Prenatal programming of the female reproductive neuroendocrine system by androgens

Jane Robinson

Division of Cell Sciences, Faculty of Veterinary Medicine, Institute of Comparative Medicine, University of Glasgow, Glasgow G63 0DW, Scotland, UK

Correspondence should be addressed to J Robinson; Email: j.robinson@vet.gla.ac.uk

Abstract

It has been clear for several decades that the areas of the brain that control reproductive function are sexually dimorphic and that the 'programming actions' of the male gonadal steroids are responsible for sex-specific release of the gonadotrophins from the pituitary gland. The administration of exogenous steroids to fetal/neonatal animals has pinpointed windows of time in an animals' development when the reproductive neuroendocrine axis is responsive to the organisational influences of androgens. These 'critical' periods for sexual differentiation of the brain are trait- and species-specific. The neural network regulating the activity of the gonadotrophin releasing hormone (GnRH) neurones is vital to the control of reproductive function. It appears that early exposure to androgens does not influence the migratory pathway of the GnRH neurone from the olfactory placode or the size of the population of neurones that colonise the postnatal hypothalamus. However, androgens do influence the number and the nature of connections that these neurones make with other neural phenotypes. Gonadal steroid hormones play key roles in the regulation of GnRH release acting largely via steroid-sensitive intermediary neurones that impinge on the GnRH cells. Certain populations of hormonally responsive neurones have been identified that are sexually dimorphic and project from hypothalamic areas known to be involved in the regulation of GnRH release. These neurones are excellent candidates for the programming actions of male hormones in the reproductive neuroendocrine axis of the developing female.

Reproduction (2006) **132** 539–547

Introduction

Just over a decade ago, David Barker proposed that the environment of the developing fetus could programme adult physiological function (Barker 1990). His hypothesis suggested that certain factors, if experienced for a discrete period during early development, could lead to dysfunctional conditions many months or even years later. Although originally focused on the effects of nutrition on the development of adult disease, it is becoming clear that other factors that impinge on the fetus may play key roles in the onset of disease states or abnormal physiological function in adulthood. Among these factors are the gonadal steroid hormones. It is now acknowledged that a short exposure of developing females to abnormally high concentrations of androgenic steroids before and/or immediately after birth may have long-lasting effects that are now regarded as 'programming' the female neuroendocrine axis to malfunction in adulthood. Such steroid exposure may occur when female fetuses (or their mothers) produce excessive concentrations of androgens (such as in

congenital adrenal hyperplasia; Barnes *et al.* 1994), are exposed to androgens via a male sibling (as in the freemartin; Parkinson *et al.* 2001), are positioned adjacent to a male sibling in the uterus of a species that produces multiple offspring, as in the rodent (Meisel & Ward 1981, Galdelman 1986) and pig (vom Saal 1989) or via an exogenous source, including endocrine disruptive agents (Colborn & Clement 1992) and certain medical interventions (Wilkins 1960, Jacobson 1962). Although the signs of *in utero* masculinisation at the level of the external genitalia are well described (Burns 1961) and abnormalities at higher levels of the reproductive axis have been acknowledged for many years (Pfeiffer 1936), the latter have received much less attention. Most importantly, the neural mechanisms underlying the programming of the reproductive neuroendocrine axis, which at time may lead to sub-fertile states, are unclear. The mechanisms by which prenatal testosterone programmes the female reproductive system, especially the gonadotrophin releasing hormone (GnRH) neuronal network to malfunction in adulthood, are the focus of this review.

