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Environmentally enriched pigs have transcriptional profiles consistent with neuroprotective effects and reduced microglial activity

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ABSTRACT

Environmental enrichment (EE) is widely used to study the effects of external factors on brain development, function and health in rodent models, but very little is known of the effects of EE on the brain in a large animal model such as the pig. Twenty-four young pigs (aged 5 weeks at start of study, 1:1 male: female ratio) were housed in environmentally enriched (EE) pens and provided with additional enrichment stimulation (a bag filled with straw) once daily. Litter, weight and sex matched controls n = (24) were housed in barren (B) conditions. Behaviour was recorded on alternate days from study day 10. After 21 days, RNA-sequencing of the frontal cortex of male piglets culled one hour after the enrichment stimulation, but not those at 4 h after stimulation, showed upregulation of genes involved in neuronal activity and synaptic plasticity in the EE compared to the B condition. This result is mirrored in the behavioural response to the stimulation which showed a peak in activity around the 1 h time-point. By contrast, EE piglets displayed a signature consistent with a relative decrease in microglial activity compared to those in the B condition. These results confirm those from rodents, suggesting that EE may also confer neuronal health benefits in large mammal models, through a potential relative reduction in neuroinflammatory process and increase in neuroprotection driven by an enrichment-induced increase in behavioural activity.

1. Introduction

Environmental enrichment (EE) paradigms are widely used in laboratory rodents as a method to study the effects of environmental influences on brain development and function [1]. EE utilises multiple methods of environmental modification to facilitate cognitive and motor stimulation in a species appropriate manner. In rodents this is often achieved through the use of novel objects, running wheels, nesting material and group housing [2].

The beneficial effects of EE on physiology and brain function in rodents are well documented (reviewed in ([3])). EE paradigms have been shown to have a neuroprotective effect through the inhibition of spontaneous apoptosis [4] and through increased cell proliferation and neurogenesis in the hippocampus [5–7] and prefrontal cortex [8]. Indeed, EE has been shown to limit neurodegeneration in models of aging [9,10], and can rescue some behavioural and physiological brain effects of early life stress [11,12]. Current evidence suggests this is most likely precipitated by the mechanisms underlying neuronal plasticity, due to increased expression of trophic factors and immediate early genes (IEG) in the brains of EE animals [13–15].

Expression of neurotrophins has also been proposed as a contributory factor in mood disorders, in particular stress-induced depression and anxiety. While the main focus has been around expression of brain derived neurotrophic factor (BDNF) [16–18], there are a number of other trophic factors and IEGs with a role in stress resilience and mood. Neuronal growth factor (*Ngf*) mRNA has been shown to be reduced in the dentate gyrus in response to restraint stress in rats [19]. Environmental deprivation during early life in rats reduces IEG expression in the prefrontal cortex both as a chronic effect [20], in response to social interaction [21], and after chronic social defeat stress in mice [22]. Levels of IEG *Arc* mRNA in the frontal cortex and hippocampus of rats exposed to predator scent stress were reduced in those animals which displayed an extreme anxiety-like behavioural response, and in the dentate gyrus *Arc* mRNA expression levels also correlated with levels of circulating corticosterone [23].

Interestingly, depressive-like behaviour in rodents induced by social isolation in early life not only results in decreased expression of a number of IEG involved in synaptic plasticity, including *Bdnf, Egr-1* and

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Arc ([24], mice), but also in increased microglial activation ([25], rats). Microglia are specialised macrophages of the central nervous system that play a role in neuromodulation, phagocytosis and inflammation (reviewed in [26]). There is increasing evidence to suggest that microglial mediated brain inflammation is a contributing factor to a number of mental health and nervous system disorders, such as multiple sclerosis [27], Alzheimer's disease [28], schizophrenia [29,30], and major depression [31]. While the exact mechanism by which microglia contribute to these disorders is not yet known, psychological stress has been shown to result in increased levels of microglia in the prefrontal cortex of rats [32] while early life stress, in the form of maternal deprivation, has also been shown to lead to increases in microglial motility [33] and phagocytic activity [34] that persist into adulthood. Anti-inflammatory drugs such as minocycline (which blocks microglial activation) produce antidepressant effects in rodent models [35]. Several anti-psychotic medications have also been shown to block microglial activation [36,37].

The physiological and behavioural effects of a number of nervous system disorders can be modulated by EE in rodent models [38], and EE is now widely used as an experimental tool in studies of neurodegeneration and neural development [39]. Due to its beneficial effects on behaviour, EE has long been used as a method to improve the welfare of production animals, by providing environmental manipulations that allow the animal to behave in a more species-typical way (reviewed in [40]). However there has been little work on the effects of EE on the brain in a large animal model, such as the pig. The aim of the present study was to investigate the effects of EE on gene expression within the frontal cortex of young pigs with the aim of identifying potential gene expression signatures of a healthy brain in a large animal model, and to potentially identify early signatures of neural ill-health in the absence of abnormal behaviours.

