Neonatal Lipopolysaccharide Exposure Delays Puberty and Alters Hypothalamic Kiss1 and Kiss1r mRNA Expression in the Female Rat

A. M. I. Knox,* X. F. Li,* J. S. Kinsey-Jones,* E. S. Wilkinson,* X. Q. Wu,* Y.S. Cheng,* S. R. Milligan,* S. L. Lightman† and K. T. O’Byrne*

*Division of Reproduction & Endocrinology, King’s College London, London, UK.
†Henry Wellcome Laboratory for Integrative Neuroscience & Endocrinology, University of Bristol, Bristol, UK.

Immunological challenge experienced in early life can have long-term programming effects on the hypothalamic-pituitary-adrenal axis that permanently influence the stress response. Similarly, neonatal exposure to immunological stress enhances stress-induced suppression of the hypothalamic-pituitary gonadal (HPG) axis in adulthood, but may also affect earlier development, including the timing of puberty. To investigate the timing of the critical window for this programming of the HPG axis, neonatal female rats were injected with lipopolysaccharide (LPS; 50 μg/kg i.p.) or saline on postnatal days 3 + 5, 7 + 9, or 14 + 16 and monitored for vaginal opening and first vaginal oestrus as markers of puberty. We also investigated the effects of neonatal programming on the development of the expression patterns of kisspeptin (Kiss1) and its receptor (Kiss1r) in hypothalamic sites known to contain kisspeptin-expressing neuronal populations critical to reproductive function: the medial preoptic area (mPOA) and the arcuate nucleus in neonatally-stressed animals. We determined that the critical period for a significant delay in puberty as a result of neonatal LPS exposure is before 7 days of age in the female rat, and demonstrated that Kiss1, but not Kiss1r mRNA, expression in the mPOA is down-regulated in pre-pubertal females. These data suggest that the mPOA population of kisspeptin neurones play a pivotal role in controlling the onset of puberty, and that their function can be affected by neonatal stress.

Key words: neonatal, LPS, puberty, kisspeptin, Kiss1, GPR54, rat.

doi: 10.1111/j.1365-2826.2009.01885.x
system was linked to the function of the HPG axis after it was noticed that mutations in the Kiss1r gene result in hypogonadotropic hypogonadism (9, 10), and that Kiss1r knockout mice exhibit the same disorder (10). Kisspeptin/Kiss1r signalling is considered part of the hypothalamic circuitry that governs the hypothalamic secretion of GnRH (11) and is strongly implicated as a ‘gatekeeper’ for the initiation of puberty. Peripheral kisspeptin administration stimulates precocious puberty in rats (12–14) and sustained gonadotropic hormone secretion in juvenile rhesus monkeys (15). Furthermore, polymorphisms in the Kiss1 gene and activating mutations in Kiss1r have been associated with precocious puberty in humans (16, 17). Although the mechanisms controlling the timing of kisspeptin/Kiss1r activation at puberty remain to be established, it has recently been shown that 17β-oestradiol (E2) is important for driving the increase in hypothalamic kisspeptin expression prior to puberty in the mouse (18).

Kisspeptin neurones are localised in several nuclei of the hypothalamus. Two of these are crucial for the regulation of gonadotrophic hormone secretion in the rat: the mPOA, which includes the anteroven tral periventricular nucleus (AVPV), and the arcuate nucleus (ARC). During the onset of puberty, Kiss1 and Kiss1r expression increases in the hypothalamus in both male and female rats (13); a further study in mice suggests that there is differential expression of Kiss1 from neurones in the ARC and AVPV during puberty: the expression shows an increase in the AVPV, but remains relatively stable in the ARC (19).

In the present study, we tested the hypotheses that a neonatal immune challenge during a certain critical time window will delay the onset of puberty, and that such an immune challenge will affect Kiss1 and Kiss1r expression in the mPOA and ARC nuclei. Our results suggest that the mPOA population of kisspeptin neurones may play a pivotal role in controlling the onset of puberty, and that their function can be affected by neonatal stress.

Materials and methods

Animals

Pregnant Sprague-Dawley rats obtained from Charles River (Margate, UK) were housed under controlled conditions (12 : 12 h light/dark cycle, lights on 07.00 h; temperature 22 ± 2 °C) and supplied with food and water ad lib. Litters were reduced to a maximum of 14 pups 3 days after birth (taking the day of birth as day 0), to standardise competition for food and maternal attention and partially correct for gender imbalance. Litters were weaned at postnatal (pnd) day 21, and female offspring were housed in groups of four to six per cage. All procedures were conducted in accordance with the United Kingdom Home Office Regulations.