A window of opportunity for programming

The idea that the brain is organised along gender-specific lines and that these organisational actions are caused by some event in early fetal life is certainly not a new idea, but it was articulated several thousand years ago by Hippocrates (460–377 BC) and Aristotle (384–322 BC). The window during which the developing human brain was given its 'soul' was estimated by both to be between about days 30 and 50 of gestation (Swaab & Hofman 1984). Relative to the reproductive axis, this idea was initially given scientific flesh with studies in the guinea pig which explored the hypothesis that male hormones were important in early development for the sexual differentiation of the brain (Phoenix *et al.* 1959). The species specific 'window' in development when the male steroid hormones are able to exert an organisational action on the GnRH neuronal network has now been defined in several mammalian species. In small rodents, this 'critical period' spans the late gestation/early postnatal period (Gorski 1971, 1985). However, in mammals that are more precocious at birth, this window falls within *in utero* life. Specifically, in the guinea pig with a gestational length of about 65 days, the period of sensitivity has been determined to be between days 30 and 37 of gestation (Goy *et al.* 1964); in the rhesus monkey, it is in the first trimester as it is in the human (Goy & Robinson 1982, Herman *et al.* 2000) while in the sheep, it extends from about 30–90 days of a 147 day pregnancy (Short 1974, Clarke *et al.* 1976, Wood *et al.* 1995). However, recent data obtained from sheep have led us to question whether this very precise delineation of the 'critical period' for sexual differentiation of the GnRH system should be more flexible. Such a doubt has arisen following the observation that chronic exposure to steroids after birth (for example, if the ewe lamb is ovariectomised and given a s.c. implant of oestrogen) appears to be able to further defeminise the GnRH neuronal network (Foster *et al.* 2002). The idea of multiple or extended critical periods has been proposed before for both mammals (Davis *et al.* 1995) and birds (Schumacher *et al.* 1989). Given that neural systems exhibit plasticity in postnatal life, steroids could continue to exert their organisational effects for much longer periods than was previously thought.

The disruption of gonadal steroid feedback by *in utero* androgen exposure

The gonadal steroid hormones exert a critically important, but sexually differentiated, role in the control of the activation of the GnRH neuronal network. While the female possesses a complex interplay among steroid feedback mechanisms that may either stimulate or inhibit GnRH release, the male exhibits a relatively

simple negative feedback mechanism mainly based on testicular androgens. Pioneering studies performed in rodent species (Gorski 1985) demonstrated that the oestrogen-stimulated GnRH surge is present in many mammals unless it has been given a signal within a specific developmental window that obviates the positive feedback mechanism. These observations have more recently been reinforced in the rodent (Connolly & Resko 1994, Rhees *et al.* 1997) and extended to larger mammalian species including the pig (Elsaesser & Parvizi 1979) and the sheep. In the ewe, studies in several laboratories across continents have clearly demonstrated that elevated testosterone concentrations, if present during the *in utero* critical period for sexual differentiation of the brain, severely compromise the positive feedback actions of oestrogen (Fabre-Nys & Venier 1991, Herbosa *et al.* 1996, Robinson *et al.* 2002, Sharma *et al.* 2002, Unsworth *et al.* 2005) and that this action impairs the ability of this steroid to stimulate the GnRH surge that is needed to trigger the luteinising hormone (LH) surge and subsequent ovulation (Fig. 1). As dihydrotestosterone (DHT) does not have this action, this strongly suggests that testosterone must be aromatised to oestrogen to have this defeminising effect (Masek *et al.* 1999). Importantly, a recent study has shown that the fetal ovine brain possess the biochemical mechanisms to aromatase androgens to oestrogens during the critical period for sexual differentiation (Roselli *et al.* 2003).

The control of episodic GnRH release is also influenced by the prenatal steroid environment such that the suppressive actions of both oestrogen (Steiner *et al.* 1976, Dumesic *et al.* 1997, Sharma *et al.* 2005) and progesterone (Robinson *et al.* 1999, Moenter *et al.* 2005) on gonadotrophin secretion are substantially reduced (Fig. 2). An elegant series of studies performed in the laboratory of Douglas Foster have demonstrated that the agonadal ewe lamb, treated chronically with oestradiol, is highly sensitive to the inhibitory actions of this steroid on LH release until she reaches about 30 weeks of age. However, the ram lamb and the androgen-treated ewe lamb escape from such oestrogen negative feedback some 20 weeks earlier when they are about 10 weeks of age (Wood *et al.* 1991). In contrast to the observation that aromatisation of androgens is necessary to defeminise the LH surge mechanisms, the rise in LH secretion at the time of neuroendocrine puberty seems to be an effect of testosterone acting via the androgen receptor (Masek *et al.* 1999).

