2005; 29(4–5):503–513. [PubMed: 15925695]
- Matthews K, Forbes N, Reid IC. Sucrose consumption as an hedonic measure following chronic unpredictable mild stress. *Physiol Behav*. 1995; 57(2):241–248. [PubMed: 7716198]
- Matthews SG. Antenatal glucocorticoids and programming of the developing CNS. *Pediatr Res*. 2000; 47(3):291–300. [PubMed: 10709726]
- Matthews SG, Owen D, Banjanin S, Andrews MH. Glucocorticoids, hypothalamo-pituitary-adrenal (HPA) development, and life after birth. *Endocr Res*. 2002; 28(4):709–718. [PubMed: 12530687]
- Mattson SN, Schoenfeld AM, Riley EP. Teratogenic effects of alcohol on brain and behavior. *Alcohol Res Health*. 2001; 25(3):185–191. [PubMed: 11810956]
- Mayberg HS. Limbic-cortical dysregulation: A proposed model of depression. *J Neuropsychiatry Clin Neurosci*. 1997; 9(3):471–481. [PubMed: 9276848]
- McArthur R, Borsini F. Animal models of depression in drug discovery: A historical perspective. *Pharmacol Biochem Behav*. 2006; 84(3):436–452. [PubMed: 16844210]
- McEwen BS. Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. *Ann N Y Acad Sci*. 2004; 1032:1–7. [PubMed: 15677391]
- McKinney WT Jr, Bunney WE Jr. Animal model of depression. I. review of evidence: Implications for research. *Arch Gen Psychiatry*. 1969; 21(2):240–248. [PubMed: 4980592]
- Meaney MJ. Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annu Rev Neurosci*. 2001; 24:1161–1192. [PubMed: 11520931]
- Mineka S, Watson D, Clark LA. Comorbidity of anxiety and unipolar mood disorders. *Annu Rev Psychol*. 1998; 49:377–412. [PubMed: 9496627]
- Mothes HK, Opitz B, Werner R, Clausen P. Effects of prenatal ethanol exposure and early experience on home-cage and open-field activity in mice. *Neurotoxicol Teratol*. 1996; 18(1):59–65. [PubMed: 8700044]
- Nash JR, Sargent PA, Rabiner EA, Hood SD, Argyropoulos SV, Potokar JP, et al. Serotonin 5-HT1A receptor binding in people with panic disorder: Positron emission tomography study. *Br J Psychiatry*. 2008; 193(3):229–234. [PubMed: 18757983]
- Nemeroff CB, Vale WW. The neurobiology of depression: Inroads to treatment and new drug discovery. *J Clin Psychiatry*. 2005; 66(Supplement 7):5–13.
- Nemeroff CB. Comorbidity of mood and anxiety disorders: The rule, not the exception? *Am J Psychiatry*. 2002; 159(1):3–4.
- Nemeroff CB, Owens MJ, Bissette G, Andorn AC, Stanley M. Reduced corticotropin releasing factor binding sites in the frontal cortex of suicide victims. *Arch Gen Psychiatry*. 1988; 45(6):577–579. [PubMed: 2837159]
- Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. *Neuron*. 2002; 34(1):13–25. [PubMed: 11931738]
- Nestler EJ, Carlezon WA Jr. The mesolimbic dopamine reward circuit in depression. *Biol Psychiatry*. 2006; 59(12):1151–1159. [PubMed: 16566899]
- Nunes EV, Rounsaville BJ. Comorbidity of substance use with depression and other mental disorders: From diagnostic and statistical manual of mental disorders, fourth edition (DSM-IV) to DSM-V. *Addiction*. 2006; 101(Suppl 1):89–96. [PubMed: 16930164]
- Nutt DJ. Relationship of neurotransmitters to the symptoms of major depressive disorder. *J Clin Psychiatry*. 2008; 69(Suppl E1):4–7.
- Nutt DJ, Stein DJ. Understanding the neurobiology of comorbidity in anxiety disorders. *CNS Spectr*. 2006; 11(10 Suppl 12):13–20.
- O'Connor MJ, Kasari C. Prenatal alcohol exposure and depressive features in children. *Alcohol Clin Exp Res*. 2000; 24(7):1084–1092. [PubMed: 10924014]
- O'Connor MJ, Paley B. The relationship of prenatal alcohol exposure and the postnatal environment to child depressive symptoms. *J Pediatr Psychol*. 2006; 31(1):50–64. [PubMed: 15802607]