Neonatal endotoxin exposure: determining a critical window for delays in puberty as a result of neonatal immune challenge

Litters were randomly divided into three groups. On pnd 3 and 5, the first group were injected with 50 µg/kg endotoxin in 0.05 ml sterile saline (LPS, serotype *E. coli* O55:B5; Sigma-Aldrich, Poole, UK), a dose sufficient to effect permanent HPA axis activation (1, 6). Half the pups in each litter were designated controls, and received an i.p. injection of sterile saline (0.05 ml). The second group were injected with LPS (50 µg/kg endotoxin in 0.15 ml) or saline on pnd 7 and 9. The third group were injected with LPS (50 µg/kg endotoxin in 0.25 ml) or saline on pnd 14 and 16. Litters were weaned as described, and females were monitored daily for vaginal opening from pnd 32. Once vaginal opening occurred, a vaginal smear was taken using a steel wire loop dipped in sterile saline. Animals were monitored until first oestrus was observed, and weighed weekly.

Neonatal endotoxin exposure: effects on Kiss1 and Kiss1r expression in the mPOA and ARC

On pnd 3 and 5, a separate group of pups was injected i.p. with 50 µg/kg LPS or saline as control as described above. The animals were killed by decapitation at various time points: pnd 14, 32, on the day of vaginal opening (dVO), or pnd 77 (11 weeks of age, hereafter ‘Adult’). Animals from the latter two groups were monitored for vaginal opening and oestrus cyclicity as described above, from pnd 32; they were also weighed weekly. The adult animals were bilaterally ovarioectomised at 10 weeks of age and implanted with a Silastic capsule (inner diameter 1.57 mm; outer diameter 3.18 mm; Sanitech, Havant, UK), filled to a length of 25 mm with E2 (Sigma-Aldrich) dissolved at a concentration of 20 µg/ml arachis oil (Sigma-Aldrich). The E2-containing capsules were assumed to produced circulating concentrations of E2 within the range observed during the dioestrous phase of the oestrous cycle (approximately 38.8 ± 1.2 pg/ml) as previously described by Maeda et al. (20). Surgical procedures were carried out under ketamine (100 mg/kg i.p.); Pharmacia and Upjohn Ltd, Crawley, UK) and Rompun (10 mg/kg i.p.; Bayer, Leverkusen, Germany) anaesthesia. The rationale for ovariectomy and E2 replacement of the adult group was to eliminate the impact of a fluctuating gonadal steroid milieu on hypothalamic Kiss1 and Kiss1r mRNA expression and in addition to allow comparison with other studies carried out in our laboratory using different stress paradigms (21).

Tissue collection and quantitative reverse transcriptase-polymerase chain reaction (RT-PCR)

Expression of Kiss1 and Kiss1r mRNA was determined by real-time quantitative RT-PCR in the mPOA and ARC from animals culled at pnd 14, pnd 32, dVO and as adults. The whole brain was carefully removed, frozen on dry ice, and stored at –80 °C. Sections were cut at 300 µm on a cryostat (Bright Ltd, Cambridgeshire, UK) for RT-PCR. Bilateral punches (1 mm in diameter) from the mPOA, which included the AVPV, were taken from bregma +0.2 to +3.0; a single midline punch (1 mm diameter) was taken from bregma –1.7 to –3.9 to include both ARC nuclei. Co-ordinates were obtained from the rat brain atlas of Paxinos and Watson (22) using the micropunch method described by Paikovits (23). The punched sections were fixed with formalin and stained with crystal violet to confirm correct punch positionining under a microscope. Total RNA was extracted from the punched mPOA and ARC tissues for each rat using TRI reagent (Molecular Research Centre, Ohio, USA) and reverse transcription was carried out using the reverse transcriptase Superscript II (Invitrogen, Carlsbad, CA) and random primer following the manufacturer’s instructions. Reverse transcription was carried out using the reverse transcriptase Superscript II (Invitrogen, Carlsbad, CA) and random primer following the manufacturer’s instructions. For the qPCR, the following primers were used: Kiss1: [sense] 5’-TGG CACCGGTGTTAAACCCGAA-3’, (antisense) 5’-ATCAGCGACTGCGGTGGCAC AC-3’, Kiss1r: [sense] 5’-TGTGCACTGCTACAATACATTCCC-3’, (antisense) 5’-AG CACCGGGCG GAAACAGCTGC-3’. 28S rRNA: [sense] 5’-TIGAAAATCCCGGG GGAAGAG-3’, (antisense) 5’-ACATGGTTCCAAATCCCGAG-3’. The primer pairs selected for Kiss1 and Kiss1r detection were designed to amplify across at least one intron, ruling out the possibility of identical size bands resulting from genomic DNA amplification. Based on the rat Kiss1 genomic sequence
Results