As the negative feedback influences of steroids on GnRH secretion are impaired, it is probable that the frequency of GnRH pulses will be elevated. Relative to this, we have noted elevated LH pulse frequency in androgenised ewes from puberty to at least 18 months of age (Robinson *et al.* 1999). Thus, such enhanced releasing hormone secretion that, as mentioned above, may be for a protracted period is likely to have

Figure 1 Schematic illustration of both the organisational and the activational effects that prenatal androgen exposure exerts on the reproductive axis of the ewe. At the level of the GnRH neuronal network (top of figure), prenatal androgens neither alter the migratory route of the GnRH cells nor the number that arrive in the preoptic area. However, androgen-exposed ewes form about half the number of synaptic connections (yellow rectangles) on the GnRH cell body (purple/red) when compared to normal ewes. Neurotransmitter systems that impinge on the GnRH neurone are also organised depending on the animals' prenatal steroid environment (pale blue, dark blue and green irregular shapes) as well as the percentage of neurones in a specific populations that are steroid sensitive (black dots). Pituitaries of androgenised animals are heavier than control tissue, while the control animals have a higher percentage of oestrogen-receptive gonadotrophs. Androgen exposure severely disrupts the steroid feedback mechanisms that modulate GnRH and LH secretion. In addition, the ovaries of the androgenised animals are significantly larger and folliculogenesis is disrupted. The ovaries shown in this figure are from 3-week-old ewe lambs (West *et al.* 2001).

substantial consequences for downstream levels of the reproductive axis, including the pituitary gland and the ovary. It is also possible that androgens have separate, but potentially cumulative, actions directly at the levels of these tissues. Recently, we have noted that the pituitaries of *in utero* testosterone treated ewes are significantly heavier than those of control ewes, even when we express hypophysial weight as a proportion of body weight. This suggests that either hypothalamic hyperstimulation or a direct action of androgens has resulted in hypertrophy of this tissue. Recent preliminary investigations have suggested that the density of immunoreactive LH β cells in the pituitary does not differ between *in utero* androgenised and control ewes. However, since the weight of the androgenised pituitary is on an average 40% greater than that of controls, these animals have a larger complement of gonadotrophs. In relation to the oestrogenic control of LH secretion, we have also determined that the percentage of oestrogen-responsive gonadotrophs is higher in the control animals (that respond to oestrogen positive feedback) than the androgenised animals (that do not exhibit oestrogen

positive feedback; Fig. 1). These findings may be important for the actions of oestrogen at the level of the hypophysis and consequently for the feedback mechanisms that modulate the activity of the ovary. At the level of the gonad, fetal androgen excess leads to abnormalities in ovarian morphology and function (Abbott *et al.* 1997, West *et al.* 2001). Specifically, the ovary of the androgen-treated ewe is significantly larger and tends to be multifollicular or contains large fluid-filled 'cysts' when compared with the control animals (Fig. 1). These ovarian abnormalities are apparent from at least as early as 3 weeks of age (West *et al.* 2001). This action requires the aromatisation of testosterone to oestrogen, because when pregnant ewes are treated with DHT there is no ovarian hypertrophy in the offspring ewe lambs and the increase in follicular number is not observed. It is currently unknown whether these morphological differences are a result of androgens actions directly on the ability of the ovary to produce normal cycles in hormone secretion (Birch *et al.* 2003), the response to abnormal gonadotrophic stimulation, or a combination of the two.

Figure 2 *In utero* androgen exposure decreases the sensitivity of the GnRH neuronal network to progesterone negative feedback. The graphs on the left show LH pulse profiles for three individual animals: a control female, a control male and an androgenised female. All the animals were gonadectomised and the blood samples were collected every 10 min for 6 h, first in the absence of progesterone and once 2 days after progesterone had been administered as a s.c. implant (early luteal phase concentrations of 1–2 ng/ml). Significant pulses of LH are identified by a red circle. The panels on the right present composite data from groups of animals ($n=7$ per group). Note that pulse frequency, mean LH concentrations and pulse amplitude are reduced by progesterone (hatched bars) only in the control females (pink bars) but not in the males (blue bars) or the androgenised females (green bars). * $P<0.05$; † $P<0.01$. Data redrawn from Robinson *et al.* (1999).