- O'Connor MJ, Shah B, Whaley S, Cronin P, Gunderson B, Graham J. Psychiatric illness in a clinical sample of children with prenatal alcohol exposure. *Am J Drug Alcohol Abuse*. 2002; 28(4):743–754. [PubMed: 12492268]
- O'Connor TM, O'Halloran DJ, Shanahan F. The stress response and the hypothalamic-pituitary-adrenal axis: From molecule to melancholia. *QJM*. 2000; 93(6):323–333. [PubMed: 10873181]
- Olson HC, Morse BA, Huffine C. Development and psychopathology: Fetal alcohol syndrome and related conditions. *Semin Clin Neuropsychiatry*. 1998; 3(4):262–284. [PubMed: 10085215]
- O'Malley KD, Nanson J. Clinical implications of a link between fetal alcohol spectrum disorder and attention-deficit hyperactivity disorder. *Can J Psychiatry*. 2002; 47(4):349–354. [PubMed: 12025433]
- Osborn JA, Kim CK, Steiger J, Weinberg J. Prenatal ethanol exposure differentially alters behavior in males and females on the elevated plus maze. *Alcohol Clin Exp Res*. 1998; 22(3):685–696. [PubMed: 9622451]
- Osborn JA, Kim CK, Yu W, Herbert L, Weinberg J. Fetal ethanol exposure alters pituitary-adrenal sensitivity to dexamethasone suppression. *Psychoneuroendocrinology*. 1996; 21(2):127–143. [PubMed: 8774058]
- Osborn JA, Yu C, Gabriel K, Weinberg J. Fetal ethanol effects on benzodiazepine sensitivity measured by behavior on the elevated plus-maze. *Pharmacol Biochem Behav*. 1998; 60(3):625–633. [PubMed: 9678645]
- Osborn JA, Yu C, Stelzl GE, Weinberg J. Effects of fetal ethanol exposure on pituitary-adrenal sensitivity to secretagogues. *Alcohol Clin Exp Res*. 2000; 24(7):1110–1119. [PubMed: 10924017]
- Palanza P. Animal models of anxiety and depression: How are females different? *Neurosci Biobehav Rev*. 2001; 25(3):219–233.
- Parker KJ, Schatzberg AF, Lyons DM. Neuroendocrine aspects of hypercortisolism in major depression. *Horm Behav*. 2003; 43(1):60–66. [PubMed: 12614635]
- Patchev VK, Hayashi S, Orikasa C, Almeida OF. Implications of estrogen-dependent brain organization for gender differences in hypothalamo-pituitary-adrenal regulation. *FASEB J*. 1995; 9(5):419–423. [PubMed: 7896013]
- Pellow S, Chopin P, File SE, Briley M. Validation of open: Closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods*. 1985; 14(3):149–167. [PubMed: 2864480]
- Pellow S, File SE. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: A novel test of anxiety in the rat. *Pharmacol Biochem Behav*. 1986; 24(3):525–529. [PubMed: 2871560]
- Pettinati HM. Antidepressant treatment of co-occurring depression and alcohol dependence. *Biol Psychiatry*. 2004; 56(10):785–792. [PubMed: 15556124]
- Phillips DI, Barker DJ, Fall CH, Seckl JR, Whorwood CB, Wood PJ, et al. Elevated plasma cortisol concentrations: A link between low birth weight and the insulin resistance syndrome? *J Clin Endocrinol Metab*. 1998; 83(3):757–760. [PubMed: 9506721]
- Phillips DI, Walker BR, Reynolds RM, Flanagan DE, Wood PJ, Osmond C, et al. Low birth weight predicts elevated plasma cortisol concentrations in adults from 3 populations. *Hypertension*. 2000; 35(6):1301–1306. [PubMed: 10856281]
- Placidi GP, Oquendo MA, Malone KM, Huang YY, Ellis SP, Mann JJ. Aggressivity, suicide attempts, and depression: Relationship to cerebrospinal fluid monoamine metabolite levels. *Biol Psychiatry*. 2001; 50(10):783–791. [PubMed: 11720697]
- Porsolt RD. Animal model of depression. *Biomedicine*. 1979; 30(3):139–140. [PubMed: 573643]
- Porsolt RD, Anton G, Blavet N, Jalfre M. Behavioural despair in rats: A new model sensitive to antidepressant treatments. *Eur J Pharmacol*. 1978; 47(4):379–391. [PubMed: 204499]
- Prut L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. *Eur J Pharmacol*. 2003; 463(1–3):3–33. [PubMed: 12600700]
- Pryce CR, Feldon J. Long-term neurobehavioural impact of the postnatal environment in rats: Manipulations, effects and mediating mechanisms. *Neurosci Biobehav Rev*. 2003; 27(1–2):57–71. [PubMed: 12732223]

