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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA12

Inter-individual variation in expression of maternal behaviour in sheep

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At birth, ewes rapidly form a selective attachment to their offspring and subsequently restrict maternal care to their own lambs. This attachment occurs during a period of intense maternal licking of the offspring, accompanied by frequent low pitched bleats (a specific maternal vocalisation). Maternal licking wanes over the first day of life and thereafter maternal behaviour is expressed as a close spatial association between ewe and lamb, distress behaviours when the ewe and lamb are separated, and frequent sucking interactions.

The expression of maternal care is impaired in primiparous ewes. These ewes show a delay in starting to lick their lambs, have a higher rate of rejection behaviours (e.g. aggressive behaviours towards the lamb), make more distress vocalisations and are more likely to show avoidance when the lamb attempts to suck. However, the total amount of licking behaviour and the frequency of low-pitched bleats are not affected by maternal experience. Furthermore, although behaviour improves with experience, individual ewes show consistency in their maternal responsiveness from one pregnancy to the next (1). These data suggest that there are underlying differences between ewes in their expression of maternal behaviour, which persist over consecutive pregnancies. To investigate the potential mechanisms underlying variation in maternal behaviour we compared the maternal responsiveness of two breeds of ewe (Suffolk, Scottish Blackface). Blackface ewes consistently show higher levels of licking behaviour over the first two hours after birth and emit more low pitched bleats than Suffolk ewes. Suffolk ewes display more fearful and aggressive behaviours towards their lambs, have higher incidences of lamb abandonment, and are more likely to move away as the lamb attempts to suck. In tests of maternal recognition 3 days after birth, Blackface ewes were better able to recognise their own lamb from a distance, and spent more time with their lamb than Suffolk ewes. Blackface ewes maintain closer spatial associations with their own lambs from birth to weaning than Suffolk ewes. Embryo transfer between breeds demonstrated that these behavioural differences were due to maternal responsiveness and not to variation in lamb behaviour (2). In addition to consistency across different parities within ewe, ewes are also consistent in their expression of maternal behaviour throughout lactation.

The onset of maternal behaviour in the sheep is primed by circulating concentrations of oestradiol and progesterone in pregnancy and triggered by the release of central oxytocin at birth. Maternal behaviour in the sheep is also potentiated by endogenous opioids. To investigate any role these factors may have on individual differences in behavioural expression, we measured oestradiol and progesterone concentration in plasma throughout gestation, and cortisol and oxytocin concentrations in plasma at parturition. A subset of ewes were administered naltrexone at the onset of labour to investigate the role of opioid modulation in mediating individual differences in behavioural expression. Blackface ewes had higher circulating oestradiol concentrations than Suffolk ewes from mid gestation onwards ($P<0.001$) and a greater oestradiol:progesterone ratio ($P<0.001$). There was no effect of previous maternal experience. Circulating oestradiol concentrations were positively correlated with maternal licking ($P=0.06$), the length of sucking bouts ($P<0.01$), and the frequency of low pitched bleats ($P<0.005$). The birth of the lamb was associated with a rapid and transitory peak in plasma oxytocin concentration, which did not differ between breeds. There was no relationship between plasma oxytocin concentration and maternal behaviour, nor were there any breed differences in the response to naltrexone treatment. Plasma cortisol concentrations were higher in Blackface ewes than Suffolk ewes in late gestation, but this difference disappeared on the day of parturition. Plasma cortisol concentration immediately after birth was negatively correlated with maternal licking behaviour.

The data suggest that maternal oestradiol concentration may be partly responsible for inter-individual variation in maternal behaviour seen in sheep, but does not appear to mediate the effects of previous maternal experience. Recent data in the sheep suggest that parity-related differences occur in the central expression of oestradiol-receptor-α (3) although receptor density decreases at parturition. Nevertheless, our data are consistent with a hypothesis that individual differences in maternal behaviour in the sheep are mediated by the effects of oestradiol on the oxytocinergic system, and that these effects continue to influence maternal behaviour expression throughout lactation.

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SA13

Nutrition Restriction and Offspring Cardiovascular and Metabolic Function

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It is increasingly realised that the nutritional environment during early life not only affects the course of development but also

