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Review

Prenatal stress and brain development

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ABSTRACT

Prenatal stress (PS) has been linked to abnormal cognitive, behavioral and psychosocial outcomes in both animals and humans. Animal studies have clearly demonstrated PS effects on the offspring's brain, however, while it has been speculated that PS most likely affects the brains of exposed human fetuses as well, no study has to date examined this possibility prospectively using an independent stressor (i.e., a stressful event that the pregnant woman has no control over, such as a natural disaster). The aim of this review is to summarize the existing animal literature by focusing on specific brain regions that have been shown to be affected by PS both macroscopically and microscopically. These regions include the hippocampus, amygdala, corpus callosum, anterior commissure, cerebral cortex, cerebellum and hypothalamus. We first discuss the mechanisms by which the effects of PS might occur. In particular, we show that maternal and fetal hypothalamic–pituitary–adrenal (HPA) axes, and the placenta, are the most likely candidates for these mechanisms. We see that, although animal studies have obvious advantages over human studies, the integration of findings in animals and the transfer of these findings to human populations remains a complex issue. Finally, we show how it is possible to circumvent these challenges by studying the effects of PS on brain development directly in humans, by taking advantage of natural or man-made disasters and assessing the impact and consequences of such stressful events on pregnant women and their offspring prospectively.

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Abbreviations: PS, prenatal stress; ADHD, attention-deficit hyperactivity disorder; HPA, hypothalamic-pituitary-adrenal; 11 β -HSD2, 11 β -hydroxysteroid dehydrogenase type 2; CRH, corticotropin-releasing hormone; GLUT-1, glucose transporter; hPL, placental lactogen; DEX, dexamethasone; 5-HT, 5-hydroxytryptamine; CNS, central nervous system; GD, gestation days; SDN-POA, sexually dimorphic nucleus of the preoptic area; MRI, magnetic resonance imaging; PTSD, post-traumatic stress disorder; Aca, anterior commissure; rAca, rostral anterior commissure; NAA, N-acetyl-aspartate; ACd, dorsal anterior cingulate cortex; OFC, orbitofrontal cortex; SDN-MPOA, sexually dimorphic nucleus of the medial preoptic area; AVPV, anteroventral periventricular nucleus

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1. Introduction

In humans, prenatal stress (PS) is linked to an increased vulnerability for developing various psychosocial problems that are observed both in childhood and adulthood. In children, PS is associated with cognitive, behavioral, physical and emotional problems (King and Laplante, 2005; King et al., 2009; Laplante et al., 2004, 2008; Talge et al., 2007) as well as with autism (Beversdorf et al., 2005; Kinney et al., 2008a,b), and attention-deficit hyperactivity disorder (ADHD) (Grizenko et al., 2008; Li et al., 2010; Linnert et al., 2003; McIntosh et al., 1995; Rodriguez and Bohlin, 2005). Our findings from Project Ice Storm (King et al., 2009; Laplante et al., 2004, 2007, 2008), the first human prospective study to use a natural disaster to study the effects of PS on developmental outcomes, finds large effect sizes (ES) of PS on play at 2 years of age (Stereotypical Play: ES=1.02; Functional Play: ES=1.24; Displayed Hypotheses: ES=1.35), cognition (IQ at 2 years: ES=0.52; IQ at 5½ years: ES=0.26), language (Productive Language at 2 years: ES=0.33; Receptive Language at 2 years: ES=0.30; Language Abilities at 5½ years: ES=0.31), and physical development (Fingerprint Assymetry: ES=1.02). In the study by Kinney et al. (2008a) of prenatal exposure to hurricanes and tropical storms in Louisiana, prevalence of autism increased from 3.72/10,000 births for the low-exposure group to 26.59/10,000 births for the group exposed to higher intensity storms, but only for individuals exposed during the 5–6 and 9–10 months of gestation. In ADHD, Rodriguez et al. found a large effect (Cohen's $d=1.20$) for global perceived stress, collected throughout pregnancy, on symptoms of ADHD in 7-year-old children (Rodriguez and Bohlin, 2005).

In adults, PS is linked to depression (Watson et al., 1999) and schizophrenia (Huttenen and Niskanen, 1978; Kinney et al., 1999a; Kinney, 2001; van Os and Selten, 1998). Exposure to a major earthquake in utero has been shown to significantly increase the rate of severe depression from 5.5% in control subjects to 13.3% in exposed individuals (Watson et al., 1999). Also, the number of individuals with a diagnosis of schizophrenia was found to be significantly higher among individuals with prenatal loss of their father (6/167) compared to individuals whose father had died during the first year of their children's lives (1/168) (Huttenen and Niskanen, 1978). Finally, van Os and Selten (1998) found that the cumulative incidence of schizophrenia was higher in individuals prenatally exposed to the 1940 invasion of the Netherlands by the German army (risk ratio (RR): 1.15, 95% CI: 1.03–1.28), especially in those exposed in the first trimester (RR: 1.28, 95% CI: 1.07–1.53).

The effects of PS are also observed in animals with increases in the latency to play (Takahashi et al., 1992), nondirected locomotor behavior (i.e., pacing) (Coe et al., 2003), anxiety (Estanislau and Morato, 2005; Fride and Weinstock, 1988; Vallee et al., 1997), and decreases in learning and memory (Gué et al., 2004; Lemaire et al., 2000; Nishio et al., 2001; Son et al., 2006; Wu et al., 2007; Yang et al., 2006), and focused exploration (Coe et al., 2003). In animals, PS is also associated with greater distractibility and attention deficits (Schneider and Coe, 1993; Schneider et al., 1999) as well as delayed object permanence (Schneider, 1992), an early indicator of cognitive development.

These effects of PS on cognitive, behavioral and psychosocial outcomes are most likely mediated by the effects of maternal stress on the structure and function of the fetal

brain, which is the control center for a multitude of systems. This is supported, as we will see in the following sections, by a wealth of animal studies that show the damaging effects of PS on the offspring's brain (Anderson et al., 1985; Barros et al., 2006; Coe et al., 2002; Coe et al., 2003; Fleming et al., 1986; Fujioka et al., 2006; Hayashi et al., 1998; Jones et al., 1997; Kawamura et al., 2006; Kerchner and Ward, 1992; Kerchner et al., 1995; Kraszpulski et al., 2006; Lemaire et al., 2000, 2006; Michelsen et al., 2007; Murmu et al., 2006; Poland et al., 1999; Rhees et al., 1999; Salm et al., 2004; Schmitz et al., 2002; Szuran et al., 1994; Ulupinar and Yucel, 2005; Ulupinar et al., 2006; Zuena et al., 2008).

There are factors, other than PS, that may compromise development of the fetus such as poor maternal diet, food restriction, maternal mental illness, drug and/or alcohol consumption, infection, exposure to environmental toxins (reviewed in (Brand and Brennan, 2009; Christian and Stewart, 2010; Schlotz and Phillips, 2009; Swanson et al., 2009; Thompson et al., 2009)). Also, diverse environmental factors such as alcohol, infection, maternal undernutrition and hypoxia can affect the activity or gene expression of placental 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2 (reviewed in (Fowden and Forhead, 2004; Fowden et al., 2008)). However, the scope of this review is to focus on the effects of PS on brain development. The objective of this review is therefore to summarize the existing literature by focusing on specific brain regions that have been reported as being affected by PS. We will first discuss the mechanisms by which the effects of PS might occur. In particular, we will show that maternal and fetal hypothalamic-pituitary-adrenal (HPA) axes, and the placenta, are the most likely candidates for these mechanisms. Because the majority of PS studies have utilized animals to study this phenomenon, we will consider the many challenges involved in integrating findings between different animal studies, as well as the difficulties in translating these results to humans. Finally, we will discuss the limitations of human studies and consider directions for future research.

2. Proposed mechanisms by which prenatal stress affects the fetus

2.1. Prenatal stress—the role of cortisol

Cortisol (corticosterone in rodents) is a glucocorticoid and the primary end product of the HPA axis, an important component of the stress system in humans, monkeys and rodents. This steroid hormone, along with the sympathetic nervous system, assists in the mobilization of the body's energy resources when a potentially threatening event requires that the individual enters into a "fight or flight" mode.

During pregnancy, women have naturally elevated levels of cortisol. This glucocorticoid is actually essential for fetal growth and the induction of certain enzymes, such as pulmonary surfactant (Garbrecht et al., 2006; Seckl and Holmes, 2007), therefore preparing the fetus for extrauterine life. However, under particular stressful conditions, maternal cortisol concentrations can reach abnormally high levels. Consequently, this excess in maternal cortisol (corticosterone in rodents), which is typically mostly transformed by the fetoplacental into its

inactive form (i.e., cortisone), reaches the fetus in high concentrations, which may potentially alter fetal development and growth (Seckl and Holmes, 2007). In fact, animal studies have clearly demonstrated, as we will see later, that fetal exposure to high levels of cortisol retards and/or alters the development of neurons in the brain, and results in smaller hippocampal sizes (Avishai-Eliner et al., 2002; Coe et al., 2003; Hayashi et al., 1998; Schmitz et al., 2002; Szuran et al., 1994).

2.2. Fetal brain programming—the role of placental CRH and 11 β -HSD2

The fetal programming hypothesis, first described by Barker (Barker et al., 1989), suggests that the intrauterine environment during critical periods of organogenesis and tissue growth may permanently alter organ structure and function. The potential importance of the link between the placenta and the fetal brain development has been emphasized (Baker and Sibley, 2006). It has been suggested that PS induces changes in placental phenotype that may have consequences later during pregnancy on fetal development (Eunson, 2006; Redline, 2006; Uno et al., 1989, 1990, 1994).

In human pregnancy, placental corticotropin-releasing hormone (CRH) activity is modulated by the maternal HPA axis (Wadhwa et al., 1998) and studies have demonstrated that placental CRH concentration is a significant predictor of spontaneous preterm birth (Glynn et al., 2001, 2004; Sandman et al., 2006) and intrauterine growth restriction (IUGR) (Wadhwa et al., 2004). This placental hormone can also influence fetal hippocampus development. PS activates the maternal HPA axis, resulting in increased production and release of placental CRH into the bloodstream (see Fig. 1). In contrast to hypothalamic CRH production, which is suppressed by stress-induced cortisol, placental CRH is increased by glucocorticoids, so that PS leads to progressively higher fetal plasma CRH levels. This placental CRH reaches the fetal brain (Kastin and Akerstrom, 2002) and could influence the fetal hippocampus, presumably by activating CRH receptors (Sandman et al., 1999; Wadhwa et al., 2001). Other parahippocampal and limbic areas rich in CRH receptors during mid- to late gestation could also be influenced by placental CRH (Sandman et al., 1999).