- Pryce CR, Ruedi-Bettschen D, Dettling AC, Weston A, Russig H, Ferger B, et al. Long-term effects of early-life environmental manipulations in rodents and primates: Potential animal models in depression research. *Neurosci Biobehav Rev*. 2005; 29(4–5):649–674. [PubMed: 15925698]
- Rainnie DG, Bergeron R, Sajdyk TJ, Patil M, Gehlert DR, Shekhar A. Corticotrophin releasing factor-induced synaptic plasticity in the amygdala translates stress into emotional disorders. *J Neurosci*. 2004; 24(14):3471–3479. [PubMed: 15071094]
- Ramsay DS, Bendersky MI, Lewis M. Effect of prenatal alcohol and cigarette exposure on two- and six-month-old infants' adrenocortical reactivity to stress. *J Pediatr Psychol*. 1996; 21(6):833–840. [PubMed: 8990727]
- Randall CL, Taylor J, Walker DW. Ethanol-induced malformations in mice. *Alcohol Clin Exp Res*. 1977; 1(3):219–224. [PubMed: 337841]
- Rathbun W, Druse MJ. Dopamine, serotonin, and acid metabolites in brain regions from the developing offspring of ethanol-treated rats. *J Neurochem*. 1985; 44(1):57–62. [PubMed: 2578063]
- Redei E, Clark WR, McGivern RF. Alcohol exposure in utero results in diminished T-cell function and alterations in brain corticotropin-releasing factor and ACTH content. *Alcohol Clin Exp Res*. 1989; 13(3):439–43. [PubMed: 2546466]
- Redei E, Halasz I, Li LF, Prystowsky MB, Aird F. Maternal adrenalectomy alters the immune and endocrine functions of fetal alcohol-exposed male offspring. *Endocrinology*. 1993; 133(2):452–460. [PubMed: 8344191]
- Redei EE, Ahmadiyeh N, Baum AE, Sasso DA, Slone JL, Solberg LC, et al. Novel animal models of affective disorders. *Semin Clin Neuropsychiatry*. 2001; 6(1):43–67. [PubMed: 11172532]
- Riley EP, Baron S, Hannigan JH. Response inhibition deficits following prenatal alcohol exposure: A comparison to the effects of hippocampal lesions in rats. In: West, JR., editor. *Alcohol and brain development*. New York: Oxford Univ Press; 1986. p. 7-102.
- Riley EP, Lochry EA, Shapiro NR. Lack of response inhibition in rats prenatally exposed to alcohol. *Psychopharmacology (Berl)*. 1979; 62(1):47–52. [PubMed: 108747]
- Riley EP, McGee CL. Fetal alcohol spectrum disorders: An overview with emphasis on changes in brain and behavior. *Exp Biol Med (Maywood)*. 2005; 230(6):357–365. [PubMed: 15956765]
- Rivier CL, Plotsky PM. Mediation by corticotropin releasing factor (CRF) of adenohipophysial hormone secretion. *Annu Rev Physiol*. 1986; 48:475–494. [PubMed: 2871808]
- Roebuck TM, Mattson SN, Riley EP. Behavioral and psychosocial profiles of alcohol-exposed children. *Alcohol Clin Exp Res*. 1999; 23(6):1070–1076. [PubMed: 10397293]
- Root AW, Reiter EO, Andriola M, Duckett G. Hypothalamic-pituitary function in the fetal alcohol syndrome. *J Pediatr*. 1975; 87(4):585–588. [PubMed: 1159590]
- Sadowski H, Ugarte B, Kolvin I, Kaplan C, Barnes J. Early life family disadvantages and major depression in adulthood. *Br J Psychiatry*. 1999; 174:112–120. [PubMed: 10211164]
- Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev*. 2000; 21(1):55–89. [PubMed: 10696570]
- Saxena S, Brody AL, Ho ML, Alborzian S, Ho MK, Maidment KM, et al. Cerebral metabolism in major depression and obsessive-compulsive disorder occurring separately and concurrently. *Biol Psychiatry*. 2001; 50(3):159–170. [PubMed: 11513814]
- Schildkraut JJ. The catecholamine hypothesis of affective disorders: A review of supporting evidence. *Am J Psychiatry*. 1965; 122(5):509–522. [PubMed: 5319766]
- Schneider ML, Moore CF, Kraemer GW, Roberts AD, DeJesus OT. The impact of prenatal stress, fetal alcohol exposure, or both on development: Perspectives from a primate model. *Psychoneuroendocrinology*. 2002; 27(1–2):285–298. [PubMed: 11750784]
- Shaywitz SE, Cohen DJ, Shaywitz BA. Behavior and learning difficulties in children of normal intelligence born to alcoholic mothers. *J Pediatr*. 1980; 96(6):978–982. [PubMed: 7373484]
- Shekhar A, Truitt W, Rainnie D, Sajdyk T. Role of stress, corticotrophin releasing factor (CRF) and amygdala plasticity in chronic anxiety. *Stress*. 2005; 8(4):209–19. [PubMed: 16423710]
- Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW. Hippocampal atrophy in recurrent major depression. *Proc Natl Acad Sci USA*. 1996; 93(9):3908–3913. [PubMed: 8632988]