Effects of LPS exposure at different time points postnatally on the timing of pubertal onset

Neonatal administration of LPS on pnd 3 + 5 resulted in a significant delay in both the day of vaginal opening (Saline: 39.5 ± 0.4; LPS: 41.4 ± 0.6; P < 0.05) and day of first vaginal oestrus (Saline: 39.4 ± 0.5; LPS: 41.9 ± 0.6; P < 0.05). There were no significant differences between the LPS and saline-treated animals for the pnd 7 + 9 or pnd 14 + 16 animals (P > 0.05), despite a slight trend towards a delay in the pnd 7 + 9 group (Fig 1). None of the LPS-treated animals showed significant differences in weight compared to their respective controls (data not shown).

Effects of neonatal LPS exposure on Kiss1 and Kiss1r expression in the mPOA and ARC across puberty

There was a significant decrease in Kiss1 mRNA expression in the mPOA on pnd 32 (pre-puberty) and adult for animals treated with LPS neonatally, in comparison to the saline controls. Neither the pnd 14 nor dVO groups showed a significant change in Kiss1 mRNA expression as a result of neonatal LPS administration. The levels of Kiss1 mRNA expression differed significantly between the pnd 14, pnd 32 and dVO groups, increasing with age; in both LPS and saline-treated groups the dVO expression of Kiss1 was significantly higher than in adults (P < 0.05; Fig 2A).

There was no significant difference in Kiss1r mRNA expression across the pubertal transition period, and no significant effect from LPS treatment for pnd 14, pnd 32 or dVO. There was, however, a significant decrease in Kiss1r mRNA expression for the adult animals in comparison to the dVO group, for both LPS and saline-treated groups, and a significant up-regulation of Kiss1r mRNA expression in the mPOA in adult animals in response to neonatal LPS treatment (P < 0.05; Fig 2B).

In the arcuate nucleus, no significant differences in Kiss1 mRNA expression were observed between the LPS and saline-treated

Statistical analysis

Comparisons between neonatal LPS and saline treatment groups on vaginal opening and first oestrus were made by subjecting data to one-way ANOVA and Dunnett’s test. A two-way ANOVA and Newman–Keuls post-hoc analysis was used to assess the effects of LPS treatment and time on the expression of hypothalamic Kiss1 and Kiss1r. P < 0.05 was considered statistically significant in all cases. Data are presented as the mean ± SEM.

Fig. 1. Effects of neonatal lipopolysaccharide (LPS; 50 μg/kg i.p.) at postnatal day (pnd) 3 + 5, 7 + 9 or 14 + 16 on the timing of vaginal opening (a) or first vaginal oestrus (b). Data are presented as the delay, in days, in vaginal opening or first oestrus due to neonatal-LPS treatment relative to neonatal saline treated controls. Only the neonatal-LPS treated rats on pnd 3 + 5 showed a significant delay in pubertal onset (P < 0.05). (mean ± SEM; n = 12–26 per group).
groups at any time point. There was a significant difference in Kiss1 mRNA expression between the pnd 14 and pnd 32 groups, and between the pnd 14 and dVO groups, with the expression increasing with age, but no significant up-regulation between pnd 32 and dVO (P > 0.05; Fig. 2c).

Kiss1r mRNA expression in the arcuate nucleus showed no significant change either with age or as a result of LPS treatment (P < 0.05; Fig. 2d). Representative examples of mPOA and ARC punched brain sections from pnd 77 (Adult) rats are shown in Fig. 3; the ARC punches shown also contain small extra-arcuate cell populations including the median eminence. None of the LPS-treated animals showed significant differences in weight compared with their respective controls (data not shown).

