individuals or women at risk for premature pregnancy termination.
In vitro data suggest a correlation between the rate of progesterone receptor expression as well as PIBF production and the success or failure of pregnancy, but provide no direct evidence for their role in maintaining gestation. To test the biological significance of our findings, we used animal systems. In vivo studies revealed that: a) The anti-abortive effect of the PIBF in vivo is manifested via inducing a Th2 dominant cytokine pattern and keeping the NK activity at a low level: b) A proper stimulation of the maternal immune system is required for the operation of the progesterone-dependent immunomodulatory pathway: c) Neutralization of endogenous PIBF results in pregnancy termination. These data allow the conclusion that the operation of progesterone-dependent immunomodulation contributes to maintaining normal gestation.

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

SA8

Cytokines and myometrial intracellular signalling

R.M. Tribe

Reproduction and Endocrinology, King's College London, London, UK

During human pregnancy the smooth muscle of the uterus, the myometrium, is relatively quiescent until the onset of contractile activity associated with labour. The cascade of events precipitating human labour remains unclear, but it is proposed that the myometrium becomes primed to contract at term by the activation of a complex array of genes encoding for proteins which include cyclo-oxygenase-II (COX-2), the oxytocin receptor and calcium regulatory proteins (TRPC isoforms and sarcoplasmic reticulum calcium ATPases). Several concordant stimuli ('physiological' inflammation, maternal and foetal endocrine signals and uterine stretch) have been implicated in this process and are proposed to drive the integration of uterine contractile activity and labour. In support of a central role for inflammatory cytokines, IL-1β, IL-8 and IL-6 have been found in myometrial tissue taken in late pregnancy, and raised concentrations are reported in amniotic fluid during human term and preterm labour. Experimental models of preterm labour have also demonstrated that introduction of bacterial products or cytokines into the amniotic cavity of pregnant animals leads to cytokine synthesis, up-regulation of Toll-like receptors and premature uterine contraction.
This presentation will discuss how the inflammatory mediator IL-1β modulates uterine excitability. It is well known that that IL-1β can stimulate myometrial prostaglandin synthesis, but our studies demonstrate for the first time that IL-1β can also enhance calcium signalling events. IL-1β treatment of human myometrial cells induces spontaneous calcium oscillations and increases resting calcium concentrations in parallel with an augmentation of store-dependent calcium entry and a substantial increase in TRPC3 protein expression. Interestingly, IL-1β treatment does not alter expression patterns of any other TrpC isoforms suggesting that TRPC3 is differentially regulated. IL-1β-treated smooth muscle cells also exhibit augmented calcium responses to a diacylglycerol analogue (OAG), a prominent activator of TrpC3 channels. These data implicate TRPC3 channels as key mediators of the IL-1β enhancement of myometrial smooth muscle calcium signalling, and provide a plausible mechanism by which uterine excitability may be augmented in term and preterm labour. This cell model may also prove useful as an endogenous 'overexpression' system with which to explore the function, regulation and role of TrpC3 proteins.

This work was supported by Tommy's the baby charity (Reg. Charity No: 1060508) and The Wellcome Trust.

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

SA9

Prolactin and the neuroendocrine adpatations of the maternal brain

D.R. Grattan

Centre for Neuroendocrinology, University of Otago, Dunedin, New Zealand

Levels of lactogenic hormones, prolactin from the maternal anterior pituitary and/or the closely related placental lactogen, are elevated during pregnancy and lactation. While these hormones are well established to have an critical role in mammary development and lactogenesis, they also exert important actions in the brain. Prolactin receptors are expressed in the choroid plexus and in several hypothalamic nuclei, and we have shown that levels of expression increase during pregnancy and lactation. Prolactin is known to influence a variety of hypothalamic functions, including regulation tuberoinfundibular dopamine (TIDA) neurons, stress responses, appetite and food intake, and fertility (1). Many of these prolactin-sensitive functions appear to change during pregnancy in a manner consistent with the influence of prolactin. Two specific examples have been examined to evaluate the role of prolactin in mediating neuroendocrine adaptation in the maternal brain: a) Decreased sensitivity of TIDA neurons to prolactin, leading to decreased secretion of dopamine and subsequent hyperprolactinaemia. b) Increased food intake and the development of leptin-resistance during pregnancy. Both changes are important maternal adaptations to pregnancy, providing high prolactin for mammary development and maternal behaviou, and increased energy storage to meet the metabolic demands of lactation, respectively.
Prolactin acts directly on prolactin receptors on TIDA neurons inducing phosphorylation of STAT5b and activation of tyrosine hydroxylase (TH, the rate limiting enzyme responsible for dopamine synthesis) and an increase in TH mRNA expression. We have measured mRNA for the long form of the prolactin receptor on TIDA neurons by in situ hybridisation and this does not change during pregnancy or lactation (2), although there is an increase in expression of met-enkephalin in prolactin-responsive TIDA neurons. Prolactin-induced phosphorylation of STAT5b in TIDA neurons is suppressed during lactation, associated with a prolactin- or suckling-dependent increase in mRNA for several endogenous inhibitors of STAT pathways (CIS, SOCS1