- Sliwowska JH, Lan N, Yamashita F, Halpert AG, Ellis L, Weinberg J. Effects of prenatal ethanol exposure on basal hypothalamic-pituitary-adrenal activity and hippocampal 5HT_{1A} receptor mRNA levels in female rats across the estrous cycle. *Psychoneuroendocrinology*. 2008 in press.
- Slone JL, Redei EE. Maternal alcohol and adrenalectomy: Asynchrony of stress response and forced swim behavior. *Neurotoxicol Teratol*. 2002; 24:173–178. [PubMed: 11943505]
- Steinhausen HC, Spohr HL. Long-term outcome of children with fetal alcohol syndrome: Psychopathology, behavior, and intelligence. *Alcohol Clin Exp Res*. 1998; 22(2):334–338. [PubMed: 9581637]
- Stratton, K.Howe, C., Battaglia, F., editors. Fetal alcohol syndrome - diagnosis, epidemiology, prevention and treatment. Washington, DC: Institute of Medicine, National Academy of Sciences; 1996.
- Streissguth, AP. The behavioral teratology of alcohol: Performance, behavioral and intellectual deficits in prenatally exposed children. In: West, JR., editor. *Alcohol and brain development*. New York: Oxford Univ Press; 1986. p. 3-44.
- Streissguth AP. Understanding the occurrence of secondary disabilities in clients with fetal alcohol syndrome (FAS) and fetal alcohol effects (FAE). 1992
- Streissguth AP, Bookstein FL, Barr HM, Sampson PD, O'Malley K, Young JK. Risk factors for adverse life outcomes in fetal alcohol syndrome and fetal alcohol effects. *J Dev Behav Pediatr*. 2004; 25(4):228–238. [PubMed: 15308923]
- Streissguth AP, O'Malley K. Neuropsychiatric implications and long-term consequences of fetal alcohol spectrum disorders. *Semin Clin Neuropsychiatry*. 2000; 5(3):177–190. [PubMed: 11291013]
- Strickland P, Deakin JFW. Cortisol, stress and depression (authors' reply). *Br J Psychiatry*. 2002; 181:348–349.
- Subcommittee on Laboratory Animal Nutrition, Committee on Animal Nutrition, Board on Agriculture, & National Research Council. *Nutrient requirements of laboratory animals*. Washington, DC: National Academy; 1995. (Fourth Revised Edition ed.)
- Swaab DF, Bao AM, Lucassen PJ. The stress system in the human brain in depression and neurodegeneration. *Ageing Res Rev*. 2005; 4(2):141–194. [PubMed: 15996533]
- Symonds ME, Stephenson T, Gardner DS, Budge H. Long-term effects of nutritional programming of the embryo and fetus: Mechanisms and critical windows. *Reprod Fertil Dev*. 2007; 19(1):53–63. [PubMed: 17389135]
- Tajuddin NF, Druse MJ. Treatment of pregnant alcohol-consuming rats with buspirone: Effects on serotonin and 5-hydroxyindoleacetic acid content in offspring. *Alcohol Clin Exp Res*. 1993; 17(1):110–114. [PubMed: 7680841]
- Taylor, AN., Branch, BJ., van Zuylen, JE., Redei, E. Maternal alcohol consumption and stress responsiveness in offspring. In: Chrousos, GP, Loriaux, DL., Gold, PW., editors. *Mechanisms of physical and emotional stress. advances in experimental medicine and biology*. 245. New York: Plenum Press; 1988. p. 311-317.
- Taylor AN, Branch BJ, Kokka N, Poland RE. Neonatal and long-term neuroendocrine effects of fetal alcohol exposure. *Monogr Neural Sci*. 1983; 9:140–152. [PubMed: 6888405]
- Taylor AN, Branch BJ, Liu SH, Kokka N. Long-term effects of fetal ethanol exposure on pituitary-adrenal response to stress. *Pharmacol Biochem Behav*. 1982; 16(4):585–589. [PubMed: 7071091]
- Thayer JF, Lane RD. A model of neurovisceral integration in emotion regulation and dysregulation. *J Affect Disord*. 2000; 61(3):201–216. [PubMed: 11163422]
- Trivedi MH, Hollander E, Nutt D, Blier P. Clinical evidence and potential neurobiological underpinnings of unresolved symptoms of depression. *J Clin Psychiatry*. 2008; 69(2):246–258. [PubMed: 18363453]
- van Praag HM, Kahn RS. L-5-hydroxytryptophan in depression and anxiety. *Schweiz Rundsch Med Prax*. 1988; 77(34A):40–46. [PubMed: 3055148]
- van Praag HM, Plutchik R. Increased suicidality in depression: Group or subgroup characteristic? *Psychiatry Res*. 1988; 26(3):273–278. [PubMed: 3265519]

