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Severity and timing: How prenatal stress exposure affects glial developmental, emotional behavioural and plasma neurosteroid responses in guinea pig offspring

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ABSTRACT

Prenatal stress has been associated with a variety of developmental changes in offspring, notably those associated with brain development and subsequent risk for neuropathologies later in life. Recently, the importance of the timing and the severity of the stressor during pregnancy has been emphasized with neurosteroids including allopregnanolone implicated in the regulation of stress and also for endogenous neuroprotection in offspring.

Prenatal stress was induced using strobe light exposure in pregnant guinea pigs (term 71 days) in three defined stress exposure groups (Gestational Age (GA)35-65, GA50-65 and GA60-65). Stress was induced for 2 h (9–11am) every 5 days via strobe light exposure. A fetal cohort were euthanased at term with fetal brains and plasma collected. Anxiety-like behaviour was evaluated at 18 days of age in a separate cohort of offspring with brains and plasma collected at 21 days of age. Markers for mature oligodendrocytes and reactive astrocytes were measured in the CA1 region of the hippocampus and the subcortical white matter. The neurosteroid allopregnanolone was measured by radioimmunoassay in offspring plasma.

In the CA1 region of the hippocampus, fetuses from all stress groups showed reduced expression of mature oligodendrocytes and reactive astrocytes. By juvenility, all male stress exposure groups had recovered to levels of unaffected controls with the exception of the GA35-65 stress group. In juvenile females, mature oligodendrocyte marker expression was reduced in all stress groups and reactive astrocyte expression was reduced in the GA35-65 and GA60-65 stress groups by juvenility. Increased reactive astrocyte expression was also apparent in the subcortical white matter in both sexes both at term and at juvenility. Prenatally stressed offspring spent less time exploring in the object exploration test and also entered the inner zone of the open field less than controls at 18 days of age. Circulating allopregnanolone concentrations were significantly reduced in GA35-65 and GA 60–65 stress exposed fetuses with those in the GA35-65 stress group remaining reduced by juvenility.

This study has shown the effects of differing levels of prenatal stress severity and timing on glial development, emotional behaviour and plasma allopregnanolone concentrations in offspring.

1. Introduction

Prenatal stress has been linked with altered pregnancy outcomes such as preterm birth and low birth weight (Class et al., 2011), as well as alterations in fetal brain development (Charil et al., 2010) that may contribute to neuropathologies later in life in offspring (King and Laplante, 2005; Laplante et al., 2004, 2008). An increasing number of studies now also highlight the importance of fetal sex as well as the type and the timing of prenatal stress experienced in determining the outcomes for offspring. Indeed, the timing at which the stressor is experienced during pregnancy not only affects the extent to which the maternal hypothalamic-pituitary-adrenal axis (HPA axis) response is activated, but also the fetal organs and systems undergoing rapid development at the time. For example, in the human fetal brain, neuronal differentiation, migration and astrogenesis occur early in gestation, with myelination beginning in the last trimester along with synaptic

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maturation and apoptotic pruning both occurring throughout late pregnancy and into adolescence (Molofsky et al., 2012; Rice and Barone, 2000). Therefore, the pathogenesis of many detrimental neurological and behavioural outcomes associated with prenatal stress may be attributable to time-dependent perturbations in fetal brain development that could confer increased vulnerability for altered health outcomes later in life.

Stress and the activation of the HPA axis may be an adaptive mechanism whereby the mother's HPA responsiveness 'programs' the fetus for an ex-utero life that is perceived to be stressful (Welberg and Seckl, 2001). However, when there are discrepancies between what is experienced prenatally and postnally, stress during pregnancy could be considered maladaptive. One hypothesised mechanism of action underlying prenatal stress-induced fetal programming involves elevated maternal circulating glucocorticoids in response to the stressor. Through placental exchange, potential perturbations of cortisol exposure on the growing fetal brain (Charil et al., 2010) could result in excitotoxicity (Sandman et al., 1999) and developmental changes. However, despite this hypothesised link between maternal glucocorticoid exposure and fetal programming, there have been very few studies specifically linking maternal stress with elevated levels of cortisol and altered perinatal outcomes. Davis and Sandman (2011) found that rises in maternal cortisol and maternal pregnancy specific anxiety predicted infant outcomes independently of each other and no positive association was found between the level of maternal cortisol and the psychological state of the mother (Davis and Sandman, 2011). This study did however show differing outcomes in terms of infant mental development when maternal cortisol concentrations were increased in early vs. late gestation. In addition, pregnancy specific anxiety was only associated with poor outcomes in infants when it was reported early in pregnancy, highlighting the importance of the timing of the stressor on perinatal outcomes. Indeed, previous studies have assessed the effect of the timing of prenatal stress exposure on the outcomes of human offspring, however a wide variation have been reported. In one observational study which used the experience of war whilst pregnant as a measure of maternal psychosocial stress, fetuses affected in the first trimester showed a 2–5 fold increase in risk for mood disorders and bipolar disorder later in life (Kleinhaus et al., 2013). First trimester prenatal stress has also been shown to predict higher scores for autism spectrum disorder at $6^{1/2}$ years of age in offspring (Walder et al., 2014). Others have found that prenatal stress exposure in the second trimester of pregnancy is associated with an increased risk for producing a small-for-gestational-age baby (Khashan et al., 2014) as well as a 2 fold increased risk for development of attention deficit hyperactivity disorder (ADHD) symptoms at 4–5 years of age, particularly in male offspring (Zhu et al., 2015).