and SOCS3) (3). The data suggest that prolactin signal transduction in TIDA neurons is specifically altered during late pregnancy and lactation. Prolactin loses the ability to induce STAT5b phosphorylation, probably due to an upregulation of SOCS proteins. Consequently, TH activity and TH mRNA levels fall, resulting in decreased dopamine release into the portal blood and increased prolactin secretion. TIDA neurons continue to express prolactin receptors, however, and may respond to the elevated prolactin with an increase in met-enkephalin expression, which could result in further suppression of dopamine secretion.
Appetite and food intake is increased during pregnancy, resulting in increased fat deposition and elevated leptin secretion. Despite high leptin levels, the increased food intake is maintained during pregnancy, suggesting leptin resistance. These changes are physiologically appropriate, providing increased energy reserves to help meet the high metabolic demands of fetal development and lactation. We have demonstrated that intracerebroventricular (i.c.v.) leptin is unable to suppress food intake in pregnant rats, as it does in non-pregnant animals (4). In addition, we have shown a specific suppression of leptin-induced phosphorylation of STAT3 (pSTAT3) in specific regions of the hypothalamus during pregnancy (5). To investigate the mechanism underlying this pregnancy-induced leptin resistance, we have investigated effects of hormone treatments on hypothalamic responses to leptin in a pseudopregnant rat model. Pseudopregnant rats were hyperphagic but did not become leptin resistant, even when given progesterone implants to extend pseudopregnancy beyond the time that resistance develops during pregnancy. Chronic i.c.v. infusion of ovine prolactin to mimic patterns of placental lactogen secretion characteristic of pregnancy, however, completly blocked the ability of leptin to suppress food intake. These data suggest that placental lactogen secretion may mediate the hormone-induced loss of response to leptin. Thus, prolactin appears to be implicated in two of the major neuroendocrine adaptations in the maternal brain, consistent with the hypothesis that prolactin acts as an important afferent signal of the pregnant state, mediating a range of adaptive responses in the maternal brain.

Grattan D.R. Behavioural significance of prolactin signalling in the central nervous system during pregnancy and lactation. Reproduction 123, 497-506 (2002)

Kokay I.C. and Grattan D.R. Expression of mRNA for prolactin receptor (long form) in dopamine and pro-opiomelanocortin neurons in the arcuate nucleus of non-pregnant and lactating rats Journal of Neuroendocrinology 17, 827-835 (2005)

Anderson GM, Beijer P, Bang AS, Fenwick MA, Bunn SJ, Grattan DR. Suppression of Prolactin-induced STAT5b Signaling and Induction of SOCS mRNA in the Hypothalamic Arcuate Nucleus of the Rat During Late Pregnancy and Lactation. Endocrinology 147, 4996-5005, (2006)

Ladyman S.R. and Grattan D.R. Region specific reduction in leptin-induced phosphorylation of STAT3 in the rat hypothalamus is associated with leptin resistance during pregnancy. Endocrinology 145, 3704-3711 (2004)

Ladyman S.R. and Grattan D.R. Suppression of leptin receptor mRNA and leptin responsiveness in the ventromedial nucleus of the hypothalamus during pregnancy in the rat. Endocrinology 146, 3868-3874 (2005)