Discussion

The present experiments demonstrate that there is a critical window for the programming of the developing HPG axis by neonatal immune challenge with LPS, and that such an insult has a significant effect on the timing of puberty and the expression of Kiss1 mRNA in the mPOA during puberty. The effect on pubertal timing was only significant when LPS treatment was given on pnd 3 + 5. Of note is the absence of effect of neonatal LPS treatment on body weight (present study, 24), a factor that is well known to affect the timing of pubertal onset.

There have been few other studies of this phenomenon, although a recent study by Iwasa et al. (25) reported that, in rats injected with LPS at postnatal day 10, there was no difference in oestrous cycle length of adult females between neonatally LPS- and saline-treated animals, supporting our findings that an immune challenge after pnd 7 does not overtly affect reproductive function.

We have shown for the first time that neonatal treatment with LPS on postnatal days 3 + 5 results in a significant decrease in Kiss1 mRNA expression in the mPOA on pnd 32, but no changes in Kiss1r mRNA expression. This decrease in Kiss1 mRNA expression could provide a mechanism for the observed delay of puberty. By contrast, the lack of effect of neonatal LPS treatment on Kiss1 or Kiss1r expression in the ARC would indicate that kisspeptin/Kiss1r signalling in this brain region is not an obvious contributing factor to the pubertal delay. The high levels of Kiss1 mRNA in the combined mPOA and ARC of the pnd 32 groups compared to the pnd 14 groups corroborate findings by Navarro et al. (13) that kisspeptin is expressed at a basal level between pnd 10 and pnd 25 in the whole hypothalamus, and is up-regulated several days before vaginal opening. Navarro et al. (13) observed high expression on pnd 30, which corresponds closely to our chosen time point of pnd 32, taking into account that we observed vaginal opening on approximately pnd 38, compared to their pnd 35 for untreated animals. By contrast to the further marked rise in Kiss1 expression between pnd 32 and the day of VO in the mPOA, the kisspeptin neurones in the ARC do not appear to undergo changes during this peripubertal period.

In the present study, Kiss1r mRNA expression showed no significant changes between pnd 14 and pnd 32, unlike the marked up-regulation of the receptor observed by Navarro et al. (13). This difference may reflect the contrast between analysing specific brain

---

**Fig. 2.** Effects of neonatal lipopolysaccharide (LPS, 50 μg/kg i.p.) or saline control at postnatal day (pnd) 3 + 5 on kisspeptin (Kiss1) mRNA expression in the medial preoptic area (mPOA) (a) and arcuate nucleus (ARC) (c), and on kisspeptin receptor (Kiss1r) mRNA expression in the mPOA (b) and ARC (d) in female rats at pnd 14, pnd 32, the day of vaginal opening (dVO) and at 11 weeks of age (Adult). Kiss1 and Kiss1r mRNA levels were measured in brain micropunch samples from the mPOA or ARC using real-time reverse transcriptase-polymerase chain reaction. Quantification for Kiss1, Kiss1r and 28S rRNA was carried out on all samples; the values are expressed as a ratio of Kiss1 mRNA and 28S rRNA, or Kiss1r mRNA and 28S rRNA (mean ± SEM). *P < 0.05 versus the respective treatment group at different time points; #P < 0.05 versus saline control at same time point; n = 5–9 per group.
nuclei rather than the entire hypothalamus, but nevertheless remains puzzling. Herbison et al. (19) have shown an absence of a change in Kiss1r expression in preoptic area GnRH neurones during pubertal development in the male mouse. However, Plant et al. (26) have reported an increase in Kiss1r mRNA expression across the pubertal transition in the mediobasal hypothalamus in female rhesus monkeys, although they show no differences in Kiss1r mRNA expression between juveniles and pubescent males despite an increase in Kiss1.

Although we have shown very early neonatal exposure to LPS may delay puberty as a results of long-term changes in Kiss1 mRNA expression in the mPOA, the underlying mechanism of action of LPS remains to be established. LPS acts to provoke a febrile immune response through the production of cytokines which stimulate the production of cyclooxygenase-2 and PGE2 (4). PGE2 administration in neonates has been shown to mimic the action of oestradiol in orchestrating sexually dimorphic neuronal organisation in the preoptic area, and is linked to masculinisation of sexual behaviour in female rats (27, 28). Because the LPS administration on pnd 3 + 5 falls within a critical window during which this sexual dimorphism in the preoptic has been shown to arise (29, 30), it is possible that there is a link between the action of PGE2 and an alteration of the normal sexual dimorphism of the kisspeptin neurones in the AVPV; it has previously been demonstrated that neonatal androgen administration in the rat plays a role in determining the sexual dimorphism in the AVPV, but not the ARC (31). It has yet to be determined whether PGE2 affects the sexually dimorphic organisation of the kisspeptin neurones, but if this is the case, it offers a possible explanation for the lack of long-term effects on the HPG axis as a result of LPS treatment on pnd 7 + 9 and 14 + 16 because the timing of these injections may fall outside the (currently unknown) critical period during which the differentiation takes place. Further work is required to address these issues.