establishes the settings of both physiological responses to the environment and the range of environments in which the offspring can live healthily in later life. When environmental challenges exceed an individual's ability to respond healthily, risk of disease increases. We describe this as an individual mismatched to their environment, and the greater the degree of mismatch the greater the risk of disease. Hence balanced nutrition in pregnancy and childhood is not only important for the health of the mother and the growth of her child, but also establishes some of the fundamental processes which determine risk of later chronic disease including the metabolic syndrome, cardiovascular disease and osteoporosis. We are now rapidly learning how these processes are established in early life, especially the role of nutritionally-induced epigenetic processes (e.g. DNA methylation and histone methylation and acetylation) which produce sustained effects on gene expression determining the offspring's cardiovascular, metabolic and neuro-endocrine responses, as well as growth of key organs such as the heart, kidney, liver and pancreas. The developmental responses additionally determine the offspring's body composition, in terms of skeletal muscle, bone and fat; these track into later life to affect risk of glucose intolerance, obesity and osteoporosis. What is striking is that these processes start to be induced even in very early development, and are then modulated by later interactions between fetus and its mother via the placenta, and then between the mother and infant during suckling. In this way factors in addition to maternal diet, such as her stature and body composition, stress and exercise levels and behaviour can influence the long-term health of her child. New evidence suggests that the effects can be passed to more than just one successive generation. In addition effects on metabolic homeostasis and obesity in females lead to increased risk of gestational diabetes, which in turn induces deleterious effects on their offspring.

There are several ways in which these insights are relevant to developed and developing societies. They include:

- rapid transitions in diet and levels of physical activity
- changes in a woman's body composition produced by dieting or by being overweight
- excessive control of weight-gain in pregnancy in some societies such as Japan
- teenage pregnancies
- increasing age at first pregnancy
- increasing proportion of primiparous pregnancies with reductions in family size
- changes in breast-feeding practices.

These socio-cultural processes, many involving changes in nutrition, will influence the degree of mismatch between the next generation and their environment. The consequences of this for incidence of non-communicable, chronic diseases are very substantial.

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SA14

Prenatal Alcohol Exposure, Fetal Programming and the HPA Axis

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Maternal alcohol consumption induces clinical abnormalities of endocrine function and neuroendocrine regulation in both the mother and fetus that may play a role in the etiology of the functional abnormalities observed in Fetal Alcohol Spectrum Disorder (FASD). The major focus of research in our lab is to understand the mechanisms underlying the effects of maternal alcohol consumption on maternal and offspring neuroendocrine function, with a particular emphasis on the hypothalamic-pituitary-adrenal (HPA) axis, a key component of the stress system.

Our data demonstrate that alcohol-induced disturbances of the reciprocal interconnections between maternal and fetal/neonatal HPA axes may provide a common pathway for fetal or early programming. We have shown that prenatal exposure to alcohol increases HPA activity in the maternal female and reprograms the fetal HPA axis such that HPA tone is increased throughout life. This is reflected in increased HPA activation and/or delayed or deficient recovery to basal levels following exposure to stressors. Investigation of mechanisms mediating this altered HPA responsiveness has demonstrated that alcohol-exposed animals exhibit HPA dysregulation under basal conditions and following stress, and that differences are further unmasked following perturbations of the system by stress, adrenalectomy and/or receptor blockade. Dysregulation occurs at multiple levels of the axis including the hippocampus, hypothalamus and pituitary, and appears to reflect changes in both HPA drive and corticosterone feedback regulation and/or in the balance between drive and feedback. Although fetal alcohol-exposed animals appear able to initiate compensatory mechanisms to maintain normal basal hormone concentrations under most circumstances, perturbations of the system reveal that tonic or basal HPA tone is in fact increased and likely plays a key role in raising the set point of responsiveness following stress. Furthermore, we have shown that prenatal alcohol exposure appears to have sexually dimorphic effects on HPA regulation, suggesting a role for the gonadal steroids, or possibly an alteration in adrenal-gonadal interactions, in mediating alcohol's effects on HPA activity and regulation.

In this presentation we will present data demonstrating dysregulation of the HPA axis in fetal alcohol-exposed offspring, and discuss potential mechanisms underlying the changes that have been observed. Recent preliminary studies examining effects of prenatal alcohol exposure on the methionine cycle, a metabolic process required for the generation of methyl groups for DNA methylation, and the implications of these effects for alcohol-induced changes in epigenetic regulation of gene expression will also be discussed. Finally, because early life events that produce enhanced reactivity to stress result in elevated cumulative exposure to endogenous glucocorticoids over the lifespan, the implications of our findings for understanding the secondary disabilities that are observed in children with FASD will be explored.