- Vieau D, Sebaai N, Leonhardt M, Dutriez-Casteloot I, Molendi-Coste O, Laborie C, Breton C, Deloof S, Lesage J. HPA axis programming by maternal undernutrition in the male rat offspring. *Psychoneuroendo.* 2007; 32(Suppl 1):S16–20.
- Walsh BT, Seidman SN, Sysko R, Gould M. Placebo response in studies of major depression: Variable, substantial, and growing. *JAMA.* 2002; 287(14):1840–1847. [PubMed: 11939870]
- Walsh RN, Cummins RA. The open-field test: A critical review. *Psychol Bull.* 1976; 83(3):482–504. [PubMed: 17582919]
- Watson S, Gallagher P, Ritchie JC, Ferrier IN, Young AH. Hypothalamic-pituitary-adrenal axis function in patients with bipolar disorder. *Br J Psychiatry.* 2004; 184:496–502. [PubMed: 15172943]
- Watson S, Gallagher P, Smith MS, Ferrier IN, Young AH. The dex/CRH test--is it better than the DST? *Psychoneuroendocrinology.* 2006; 31(7):889–894. [PubMed: 16701957]
- Weinberg J. Prenatal alcohol exposure: Endocrine function of offspring. In: Zakhari, S., editor. *Alcohol and the endocrine system.* Bethesda, MD: NIH (NIAAA); 1993. p. 363-382. Research Monograph No 23
- Weinberg J. In utero alcohol exposure and hypothalamic-pituitary-adrenal activity: Gender differences in outcome. In: Zakhari, S., Hunt, WA., editors. *Stress, gender and alcohol-seeking behavior.* Bethesda: National Institute on Alcohol Abuse and Alcoholism; 1995. p. 241-256. Research Monograph 29
- Weinberg J., Nelson, LR., Taylor, AN. Hormonal effects of fetal alcohol exposure. In: West, JR., editor. *Alcohol and brain development.* New York: Oxford Univ Press; 1986. p. 310-342.
- Weinberg J. Nutritional issues in perinatal alcohol exposure. *Neurobehav Toxicol Teratol.* 1984; 6(4): 261–269. [PubMed: 6514087]
- Weinberg J. Effects of ethanol and maternal nutritional status on fetal development. *Alcohol Clin Exp Res.* 1985; 9(1):49–55. [PubMed: 3887968]
- Weinberg J. Hyperresponsiveness to stress: Differential effects of prenatal ethanol on males and females. *Alcohol Clin Exp Res.* 1988; 12(5):647–652. [PubMed: 3067609]
- Weinberg J. Prenatal ethanol exposure alters adrenocortical development of offspring. *Alcohol Clin Exp Res.* 1989; 13(1):73–83. [PubMed: 2538092]
- Weinberg J. Prenatal ethanol effects: Sex differences in offspring stress responsiveness. *Alcohol.* 1992a; 9(3):219–223. [PubMed: 1605890]
- Weinberg J. Prenatal ethanol exposure alters adrenocortical response to predictable and unpredictable stressors. *Alcohol.* 1992b; 9(5):427–432. [PubMed: 1418669]
- Weinberg J. Neuroendocrine effects of prenatal alcohol exposure. *Ann NY Acad Sci.* 1993; 697:86–96. [PubMed: 8257025]
- Weinberg J, Gallo PV. Prenatal ethanol exposure: Pituitary-adrenal activity in pregnant dams and offspring. *Neurobehav Toxicol Teratol.* 1982; 4(5):515–520. [PubMed: 7177301]
- Weinberg J, Sliwowska JH, Lan N, Hellemans KG. Prenatal alcohol exposure: Foetal programming, the hypothalamic-pituitary-adrenal axis and sex differences in outcome. *J Neuroendocrinol.* 2008; 20:470–488. [PubMed: 18266938]
- Weinberg J, Taylor AN, Gianoulakis C. Fetal ethanol exposure: Hypothalamic-pituitary-adrenal and beta-endorphin responses to repeated stress. *Alcohol Clin Exp Res.* 1996; 20(1):122–131. [PubMed: 8651441]
- Welberg LA, Seckl JR. Prenatal stress, glucocorticoids and the programming of the brain. *J Neuroendocrinol.* 2001; 13(2):113–128. [PubMed: 11168837]
- Wigger A, Neumann ID. Periodic maternal deprivation induces gender-dependent alterations in behavioral and neuroendocrine responses to emotional stress in adult rats. *Physiol Behav.* 1999; 66(2):293–302. [PubMed: 10336157]
- Wilcoxon JS, Redei EE. Maternal glucocorticoid deficit affects hypothalamic-pituitary-adrenal function and behavior of rat offspring. *Horm Behav.* 2007; 51(3):321–327. [PubMed: 17275820]
- Willner P. Validity, reliability and utility of the chronic mild stress model of depression: A 10-year review and evaluation. *Psychopharmacology (Berl).* 1997; 134(4):319–329.