Stress in the mother during pregnancy not only increases her own circulating cortisol, it also reduces the expression and activity of the glucocorticoid barrier enzyme, 11 β -HSD2, in the placenta, leaving the fetus less protected (Avishai-Eliner et al., 2002; Mairesse et al., 2007; Welberg et al., 2005). Down-regulation of placental 11 β -HSD2 activity increases glucocorticoid exposure of the placenta and the fetus. In turn, this influences the placental production and metabolism of other glucocorticoid-sensitive proteins and hormones such as the prostaglandins, progesterone, estrogens, glucose transporter (GLUT-1) and placental lactogen (hPL) (Mairesse et al., 2007). Moreover, inhibition of 11 β -HSD2 by PS might contribute to low birthweight, IUGR and to pregnancy disorders, such as preterm birth and preeclampsia (reviewed in (Causevic and Mohaupt, 2007; Michael and Papageorghiou, 2008)). Finally, although this is not the focus of this review, it is worth noting that hypoxia has also been shown to inhibit the increase in expression of 11 β -HSD2 normally observed in trophoblast

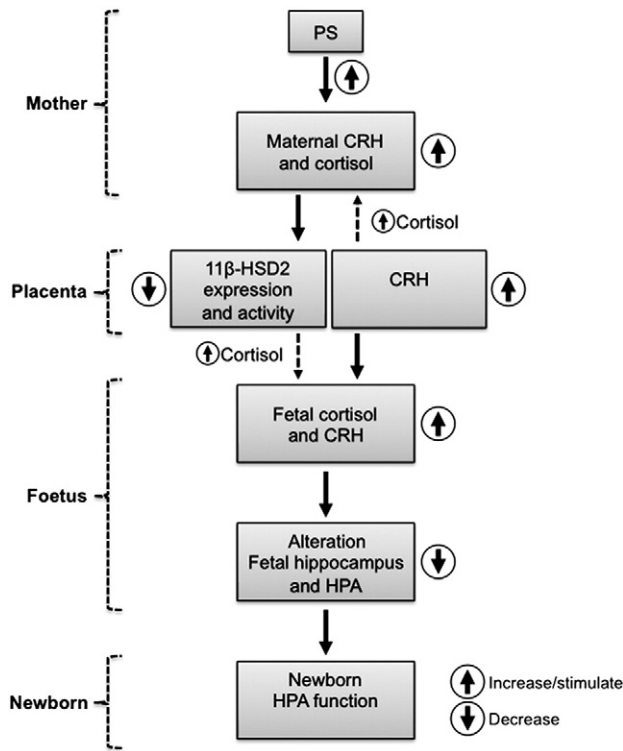


Fig. 1 – Role of PS on the mother-placenta-fetus unit. PS activates the maternal HPA axis, which increases levels of circulating maternal CRH and cortisol. This, in turn, increases the production and release of placental CRH into the bloodstream. In contrast to hypothalamic CRH production, which is suppressed by stress-induced cortisol, placental CRH is increased by glucocorticoids, so that PS leads to progressively higher fetal plasma cortisol and CRH levels. This placental CRH reaches the fetal brain and could influence the fetal hippocampus, presumably by activating CRH receptors. PS also reduces the expression and activity of 11β -HSD2, in the placenta, leaving the fetus less well protected. Down-regulation of placental 11β -HSD2 activity increases glucocorticoid exposure of the placenta and the fetus. Alterations of fetal HPA axis remain present in the newborn. PS=prenatal stress; HPA=hypothalamic-pituitary-adrenal; CRH=corticotropin-releasing hormone; 11β -HSD2= 11β -hydroxysteroid dehydrogenase type 2.

development. Hardy and Yang (2002) suggest that this effect of hypoxia on placental 11β -HSD2 may be a factor contributing to the decrease of this enzyme observed in placenta from pregnancy complicated by preeclampsia and IUGR (Hardy and Yang, 2002).

Thus, placental CRH and 11β -HSD2 play important roles in modulating the programming effects of PS (Mairesse et al., 2007; Seckl, 2001; Seckl, 2004; Seckl and Meaney, 2004). Moreover, as stated by O'Donnell et al. (2009), since the placenta controls fetal exposure to placental and maternal hormones (such as cortisol), as well as to environmental factors, this organ may be viewed as a primary programming vector. A number of functions of the placenta have been shown to be compromised by PS (reviewed in (Robinson et al., 1997)). It has been shown that PS affects the capacity of the

placenta to deliver nutrients and oxygen to the fetus by altering placental morphology and growth (Angiolini et al., 2006). Blood flow, a critical determinant of placental function and fetal growth is also affected by environmental factors, such as stress. For example, catecholamines affect blood flow to the placenta by increasing vascular resistance. Fluctuations in oxygenation are a powerful inducer of placental oxidative stress which leads to activation of stress-activated pathways such as pro/anti-inflammatory cytokines. There is also evidence that PS increases pro-inflammatory cytokines and oxidative stress in placental tissues, which have been found to be associated with neurodevelopmental disorders (Crocker, 2006).

The potential importance of the link between the placenta and the fetal brain has been well noted (Baker and Sibley, 2006). Studies have found associations between placental inflammation and neurodevelopmental disorders (Crocker, 2006) and brain damage (Eunson, 2006; Redline, 2006). Other utero-placental characteristics that have been associated with neurological abnormality include a short umbilical cord, abnormal placental weight, macrophages in placental deciduas, and neutrophilic infiltration of amnio and chorionic surface (Baker and Sibley, 2006). Placental programming may lead to mental illness such as schizophrenia (Abel and Allin, 2006). However, placental damage has been poorly studied in humans as a mediator between PS caused by exposure to a major independent stressor and neurodevelopmental disorders and thus the role of this organ in PS effect on fetal brain development remain to be investigated.

2.3. Prenatal stress and alterations in the offspring's HPA axis and hippocampus

A number of studies suggest that PS is associated with alterations in the offspring's HPA axis activity. Some studies find PS to be associated with higher basal glucocorticoid secretion. For example, baseline corticosterone release is elevated in rat pups whose mothers were stressed during gestation with either restraint under intense illumination, handling, or intermittent flashing lights and/or noise (Maccari et al., 1995; Ward et al., 2000; Weinstock et al., 1992) or when stress hormones were administered to the mothers during pregnancy (Fameli et al., 1994). Ward et al. (2000) produced mild stress by handling and exposing pregnant rat dams to novel cages during the last week of pregnancy and reported a 64% increase in the level of plasma corticosterone in the offspring. Furthermore, increases in plasma corticosterone levels in the offspring are obtained even after only one day of stress exposure to the pregnant dam (Weinstock et al., 1992), suggesting that in rodents, the fetal HPA axis is highly susceptible to stress. The same is true in non-human primates, with similar elevations in cortisol levels being observed following either injection of dexamethasone (DEX, a synthetic glucocorticoid; Uno et al., 1994, see Table 1) or after the administration of an acoustic startle stressor (Clarke et al., 1994; Coe et al., 2003).

Beyond alterations in baseline cortisol, there are also studies showing that PS is associated with alterations in stress reactivity. In Rhesus monkeys, prenatal exposure to either glucocorticoids or maternal stress is associated with

Table 1 – Summary of studies examining the effects of prenatal stress on the offspring's brain development.

Reference	Species/strain; samples	Type of stressor	Timing of the stressor and assessment	Structure of interest investigated	Findings in the brain
Coe et al. (2002)	Rhesus monkeys PS=6♂, 5♀ C=3♂, 2♀	Relocation to a darkened room + Acoustical startle protocol (3×1 sec/10 min, randomly; 5 d/w)	90–140 GD; 7–11 m	Corpus callosum	↓ Callosal area on MRI in PS ♂ ↑ Callosal area on MRI in PS ♀ Changes in corpus callosum volumes accompanied by changes in corpus callosum shape
Coe et al. (2003)	Rhesus monkeys Early PS=2♂, 2♀ Late PS=2♂, 2♀ C=2♂, 2♀	Relocation to a darkened room + Acoustical startle protocol (3×1 sec/10 min, randomly; 5 d/w)	Early PS=50–92 GD Late PS=105–147 GD; 2.5–3 y	Hippocampus	↓ Neurogenesis (by 32%) in DG (no differential timing effect) ↓ HC volume (Early PS=12%; Late PS=10%) (no differential timing effect) Similar proportions of proliferating cells mature into neurons in all 3 groups (C, Early and Late PS)
Uno et al. (1990)	Rhesus monkeys (n = ?)	Dexamethasone administration	132–133 GD; 135 or 165 GD	Hippocampus	Degeneration and depletion of HC pyramidal and dentate granular neurons
Uno et al. (1994a,b)	Rhesus monkeys PS=5 (♂/♀=?) C=3 (♂/♀=?)	Dexamethasone administration	132–133 GD; 20 m	Hippocampus	↓ HC volume (by ~30%; determined by MRI)
Szuran et al. (1994)	Rats (W) PS=21♂, 20♀ C=26♂, 24♀	Restraint (3×30 min/d, randomly)	15–19 GD; 90 d	Hippocampus	↓ HC wet weight in PS ♂ (by 15.4%) and ♀ (by 8.2%)
Hayashi et al. (1998)	Rats (W) PS=4 (♂/♀=?) C=5 (♂/♀=?)	Crowding+pain (saline intramuscular injection once a day)	15–21 GD; 35 d	Hippocampus	↓ Synaptic density (by 32%) in CA3 in PS animals
Lemaire et al. (2006)	Rats (W) PS=18♂ C=19♂ Handling=19♂ PS+ Handling=20♂	Bright light+restraint (3×45 min/d)	15–21 GD; 4 or 26 m	Hippocampus	↓ Neurogenesis (by 46–47%) in DG of PS adult and senescent rats Neonatal handling reverses these effects
Barros et al. (2006)	Rats (W) PS=4♂ C=4♂	Restraint (3×45 min/d)	14–21 GD; 90 d	Hippocampus Frontal Cortex	↓ Dendritic arborization in frontal cortex (by 46%) and in HC CA1 (by 73%) Synaptic loss in frontal cortex (by 52%) and HC CA1 (by 50%)
Schmitz et al. (2002)	Rats (LE) PS=6♂, 6♀ C=6♂, 6♀	Restraint (1×20 min/d)	18 GD; 75 d	Hippocampus	↓ HC volume in PS ♀ ↓ Number of granule cells (by 24.3%) in PS ♀ (relative to control ♀) not in PS ♂ No significant difference in number of pyramidal cells (CA1–3) between PS and C for both ♂ and ♀
Lemaire et al. (2000)	Rats (SD) PS=25♂ C=20♂	Bright light+restraint (3×45 min/d)	15–21 GD; 28d, 3, 10 or 22 m	Hippocampus	↓ Neurogenesis in left DG of PS rats at every age (by 38.4% at 28d, by 59.3% at 3 m, by 42.3% at 10 m and by 55.2% at 22 m) ↓ Total number of granule cells in left DG from 3 m and after
Fujioka et al. (2006)	Rats (SD) PS=10 (♂/♀=?) C=5 (♂/♀=?)	Restraint (30 min or 240 min/d)	15–17 GD; 1, 15 d or 11 w	Hippocampus	↑ Neurogenesis in DG and ↑ neonatal differentiation of processes for 30 min PS group ↓ Neurogenesis in DG and ↓ neonatal morphology of processes for 240 min PS group
Zuena et al. (2008)	Rats (SD) PS=5 (♂/♀=?) C=5 (♂/♀=?)	Heat, bright light+restraint (3×45 min/d)	11–21 GD; 110 d	Hippocampus	↓ Neurogenesis (by ~24%) in DG of PS ♂, but not in PS ♀
Kawamura et al. (2006)	Rats (SD) PS=6 (♂/♀=?) C=6 (♂/♀=?)	Bright light+restraint (3×45 min/d)	13–17 GD; 10 d	Hippocampus Amygdala	↓ Neurogenesis in hippocampus (by 60%) and amygdala (by 30%)
Szuran et al. (2000)	Rats (W) PS=7–8 ♂, 7–8♀ C=7–8♂, 7–8♀	Restraint (3×30 min/d, randomly)	15–19 GD; 12 m	Hippocampus	↓ HC glucocorticoid receptor densities in ♀ only relative to control ♀

Table 1 (continued)