GnRH neurones and sexual dimorphism in their distribution

As the GnRH neuronal network is programmed to function in a sexually differentiated manner, a major question to be resolved is what neural substrate is responsible for these differences. The first port of investigation must be the GnRH neurone itself. As this neural phenotype originates outside the brain and migrates, in early embryonic life, from the developing olfactory placode into the hypothalamus (Schwanzel-Fucuda 1999), it is possible that prenatal androgens disrupt some aspect of the migratory pathway. However, despite early embryonic androgen exposure, it appears that GnRH neurones are not substantially perturbed on their migratory trek, but colonise the hypothalamus/preoptic area in similar numbers and locations to control animals in the sheep (Wood *et al.* 1992), rat (Wray & Hoffman 1986) and rhesus monkey (Goldsmith & Song 1987). Therefore, this suggests that the neural mechanisms that impinge on the GnRH neurone may be altered by prenatal androgens and by the implication that the androgens act on the area to which the GnRH neurones are travelling and/or the neural connections that they form when they arrive at their destination rather than on their migratory route or the number of cells that successfully complete the

journey (Fig. 1). It has previously been proposed that steroidal influences on the migration of certain neurotransmitter-synthesising cells in the developing hypothalamus could contribute to the sexually differentiated functioning of the brain (Tobet 2002).

Sexually dimorphic input to GnRH neurones

It is clear that the GnRH neurone is influenced not only by its steroidal environment, but also by other sensory and endocrine cues that include the length of the day, level of stress, metabolic factors, ageing and the presence of a mate. Information about the animals' internal and external environment is relayed to the GnRH cell by various neural inputs to influence its activity. However, determining the chemical and structural nature of such inputs is technically extremely challenging in part because such information transduction may take place at various points of the neurone from the cell body to the web of neural processes and the terminal field where GnRH is released into the portal vessels. Although this is a largely unexplored field, there are reports of sexually dimorphic inputs to GnRH neurones at the level of the cell body (rat; Chen *et al.* 1990; sheep; Kim *et al.* 1999). In the latter case, the GnRH perikarya and proximate dendrites of female ovine GnRH neurones received twice the number of

synaptic contacts as those of male neurones. Significantly, this input is influenced by the prenatal androgen environment of the developing animal, as the number of synapses on the androgenised ewe lambs was similar to that of males but significantly fewer than that of non-androgen-exposed ewe lambs (Fig. 1). Recent studies in mice using biocytin to fill the GnRH neurones of adult mice have suggested that the number of synaptic inputs to GnRH neurones may have been substantially underestimated in these earlier studies. Specifically, the long dendritic processes and cell bodies of these biocytin-filled neurones possess many spiny processes which, in the mouse, were not sexually differentiated (Campbell *et al.* 2005). Whether a similar situation is present in the ovine preoptic area has not yet been explored. Although the GnRH neurones and proximal dendrites of some species may not show sexually differentiated inputs, the activity of the GnRH neurone can potentially be altered at other locations. In relation to this, two areas of the brain that play critical roles in the control of gonadotrophin secretion and female sexual behaviour in the rodent (the arcuate nucleus (ARC) and the ventromedial nucleus (VMN)) have been shown to have sexually differentiated patterning of synaptic spines. This is highly region-specific, with the female exhibiting more axo-dendritic spine synapses than the male in the ARC, while the opposite sex dominance is observed in the ventrolateral VMN. Importantly, this spine pattern is altered by steroid exposure during the critical period for sexual differentiation (Matusomoto & Arai 1980, 1986). A continued focus on the sexually differentiated synaptic inputs to GnRH neurones at whatever level must attempt to determine the chemical nature of these inputs and identify those conveying steroidal information to the reproductive neuroendocrine axis. However, this undertaking is technically difficult and labour intensive and so many researchers have embarked on an alternative approach. This involves identification of the location and chemical nature of steroid-sensitive, sexually dimorphic neuronal populations that project from areas of the brain known to be involved in the hormonal control of GnRH release to the hypothalamic site of the GnRH neurones themselves.

Steroid hormone receptors in the brain

As the steroid control of the GnRH neurone is clearly sexually differentiated in several species because of the prenatal experience of the fetus, an understanding of how this is achieved is obviously paramount. A major (but not exclusive) mechanism by which steroids alter GnRH neuronal activity is by altering the activity of steroid sensitive inputs. This is because GnRH neurones themselves are largely devoid of progesterone, androgen and oestrogen (α) receptors (Herbison 1995, Skinner *et al.* 2001). Sex differences in the distribution of steroid receptive neurones in brain areas that influence

reproductive function have been described in several species including the human (Kruijver *et al.* 2003), rodent (Orikasa & Sakuma 2004, Foecking *et al.* 2005), bird (Gahr 2001) and sheep (Scott *et al.* 2000, 2004, Robinson *et al.* 2003). However, sex steroids are involved in physiological functions other than the control of GnRH release and so the identification of specific neurones that convey information to the reproductive neuroendocrine axis is inherently complicated.