In adult animals, we found a significant increase in mPOA Kiss1r mRNA expression in response to neonatal LPS administration. This up-regulation of kisspeptin's receptor may represent a compensatory effect to allow some reproductive function despite reduced Kiss1 expression possibly through increased detection of the ligand by increased receptor presence, although, at the present time, this is just speculation. Nevertheless, we observed the same down-regulation of Kiss1 and corresponding up-regulation of Kiss1r in response to both acute and chronic corticosterone administration in ovariectomised adults, although, in contrast to neonatal LPS treatment, corticosterone significantly alters expression profiles in both the mPOA and ARC (21). We have previously shown that adult animals treated neonatally with LPS exhibit chronic hypercortisolaeemia, as well as persistent elevation in response to a mild stressor (6), but neither acute nor chronic stress levels of corticosterone had any effect on pulsatile LH secretion (21). The possible compensation mechanism for up-regulating Kiss1r in the presence of low Kiss1 levels in adults would agree with previous reports that, under nonstressed conditions, pulsatile LH secretion is unaffected by neonatal treatment with LPS in ovariectomised females (7), suggesting that the altered expression of Kiss1 and its receptor in the mPOA does not affect the basal operation of the HPG axis, at least in agonadal female rats. However, we have observed that pnd 3 + 5 LPS treatment has a disruptive effect on oestrus cyclicity, with many more LPS than saline-treated animals exhibiting irregular oestrus cycles, both immediately post-puberty and as adults (32), indicating that the change in Kiss1r/Kiss1r expression profiles as a result of neonatal LPS may be the first manifestation of more long-lasting changes to the HPG axis, namely the sensitisation to stress-induced suppression of the GnRH pulse generator in adulthood (7).

The present study is the first to demonstrate that a neonatal immune challenge can have direct effects on the timing of puberty, concordant with altered hypothalamic Kiss1 expression. That such an early insult is capable of such long-term effects is not entirely unexpected. Many studies of perinatal programming (33) have contributed to the understanding that adverse early environments can have long-lasting effects on adult health, with these changes corresponding to the stages of brain development that continue to mature both before and after birth. Similar to many epigenetic factors that influence development, immunological challenge has come under scrutiny recently, and neonatal LPS administration has been discovered to programme a wide range of adult phenotypes, including an attenuation of the febrile response (34), insulin sensitivity (35), susceptibility to stress-induced suppression of reproductive function (7), and adult anxiety-related behaviour (36). It is apparent...
that a stressful environment in the perinatal period can program an animal’s immune, metabolic and reproductive functions later in life; it remains to be shown which changes are adaptations that allow the animals to better cope with a stressful adult environment, and which represent disadvantages resulting from disruption at key points in development.

Acknowledgements

The authors wish to thank Dr Parlow of the National Institute of Diabetes, Digestive and Kidney Diseases for providing the LH radioimmunoassay kit. This work was supported by the BBSRC and Wellcome Trust. Y.S.C. was sponsored by a Summer Vacation Scholarship from the Society for Reproduction and Fertility.