Reference	Species/strain; samples	Type of stressor	Timing of the stressor and assessment	Structure of interest investigated	Findings in the brain
Henry et al. (1994)	Rats (W) PS ~ 15 ♂ (3 and 21 d), 7 ♂ (90 d) C ~ 15 ♂ (3 and 21 d), 7 ♂ (90 d)	Bright light + restraint (3 × 45 min/d)	14–21 GD; 3, 21 and 90 d	Hippocampus	↓ HC glucocorticoid receptor densities at 21 and 90 d of life; no change at 3 days after birth
Weinstock et al. (1992)	Rats (S) PS = 12–16 ♂, 12–16 ♀ C = 12–16 ♂, 12–16 ♀	Noise + flashing lights (alternating 5 min periods for 4 h, 3 times a week)	0–21 GD; 60 d	Hippocampus	↓ HC glucocorticoid receptors in ♀ only relative to control ♀
Kraszpulski et al. (2006)	Rats (SD) ♂ (n = ?)	Handling + novel environment + saline intramuscular injection (once a day, randomly)	14–21 GD; 7, 25, 45 or 60 d	Amygdala	↓ Volume for all 3 amygdalar nuclei (left basolateral, central and lateral) at 25 days, which resolved at 45 days ↓ Anterior-posterior lengths of lateral and basolateral nuclei at 25 days with continued elongation after this time unlike controls ↓ Number of neurons for all 3 nuclei at 7 (Lateral) or 25 days (basolateral and central), which resolved at 45 days ↓ Number of glial cells for all 3 nuclei at 7 (Lateral) or 25 days (basolateral and central), which resolved at 45 days
Kerchner et al. (1995)	Rats (SD) PS = 31 ♂ ejaculators, 12 ♂ non ejaculators C = 35 ♂ ejaculators, 17 ♂ non-ejaculators, 8 ♀	Heat, bright light + restraint (3 × 45 min/d)	14–21 GD; 73–176 d	Amygdala	Medial amygdala is bigger in C ♂ than ♀ PS has no effect on the sexual differentiation of the total medial amygdala, posterior dorsal or posterior ventral medial amygdala in ♂
Salm et al. (2004)	Rats (SD) PS = 9 ♂ C = 8 ♂	Handling + novel environment + saline intramuscular injection (once a day, randomly)	14–21 GD; 80 or 120 d	Amygdala	↑ Volume of lateral nucleus (by 30%) in PS; no difference between PS and C in the other amygdalar nuclei volumes (basolateral, dorsal endopiriform, and central) ↑ Neuronal density (by 22%), but not glial density, in lateral nucleus in PS; no difference in neuronal or glial densities for central or basolateral amygdalar nuclei ↑ Number of neurons (by 49%) and number of glial cells (by 43%), in lateral nucleus; no difference in neuron or glial cells numbers for central or basolateral amygdalar nuclei No difference in ratio glia/neurons between any of the groups
Jones et al. (1997)	Rats (SD) PS = 10 ♂, 10 ♀ C = 10 ♂, 10 ♀	Heat, bright light + restraint (3 × 30 min/d)	15–22 GD; 90–100 d	Rostral Anterior Commissure	PS reverses sexual dimorphism of rostral anterior commissure (rAca): rAca area in C ♀ > C ♂ rAca area in PS ♂ > PS ♀ rAca area in PS ♂ > C ♂ rAca area in PS ♀ > C ♀ rAca area in C ♂ = PS ♀ rAca area in C ♀ = PS ♂
Poland et al. (1999)	Rats (SD) PS = 4 ♂ C = 6 ♂	Restraint (2 × 1 hour/d)	14–21 GD; 120 d	Frontal Cortex	↓ NAA concentration (by 21%) in left frontal cortex in PS No significant NAA concentration difference between PS and C in right frontal cortex, left and right hippocampus
Fleming et al. (1986)	Rats (SD) PS = 10 ♂ C = 11 ♂	Heat, bright light + restraint (3 × 45 min/d)	14–21 GD; 130–140 d	Cerebral Cortex	C ♂ have sexual dimorphic right > left thickness asymmetries in the cerebral cortices PS ♂ have a non-significant reversed pattern that is characteristic of the ♀ cortex

(continued on next page)

Table 1 (continued)

Reference	Species/strain; samples	Type of stressor	Timing of the stressor and assessment	Structure of interest investigated	Findings in the brain
Murmu et al. (2006)	Rats (SD) PS=6♂, 6♀ C=6♂, 6♀	Restraint (45 min on days 15 and 18) Crowding (8 hrs on days 16 and 19) Forced swimming (15 min on days 17 and 20)	15–20 GD; 23 d	ACCd OFC	PS ♂: ↓ Dendritic spine densities on the apical dendrite of pyramidal neurons in ACCd (20%) and in OFC (25%) ↓ Dendritic spine densities on the basal dendrites of pyramidal neurons in OFC (20%), but not in ACCd ↓ Apical dendritic length of pyramidal neurons, but not basal in ACCd (30%) and in OFC (26%) ↓ Complexity of the dendritic trees of pyramidal neurons in both ACCd and OFC PS ♀: ↓ Dendritic spine densities on the apical dendrite of pyramidal neurons in ACCd (21%) and in OFC (21%) ↓ Dendritic spine densities on the basal dendrite of pyramidal neurons ACCd (21%) and in OFC (20%) No dendritic atrophy of pyramidal neurons was detected in the PS ♀, unlike in PS ♂ ↓ Ratio of mushroom spines of pyramidal neurons in the mPFC of PS animals PS did not affect spine density of pyramidal neurons in the mPFC
Michelsen et al. (2007)	Rats (SD) PS=4♂ C=4♂	Bright light+restraint (3×45 min/d)	14–21 GD; 100 d	mPFC	PS and C have similar brain and cerebellum weights or brain to body ratios ↓ Volume fraction of granule cell nuclei in granular layer in PS rats (by 11%) related to a ↓ in mean granule cell diameter No difference in the volume fraction of the granule cell layer to whole cerebellar cortex between PS and C No difference between PS and C in the numerical densities of granule cells ↓ Numerical densities of synapses in granular layer in PS (by 49%) ↓ Number of synapses per neuron in granular layer in PS (by 51%)
Ulupinar and Yucel (2005)	Rats (SD) PS=3 (♂/♀=?) C=3 (♂/♀=?)	Restraint (6hours/d)	7+14 GD; 30 d	Cerebellum	↓ Cerebellar granule-to-Purkinje cell ratio in PS (by 24%), reflecting an ↑ in the numerical density of Purkinje cells
Ulupinar et al. (2006)	Rats (SD) PS=5 (♂/♀=?) C=5 (♂/♀=?)	Restraint (6hours/d)	7+14 GD; 30 d	Cerebellum	In C, cross-sectional area of SDN-POA is bigger in ♂ than ♀ at 20 and 60 days, but not at birth PS ♂ have a cross-sectional area of SDN-POA at birth that is almost twice the one of C males ↓ 50% of cross-sectional area of SDN-POA in PS ♂ rats at 20 and 60 days, relative to C of same age, reaching values similar to C ♀ Cross-sectional area of SDN-POA in PS ♀ did not differ from C ♀ at birth, 20 and 60 days C ♂ have a larger SDN-MPOA than both PS ♂ and C ♀ PS ♂ have a larger SDN-MPOA than ♀
Anderson et al. (1985)	Rats (SD) PS=5–6♂, 5–6♀ C=5–6♂, 5–6♀	Heat, bright light+restraint (3×45 min/d)	14–20 GD; 0, 20 or 60 d	Hypothalamus	↓ SDN-POA volumes (feminized) in non-sexually active PS ♂ relative to sexually active C ♂ ↓ SDN-POA volumes in non-sexually active C ♂ relative to sexually active C ♂ No difference in SDN-POA volumes between sexually active PS ♂ and sexually active C ♂, or between non-sexually active PS ♂ and non-sexually active C ♂
Kerchner and Ward (1992)	Rats (SD) PS=44♂ C=56♂, 10♀	Heat, bright light+restraint (3×45 min/d)	14–21 GD; 73–176 d	Hypothalamus	
Rhees et al. (1999)	Rats (SD) PS=10♂ sexually active, 10♂ sexually non-actives C=10♂ sexually active, 6♂ sexually non-active, 10♀	Heat, bright light+restraint (3×45 min/d)	14–21 GD; 90 d	Hypothalamus	

Table 1 (continued)

Reference	Species/strain; samples	Type of stressor	Timing of the stressor and assessment	Structure of interest investigated	Findings in the brain
Rhees et al. (1999)					<p>↑ AVPV (feminized) in non-sexually PS ♂ relative to sexually active C ♂</p> <p>↑ AVPV volumes in non-sexually active C ♂ relative to sexually active C ♂</p> <p>No difference in AVPV volumes between sexually active PS ♂ and sexually active C ♂, or between non-sexually active PS ♂ and non-sexually active C ♂</p>
<p>Abbreviations: W=Wistar; LE=Long-Evans; SD=Sprague-Dawley; S=Sabra; d=days; w=weeks; y=years; C=controls; PS=prenatally stressed; HC=hippocampus; DG=dentate gyrus; OFC=Orbitofrontal cortex; ACCd=Dorsal anterior cingulate cortex; mPFC=medial prefrontal cortex; SDN-POA=sexually dimorphic nucleus of the preoptic area; SDN-MPOA=sexually dimorphic nucleus of the medial preoptic area; AVPV=anteroventral periventricular nucleus; MRI=magnetic resonance imaging; GD=gestational day.</p>					

greater increases in cortisol following a stressor (Uno et al., 1994). Additionally, the offspring show a prolonged stress-induced cortisol secretion reflecting a slower recovery following stress (Uno et al., 1994). Similarly, greater stress reactivity is found in PS rats (Koehl et al., 1999; Takahashi and Kalin, 1991; Weinstock et al., 1992) who also show a slower recovery from the stressor (Fride et al., 1986; Maccari et al., 1995).

In addition to alterations in the offspring's HPA axis activity, hippocampal glucocorticoid receptors are also affected. These receptors are involved in the negative feedback loop that inhibits the hormonal stress response and restores the system to a steady state (Avishai-Eliner et al., 2002). In rodents, studies indicate that stressing pregnant dams with restraint, with or without bright light, or a procedure consisting of flashing lights and noise, alters the density of hippocampal glucocorticoid receptors in the pups (Henry et al., 1994; Szuran et al., 2000; Weinstock et al., 1992) (Table 1). After restraining pregnant rat dams for 30 minutes/day during gestation days (GD) 15–19, the density of hippocampal glucocorticoid receptors was lower by approximately 50% in PS female offspring; however, no difference were observed between PS males and control males (Szuran et al., 2000). This female-specific decrease in hippocampal glucocorticoid receptors has also been reported by Weinstock et al. (1992), although Henry et al. (1994) have found similar decreases in males as well (these authors only studied male offspring). Male/female differences are further discussed in Section 3.5.

As described above, exposure to excess CRH released from the placenta during sub-acute and chronic PS may penetrate the blood-brain barrier of the fetus, and subsequently influence both the function and the integrity of the hippocampus (Kastin and Akerstrom, 2002). It should be noted that in humans, the blood-brain barrier forms during early development and cerebral endothelial junctions are evident at 8 weeks of gestation (Mollgard and Saunders, 1986), but they remain immature and more vulnerable than adult blood-brain barriers to insults. Prenatal treatment with corticosteroids (DEX) reduces blood-brain permeability in the ovine fetus (Stonestreet et al., 1999). This interaction between the HPA axis and hippocampus is important in that the hippocampus is believed to play significant roles in memory and learning via the cholinergic

system (Messer, 2002), and in mood disorders, anxiety, aggression, and impulsivity disorders via the serotonergic system (Gorman, 2002). PS increases the release of acetylcholine in the hippocampus, inhibiting the influence of cholinergic receptors on the activity of the HPA axis (Sithichoke and Marotta, 1978), thereby down-regulating hippocampal glucocorticoid receptors (Alema et al., 1995; Avishai-Eliner et al., 2002; Yau et al., 1992). It is believed that this process influences the memory and learning abilities of exposed offspring. In terms of serotonergic function, 5-hydroxytryptamine (5-HT) is believed to play a major role in early brain development through facilitating synapse formation and maintenance (Hayashi et al., 1998). PS results in the reduction of 5-HT binding sites in the hippocampus, thereby suppressing the negative feedback of corticosteroid receptors in the hippocampus (Avishai-Eliner et al., 2002; Huizink et al., 2004).