Sexually dimorphic neurotransmitter input

The identification of the specific steroid-responsive neurotransmitter systems that modulate the activity of the GnRH neurone has been a quest for reproductive neuroendocrinologists for several decades. The fact that steroid-sensitive inputs to GnRH neurones may be indirect and mediated by one or more intermediate neural population adds further complication. In addition, other neural cells, for example glia, may be extremely important in altering the ability of input neurones to form synapses. It has been shown that glial morphology is modified by steroid hormones and this alters the ensheathment of the GnRH neurone (Mong *et al.* 1999).

A major leap forward has been made in the identification of specific brain sites that appear to be key in the feedback mechanisms of both oestrogen and progesterone and are sexually dimorphic. In rodents, the brain regions that are gender-specific and implicated in the steroidal control of GnRH are found in the preoptic area/anterior hypothalamic areas, including the anteroventral periventricular nucleus (AVPv) and the medial preoptic nucleus (Gorski *et al.* 1980, Hammer 1984, Simerly *et al.* 1984, 1985, Sumida *et al.* 1993, Davis *et al.* 1996). Sex differences have been reported in the existence/position and/or size of specific cell groups (Bleier *et al.* 1982, Henderson *et al.* 1999), the dimensions of cell perikarya (Brown *et al.* 1999) and the chemical phenotype of these cells (De Vries 1990, Park *et al.* 1997, McCarthy & Auger 2002, Simerly 2002, Otten *et al.* 2004, Wolfe *et al.* 2005). However, one limitation of many of these studies in the context of this review is the confirmation that these sexually dimorphic features of the hypothalamus are a consequence of steroidal programming of the brain.

In an attempt to identify specific neural populations that might have sex-specific steroid inputs to GnRH neurones to induce the GnRH surge, we have focused our efforts on the ARC/VMN area of the ovine hypothalamus. This is because microimplants of oestrogen placed in this region have been shown to induce the preovulatory GnRH surge (Caraty *et al.* 1998) as well as stimulating the proceptive and receptive behaviours that accompany it (Blache *et al.* 1991). Track tracing studies have concluded that a trans-synaptic pathway links this

region of brain with the preoptic area, where the majority of ovine GnRH neurones reside (Goubillon *et al.* 2002), providing a potential conduit for information about an animals' steroidal status to be relayed to the reproductive neuroendocrine axis. Within the ovine VMN, initial studies were focused on a population of neurones that synthesise somatostatin (Fig. 3a), largely because these comprise about 70% of the oestrogen receptive cells in this region (Herbison 1995). Further, somatostatin fibres are found in close apposition to GnRH neurones (Fig. 3b). Although the percentage of oestrogen-responsive somatostatin neurones is not sexually differentiated, we have recently determined that these neurones are 'activated' in the ewe in a manner that is dependent on the prenatal androgen environment of the fetus. Specifically, neurones in the VMN that were activated by oestrogen were identified using immunocytochemistry for the protein product of the immediate early gene *c-fos* (Fig. 3a). Ovariectomised ewes were exposed to late follicular phase concentrations of oestrogen that triggered an LH surge in the control, but not the androgenised ewes. On examination of their hypothalami, we noted that the percentage of cells in the VMN of the control ewes that were immunoreactive for *fos* following oestrogen administration was approximately double that of androgenised

animals (Fig. 3c; Robinson *et al.* 2003). Further, the androgenised ewes had a similar number of *fos*-positive neurones in the VMN to a control group that had not been exposed to exogenous oestrogen. On further examination, we found that approximately 20% of somatostatin neurones were 'activated' by oestrogen in the controls but significantly fewer in the androgenised animals and controls not given exogenous steroids. These data support the suggestion that the activation of steroid responsive somatostatin neurones in the VMN is one of the early events in a chain by which oestrogen triggers the preovulatory surge of GnRH. This conjecture is also supported by a study by Pilon *et al.* (2004) in the normal ewe. Further, our data suggest that this mechanism is compromised in animals that have been treated with testosterone *in utero*, which do not exhibit an LH surge. Our future studies include those in which somatostatin will be infused directly into the brain in an attempt to alter GnRH surge dynamics. Similar studies have been carried out in the rat, where centrally administered somatostatin has been shown to inhibit the oestrogen-induced LH surge by preventing activation of the GnRH neurones (Van Vugt *et al.* 2004). Furthermore, it is clear that other neural phenotypes in the region of the ovine VMN are differentially activated by oestrogen and these will be the focus of future studies.