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SA15

Uterine Heterogeneity: Toward a molecular and biophysical basis of activation

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The transition of myometrium from relative quiescence throughout pregnancy to powerful contractions during labour is a very complex and precisely timed process. This process of activation occurs in preterm as well as term labour irrespective of the aetiology. An increase in resting membrane potential, contraction frequency, intercellular coupling through gap-junctions and paracrine interactions is thought to be important for synchronising the activity of uterine myocytes at the end of pregnancy, yet the mechanism of myometrial autorhythmicity remains unclear. An increase in intracellular concentration of Ca2+ ([Ca2+]i) initiates contraction in all types of smooth muscle cells including uterine myocytes. On a tissue level, temporal and spatial summation of [Ca2+]i transients in individual cells triggers contraction patterns specific to particular types of smooth muscle. The mechanisms responsible for the initiation and spread of tissue-level Ca2+ signals differ in different smooth muscles, involving in some cases, a specialised type of cell called interstitial cells of Cajal (ICCs). Recent work has established that ICC-like cells are present in both rodent and human myometrium, although they are unlikely to be the pacemakers, at least in the rat myometrium [1]. We have used laser scanning confocal microscopy to investigate the initiation and propagation of Ca2+ signals between uterine myocytes in their natural environment (i.e. within thin slices of intact myometrium). We observed synchronous and large rises in [Ca2+]i elicited by action potentials in bundles of smooth muscle cells. This was followed by non-propagating asynchronous Ca transients of smaller amplitude, presumably due to spontaneous Ca2+ release from the sarcoplasmic reticulum. Immediately preceding the high-amplitude [Ca2+]i transient in the smooth muscle cell bundle, there were spikes of [Ca2+]i originating outside the bundle, in the interstitial space and propagating towards the muscle bundle. Immunohistochemistry of fixed myometrial slices revealed vimentin-positive ICC-like cells residing within the interstitium and surrounding the smooth muscle cell bundles. Communication of the tissue level action potential is quickly lost during cell culture with spontaneous activity occurring only within the first day of culture. Upon loss on intercellular communication individual cells that spontaneously oscillate are revealed, as well as cells which respond differently to agonists.We further demonstrate that within the population of smooth muscle cells expression of important contraction associated proteins is not uniform. Specifically we show that myometrial smooth muscle cells express the Cav3.1(alpha1G) subunit of the T-type calcium channel in approximately 50% of cells, which is in contrast to the L-type channel being expressed in 100% of cells. In current clamp experiments we observed low voltage activated (LVA) spikes that could be elicited after short hyperpolarizing pulses. These LVA spikes were blocked by 100μM Nickel. We determined that cells that express both LVA and HVA current demonstrate an extended "window current" that may allow for slow depolarization when resting membrane potential approaches -55mV. We therefore hypothesise that T-type mediated calcium entry may act a pacemaker current by setting contraction frequency when resting membrane potential increases during myometrial activation. In agreement with this hypothesis 100 μM nickel produced a four fold decrease in contraction frequency, an effect that was reversible on wash out.

The implications of cellular heterogeneity in the process of activation and the genesis of spontaneous rhythmicity will be discussed.

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SA16

Antenatal stress/anxiety, the fetal environment, and the neurodevelopment of the child

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There is good evidence, from independent prospective studies, that if the mother is anxious /stressed while pregnant, her child is substantially more likely to have behavioural, emotional or cognitive problems. An increased risk of attention deficit/hyperactivity, anxiety and language delay have been described. However these studies all have design flaws, such as relying on maternal report for child outcome or not controlling for postnatal maternal mood.

In a recent study we have recruited women awaiting amniocentesis and followed their child until 18 months. We have found that maternal exposure to antenatal life events was negatively associated with the child's Bayleys' Mental Developmental Index (MDI) (r_s= - 0.39, $p < 0.001$, n=123). There was also a positive association between antenatal life events and the child's observed fear reactivity (r_s= 0.39, $p < 0.001$, n=106), although the two outcomes were not associated (=-0.09,n=108, ns). There were no such associations between postnatal life events and child outcome. This provides new evidence for the occurrence of fetal programming. With both outcomes it was antenatal stress due to problems in the relationship with the partner which had the largest effect on the child. There was an inverse relation between amniotic fluid cortisol level and the child MDI (r= - 0.25, p=0.01, n=125). This is the first evidence in humans that the level of fetal exposure to cortisol in utero is related to the neurodevelopmental outcome of the child.

The fetal environment may be altered if stress in the mother changes her hormonal profile; there is a strong correlation between maternal plasma and amniotic fluid cortisol levels ($r=0.43$ $p<0.001$, $n=290$). However many problems remain in understanding the mechanisms. We do not know the gestational age of greatest susceptibility. As pregnancy advances the sensitivity of the maternal HPA axis to stress or anxiety diminishes. It may be that some women maintain a cortisol response to stress depending on their individual genetic constitution, and it is the fetuses of these women that are affected. It may also be that other hormones such as noradrenaline also play a part in mediating links antenatal maternal stress and anxiety and fetal development.

It has been suggested that the evolutionary function of fetal programming has been to prepare the child for the particular environment in which he/she is going to find themselves, the predictive adaptive response. Extra vigilance, increased fear reactivity, or readily distracted attention may have been adaptive in a prehistoric stressful environment, but are maladaptive in our society.

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