This research was supported by the Royal Society of New Zealand Marsden Fund

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

SA10

Neurohypophyseal Peptides and Maternal Aggression

S. Meddle

Centre for Integrative Physiology, University of Edinburgh, Edinburgh, UK

Reproductive and stress axis hormones have profound influences on social behaviour, and dramatic changes in the hormonal milieu within the brain following parturition may underlie emotional instability at this time. Female rats are not normally aggressive, but post-partum they become fiercely aggressive towards other rats. Aggressive behaviour is part of the complex suite of maternal behaviour that ensures protection of the offspring, for example, by preventing an intruder from attacking or even killing the pups [1]. Thus the lactating rat is a useful model to study the neuroendocrine mechanisms that control this behavioural switch.
The central oxytocin and vasopressin systems are putative modulators of maternal aggression since they undergo regulatory changes that are temporally synchronised with the expression of maternal behaviour. As the regulation of these neuroendocrine systems peripartum may be relevant to direct central effects of these neuropeptides on behaviour, we investigated receptor expression in the brain from pregnancy and through to lactation. Quantitative in situ hybridisation for oxytocin receptor and vasopressin receptor mRNA expression has revealed dynamic changes around the time of birth. Changes in receptor expression distribution and density patterns in hypothalamic and limbic brain areas point to the crucial role neurohypophyseal hormones play in orchestrating parturition, but importantly these changes may also contribute to observed maternal aggression levels.
In a recent study we sought to identify the neural basis of maternal aggression by using immunocytochemistry for the expression of the immediate early gene Fos protein (an indicator of neuronal activation). In a resident intruder paradigm, Fos expression was significantly increased in brain regions such as the olfactory bulbs, lateral septal nucleus, bed nucleus of the stria terminalis (BnST), supraoptic nucleus (SON), paraventricular nucleus (PVN), central amygdala (CeA) and ventromedial hypothalamus of aggressive lactating rats compared to controls. Activated cells in the PVN and SON, were additionally double labelled for vasopressin or oxytocin. Further double labelling studies indicate that vasopressin receptor (V1a and V1b) expressing neurones and corticotropin releasing factor (CRF) cell populations in the CeA and BnST are also activated. It is unclear how central CRF relates to maternal aggression, but CRF neurone activation in the CeA and BnST may serve as a response to fear particularly since CRF has putatively anxiogenic properties [2, 3].
In a separate study using resident intruder tests on lactating rat dams selectively bred for extremes in emotionality, i.e. for high (HAB) and low (LAB) anxiety-related behaviour [4] we have shown that innate anxiety levels of the dam persist during lactation and underlie the intensity of maternal aggressive behaviour [5]. HAB dams demonstrate overprotective behaviour by spending significantly more time in direct pup contact and are

more aggressive during the resident intruder paradigm compared to LAB dams.

To test the hypothesis that intracerebral oxytocin and vasopressin release patterns are crucially involved in determining levels of maternal aggression we experimentally manipulated brain oxytocin and vasopressin systems. Oxytocin and vasopressin release, as measured by intracerebral microdialysis, increased within the CeA of HAB but not LAB rats during the resident intruder paradigm. Local oxytocin blockade by bilateral retrodialysis of an oxytocin receptor antagonist into either the PVN or the CeA of HAB dams resulted in reduced maternal aggression, whereas the infusion of oxytocin into the PVN or vasopressin into the CeA of LAB dams tended to increase their aggression. These findings lead to the assumption that high local oxytocin levels transiently reduce anxiety to enable the expression of aggressive behaviour towards the potentially dangerous intruder; however this hypothesis remains to be tested. Involvement of the vasopressin system in male rodent aggression is increasingly understood however the role of vasopressin in maternal aggression warrants further investigation.

In summary, we have identified several populations of neurones, including those in the limbic system that are involved in maternal aggression. Moreover the present results provide evidence that maternal aggressive behaviour in the rat is dependent on inborn emotionality and its regulation involves central oxytocin, vasopressin and CRF systems.

Gammie SC (2005) Behav Cogn Neurosci Rev 4: 119-135.

Jasnow AM et al (2006) Horm Behav 49: 197-205.

D'Anna KL et al (2005) Behav Neurosci 119: 1061-1071.

Landgraf R & Wigger A (2003) Stress 6: 111-119.

Bosch OJ et al (2005) J Neurosci 25: 6807-6815.

Acknowledgements: This work was supported by the BBSRC.