- Willner P. Chronic mild stress (CMS) revisited: Consistency and behavioural-neurobiological concordance in the effects of CMS. *Neuropsychobiology*. 2005; 52(2):90–110. [PubMed: 16037678]
- Wong ML, Kling MA, Munson PJ, Listwak S, Licinio J, Prolo P, et al. Pronounced and sustained central hypernoradrenergic function in major depression with melancholic features: Relation to hypercortisolism and corticotropin-releasing hormone. *Proc Natl Acad Sci USA*. 2000; 97(1): 325–330. [PubMed: 10618417]
- Young AH. Cortisol in mood disorders. *Stress*. 2004; 7(4):205–208. [PubMed: 16019585]
- Young AH. Antiglucocorticoid treatments for depression. *Aust NZJ Psychiatry*. 2006; 40(5):402–405.
- Young EA, Abelson JL, Cameron OG. Effect of comorbid anxiety disorders on the hypothalamic-pituitary-adrenal axis response to a social stressor in major depression. *Biol Psychiatry*. 2004; 56(2):113–120. [PubMed: 15231443]
- Young EA, Altemus M. Puberty, ovarian steroids, and stress. *AnnNY Acad Sci*. 2004; 1021:124–133.
- Zafar H, Shelat SG, Redei E, Tejani-Butt S. Fetal alcohol exposure alters serotonin transporter sites in rat brain. *Brain Res*. 2000; 856(1–2):184–192. [PubMed: 10677625]
- Zilberman ML, Tavares H, Blume SB, el-Guebaly N. Substance use disorders: Sex differences and psychiatric comorbidities. *Can J Psychiatry*. 2003; 48(1):5–13. [PubMed: 12635558]