Received: 3 March 2009, revised 16 April 2009, 5 May 2009, accepted 6 May 2009

References

1 Shank N, Larocque S, Meaney MJ. Neonatal endotoxin alters the development of the hypothalamic-pituitary-adrenal axis: early illness and later responsivity to stress. J Neurosci 1995; 15: 376–384.
2 Brevik T, Stephan M, Brabant GE, Straub RH, Pabst R, von Hörsten S. Postnatal lipopolysaccharide-induced illness predisposes to periodontal disease in adulthood. Brain Behav Immun 2002; 16: 421–438.
3 Boisse L, Mouihate A, Ellis S, Pittman QJ. Long-term alterations in neuroimmune responses after neonatal exposure to lipopolysaccharide. J Neurosci 2004; 24: 4928–4934.
4 Cao C, Matsumura K, Yamagata K, Watanabe Y. Induction by lipopolysaccharide of cyclooxygenase-2 mRNA in rat brain; its possible role in the febrile response. Brain Res 1995; 697: 187–196.
5 Blatteis CM, Li S, Li Z, Feleder C, Perlik V. Cytokines, PGE2 and endotoxic fever: a re-assessment. Prostaglandins Other Lipid Mediat 2005; 76: 1–18.
6 Shank N, Windle RJ, Perks PA, Harbuz MS, Jessop DS, Ingrain CD, Lightman SL. Early life exposure to endotoxin alters hypothalamic-pituitary-adrenal function and predisposition to inflammation. Proc Natl Acad Sci USA 2000; 97: 5645–5650.
7 Li XF, Kinsey-Jones JS, Knox AM, Wu QX, Tahsinsoy D, Brain SD, Lightman SL, O’Byrne KT. Neonatal lipopolysaccharide exposure exacerbates stress-induced suppression of luteinizing hormone pulse frequency in adulthood. Endocrinology 2007; 148: 5984–5990.
8 Messager S, Chatzidaki EE, Ma D, Hendrick AG, Zahn D, Dixon J, Thresher RR, Malling I, Lomet D, Carlton MB, Colledge WH, Caraty A, Aparicio SA. Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. Proc Natl Acad Sci USA 2005; 102: 1761–1766.
9 de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E. Hypogonadotropic hypogonadism due to loss of function of the Kiss1-derived peptide receptor GPR54. Proc Natl Acad Sci USA 2003; 100: 10972–10976.
10 Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acienno JS Jr, Shagoury JK, Bo-Abbas Y, Kuchung W, Schwindof KM, Hendrick AG, Zahn D, Dixon J, Kaiser UB, Slaugenhaupt SA, Gusella JF, O’Rahilly S, Carlton MBL, Crowley W, Aparicio AJR, Colledge WH. The GPR54 gene as a regulator of puberty. N Engl J Med 2003; 349: 1614–1627.
11 Gottsch ML, Cunningham MJ, Smith JT, Popa SM, Acohido BV, Crowley WF, Seminara S, Clifton DK, Steiner RA. A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. Endocrinology 2004; 145: 4073–4077.
12 Matsui H, Takatsu Y, Kumano S, Matsumoto H, Ohtaki T. Peripheral administration of metastin induces marked gonadotropin release and ovulation in the rat. Biochem Biophys Res Commun 2004; 320: 383–388.
13 Navarro VM, Castellano JM, Fernandez-Fernandez R, Barreiro ML, Roa J, Sanchez-Criado JE, Aguilar E, Dieguez C, Pinilla L, Tena-Sempere M. Developmental and hormonally regulated messenger ribonuclease acid expression of Kiss-1 and its putative receptor, GPR54, in rat hypothalamus and potent luteinizing hormone-releasing activity of Kiss-1 peptide. Endocrinology 2004; 145: 4565–4574.
14 Navarro VM, Fernandez-Fernandez R, Castellano JM, Roa J, Mayen A, Barreiro ML, Gaytan F, Aguilar E, Pinilla L, Dieguez C, Tena-Sempere M. Advanced vaginal opening and precocious activation of the reproductive axis by Kiss-1 peptide, the endogenous ligand of GPR54. J Physiol 2004; 561: 379–386.
15 Plant TM, Ramaswamy S, DiPietro MJ. Repetitive activation of hypothalamic G protein-coupled receptor 54 with intravenous pulses of kisspeptin in the juvenile monkey (Macaca mulatta) elicits a sustained train of gonadotropin-releasing hormone discharges. Endocrinology 2006; 147: 1007–1013.
16 Luan X, Zhou Y, Wang W, Yu H, Li P, Gan X, Wei D, Xiao J. Association study of the polymorphism in the KISS1 gene with central precocious puberty in Chinese girls. Eur J Endocrinol 2007; 157: 113–118.
17 Teles MG, Bianco SD, Brito VN, Trarbach EB, Kuhung W, Xu S, Seminara SB, Mendozaa BK, Kaiser UB, Latronico AC. A GPR54-activating mutation in a patient with central precocious puberty. N Engl J Med 2008; 358: 709–715.
18 Clarkson J, Boon WC, Simpson ER, Herbison AE. Postnatal development of an estradiol-kisspeptin positive feedback mechanism implicated in puberty onset. Endocrinology 2009 Mar 19; doi: 10.1210/en.2008-1733.
19 Han SK, Gottsch ML, Lee KJ, Popa SM, Smith JT, Jakawich SK, Clifton DK, Steiner RA, Herbison AE. Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. J Neurosci 2005; 25: 11349–11356.
20 Cagampang FR, Maeda KJ, Tsukamura H, Ohkura S, Ota K. Involvement of ovarian steroids and endogenous opioids in the fasting-induced suppression of pulsatile LH release in ovariectomized rats. J Endocrinol 1991; 129: 321–328.
21 Kinsey-Jones JS, Li XF, Knox AM, Wilkinson ES, Zhu XL, Chaudhary AA, Milligan SR, Lightman SL, O’Byrne KT. Down-regulation of hypothalamic kisspeptin and its receptor, Kiss1r, mRNA expression is associated with stress-induced suppression of luteinizing hormone secretion in the female rat. J Neuroendocrinol 2009; 21: 20–29.
22 Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates, 2nd edn. London: Academic Press, 1986.
23 Palkovits M. Isolated removal of hypothalamic or other brain nuclei of rats does not affect body weight regulation in rats. Proc Natl Acad Sci USA 1973; 70: 449–450.
24 Spencer SJ, Mouihate A, Galic MA, Ellis SL, Pittman QJ. Neonatal immune challenge does not affect body weight regulation in rats. J Physiol Regul Integr Comp Physiol 2007; 293: R581–R589.
25 Iwasa T, Matsuzaki T, Murakami M, Krouchii R, Shimizu F, Kuwahara A, Yasui T, Irahara M. Neonatal immune challenge affects the regulation of estrus cyclicity and feeding behaviour in female rats. Int J Devl Neurosci 2009; 27: 111–114.
26 Shahab M, Mastronardi C, Seminara SB, Crowley WF, Ojeda SR, Plant TM. Increased hypothalamic GPR54 signalling: a potential mechanism for initiation of puberty in primates. Proc Natl Acad Sci USA 2005; 102: 2129–2134.
27 Amateau SK, McCarthy MM. A novel mechanism of dendritic spine plasticity involving estradiol induction of prostaglandin-E2. J Neurosci 2002; 22: 8586–8596.