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

SA11

Maternal-Induced Neurobiological and Behavioral Plasticity

K. Lambert

Psychology, Randolph-Macon College, Ashland, Virginia, UK

Perhaps more than any other time in her life, the female rat must exhibit efficient responses as she balances the challenges of caring for her offspring with the continued need to meet her own metabolic demands. In addition to the more traditionally viewed maternal behaviors including nursing, retrieving, and grooming her pups, successful maternal rats must engage in efficient foraging responses in order to minimize time spent off the nest at a time when her offspring are vulnerable to predation. Indeed, our laboratory* has found that both maternal (primiparous and multiparous) and pup-sensitized females exhibit enhanced learning ability in a foraging task (Kinsley et al., 1999). Longitudinal explorations confirmed the long-lasting nature of these effects. Reductions in anxiety-like behaviors have also been observed in maternal rats; specifically, primiparous and multiparous animals are more curious and exploratory in the elevated plus maze, an effect that also appears to persist throughout the maternal rat's life (Love et al., 2005; Kinsley & Lambert, 2006). After finding that the maternal rats were also successful in more complex and ecologically relevant foraging tasks including social competition and hunting live crickets, we are currently focusing on the cognitive strategy of attention set-shifting. Results suggest that maternal rats, especially the multiparous animals, demonstrate faster latencies and fewer errors in this task, requiring them to focus their attention on salient cues (e.g., a specific odor) while ignoring distracting cues (e.g., digging medium) in order to more quickly obtain the food reward.

Neurobiological examinations have also been conducted to determine the specific mechanisms underlying these maternal-induced behavioral alterations. During pregnancy, we have found increased dendritic spines in the CA1 area of the hippocampus and increased hippocampal glial fibrillary acidic protein (GFAP) immunoreactivity (continuing through lactation). Although we did not find alterations in CA1 morphology in old-age maternal rats in the longitudinal studies, we found fewer deposits of amyloid precursor protein (a hallmark of Alzheimer's Disease) in older rats with maternal experience (Gatewood et al., 2005). Finally, our most recent work suggests that maternal rats exhibiting enhanced performance in the attention tasks have more nestin-immunoreactivity (Nestin is a class IV intermediate filament protein typically used as a marker for uncommitted progenitor cells but has more recently been found in mature neurons—perhaps indicating cytoskeletal plasticity in these nestin-expressing neurons; Rao et al., 2004) in the CA1 and CA3 areas of the hippocampus, indicating structural changes in these brain areas critical for spatial learning and problem solving. Related to the enhanced boldness observed in maternal animals, less c-fos immunoreactivity was observed in the CA3 area of the hippocampus and basolateral amygdala (both known for their involvement in stress responses). Further, a histological assessment of wild-caught pregnant and lactating rats revealed larger neuronal cell bodies in the basolateral amygdala than observed in the nonreproductive animals, perhaps related to maternal defensive responses. These histological data corroborate the aforementioned behavioral findings, suggesting that the maternal rat's brain is altered in ways leading to enhanced efficiency in foraging and problem solving tasks, as well as reductions in metabolically costly anxiety/stress responses.

In addition to our explorations of maternal rats, we are currently exploring the effects of parental experience on the brain by examining paternal behavior in the monogamous and bi-parental California Deer Mouse (Peromyscus californicus). Thus far, the paternal deer mice exhibit enhanced foraging and diminished anxiety in exploratory tasks as observed in the maternal rats. When exposed to pups restrained in a small enclosure, mice with paternal experience exhibited greater c-fos activation in the CA1 and prefrontal cortical areas than their nonpaternal counterparts. These initial findings suggest that paternal, as well as maternal, behavior contributes to significant neurobiological and behavioral alterations leading to adaptive responses for successful nurturing of offspring.

* This research was conducted in collaboration with Dr. Craig Kinsley and his students at the University of Richmond, Richmond, VA USA.

Gatewood, J.D. et al. (2005). Brain Res Bull 66, 91-98.

Love, G., et al. (2005). Behav Neurosci 119, 1084-1096

Kinsley, C.H., et al. (1999). Nature 402, 137.

Kinsley, C.H., & Lambert, K.G. (2006). Sci Am 294, 72-79.

Rao, A.J. et al. (2004). Society for Neuroscience Abstracts #719.17.

The current research was supported by NIH (1-R15-HD37578-01) and the Psychology Departments at Randolph-Macon College and the University of Richmond.

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

C. Dwyer

Sustainable Livestock Systems, Scottish Agricultural College, Edinburgh, UK

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.

(1) Dwyer CM & Lawrence AB (2000), Behav 137, 1391-1413

(2) Dwyer CM & Lawrence AB (1999), Behav. 136: 367-389

(3) Meurisse M et al. (2005), Horm Behav 48: 34-43

These studies was supported by the Scottish Executive Environment and Rural Affairs Department.

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

SA13

Nutrition Restriction and Offspring Cardiovascular and Metabolic Function

M. Hanson[1], P.D. Gluckman[2], K.M. Godfrey[1], H. Inskip[3] and C. Cooper[3]

[1]Division of Developmental Origins of Health and Disease, University of Southampton, Southampton, UK, [2]Liggins Institute, University of Auckland, Auckland, New Zealand and [3]Epidemiology Resource Centre, University of Southampton, Southampton, UK

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