Figure 1.

Effects of Prenatal treatment, Sex, and Chronic Mild Stress (CMS) on: A) percent time spent in the open arms (%); B) frequency of total (full + partial) open arm entries; and C) frequency of total (closed + open) arm entries on the EPM. Black bars represent prenatal alcohol-exposed (PAE), hatched bars represent pair-fed (PF), white bars represent control (C) animals. (*) denotes significantly different from the Non-CMS group, $p < 0.05$. (#) denotes significantly different from other prenatal groups, $p < 0.05$. (^) denotes significant differences between sexes, $p < 0.05$. n 's=7–10 in each condition.

Figure 2. Effect of Prenatal treatment, Sex, and CMS on plasma corticosterone concentration (µg/dL) 30 min post-EPM testing. Black bars represent PAE, hatched bars represent PF, white bars represent C animals. (*) denotes significantly different from the Non-CMS group, $p < 0.05$. (#) denotes significantly from other prenatal groups, $p < 0.05$. n 's=7–10 for each condition.

Figure 3.

Effect of Prenatal treatment and CMS on plasma levels of: A) testosterone; B) estradiol; and C) progesterone (ng/mL) post-EPM testing in male (A) and female (B and C) rats. Black bars represent PAE, hatched bars represent PF, white bars represent C animals. (*) denotes significantly different from the Non-CMS group, $p < 0.05$. (#) denotes significantly from other prenatal groups, $p < 0.05$. n 's=7–10 for each condition.

Figure 4.

Effects of Prenatal group and Sex on duration of immobility in the forced swim test. Data represent the time (sec) spent immobile in A) males and B) females on Days 1 and Day 2. (*) denotes PAE significantly different from C animals. (#) denotes PAE and PF significantly different from C animals. (&) denotes that C males significantly more immobile than C females. n's=8–10 for each condition; p 's<0.05. n's=7–10 for each condition.

Males

First 5 min

Females

Last 5 min

Figure 5.

Effect of Prenatal treatment on duration and frequency of affiliative and non-affiliative behaviour in the social interaction test. Top figure represent the time spent in either behaviour in the first 5 min of the test among males. Bottom figure represents the frequency of behaviours in the last 5 min of testing among females. Black bars represent PAE, hatched bars represent PF, white bars represent C animals. (*) denotes significantly different from

other prenatal groups, $p < 0.05$. (%) denotes significantly from affiliative behaviors, $p < 0.05$. $n's = 7-10$ for each condition.