Thus, while exposure to glucocorticoids is necessary for the normal development of the HPA axis and, in particular, the hippocampus, excessive levels observed in offspring of stressed rat dams have direct deleterious effects on the fetal HPA axis and on the development of the hippocampus resulting in observable negative influences on postnatal development (Avishai-Eliner et al., 2002).

3. Factors that may contribute to differences in the effects of prenatal stress in animal studies

Unlike research with human populations, animal studies offer the possibility to randomly assign pregnant dams to stress groups, and to control the type, intensity, duration and timing of the stressor, as well as the time when offspring are evaluated or sacrificed in order to observe effects in the brain. These animal studies help us understand, at least in part, the mechanisms by which PS might affect the animals' developing brain and behavioral outcomes, and generate working hypotheses about the underlying mechanisms occurring in humans. However, in reviewing the research on PS and its effects on brain development, a number of parameters vary from study to study, and needs improving before drawing conclusions. These parameters include species, timing of

stress administration during gestation, types of stressors, timing of postnatal assessment, and the moderating effect of sex.

3.1. *Different species with varying gestational lengths*

The challenge in trying to bridge animal and human studies lies, on the one hand, in the ability to integrate the various results from controlled animal studies that use different non-human animal species and, on the other hand, in the ability to extrapolate those findings to humans. Animal studies on the effects of PS on brain development have been conducted mainly in Sprague–Dawley, Wistar, and Long–Evans rat strains, but also in Rhesus macaques. Rodents are clearly different from primates (including humans) in a number of ways, two of those being gestational duration (i.e., rats=21.5 days; monkeys=165 days; humans=270 days) and associated levels of brain maturation at birth. Fig. 2 (adapted from Clancy et al. (2001)) represents normalized (i.e., percent of total gestation) developmental time-lines in rats, monkeys, and humans; some neurodevelopmental events that have been shown to be affected by PS are indicated. The figure shows that several major brain developmental events (e.g., peak of amygdalar development, appearance of the corpus callosum) occur during the final stages of gestation in rats, whereas in monkeys and humans the same events take place during the first half of gestation. This difference is important since it shows that an intuitive direct comparison between rodents and humans of the relative periods of exposure to a stressor can be incorrect, while the timing periods are much more similar between non-human primates and humans. In other words, exposure in the last third of gestation in rats does not correspond to exposure in the last third of gestation in humans, with regards to developmental events taking place at these times. Additionally, the level of brain maturity at birth differs between species. For example, compared to humans, rodents have a relatively immature brain at birth, and a considerable amount of the brain development occurs during the postnatal period, rather than prenatally, as in humans (Rice and Barone, 2000). Finally, neurodevelopment is a much longer process than is gestation and birth is a relatively arbitrary event in the process that does not represent the end of brain development. Thus, birth is relevant as the end of the physical link (i.e., umbilical cord and placenta) between the mother and infant, but neurodevelopment remains ongoing from the earliest stages in gestation until death. As such, this has implications for the timings of both exposure and assessment since the point of assessment in one species (e.g., rodents) might correspond to prenatal exposure in another (e.g., humans).

3.2. *Exposure timing differences*

The timing of PS exposure may be the most important moderator for effects on the fetus. It may be less the type of disruption to fetal development than the timing that determines risk for negative outcomes (Mednick et al., 1988). Timing effects are a function of (a) the ontogeny of fetal development, (b) alterations in maternal biological and psychological reactivity to PS exposure during pregnancy, and (c) fetal exposure to PS-related biological processes through placental function and/or placental transfer. As

well, the expression and activity of placental 11 β HSD2 is regulated in a cellular and gestational age-specific manner in humans (Pepe et al., 2001; Rosenthal et al., 2001).

The central nervous system (CNS) is a dynamic and complex structure that develops and changes by way of a long-lasting process that begins in utero and continues until death. In fact, prenatal brain development represents only one part of the lengthy developmental process, and early postnatal life, childhood and adolescence represent windows of tremendous brain changes and maturational processes (Marsh et al., 2008; Paus et al., 2001, 2008; Paus et al., 1999). Mammals have a highly conserved order of developmental events (Finlay and Darlington, 1995; Finlay et al., 1998) and while regional brain development is somehow comparable between mammals, there are, however, many differences such as the relative duration of these neurodevelopmental events during gestation (Bayer et al., 1993; Clancy et al., 2001, 2007a,b), the neurodevelopmental time scales (e.g., days for rats versus weeks/months for humans), as well as the ratio of specific brain structure volumes to total brain volume in different species (Rilling and Insel, 1998; Schoenemann et al., 2005; Semendeferi and Damasio, 2000).

It would be beyond the scope of this review to fully describe the mechanisms that are involved at each step of the ontogeny of the CNS (see review by Rice and Barone (2000)). However, to better understand the importance of the timing of the exposure in determining the effects of PS on the developing brain, it is important to review some of the main processes involved during the ontogeny of the CNS, which includes cell proliferation, migration, differentiation, synaptogenesis, myelination and apoptosis.

Shortly after the neural tube is formed (rats ~GD 11; humans ~ GD 26 to 28; (Rice and Barone, 2000)) regions of cell proliferation appear in the ventricular and subventricular zones (de Graaf-Peters and Hadders-Algra, 2006). Subsequently, neurogenesis and migration of neuroblasts in the forebrain, midbrain, and hindbrain mark the initial formation of specific areas of the CNS (Rice and Barone, 2000), following a pattern and progression of regional neurodevelopment that are relatively parallel between rats and humans (Bayer et al., 1993). The undifferentiated migrating neuroblasts will later differentiate into neurons or glial cells depending on the interaction with intra- and extracellular cues. Both differentiation and synaptogenesis continue into the postnatal period (Bayer et al., 1993; de Graaf-Peters and Hadders-Algra, 2006; Rice and Barone, 2000), and synaptic maturation (Waites et al., 2005) takes place postnatally during the first 3 weeks in rats and through adolescence in monkeys and humans (de Graaf-Peters and Hadders-Algra, 2006; Rice and Barone, 2000). Following cell proliferation, migration and differentiation, myelination occurs during the second postnatal week in rats and begins during the last trimester of gestation in humans and continues through adolescence in both rodents and humans (de Graaf-Peters and Hadders-Algra, 2006; Rice and Barone, 2000). Apoptosis is another mechanism that participates in the normal development of the brain by removing large numbers of neurons in some regions during both pre- and postnatal development. It is important to understand that if cell proliferation is affected, then migration can be affected, which may in turn alter cell differentiation (Rice and Barone,

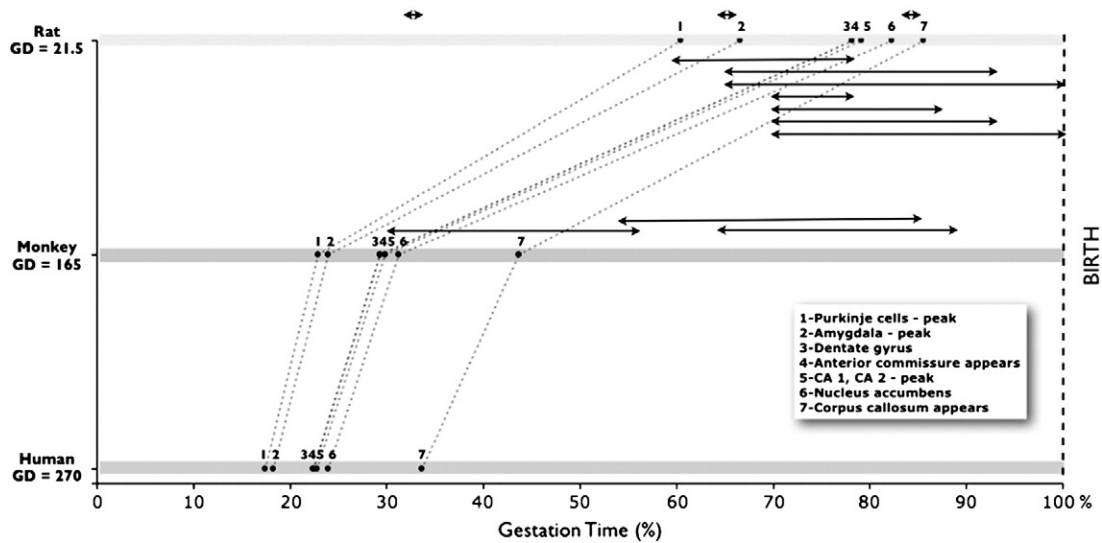


Fig. 2 – Normalized developmental timelines during gestation for the rat, monkey and human. Adapted from Clancy et al. (2001). Horizontal arrows represent the duration of the prenatal stressors used in the various studies that are reviewed in this article (see Table 1). Numbers 1 to 7 represent some major brain developmental events. GD=gestational days.

2000). In other words, cell differentiation in a particular structure of the brain could be affected, for example, by PS occurring at the time of cell differentiation or by the same stressor occurring earlier at the time of cell proliferation or migration, all of these occurrences resulting potentially in the same localized brain morphological changes. This possibility further complicates the determination of a relationship between timing of a stressor and affected neurodevelopmental process.

A good illustration of the complexity and the importance of the timing of exposure come from studies that have looked at the effect of maternal undernutrition on placental and fetal weight. The effect differs depending on when the insult occurs during gestation (Belkacemi et al., 2009; Heasman et al., 1998; Lumey, 1998). Pregnant women who experienced starvation during their 3rd trimester had a smaller placenta and a low birthweight newborn but an unaltered placenta/birthweight ratio compared to mothers who did not experience starvation during this same period of gestation (Lumey, 1998). However, maternal starvation during the first trimester of pregnancy increased placental weight without any impact on newborn weight compared to women who did not experience starvation during this same period of gestation (Lumey, 1998). This study suggests that placental adaptation in early pregnancy can overcome environmental stressors, so that fetal supply (e.g., nutrients, oxygen) is maintained in late gestation; thus sustaining normal fetal growth and development. In sheep, maternal undernutrition during early to mid-pregnancy increased placental weight at term without altering fetal weight—thus increased the placental to fetal weight ratio; a finding similar to humans (Heasman et al., 1998). Finally, undernutrition during the second half of pregnancy in rodents induces a decrease in placental to the fetal weight ratio, suggesting that undernutrition irreversibly affects placental weight when nutrient deprivation occurs at a time when fetal nutrient demand is maximal (Belkacemi et al., 2009).

In terms of the relationship between timing of a stressor and outcomes, human studies suggest that stressors occurring during mid-gestation are particularly associated with the worst behavioral outcomes (Huttenen and Niskanen, 1978; Kinney et al., 2008a; Mednick et al., 1999; Watson et al., 1999). Interestingly, however, in the case of non-human primates, both birth weight and motor functioning seem to be more affected if PS occurs in early gestation, versus mid-late gestation (Schneider et al., 1999). However, some researchers have found effects of 3rd trimester PS on obstetric complications (Crandon, 1979), autism (Kinney et al., 2008a,b), and inattention and hyperactivity in 4- and 6-year-old boys (O'Connor et al., 2002, 2003). Thus, the question of timing of PS is intimately associated with the outcome factor of interest and closely related to the developing brain structures being affected.

The timing of a stressor during pregnancy might also moderate the mothers' stress reactions. It has been demonstrated that women rated the stress of an earthquake as less severe if they were exposed during the 3rd trimester than if they were exposed in the 1st trimester, or postpartum (Glynn et al., 2001; Kammerer et al., 2002).