28 Amateau SK, McCarthy MM. Induction of PGE2 by estradiol mediates developmental masculinization of sex behavior. Nat Neurosci 2004; 7: 643–650.

29 Davis EC, Popper P, Gorski RA. The role of apoptosis in sexual differentiation of the rat sexually dimorphic nucleus of the preoptic area. Brain Res 1996; 734: 10–18.

30 Davis EC, Shryne JE, Gorski RA. Structural sexual dimorphisms in the anteroventral periventricular nucleus of the rat hypothalamus are sensitive to gonadal steroids perinatally, but develop peripubertally. Neuroendocrinology 1996; 63: 142–148.

31 Kauffman AS, Gottsch ML, Roa J, Byquist AC, Crown A, Clifton DK, Hoffman GE, Steiner RA, Tenia-Sempere M. Sexual differentiation of Kiss1 gene expression in the brain of the rat. Endocrinology 2007; 148: 1774–1783.

32 Wu XQ, Li XF, Popat N, Milligan SR, O’Byrne KT. Neonatal Exposure to Lipopolysaccharide Disrupts Ovarian Function in the Adult Rats. Programme of the Annual Meeting of The Society for Reproduction and Fertility. Edinburgh, 2008; (Abstract P48).

33 Seckl JR, Meaney MJ. Glucocorticoid programming. Ann NY Acad Sci 2004; 1032: 63–84.

34 Spencer SJ, Martin S, Mouihate A, Pittman QJ. Early life immune challenge: defining a critical window for effects on adult responses to immune challenge. Neuropsychopharmacology 2006; 31: 1910–1918.

35 Nilsson C, Jennische E, Ho HP, Eriksson E, Bjorntorp P, Holmang A. Postnatal endotoxin exposure results in increased insulin sensitivity and altered activity of neuroendocrine axes in adult female rats. Eur J Endocrinol 2002; 146: 251–260.

36 Spencer SJ, Heida JG, Pittman QJ. Early life immune challenge–effects on behavioural indices of adult rat fear and anxiety. Behav Brain Res 2005; 164: 231–238.