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Prenatal stress produces anxiety prone female offspring and impaired maternal behaviour in the domestic pig

Kenneth M.D. Rutherford [a,*], Agnieszka Piastowska-Ciesielska [b,c], Ramona D. Donald [a], Sheena K. Robson [a], Sarah H. Ison [a], Susan Jarvis [a], Paula J. Brunton [c,1], John A. Russell [c], Alistair B. Lawrence [a]

[a] Animal Behaviour and Welfare, Animal and Veterinary Sciences Research Group, SRUC, West Mains Rd., Edinburgh EH9 3JG, Scotland, United Kingdom
[b] Department of Comparative Endocrinology, Faculty of Biomedical Sciences and Postgraduate Training, Medical University of Lodz Zeligowskiego 7/9, Lodz 90-752, Poland
[c] Centre for Integrative Physiology, CMVM, University of Edinburgh, Hugh Robson Building, George Square, Edinburgh EH8 9XD, Scotland, United Kingdom

HIGHLIGHTS

- Prenatal stress can shape postnatal behaviour and well-being.
- Prenatal stress altered corticotropin releasing hormone receptors in the amygdala.
- Maternal behaviour was also negatively affected by prenatal stress.
- Implications for pig welfare and relevant model for human prenatal stress effects

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ABSTRACT

Numerous studies have shown that prenatal stress (PNS) can have profound effects on postnatal well-being. Here, the domestic pig (*Sus scrofa*) was used to investigate PNS effects owing to the direct relevance for farm animal welfare and the developing status of the pig as a large animal model in translational research. Pregnant primiparous sows were exposed, in mid-gestation, to either a social stressor (mixing with unfamiliar conspecifics) or were kept in stable social groups. The ratio of levels of mRNAs for corticotropin releasing hormone (CRH) receptors 1 and 2 in the amygdala, measured for the first time in the pig, was substantially increased in 10-week-old female, but not male, PNS progeny indicating a neurobiological propensity for anxiety-related behaviour. Mature female offspring were observed at parturition in either a behaviourally restrictive crate or open pen. Such PNS sows showed abnormal maternal behaviour in either environment, following the birth of their first piglet. They spent more time lying ventrally, more time standing and showed a higher frequency of posture changes. They were also more reactive towards their piglets, and spent longer visually attending to their piglets compared to controls. Associated with this abnormal maternal care, piglet mortality was increased in the open pen environment, where protection for piglets is reduced. Overall, these data indicate that PNS females have their brain development shifted towards a pro-anxiety phenotype and that PNS can be causally related to subsequent impaired maternal behaviour in adult female offspring.

1. Introduction

It is increasingly evident that early life events can result in long-term changes in biological function. Interest in early life effects has been stimulated by human epidemiological evidence indicating the important influence that the foetal environment can exert on disease susceptibility in later life [1]. In particular, stress experienced by pregnant mothers has been shown to have wide-ranging and important effects on their offspring's later physiology and behaviour. Prenatal effects may occur due to pathological alterations to normal development, or represent instances where foetal biological adjustments to cope with a challenge have long-term effects [2]. Alternatively, in some instances such effects may have an adaptive basis but may produce maladaptive outcomes when there is a mismatch between the predicted environment and the reality of the actual postnatal environment [3]. This may be particularly true for captive animals where the environment experienced during postnatal life is often highly artificial. Moreover, some data

* Corresponding author. Tel.: +44 131 651 9345; fax: +44 131 535 3121.
E-mail addresses: kenny.rutherford@sruc.ac.uk (K.M.D. Rutherford), agnieszka.piastowska@umed.lodz.pl (A. Piastowska-Ciesielska), ramona.donald@hotmail.co.uk (R.D. Donald), sheena.robson@sruc.ac.uk (S.K. Robson), sarah.ison@sruc.ac.uk (S.H. Ison), susan.jarvis@sruc.ac.uk (S. Jarvis), p.j.brunton@ed.ac.uk (P.J. Brunton), j.a.russell@ed.ac.uk (J.A. Russell), alistair.lawrence@sruc.ac.uk (A.B. Lawrence).
[1] Present address: The Roslin Institute and R(D)SVS, University of Edinburgh, Division of Neurobiology, Roslin, EH25 9RG, Scotland, United Kingdom.

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indicate that domestication may have resulted in increased sensitivity to prenatal effects [4,5]. Overall, the implication for captive domesticated species is that variation in the conditions for development provided by the reproductive tract or egg, for instance by altered nutritional supply or hormonal milieu, may explain a large degree of variation in many aspects of biology, some of which may impact on health and welfare [6]. Indeed, research across a wide range of farmed species has shown negative impacts of prenatal stress [7]. Prenatal stress studies in pigs have also shown many diverse outcomes in the offspring of stressed mothers [8–15]. There is clear evidence that aspects of stress physiology can be affected in the offspring of stressed mothers. For example, social stress experienced by primiparous sows results in a state of stress hyperreactivity in the female offspring, with disturbed behaviours, increased corticotropin-releasing hormone (CRH) mRNA expression in the amygdala (a key brain region involved in mediating behavioural responses to stress, including anxiety and fear responses [16]) and in the paraventricular nucleus (PVN) of the hypothalamus (the brain region that mediates the neuroendocrine response to stress [17]) [10]. Haussmann and colleagues [8] also found evidence of increased stress reactivity in prenatally stressed pigs. However, other studies [9,11–13] have found no such effect. These differences may reflect different animal genotypes, maternal stress models, offspring stressors, outcomes measurements and animal ages.