3.3. Different types of stressors

Animal studies of the effects of PS on the offspring's brain vary in the kinds of stressors they use. In rodent studies saline injection, forced swimming, crowding, and most often, restraint alone or restraint with heat and bright light are frequently used stressors, whereas in non-human primate studies an acoustical startle protocol is preferred (Table 1). The common thread among these stressors is that they are relatively unpredictable and uncontrollable events, with sudden onsets that trigger an HPA response in the animals (Clarke and Schneider, 1993; Coe et al., 1996, 2003; Kapoor et al., 2008; Murmu et al., 2006; Peters, 1982; Szuran et al., 1994;

Ward and Weisz, 1980). It is, however, important to note that the level of the stress response in the pregnant dam is likely to be dependent on the type of stressor. For example, Murmu and colleagues (2006) show that in pregnant dams, restraint, forced swimming and crowding each significantly increase plasma levels of corticosterone. However, and most importantly, the increases vary by stressor, with restraint showing greatest increase (522 ± 55 ng/mL), forced swimming a lesser increase (438 ± 85 ng/mL), and crowding the weakest increase (247 ± 61 ng/mL). The control pregnant rat, that was left undisturbed in her home cage, had a much lower plasma level of corticosterone of 52 ± 9 ng/mL. Thus, restraint protocols have almost a twofold greater effect on the pregnant dams' HPA axis response relative to crowding protocols. Interestingly, one of the factors that differs among these stressors is the degree of control that an animal has over that event, i.e. restraint stress can heuristically be seen as the stressor that leaves the animal with absolutely no control over the situation resulting in the highest HPA axis response, whereas both forced swimming or crowding might represent stressful events that the animal can "act on" and therefore exert a certain degree of control over it. Thus, the type of stressor seems to influence the animal's resiliency to stress (Murmu et al., 2006). Moreover, differences in the degree of resiliency to stress among animals of the same species or between animals of different species might represent another factor that is likely to contribute to differences in the effects of PS in animal studies.

More importantly, for this discussion on the compatibility of animal and human PS studies, stressors used in animal studies are very different from those that might affect humans. Unlike animal studies, it is unethical to knowingly stress pregnant women, thus researchers must rely on more naturally occurring stressful events. As such, stressors that evoke an HPA reaction in pregnant women, and are the subject of research, are either of a psychosocial nature or related to natural (e.g., earthquakes, hurricanes, ice storms) or man-made (e.g., terrorist attacks on civilian populations, oil spills, nuclear power plant explosions) disasters. These stressors may be novel, perceived as uncontrollable, and/or threatening. It is important to note that most studies do not use natural or man-made disasters as stressors because of their relatively rare occurrence compared to stressors of a psychosocial nature. Another important difference between animal and human studies is that in humans it is possible to assess not only the objective exposure and hormonal response to a stressor, but also the subjective distress resulting from a particular stressful event. This is important since humans can have very different cognitive attributions with respect to the same stressor (Lazarus and Folkman, 1984). In other words, what might seem totally uncontrollable to some might be perceived as a controllable event to others. This subjective component in the face of a stressor has direct physiological implications such that having a feeling of control over a stressful event triggers a chain of physiological reactions different from the one that is triggered when the stressful event appears to be uncontrollable (Huether, 1998). The different nature of stressors affecting experimental animals and humans also contributes to the challenge in bridging animal and human studies.

3.4. Different timings of postnatal assessment

Another factor that complicates the comparison and integration of findings among animal studies is the difference in postnatal ages when offspring are assessed or sacrificed (Table 1). This element is particularly relevant as illustrated in the study by Anderson et al. (1985) in which the pattern of differences in the size of the sexually dimorphic nucleus of the preoptic area (SDN-POA) between PS and control animals is different at different times (birth, postnatal day 20 and 60). Similarly, Kraszpulski et al. (2006) report reductions in amygdalar nuclei volumes, together with decreases in neurons and glial cell numbers at either 7 or 25 days postnatally in PS rats, which then resolved at 45 days. This suggests that assessment time is an important factor that can affect the likelihood of observing a PS-related effect in the offspring's brain.

Finally, as there are differences in developmental timings during gestation between rodents and humans, there are also differences in developmental timings postpartum. As noted by Quinn (2005) "Just because the rat's lifespan is 3 years does not mean it lives a miniature human lifetime within those 3 years" (p. 776). Certainly, both rats and humans go through the same order of developmental stages including infancy, weaning, puberty, adulthood, reproductive senescence and post-senescence. However, the timing of these periods is different (Quinn, 2005). Therefore, a straight age translation between rats and humans is not reasonable (Clancy et al., 2001) although approximations might be possible within known periods of development (e.g., nursing, prepubescent, adolescent, adult and aged; (Quinn, 2005)). This is an important issue to keep in mind when trying to extrapolate findings from animals to humans.

3.5. Sex moderating effects

Some studies assess the effects of PS on brain development in both male and female offspring (e.g., (Coe et al., 2002, 2003; Jones et al., 1997; Mandyam et al., 2008; Schmitz et al., 2002; Szuran et al., 1994). Others, however, only assess males (e.g., (Barros et al., 2006; Kraszpulski et al., 2006; Lemaire et al., 2000; Lemaire et al., 2006; Salm et al., 2004), and some others present their findings without specifying the sex of the animals that were studied (e.g., (Fujioka et al., 2006; Hayashi et al., 1998)). Since many findings differ between males and females (Table 1), it is important to consider the sex of the animals from which these findings originate. These differences are likely due to the fact that sex hormone activity during gestation influences male and female brains in a number of different ways. More specifically, animal studies have shown that specific regions of the normal male and female brains differ in structure volumes, cell numbers, neuronal morphology, synaptic connections, and numbers of connecting fibers between structures (Schwarz and McCarthy, 2008). For example, in rats, males have a larger SDN-POA of the hypothalamus than females (Anderson et al., 1985; Gorski et al., 1978) and a right cerebral cortex that is thicker than the left (Diamond et al., 1983; Fleming et al., 1986), whereas females have a larger rostral anterior commissure (rAca) than males (Jones et al., 1997). In humans, structural sex-specific differences are also

present in the brain (e.g., hypothalamus, cerebellum, hippocampus, amygdala, etc. (Cosgrove et al., 2007)).

These sex-specific differences in the brain result from the activity of sex hormones, in particular androgens, during critical periods of brain development (Morris et al., 2004; Schwarz and McCarthy, 2008; Weinstock, 2007). In males, testosterone is produced by the testes in considerable amounts at the end of gestation in rats, with a peak production on gestation days 18–19 (Ward and Weisz, 1980; Weisz and Ward, 1980), and during mid-pregnancy in humans, between weeks 12 and 18 (Abramovich, 1974). The enzyme aromatase then converts testosterone into estradiol, which permits the masculinization of the brain (Morris et al., 2004; Schwarz and McCarthy, 2008). These steroid hormones, rather than promoting cell genesis in future sexually dimorphic regions, determine whether or not pre-existing cells will undergo apoptosis (McCarthy and Konkle, 2005). For example, volumetric differences in the SDN-POA of the hypothalamus are absent at birth between male and female rodents, but during the first postnatal week, cells in this region undergo apoptosis in females only, resulting subsequently in smaller volumes (Anderson et al., 1985).

Interestingly, PS resulting from three 45-min periods of restraint under intense illumination daily from GD 14–21 shifts the peak production of testosterone in male fetuses one day earlier to GD 17 rather than the normal GD 18 (Ward and Weisz, 1980, 1984). This shift in the peak production of testosterone translates into both neurodevelopmental, cognitive and behavioral changes observed later in adulthood (Anderson et al., 1985; Coe et al., 2002; Jones et al., 1997; McCarthy and Konkle, 2005; Schwarz and McCarthy, 2008). This shift in the peak production of testosterone might be related to increased levels of cortisol in the fetus following the implementation of the stressor as suggested by positive correlations found between testosterone and cortisol in both amniotic fluid and fetal plasma in humans (Gitau et al., 2005; Sarkar et al., 2007).

In many animal models of fetal programming a disparity between male and female disease outcomes in the timing of onset and severity has been noted. Indeed, the same prenatal insult does not always affect males and females in the same manner or to the same degree. It has been suggested that the male fetus is more vulnerable to the effect of PS (Mueller and Bale, 2008). However, to date there has been a paucity of studies on sex differences in the placental response to PS. Sexual dimorphism has been shown for the human 11 β -HSD2 showing a significant decrease in expression and activity in male compared to female fetuses (Stark et al., 2009). Also, in rodents and humans, the activity and sensitivity of the placental 11 β -HSD2 to stimuli appears to be sex-linked, with greater vulnerability in males (Burton and Waddell, 1994; Kerzner et al., 2002). These studies suggest that male fetuses are exposed to higher levels of cortisol and may partly explain the gender specificity of the phenotype programmed in utero. Moreover, epidemiological studies have shown that pregnancy complications, fetal morbidity/mortality and neurodevelopmental alterations are more often associated with male than female fetuses (e.g. Draper et al., 1999; Johnson and Breslau, 2000; Shankaran et al., 1996; Sizun et al., 1998; Stevenson et al., 2000; Vatten and Skjærven, 2004)). The mechanisms that are implicated in those differences are still unknown.

3.6. Summary of the section

In light of these considerations it appears that integrating the results obtained from animal studies and then extrapolating them to humans is an extremely challenging task. However, animal studies provide an invaluable source of hypotheses that need to be tested in humans.

With these many considerations in mind, the following sections describe findings from animal studies on the effects of PS on the developing brain.

4. Effects of prenatal stress on the offspring's brain

Animal studies on the effects of PS in the offspring have mostly looked at alterations in behavioral and cognitive outcomes (Weinstock, 2008). Comparatively, PS-related changes in the offspring's brain, which might explain, at least in part, the observed behavioral and cognitive changes, have been the subject of fewer studies. Brain regions that have received attention, so far, are those that are known to subserve these PS-related affected behavioral and cognitive functions (e.g., hippocampus for learning and memory, amygdala for emotions, etc.). Since the choice of brain regions studied is driven by previous behavioral and cognitive findings on the effects of PS in the offspring, there might possibly exist other regions that are equally affected by PS. Here we review the various regions that have been shown to be susceptible to PS including the hippocampus, amygdala, corpus callosum, anterior commissure, cerebral cortex, cerebellum and hypothalamus. Brief summaries of the methods and findings of studies examined for this review can be found in Table 1.

4.1. Hippocampus

The hippocampus develops primarily during the fetal period in both rodents and primates (Rice and Barone, 2000; Seress et al., 2001). The dentate gyrus, which contains granule cells, is a very particular region of the hippocampus in that active neuronal proliferation continues into adulthood (Balu and Lucki, 2009; Piatti et al., 2006). The physiological and behavioral role of adult hippocampal neurogenesis remains the subject of much debate (Amrein and Lipp, 2009), but it may participate in learning and memory. Rodents and primates differ regarding the timing of the production of the majority of dentate's granule cells (i.e., around 85% are produced postnatally in rodents while a similar ratio is produced prenatally in primates (Bayer, 1980, 1982; Rakic and Nowakowski, 1981)). The same applies to the neurons of the CA1–3 areas (i.e., during the first half of pregnancy in primates (Rakic and Nowakowski, 1981), whereas in rodents this occurs during the last days of gestation (Bayer, 1980)).

Uno and colleagues (Uno et al., 1989, 1990, 1994) have conducted a number of studies on the effects of pre- and post-natal stress on hippocampal functioning in non-human primates. They reported that chronic stress in adult primates is associated with alterations within the hippocampus, suggesting that the hippocampus is a primary target for the effect of glucocorticoid steroids following stressful experiences (Uno et

al., 1989). By mimicking the effects of PS through intramuscular injection of DEX to pregnant dams, the same authors demonstrated a dose-dependent degenerative change and reduction of the offspring's hippocampal neurons (Uno et al., 1990) and an overall 30% reduction in hippocampal volume in DEX-treated 9-month-old juvenile monkeys, even though the total brain volume remained unchanged in these animals (Uno et al., 1994). Finally, magnetic resonance imaging (MRI) studies indicate that this hippocampal volume loss in the infant monkey is maintained 2-years postnatally (Uno et al., 1994). Coe and colleagues (Coe et al., 2003) report similar findings using a more "natural" acoustic startle stressor. Both early (i.e., GD 50 to 92) and late (i.e., GD 105 to 147) stress to pregnant Rhesus monkey dams resulted in reduced hippocampal volumes in the offspring (decreases of 12% and 10% for early and late PS, respectively) and a 32% inhibition of postnatal neurogenesis in the dentate gyrus (Coe et al., 2003). This study suggests that, at least in non-human primates, the same stressor administered at different periods during gestation produces similar effects on the offspring's developing brain.