In the brains of offspring, pregnancy-specific anxiety at 19 weeks of gestation has also been associated with reductions in grey matter volume in offspring at 6–9 years of age, with maternal anxiety reported at 25 and 31 weeks showing no change in the offspring (Buss et al., 2010), indicating the highly specific effects of the type and timing of prenatal stress on outcomes. In non-human primates, prenatal stress in both early and late pregnancy has been shown to decrease neurogenesis in the hippocampus and produce emotional alterations in offspring (Coe et al., 2003), implicating damage to the hippocampus as a potential underlying cause of emotional outcomes in offspring.

Indeed, the hippocampus has been shown to be particularly vulnerable to the effects of prenatal stress in a number of studies. The CA1 region of the hippocampus contains a high concentration of important efferent projections and pyramidal cells involved in cognitive functioning (Barros et al., 2006; Bartsch et al., 2011; Uno et al., 1989) and also has been shown to be selectively vulnerable to excitotoxic insults (Butler et al., 2010; Herman and Spencer, 1998; Kadar et al., 1998). This may be attributable in part, to the high levels of expression of glucocorticoid receptors (Herman and Spencer, 1998) and mineralocorticoid in this region (Zhe et al., 2008) and therefore is of particular importance in the experience of stress as an agonist for these receptors (Fujioka et al., 2006). In rats, prenatal stress in mid-late pregnancy has been shown to disrupt the myelin sheaths of cells in the hippocampus (Xu et al., 2013), a process known to occur towards the end of pregnancy (Rice and Barone, 2000), which highlights that myelination may also be vulnerable the effects of prenatal stress, however the differences between the relative timings of rat and human brain development must be considered.

The guinea pig is a precocious species (Clancy et al., 2007; Dobbing and Sands, 1979) with a relatively long gestation compared to rats and mice (approximately 71 days), which allows for diverse manipulations in fetal life. In the guinea pig, relative brain to body weight reaches its peak at around gestational age (GA) 29–35 and declines with increasing GA which is characteristic of many mammals during development (Dobbing and Sands, 1970). A peak in brain growth by wet weight occurs around GA50 in this species, with maximal cholesterol and DNA polymerase (glial cell multiplication) accumulation occurring shortly before birth at around 60 days of gestation (Dobbing and Sands, 1970). Therefore, prenatal stress in this study beginning at GA35, GA50 and GA60 targets potentially vulnerable windows of neurodevelopment in the guinea pig which may be similar to that of human pregnancy (Clancy et al., 2007; Dobbing and Sands, 1979). Specifically, a myelination or neurogenesis event in the developing guinea pig brain at GA35 approximately corresponds to GA111 in humans (term 270 days) which equates to 50% and 40% of pregnancy length respectively (Workman et al., 2013). At this time, neurogenesis in the CA1 region of the hippocampus and the cortex is rapid (Workman et al., 2013). At GA50 in the guinea pig (70% of pregnancy), similar neurodevelopmental events such as the onset of myelination in the hippocampus and cerebellum are occurring at approximately GA257 (95% pregnancy) in the human fetus (Workman et al., 2013). Finally brain development in the guinea pig at GA60 (80% of pregnancy) is equivalent to postnatal day 126 in human babies, approximately 4 months of age, which is a time critical for plasticity in the early brain (Workman et al., 2013). Furthermore, body weight in guinea pigs reaches its fastest growth peak at 21 days postnatal age (Dobbing and Sands, 1970), indicating that this time may be important for determining developmental changes in the preceding time.

In the precocious guinea pig, prenatal stress in mid gestation has been shown to be associated with anxious behaviour in male offspring (Kapoor and Matthews, 2005) and we have previously shown, using the same model of prenatal stress, that prenatal stress may also affect key inhibitory neurosteroid systems in offspring (Bennett et al., 2013). Neurosteroids, including allopregnanolone, have been shown to be important for optimal neurodevelopment and are also responsive to stressors during pregnancy, indicating that neurosteroids may provide endogenous neuroprotection against prenatal stress (Hirst et al., 2006). The synthetic glucocorticoid betamethasone, when administered to pregnant guinea pigs, has been shown to result in a male-specific reduction in the neurosteroid synthesising enzyme which is further associated with reduced markers of brain development (McKendry et al., 2010), again highlighting the potential importance of this system in providing protection to the vulnerable fetal brain, particularly in male offspring. To the best of our knowledge however, there have been no studies assessing the impact of the timing of prenatal stress exposure on neurodevelopment, behavioural parameters and their interaction on the neurosteroid system in a translational guinea pig model of stress in pregnancy. The timing of stress exposure in this study will reflect the types of exposures that affect many human