CRH receptor-1 (CRH-R1) and -2 (CRH-R2) in the amygdala play a critical, and largely opposing, role in regulating emotionality and stress responses in vertebrate species [18,19]. Broadly, activation of CRH-R1 by its principal ligand CRH increases behavioural indications of fear/anxiety and physiological stress responding, whilst activation of CRH-R2 by its main ligands (urocortins II and III) has the opposite effect [19]. Here we investigated the hypothesis that an altered balance in the relative expression of mRNAs for CRH-R1 and CRH-R2 in the amygdala, measured for the first time in the pig, may underlie the prenatal stress phenotype seen previously [10]. We also investigated the separate and interacting effects on these measures of a postnatal painful challenge, using tail-docking, which is a common commercial practice within pig farming.

We furthermore sought to examine the hypotheses that prenatal stress (PNS) exposure would impact adversely on subsequent maternal behaviour in the adult female offspring and that this effect would be more substantial in a restrictive environment. Maternal behaviour was a particular focus of the study given our previous findings indicating that prenatal stress increases the likelihood of primiparous sows showing piglet-directed aggression [10] and rodent studies demonstrating impaired maternal behaviour in females exposed to prenatal stress [20]. Also, the pig has been suggested as a possible large animal model for harmful human maternal behaviour [21,22] and the role of early life experiences in later maternal behaviour deserves consideration. This is particularly important as social stress during pregnancy represents a relevant paradigm for comparison with the experiences of human females [23].

2. Materials and methods

2.1. Experimental over-view

The work detailed here consisted of two phases (Fig. 1). Phase one involved pregnant sows being exposed to a social stressor during pregnancy. In phase two, individual male and female prenatally stressed and control offspring were euthanized, at around nine weeks of age, and brain sections were collected for measurement of CRH-R1 and CRH-R2 mRNA levels in the amygdala. In the second stage of phase two, female offspring from PNS or control litters were kept to maturity and observed when they themselves gave birth. Other data from this experiment have been reported separately [14].

All work was carried out in compliance with EC Directive 86/609/EEC, under UK Home office licence where appropriate, and following ethical approval by the Animal Experiments Committee at Scotland's Rural College (SRUC).

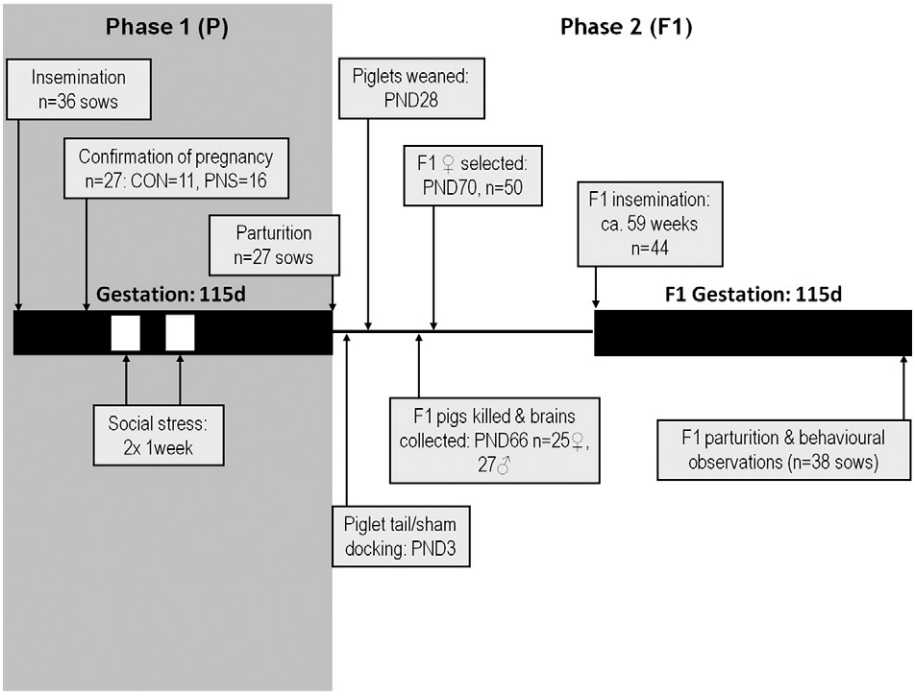

Fig. 1. Diagram of experimental timeline. Phase 1: sow gestation, showing sample sizes and timing of social stress treatment. Phase 2: offspring measures, including F1 gestation and subsequent observation of maternal behaviour.