The PS-induced global decrease in hippocampal volume and localized inhibition in postnatal neurogenesis are also demonstrated in studies conducted in rodents. After subjecting pregnant dams to restraint stress during GD 15–19, hippocampal wet weights of 3-month-old offspring are reduced by 15.4% in PS males and by 8.2% in PS females (Szuran et al., 1994). However, Schmitz et al. (2002) observed decreased hippocampal volumes, that were related to a reduced number of hippocampal granule cells, in 2½-month-old PS female rats relative to control female rats, whereas no differences were observed between PS and control males. It is worth noting that, unlike the study by Szuran et al. (1994) in which restraint stress 3 times for 30 min per day during GD 15–19 was used, Schmitz et al. (2002) used a milder stressor (i.e., 20-min restraint stress on a single day of gestation (GD 18)) than that used by Szuran et al. (1994). Thus, the difference in the magnitude of the stressor might explain why differences in PS and control males in hippocampal volumes were found in the Szuran et al. (1994) study.

The aforementioned changes in hippocampal volumes represent macroscopic manifestations of the effects that PS has on the developing brain. At a more microscopic level, a number of alterations, that are likely related to macroscopic changes, have also been demonstrated. In one such study, Lemaire and colleagues (Lemaire et al., 2000) reported a reduction in the number of granule cells within the hippocampal dentate gyrus of PS rats at 28 days postnatally that continued to be seen 22 months following birth, resulting in a 55% decrease in granule cell production by this age. These authors also reported similar findings in Wistar rats at 4 and 26 months (i.e., 46–47% decreases in neurogenesis in the dentate region (Lemaire et al., 2006)). This finding is important since, as mentioned earlier, approximately 85% of these cells are usually formed postnatally in the rodent. Therefore, it appears that the effects of higher-than-normal plasma corticosterone levels observed in the offspring's stressed mother are not restricted to the fetal hippocampal development, but extend long after birth. Other rodent studies report similar PS-induced decreases in cell proliferation in the hippocampus by 60% at 10 days postnatally (Kawamura et al., 2006) or by about 24% (only in

males) at 110 days postnatally (Zuena et al., 2008). These differences in the ratios of decreased adult hippocampal neurogenesis could arise from different timings of the stressor and/or different assessment times postnatally.

PS has also been found to induce a reduction in dendritic arborization by 73%, with synaptic loss by 50%, in the CA1 hippocampal area, in 90-day-old adult rats (Barros et al., 2006). This is in agreement with an earlier study by Hayashi and colleagues reporting that PS Wistar rats have a significant 32% reduction in synaptic density ($p < 0.0001$) within the hippocampal CA3 area as measured on postnatal day 35 (Hayashi et al., 1998).

Most interestingly, PS seems to have both enhancing and suppressing effects on the development of hippocampal neurons depending on the intensity of the stressor (Fujioka et al., 2006). Short-lasting (i.e., 30 min), mild PS seems to enhance neonatal neurogenesis and differentiation of processes of hippocampal neurons, whereas, long-lasting (e.g., 240 min), severe PS impairs their morphology. This phenomenon known as hormesis can be defined as "a dose–response phenomenon that is characterized by a low-dose stimulation and a high-dose inhibition" (Calabrese and Baldwin, 2002; Calabrese, 2008). Therefore, while PS may have detrimental effects on the brain at high levels, it appears to have some beneficial effects at low levels. This study also shows that mineralocorticoid receptors in the hippocampus mediate the enhancement of neurogenesis and differentiation of processes of cultured hippocampal neurons whereas glucocorticoid receptors are involved in the suppression of their morphology (Fujioka et al., 2006).

In contrast to animal studies, no human study has to date examined the potential impact of PS on the structural development of the hippocampus prospectively. Some, often conflicting, evidence that a severe stressor might alter hippocampal structure in humans comes from studies conducted in the field of post-traumatic stress disorder (PTSD). Early studies indicate that the hippocampal volumes of individuals with PTSD are substantially lower than those of matched controls (Bremner et al., 1995, 1997; Gurvits et al., 1996; Schuff et al., 1997; Stein et al., 1997). However, more recent studies have not replicated this finding (Agartz et al., 1999; Bonne et al., 2001; DeBellis et al., 1999; Schuff et al., 2001; Yamasue et al., 2003). Moreover, Pitman et al. (2006) report smaller hippocampal size in both Vietnam soldiers with PTSD and their monozygotic twins who did not go to war suggesting that the smaller hippocampal size was pre-existing. Regardless of whether traumatic stress results in smaller hippocampi, PTSD patients have a significantly reduced number of active neurons within their hippocampi relative to matched controls (Corbo and Brunet, 2003), indicating that while volume may not be altered by severe stress, the functionality of the hippocampus is possibly altered.

4.2. Amygdala

The amygdala is part of the limbic system and plays a central role in memory and emotions (Seymour and Dolan, 2008). Several nuclei constitute the amygdala including the medial, central, lateral, basolateral, basomedial and cortical amygdalar nuclei.

Offspring of Sprague–Dawley dams who were handled, exposed to a novel environment and injected with saline once

a day between days 14 and 21 of gestation have smaller amygdalar nuclei volumes (basolateral, central and lateral; by ~20–25%), smaller anterior-posterior lengths of lateral and basolateral nuclei (by ~10%), and decreased number of neurons (by ~25–30%) and glial cells (by ~30%) (basolateral, central and lateral) relative to controls on postnatal days 7 or 25 (Kraszpulski et al., 2006). This finding is consistent with another study showing a global, albeit non-significant, 30% decrease in total amygdalar neurogenesis in 10-day-old PS Sprague–Dawley rats (Kawamura et al., 2006). Interestingly, the decrease in volumes and cell numbers seems to resolve after 45 days (Kraszpulski et al., 2006) or 80 days (Salm et al., 2004). At this latter postnatal time the volume of the lateral amygdalar nucleus in PS-exposed offspring is increased by 30%, the number of its neurons by 49%, its glial cells by 43%, and its neuronal density by 22% compared to control offspring (Salm et al., 2004). The postnatal volume increase occurs exclusively for this lateral amygdalar nucleus since no changes are observed for the dorsal endopiriform, central or basolateral (Salm et al., 2004) or medial (Kerchner et al., 1995) amygdalar nuclei. This suggests that PS does not alter the developmental trajectory of all amygdalar nuclei in the same way. During the early postnatal developmental stages a decreased volume and number of neurons and glial cells is observed in the lateral amygdalar nucleus, whereas during the following stages of development, there is an increased volume and number of neurons and glial cells in this nucleus, relative to control animals. This emphasizes the importance of taking into account assessment times when interpreting findings from animal studies as well as investigating sub-regions of a particular structure when possible.

4.3. Corpus callosum and anterior commissure

The corpus callosum appears around GD 18 in rats, GD 72 in non-human primates and beginning of the 2nd trimester in humans (Clancy et al., 2001). To date, the only study that has investigated the effects of PS on corpus callosum morphology has been conducted in Rhesus monkeys (Coe et al., 2002). The authors performed structural MRI in both PS and control offspring when they were between 7 and 11 months of age and obtained morphometric measures from sagittal and coronal scans. PS, in the form of an acoustical startle protocol administered to pregnant dams between days 90 and 140 of gestation, differentially alters the corpus callosum for males and females. While PS males exhibit a decreased corpus callosum area relative to control males, females show an increase in the total callosal area when compared to control females. This is accompanied by a shift in the anterior-to-posterior shape from the genu back toward the splenium in both males and females. Despite these localized structural changes, PS animals do not exhibit abnormal behavior.

In humans, alterations of the corpus callosum have been observed in a number of disorders including autism (Egaas et al., 1995), schizophrenia (Innocenti et al., 2003) and ADHD (Seidman et al., 2005). Considering the association that exists between PS and these disorders (Beversdorf et al., 2005; Grizenko et al., 2008; Huttenen and Niskanen, 1978; Kinney et al., 1999a, 2008a,b; Kinney, 2001; Linnert et al., 2003; McIntosh et al., 1995; Rodriguez and Bohlin, 2005; van Os and

Selten, 1998), it could be argued that this association represents indirect evidence suggesting that PS might affect the development of the corpus callosum in humans as well.

The anterior commissure (Aca), a white matter tract located in front of the anterior columns of the fornix, conveys mostly olfactory information between the hemispheres (Jones et al., 1997). The rostral Aca (rAca) is a sexually dimorphic structure, with females having a larger coronal rAca area than males, that has been shown to be affected by PS (Jones et al., 1997). PS in rats reverses this sexual dimorphism causing the rAca area in males to be larger than that of females (Jones et al., 1997). This is consistent with other studies that have shown that PS also reverses the sexual dimorphism of structures such as the SDN-POA (Anderson et al., 1985; Rhees et al., 1999).

In humans, decreases in white matter density of the Aca, as determined by MRI, have been found in patients with schizophrenia (Hulshoff Pol et al., 2004) as well as in post-mortem brains of females with schizophrenia, but not males (Highley et al., 1999). This evidence supports the notion that in schizophrenia there is an alteration of interhemispheric connectivity.

4.4. Cerebral cortex

The neocortex, which accounts for about 90% of the cerebral cortex, is organized as a six-layer mantle of gray matter containing neuronal cell bodies and unmyelinated fibers. Humans and other primates have a highly convoluted cerebral cortex with gyri (ridges) and sulci (grooves) that confer a large surface area, while that of the rodents is lissencephalic with a relatively smaller surface area (Rice and Barone, 2000). The cerebral cortex is responsible for a variety of higher order brain processes such as memory, planning, problem solving, sensation, voluntary muscle movement and, in humans, language.

In 90- and 185- to 190-day-old control Long–Evans rats, the right cerebral cortex is thicker than the left in several areas in males, while in females the left cerebral cortex is generally thicker than the right, in the same areas, although these differences do not reach statistical significance (Kreig, 1946). In PS Sprague–Dawley males, the cerebral cortex appears more female-like compared to control males, although results fail to reach significance (Fleming et al., 1986). This evidence is in line with the demasculinized and feminized sexual behavior patterns exhibited by adult PS males (Fleming et al., 1986). In addition, a 46% dendritic arborization reduction and a 52% synaptic loss is found in the frontal cortex of PS males (Barros et al., 2006), and N-acetyl-aspartate (NAA) is reduced by 21% in the left frontal cortex in PS males, but not in the right, reflecting a decrease in neuronal integrity (Poland et al., 1999). Moreover, dendritic spine densities on both the apical and basal dendrites of pyramidal neurons are reduced by approximately 20% in the dorsal anterior cingulate cortex (ACd) and orbitofrontal cortex (OFC) for both males and females (although results failed to reach significance for the basal dendrites of pyramidal neurons in the ACd, in males) (Murmur et al., 2006). In PS males, apical, but not basal, dendritic length of pyramidal neurons is reduced by 30% in the ACd and 25% in the OFC. In both of these cortical regions,

the complexity of dendritic arborization is also reduced in PS males. In PS females, however, no dendritic atrophy of pyramidal neurons is found (Murmu et al., 2006). In contrast, Michelsen et al. (2007) do not find that PS affects dendritic spine density of pyramidal neurons in the medial prefrontal cortex, although they do report a decrease in the ratio of mushroom spines of pyramidal neurons. The difference in the findings of these two aforementioned studies in the presence or absence of reduced dendritic spine densities of pyramidal neurons in regions of the frontal cortex could arise from differences in the stressors that were used namely, varied stressors over time (Murmu et al., 2006) versus the same repeated stressor (Michelsen et al., 2007). Perhaps most importantly, differences could arise from the fact that assessment times were very different with a relatively early assessment at 23 days in the study by Murmu et al. (2006) and a later assessment, at 100 days, for Michelsen et al. (2007). Reductions in dendritic spine densities of pyramidal neurons in the medial frontal cortex may have been present at the early stages of postnatal development and may have resolved at later stages.