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Table 1

Diagnostic Criteria for Fetal Alcohol Syndrome with or without Confirmed Maternal Alcohol Exposure
(adapted from Stratton et al., 1996).

Fetal Alcohol Syndrome	
1. Fetal Alcohol Syndrome with Confirmed Maternal Alcohol Exposure	
A.	Confirmed maternal alcohol exposure
B.	<i>Evidence of a characteristic pattern of facial anomalies</i> that includes features such as short palpebral fissures and abnormalities in the premaxillary zone (e.g. flat upper lip, flattened philtrum, and flat midface).
C.	Evidence of growth retardation, as in at least one of the following: <ul style="list-style-type: none"> • low birth weight for gestational age • decelerating weight over time not due to nutrition • disproportional low weight to height
D.	Evidence of CNS neurodevelopment abnormalities, as in at least one of the following: <ul style="list-style-type: none"> • decreased cranial size at birth • structural brain abnormalities (e.g. microencephaly, partial or complete agenesis of the corpus callosum, cerebellar hypoplasia) • neurological hard or soft signs (as age appropriate), such as impaired fine motor skills, neurosensory hearing loss, poor tandem gait, poor hand eye coordination
2. FAS without Confirmed Maternal Alcohol Exposure	
<i>B, C, and D as above.</i>	
3. Partial FAS with confirmed maternal alcohol exposure	
A.	Confirmed maternal alcohol exposure
B.	Evidence of some components of the pattern of characteristic facial anomalies <i>Either C or D or E</i>
C.	Evidence of growth retardation, as above
D.	Evidence of CNS neurodevelopmental abnormalities, as above
E.	<i>Evidence of a complex pattern of behavior or cognitive abnormalities</i> that are inconsistent with developmental level and cannot be explained by familial background or environment alone, such as hearing difficulties; deficits in school performance; poor impulse control; problems in social perception; deficits in higher level receptive and expressive language; poor capacity for abstraction or metacognition; specific deficits in mathematical skill; or problems in memory, attention or judgment.
4. Alcohol-Related Effects	
Clinical conditions in which there is a history of maternal alcohol exposure, and where clinical or animal research has linked maternal alcohol ingestion to an observed outcome. There are 2 categories, which may co-occur.	
5. Alcohol-Related Birth Defects (ARBD)	
List of congenital anomalies, including malformations and dysplasias, involving various systems including cardiac, skeletal, renal, ocular, and auditory.	
6. Alcohol-Related Neurodevelopmental Disorder (ARND)	
Presence of	
A.	<i>Evidence of CNS neurodevelopmental abnormalities, as above</i> and/or
B.	<i>Evidence of a complex pattern of behavior or cognitive abnormalities, as above</i>

Table 2

Clinical Features of Depression/Anxiety and Neurobehavioral and Neuroendocrine Endpoints Measured in Animal Models

Clinical Symptom	Neurobehavioural / Neuroendocrine Endpoint
Depressed or irritable mood	Behavior in Porsolt forced swim test ('behavioral despair')
Decreased interest in pleasurable activities and ability to experience pleasure (anhedonia)	Behavior in sucrose preference or sucrose contrast tests
Significant weight gain or loss	Changes in body weight
Insomnia / hypersomnia	Changes in sleep patterns / circadian rhythms
Psychomotor agitation / retardation	Behavior in open field, elevated plus maze
Excessive anxiety and worry	Behavior in open field, elevated plus maze
Sex differences in presentation	Sex differences in response
Decreased interest in social activities	Altered social interactions
Increased HPA tone (break-through in the DST; increased responses to the DEX/CRH test)	Altered basal and / or stress levels of corticosterone, ACTH; altered expression of HPA markers in key brain areas (hypothalamus, hippocampus, amygdala, prefrontal cortex); altered corticosterone responses to the DST and DEX/CRH test under basal and stress conditions