2. Materials and methods

2.1. Ethical review

All work was carried out in accordance with the U.K. Animals (Scientific Procedures) Act 1986 under EU Directive 2010/63/EU following ethical approval by SRUC (Scotland's Rural College) Animal Experiments Committee. All routine animal management procedures were adhered to by trained staff and health issues treated as required. All piglets remaining at the end of the study were returned to commercial stock.

2.2. Animals and general experimental procedures

Post-weaning behavioural observations were carried out on litters from six commercial cross-bred mothers (Large White x Landrace); the boar-line was American Hampshire. Artificial insemination was performed using commercially available pooled semen. Litters were born within a 72 h time window in free-farrowing pens which allowed visual and physical contact (nasal contact through bars) with neighbouring litters. No tooth resection was performed and males were not castrated. In line with EU Council Directive 2008/120/EC tail docking is not routinely performed. Weaning occurred at 24–27 days of age. At weaning piglets were weighed, vaccinated against Porcine Circoviral Disease and ear tagged for identification. Post-wean diet was in the form of pelleted feed (Primary Diets Prime Link Extra) provided *ad libitum*. At 4 days post weaning, 8 piglets per litter were selected as being as close to the litter average weight as possible while balancing for sex (1:1 male to female). These 8 piglets per litter were split into sex balanced quartets to give final group sizes of 4 piglets per matched group. One group was then housed in an enriched (EE) pen and one in a barren (B) pen, all in the same room (Fig. 1). Post weaning pens did not allow for physical or visual contact between neighbours. All pens contained feed troughs ~80 cm in length and piglets had access to one water nipple per pen.

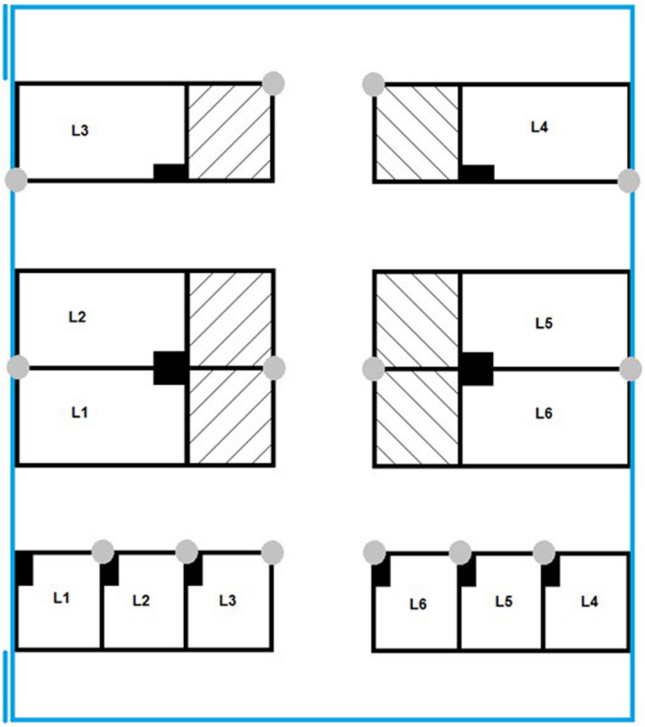

Fig. 1. Diagram of the layout of the experimental room. Double lines signify the outer room doors used for access. Grey circles indicate the location of cameras and filled black boxes show the location of feeders. Litter of origin is identified as L1-L6. Empty pens are indicated by hatched lines. EE pens were twice the size of B pens as indicated in the diagram. All pens were solid floored. EE pens had the addition of straw over two thirds of the flooring.

All piglets were marked on the back with spray marker paint to allow individual identification. B pens measured 1.8 m × 1.8 m with concrete floors and rubber matting (1.8 m × 1.2 m) in one corner to provide some thermal insulation. Kennels were provided for the first 10 days to enhance thermal comfort as B housed piglets had no opportunity to use straw to control their thermal environment as the EE pigs could. EE pens measured 3.6 m x 1.8 m also with concrete flooring and with large quantities of straw covering the floor (covering half the pen to a depth of 0.15 m). The same rubber matting was also provided in the enriched pens. Piglets in the six EE pens were provided with additional enrichment once daily (enrichment stimulus) in the form of a plastic bag (approximately 400 mm × 750 mm × 150 mm) filled with straw (approximately 2 kgs). This bag was placed in the EE pens after morning husbandry was complete (at around 10 a.m.). At the same time the gate of the matched B pen was approached to account for the stimulating effect of human presence. The bag was removed at the end of each day (~1700 h) and a new bag used on the following day. The bag was open at one end to allow straw to be extracted by the piglets and was the only source of fresh straw given to the EE pigs daily. The bag was first introduced at day 5 post weaning and thereafter daily. Pen cleaning and enrichment order was randomised daily. In accordance with the Defra Code of Recommendations for the Welfare of Livestock [41], temperature within the room was automatically controlled at 22 °C and artificial lighting was maintained between the hours of 0800–1600, with low level night lighting at other times.

2.3. Behavioural observations

Piglets were digitally recorded in their home pen using Sony LL20 low light cameras with infra-red and a Geovision GV-DVR. Two cameras were set up per EE pen, one at the rear and one at the front to