All of these findings (Barros et al., 2006; Fleming et al., 1986; Michelsen et al., 2007; Poland et al., 1999), except for the study by Murmu et al. (2006), were obtained in male rats only. Therefore, because of possible sex-specific differences in the effects of PS in the offspring's brain, these results would need to be replicated in female rats.

4.5. Cerebellum

The cerebellum participates in the integration of sensory perception, coordination and motor control. There is also increasing evidence that the cerebellum is involved in cognition and emotion in humans (Schmahmann, 2004; Schmahmann and Caplan, 2006; Schmahmann et al., 2007; Schutter and van Honk, 2005; Schutter and van Honk, 2009). Furthermore, the cerebellum might be involved in the pathophysiology of several psychiatric disorders (reviewed by Hoppenbrouwers et al., 2008) such as autism, schizophrenia, ADHD, and mood and anxiety disorders.

So far, two animal studies by Ulupinar and colleagues (Ulupinar and Yucel, 2005; Ulupinar et al., 2006) have looked at the effects of PS (restraint) on the cerebellum of rats. At postnatal day 30, PS and control animals do not differ in their brain and cerebellum weights, brain-to-body ratios, volume fraction of the granule cell layer to whole cerebellar cortex, or numerical densities of granule cells. However, PS animals have an 11% reduction in volume fraction of granule cell nuclei in granular layers that are related to a decrease in mean granule cell diameter (Ulupinar and Yucel, 2005). This suggests that it is the morphology of granule cells that is affected by PS, and not their number or the volume proportion of the granular layer. Synaptic density and number of synapses per neuron are also reduced by half in the granular layer of PS animals, consequently affecting interneuronal connectivity (Ulupinar and Yucel, 2005). The authors subsequently found a 24% decrease in cerebellar granule-to-Purkinje cell ratio in PS animals due to an increase in the numerical density of Purkinje cells rather than a decrease in granule cells (Ulupinar et al., 2006). The timing of the stressor in this study (i.e., GD 14) corresponds to

the time when Purkinje cells are generated (Fig. 2). Therefore, it seems that PS increases the numerical density of Purkinje cells in the cerebellum.

4.6. Hypothalamus

The hypothalamus develops during the late embryonic/early fetal periods in both rats and humans. It is located medially in the brain, just below the thalamus and is composed of several nuclei, some of which are sexually dimorphic. One of these nuclei, the SDN-POA (see review by Hofman (1997)), first described in the rat brain by Gorski et al. (1978), is involved in aspects of male sexual behavior (i.e., mounting, intromission, and ejaculation) (De Jonge et al., 1989).

In control rats, the cross-sectional area of the SDN-POA is bigger in males than in females at 20 and 60 postnatal days, but not at birth (Anderson et al., 1985), as sexual differentiation of the SDN-POA occurs within the first 10 days of postnatal life (Jacobson et al., 1980) resulting from differences in perinatal steroid levels (Kawata, 1995). Similarly, in the adult human brain the SDN-POA is twice as large in males as in females and contains twice as many cells, a difference that arises around the age of four years when cell numbers start to decline in girls but remain stable in boys (Hofman, 1997).

PS induces a marked difference between PS and control animals in the size of SDN-POA at birth, but only in males (Anderson et al., 1985). Indeed, the SDN-POA of PS males is almost twice as large as in control rat males. Later, at 20 and 60 days of age, the SDN-POA size of PS males is 50% smaller than control animals, which brings their values closer to those of control females of the same age (Anderson et al., 1985). Females, on the other hand, show no difference between PS and control animals in the size of SDN-POA at birth, 20 and 60 days suggesting that this structure in females is not sensitive to the effects of PS. Findings by Kerchner and Ward (1992) on the SDN of the medial preoptic area (SDN-MPOA) are consistent with those reported by Anderson et al. (1985). While Kerchner and Ward found smaller SDN-MPOA in PS males aged between 73 and 176 days relative to control males, PS males had larger SDN-MPOA than females. Together these results suggest that PS affects the sexual differentiation of SDN-POA but only in males.

When the males' sexual behavior is introduced into the analyses (Rhees et al., 1999), the SDN-POA volumes of PS male rats that do not copulate are found to be significantly reduced, therefore feminized, whereas the SDN-POA volumes in PS males that do copulate are not altered (i.e., similar to sexually active control males). Interestingly, the few sexually non-active control males had significantly reduced SDN-POA volumes compared to the control males that copulate. In opposition to the decrease in SDN-POA in PS males that do not copulate, the volume of the anteroventral periventricular nucleus (AVPV), another hypothalamic nucleus, is significantly increased (feminized) in PS males that are sexually non-active compared to AVPV volumes in sexually active males. Therefore it seems that PS can have differential effects on the morphology of certain hypothalamic nuclei by either increasing or decreasing their volumes, and that these volumes are associated with specific observable behaviors.

4.7. Summary of the section

PS affects a number of brain regions in exposed offspring including the hippocampus, amygdala, corpus callosum, neocortex, cerebellum, and hypothalamus. These PS-induced changes are visible both macroscopically and microscopically but the relation between the two is still unclear. Because the choice to investigate those regions is based on pre-existing literature showing a link between PS and an affected behavioral outcome, subserved by these brain regions, it is plausible that using exploratory approaches to look at the whole brain could help uncover the full extent of PS-related brain changes in the offspring.

Notwithstanding the value of these results in providing working hypotheses on the mechanisms by which PS might affect brain development in humans, it seems that the ideal solution to circumvent the many challenges and limitations involved in animal studies would be to study the effects of PS directly in humans, while remaining within ethical bounds. Thus, disaster research might represent a unique opportunity to study the effects of PS on brain development in humans.

5. Prenatal stress studies in humans: The present and the future

A certain amount of life stress is unavoidable. However, when sudden stressors occur to pregnant women, effects might be observed in the subsequent cognitive, behavioral, emotional, and physical attributes of their children. The sequelae of prenatal exposure to maternal stress may well have long-lasting consequences on academic performance, physical and mental health. The root of these delays, deficits, and alterations may be through direct effects of PS on fetal brain morphology. However, to date, no study in humans has been able to relate the severity and timing of PS to effects on specific brain regions. Recently, however, a prospective longitudinal study of 35 women (Buss et al., 2010) has examined the effects of pregnancy anxiety (available at 19, 25 and 31 weeks gestation) on their offspring's brain at 6 to 9 years of age using MRI and voxel-based morphometry. This study shows regional reductions in gray matter density associated with pregnancy anxiety at 19 weeks gestation, but not at 25 and 31 weeks gestation, in the prefrontal and premotor cortices, the medial temporal lobe, the lateral temporal cortex, the postcentral gyrus, the cerebellum, the middle occipital gyrus and the fusiform gyrus (Buss et al., 2010), regions particularly involved in cognitive processing.

Despite the paucity of similar human studies, there is, however, direct evidence from animal experimentation, and indirect evidence from correlational behavioral studies in humans. Both retrospective studies with humans, and experimental research with animals, suggest that psychosocial stressors during pregnancy can influence the physical, cognitive and behavioral outcomes of the offspring. The expression of stress exposure, however, may be contingent upon the nature of the individual's pre-existing vulnerability, the timing and severity of the stressor, and maternal psychosocial characteristics such as socio-economic status, social support and personality traits. There are important gaps

in the existing literature, however, that obscure the nature of the underlying mechanisms.

5.1. Limitations of human studies

The manner in which animal studies are designed allows for the direct assessment of the effects of PS on brain development (e.g., random assignment to various controlled stress conditions, sacrifice of the animal for assessment). In contrast to the growing number of animal studies to date, there have been no studies that allow us to look at the extent, longevity, nature, and mechanisms of the effects of PS in the developing human brain. Although animal studies can provide valuable working hypotheses on the mechanisms by which PS might affect brain development in humans, direct comparison between experimental animals and humans remains, as we have seen, is hampered by many challenges. Human studies also have many limitations, including: (1) the challenge of separating state versus trait anxiety, the latter of which may be passed on to the offspring, in part, genetically (Rowe, 1994), (2) the fact that severe life events (e.g., job loss or divorce) are not always "independent" life events but instead reflect, at least in part, heritable personality traits, (3) the relatively small variance and statistical power of studies that would ideally need to include extremely large numbers of pregnant women in order to obtain a large enough group that had suffered from high levels of PS, and (4) the fact that independent life events, assessed in large-scale retrospective studies (e.g., death of father, tornado, foreign invasion) (Huttenen and Niskanen, 1978; Kinney et al., 1999a; van Os and Selten, 1998), happened too long ago to properly assess the pregnant woman's appraisal or hormonal response to that event. Furthermore, any explanations of the potential underlying brain mechanisms responsible for the link between PS and infant and childhood outcomes are forced to rely heavily on animal studies. Thus it is clear that in order to fully understand the mechanisms by which PS may lead to abnormal human brain development, prospective research designs with humans have to be undertaken. But how exactly can we conduct human studies of the effects of PS on brain development that use an experimental protocol similar to those used in animal studies and still remain in accordance with ethical principles?

One unique solution is to take advantage of natural or man-made disasters by assessing the impact and consequences of such stressful events on pregnant women and their offspring prospectively. This type of study would help circumvent many of the limitations discussed above, and therefore allow the researcher to disentangle the objective, subjective and hormonal components of a stressful event.

According to Lechat (1979) disasters are characterized by "disruption exceeding the adjustment capacity of the affected community". They represent stressful life events that are by definition "independent" from any influence of their victims (unlike events such as divorce or job loss) and that can affect a large number of pregnant women in a quasi-random fashion. Natural (e.g., tornados, earthquakes, tsunamis, ice storms), but also man-made disasters (e.g., oil spills, terrorist attacks, nuclear plant explosions), act as "natural experiments" that

randomize distribution of exposure in the same way as in animal studies.

5.2. Project Ice Storm—results to date

In January 1998, southern Québec was hit by an ice storm that resulted in electrical power failures for more than three million individuals for as long as 6 weeks during the coldest month of the year. The ice storm caused about 27 deaths, and created financial and logistical hardships, especially in the Montérégie area, southeast of Montreal. Both the Insurance Bureau of Canada and Environment Canada count the 1998 ice storm as Canada's worst and most costly natural disaster in history. This natural disaster randomly exposed a large number of pregnant women in various stages of pregnancy to varying degrees of storm-related hardship. Project Ice Storm was initiated soon after the event by recruiting a sample of women that were either pregnant during the ice storm or who became pregnant within 3 months after the storm. Project Ice Storm represents a unique opportunity to study prospectively the effects of PS on the developing human brain (King and Laplante, 2005). Apart from the obvious advantage of studying humans rather than animals, the advantages of Project Ice Storm over other human studies are that (1) the stressor was completely “independent” of the pregnant woman's temperament, unlike in other studies that count antenatal anxiety or life events such as divorce and job loss as “stressors” (Lou, 1993; Lou et al., 1994; Wurmser et al., 2006); (2) there was a wide variation in both the timing and the severity of exposure among subjects; (3) it was possible to separate “stress” into degrees of objective exposure (what events did the women experience), subjective distress (what was their psychological reactions to the ice storm), and hormonal stress response; (4) there was a large number of pregnant women exposed to this stressor; (5) it was possible to assess exposure and its effects fairly soon after they occurred and to follow the offspring prospectively; (6) the objective hardship due to the ice storm was randomly distributed and uncorrelated with socioeconomic status; (7) the sample is Canadian, which means that prenatal medical care was available to all, irrespective of social class; (8) even after 10 years, the level of family participation is still high; and (9) there is already overwhelming evidence that the ice storm stress was great enough to produce physical, cognitive and behavioral effects in the children born to ice storm-exposed women (King and Laplante, 2005; Laplante et al., 2004, 2007, 2008).

Findings to date from this ongoing study of the effects of PS on child outcomes indicate that children exposed in utero to high levels of objective PS, independent of the mother's own anxiety levels, exhibit poorer cognitive, linguistic and play abilities relative to children in the low PS group at 2 and 5½ years of age (King and Laplante, 2005; King et al., 2009; Laplante et al., 2004, 2007, 2008), as well as at 8½ years of age (unpublished results). Likewise, greater dermatoglyphic asymmetries are observed in the fingerprints of children whose mothers were without electricity at any time between weeks 14 and 22 of pregnancy, the period of fingerprint development, relative to children exposed during other periods of prenatal development (King et al., 2009). Interestingly, during this period, the development of fingerprints (Mulvihill and Smith, 1969) and hippocampus (Bayer et al., 1993; van Oel et al., 2001)

partially overlap. Thus, fingerprint anomalies may reflect disruptions in normal fetal development that may be clues to disruptions in brain development, in particular the hippocampus. This idea is supported by the fact that fingerprints and the brain develop out of the same fetal ectoderm (van Oel et al., 2001).

Findings from Project Ice Storm suggest that the timing of the exposure to a natural disaster is important: in most instances, poorer initial outcomes were associated with 1st or 2nd trimester exposure (King et al., 2009; Laplante et al., 2004), a period of rapid brain development. Importantly, our finding that greater ridge count asymmetry is observable for children exposed to the ice storm during the critical period for fingerprint development strongly suggests that the level of stress experienced by the pregnant mothers was sufficiently high to result in permanent alterations in the children's physical development.

5.3. Project Ice Storm—ongoing MRI study

In order to determine whether the observed differences in intellectual, linguistic, behavioral/emotional and physical development between children of mothers who experienced high PS and those whose mothers experienced less severe levels of PS could be mediated by differences in brain morphology in the children we obtained structural MRI data in 2008–2009 from a newly recruited control cohort born one year before the ice storm, and from our Project Ice Storm cohort in 2009–2010. More specifically, this study will determine the extent to which varying degrees of PS, and its timing during pregnancy, affects brain morphology, dermatoglyphic asymmetry, and cognitive and behavioral outcomes in 11½-year-old children. One specific goal is to better understand the unique and combined effects of three aspects of the mother's stress experience: the objective severity of the hardship imposed by the ice storm, her subjective reaction to the ice storm, and her hormonal response (i.e., cortisol). Moreover, we wish to explore whether a direct relationship exists (1) between brain morphology and dermatoglyphic asymmetry, and (2) between brain morphology and cognitive or behavioral outcomes.

This study compares approximately 100 (equal number of boys and girls) from the Project Ice Storm cohort, born between January and September 1998, with 60 control subjects (matched for birth month, sex, and socio-economic status) born one year earlier (1997), assessed at age 11½ years. Controls are selected to be born one year before, rather than after, the ice storm in order to avoid any effects of lingering stress in mothers who became pregnant after the storm, since natural disasters can have physiological effects lasting years (Adams et al., 2002); although children born before 1998 will have been exposed to the ice storm in infancy there would not be the same physical connection (i.e., placenta and umbilical cord) as with the Ice Storm cohort, linking maternal stress and its hormones to the child. All subjects are from the Montérégie area of Québec, where the mothers would have been living, pregnant or not, during the January 1998 ice storm.

Computational analysis of high-resolution (1 mm-isotropic at 3.0 Teslas) structural MR images will be used to assess group differences in brain structures. Structures of interest such as the hippocampus will be segmented on these images and their

volumes will be compared between the different groups. Based upon the available literature, and upon our results to date, we hypothesize, specifically for the hippocampus, that: 1) more severe objective stress exposure in the mothers will predict decreased volumes of the hippocampi of their 11½-year-old children; (a) smaller hippocampi will be seen in children exposed to high (vs. low) levels of objective PS; (b) smaller hippocampi will be found in the Project Ice Storm cohort compared to the control cohort; (c) these effects will be greatest in children who were exposed to high levels of objective PS during the first half of pregnancy; (2) greater dermatoglyphic (fingerprint) asymmetry will be observed in the Project Ice Storm cohort children exposed to the ice storm during gestation weeks 14–22 relative to the control cohort children; (3) dermatoglyphic asymmetry will correlate with hippocampal volumes (i.e., increased asymmetry will be associated with smaller hippocampal volumes); and finally, (4) the children's intellectual and language abilities and their behavioral/emotional problems at age 11½ will correlate with hippocampal volumes, i.e. poorer intellectual and linguistic abilities and higher levels of behavioral/emotional problems will be related to smaller hippocampal volumes. Other regions of interest, including for example the amygdala, cerebellum, corpus callosum and neocortex, will also be investigated.

6. Conclusions

Prenatal stress has been linked to abnormal outcomes in rodents, non-human primates, and humans (Anderson et al., 1985; Barros et al., 2006; Beversdorf et al., 2005; Coe et al., 2002, 2003; Crandon, 1979; Estanislau and Morato, 2005; Fleming et al., 1986; Fride and Weinstock, 1988; Fujioka et al., 2006; Grizenko et al., 2008; Gué et al., 2004; Hayashi et al., 1998; Henry et al., 1994; Huttenen and Niskanen, 1978; Jones et al., 1997; Kawamura et al., 2006; Kerchner and Ward, 1992; Kerchner et al., 1995; King and Laplante, 2005; Kinney et al., 1999a,b; Kinney, 2001; Kinney et al., 2008a,b; Kraszpulski et al., 2006; Laplante et al., 2004, 2008; Lemaire et al., 2000, 2006; Linnert et al., 2003; McIntosh et al., 1995; Mednick et al., 1999; Michelsen et al., 2007; Murmu et al., 2006; Nishio et al., 2001; O'Connor et al., 2002, 2003; Poland et al., 1999; Rhees et al., 1999; Rodriguez and Bohlin, 2005; Salm et al., 2004; Schmitz et al., 2002; Schneider, 1992; Schneider and Coe, 1993; Schneider et al., 1999; Son et al., 2006; Szuran et al., 1994, 2000; Talge et al., 2007; Ulupinar and Yucel, 2005; Ulupinar et al., 2006; Uno et al., 1994; Vallee et al., 1997; van Os and Selten, 1998; Watson et al., 1999; Weinstock et al., 1992; Wu et al., 2007; Yang et al., 2006; Zuena et al., 2008).

It is clear from animal studies that PS affects the morphology of the offsprings' brains, but the mechanisms that lead to these effects remain poorly understood. However, maternal and fetal HPA axes and the placenta seem to represent primary candidates for these mechanisms; although other systems have also been implicated, but less well studied (i.e. sympathetic adrenomedullary system (Kaiser and Sachser, 2005), hypoxia (Hardy and Yang, 2002)). Most likely, the effects of PS will involve most, or all, of the systems.

The vast majority of what is known about the effects of PS on neurodevelopmental outcomes stems from animal studies. However, as we have seen, it is quite challenging to integrate

these results and extrapolate them to humans for the following reasons: (1) although neurodevelopmental events follow a general order and pattern that is similar across species, differences exist in developmental timelines and maturation levels at birth between species; (2) differences in the timing of exposure to the stressor might contribute to differences in the effects of PS; (3) different types of stressors may have different effects on the HPA axis response in pregnant dams; (4) differences in postnatal ages when offspring are assessed or sacrificed might also influence the likelihood of observing a PS-related effect in the offspring's brain; and finally, (5) the effects of PS appear to vary by sex in a number of brain regions, which is important since many studies assess and, therefore, report findings only in males.

Different brain structures do not develop in synchrony but rather as a result of the multitude of developmental processes in distinct regions, each with their own developmental rates and timings, during the pre- and postnatal periods. As such, this process creates different and partially overlapping windows of vulnerability to stressors. It is therefore difficult to determine the period of highest susceptibility to an independent stressors during gestation since the majority of animal studies of the effects of PS on brain development involve rodents and stress of the pregnant dams during the last week of gestation, which is a period of active neurodevelopment in a number of regions of the rodent's brain. Moreover, the study by Coe and colleagues (2003) conducted with Rhesus monkeys suggests that PS insults can have similar effects in the offspring's brain. This finding suggests that if proliferation is affected, then subsequent events, such as migration may be affected. Ultimately, cell differentiation may be affected (Rice and Barone, 2000).

PS affects many brain regions including the hippocampus, amygdala, corpus callosum, neocortex, cerebellum, and hypothalamus, most often resulting in reduced tissue volumes. These changes are visible both at the macroscopic and microscopic level (Anderson et al., 1985; Barros et al., 2006; Coe et al., 2002, 2003; Fleming et al., 1986; Fujioka et al., 2006; Hayashi et al., 1998; Jones et al., 1997; Kawamura et al., 2006; Kerchner and Ward, 1992; Kerchner et al., 1995; Kraszpulski et al., 2006; Lemaire et al., 2000, 2006; Michelsen et al., 2007; Murmu et al., 2006; Poland et al., 1999; Rhees et al., 1999; Salm et al., 2004; Schmitz et al., 2002; Szuran et al., 1994; Ulupinar and Yucel, 2005; Ulupinar et al., 2006; Zuena et al., 2008), however the relation between the two remains to be fully understood. The current findings suggest that PS affects the sexual differentiation of various brain regions which has been related, for example, to altered sexual behaviors in rats (Fleming et al., 1986; Rhees et al., 1999).

The understanding of the effects of PS in humans on the developing fetus is woefully incomplete. While it has been speculated that PS affects the brains of exposed fetuses, no study to date has examined this possibility prospectively using a stressor that is outside of the pregnant women's control. Our current program of research will soon be able to provide information about the effects of PS on neurodevelopmental outcomes by analyzing the brain MRI scans of 11½-year-old children that were exposed to a natural disaster while in utero. The recent NIH study of normative brain development (Evans, 2006) will be instrumental in identifying brain regions affected by PS. Combining PS research with those obtained from the NIH study will aid in determining how PS affects brain morphology.

Future animal studies should use exploratory approaches to look at the effects of PS in the whole CNS. Regions that have been investigated so far have been chosen based on pre-existing literature showing a link between PS and affected behavioral outcomes (Anderson et al., 1985; Barros et al., 2006; Coe et al., 2002, 2003; Fleming et al., 1986; Fujioka et al., 2006; Hayashi et al., 1998; Jones et al., 1997; Kawamura et al., 2006; Kerchner and Ward, 1992; Kerchner et al., 1995; Kraszpulski et al., 2006; Lemaire et al., 2000, 2006; Michelsen et al., 2007; Murmu et al., 2006; Poland et al., 1999; Rhees et al., 1999; Salm et al., 2004; Schmitz et al., 2002; Szuran et al., 1994; Ulupinar and Yucel, 2005; Ulupinar et al., 2006; Zuenka et al., 2008). Finally, the question of whether or not changes in brain morphology due to PS might be prevented or reversed is of utmost importance. Lemaire et al. (2006) found that PS reduces hippocampal cell proliferation in rats throughout life (assessed at 4 or 26 months). Furthermore, the survival rate of newborn cells, the number of immature neurons and the number of differentiated new neurons are reduced in young and older PS rats. However, all those deleterious effects are counteracted by neonatal handling (Lemaire et al., 2006). This opens the door to ways to counteract the devastating effects of PS in the offspring with important implications for humans. For example, one area of research that deserves more attention is infant massage as a way to potentially improve outcomes in infants whose mother suffered from stress during pregnancy. This technique seems to have beneficial effects on mother–infant interactions, sleeping and crying, anxiety, and on hormones influencing stress levels (Beider et al., 2007; Underdown et al., 2006). This however, would need to be experimentally tested in humans as a possible intervention strategy following a major stressor during pregnancy.

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