Figure 3 (a) Cells in the ventrolateral aspect of the ventromedial nucleus (vVMN) of the ewe stained for somatostatin (brown cytoplasmic stain) or fos (black nuclear staining). (b) A cell in the preoptic area that is immunoreactive for GnRH (brown cytoplasmic stain). An axon immunoreactive for somatostatin is in close apposition to the GnRH cell body. (c) Control ewes given high follicular phase concentrations of oestrogen (+E) have significantly more *fos*-positive cells in the vVMN than similarly treated androgenised ewes or control animals not treated with oestrogen (–E). (d) About 20% of the *fos*-positive cells in the oestrogen-treated control animals are somatostatin. The oestrogen-treated androgenised animals and the non-steroid-treated controls have significantly fewer *fos*-positive somatostatin neurones in the vVMN. See text for further details. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Conclusion

Exposure of the developing female to male steroid hormones has disruptive actions on the organisation of all the levels of reproductive axis from the GnRH neuronal network to the gonad. These organisational actions take place during specific windows of time during an individuals' development and these so-called 'critical periods' are species-specific. The precise mechanism by which early exposure to male hormones programmes the reproductive neuroendocrine axis of the female to malfunction in postnatal life is unclear. However, we do know that the gonadal steroid feedback mechanisms that modulate GnRH release are central to this process. The challenge is to identify those steroid-responsive inputs. This will facilitate a better understanding of the neural mechanisms by which the activity of the GnRH neurone is regulated in normal conditions as well as which specific circuits are disrupted by the programming influences of neonatal steroids.

Acknowledgements

The author wishes to thank the BBSRC and Wellbeing and the Royal College of Obstetricians and Gynaecologists for their financial support. Several collaborators contributed substantially to the work described in this manuscript: Neil Evans, Rachel Forsdike, Douglas Foster, Jo Grindrod, Vasantha Padmanabhan, James Taylor and Will Unsworth. Andrew Dady, Jonah Jones, Malcolm McColl and Martin White who took care of the animals made these studies possible. The author declares that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References

- Abbott DH, Dumesic DA, Eisner JR, Kemnitz JW & Goy RW 1997 The prenatally androgenised female rhesus monkey as a model for PCOS. In *Androgen Excess Disorders in Women*, pp 369–382. Eds R Azziz, JE Nestler & D Dewailly. Philadelphia: Lippincott-Raven.
- Barker DJP 1990 Fetal and infant origins of adult disease. *British Medical Journal* **301** 1111.
- Barnes RB, Rosenfeldt RL, Ehrmann DA, Cara JF, Cuttler L, Levitsky LL & Rosenthal IM 1994 Ovarian hyperandrogenism as a result of congenital adrenal virilizing disorders: evidence for perinatal masculinization of neuroendocrine function in women. *Journal of Clinical Endocrinology and Metabolism* **79** 1328–1333.
- Birch RA, Padmanabhan V, Foster DL, Unsworth WP & Robinson JE 2003 Prenatal programming of reproductive neuroendocrine function: fetal androgen exposure produces progressive disruption of reproductive cycles in sheep. *Endocrinology* **144** 1426–1434.
- Blache D, Fabre-Nys CJ & Vernier G 1991 Ventromedial hypothalamus as a target for estradiol action on proceptivity, receptivity and luteinizing hormone surge of the ewe. *Brain Research* **546** 241–249.
- Bleier R, Byne W & Siggelkow I 1982 Cytoarchitectonic sexual dimorphism of the medial preoptic and anterior hypothalamic areas in guinea pig, rat, hamster, and mouse. *Journal of Comparative Neurology* **212** 118–130.
- Brown AE, Mani S & Tobet SA 1999 The preoptic area/anterior hypothalamus of different strains of mice: sex differences and development. *Developmental Brain Research* **115** 171–182.
- Burns RK 1961 Role of hormones in the differentiation of sex. In *Sex and Internal Secretions*, 3rd edn, vol 1, pp 76. Baltimore: Wilkinson and Co.
- Campbell RE, Han S-K & Herbison AE 2005 Boicytin filling of adult gonadotropin-releasing hormone neurones *in situ* reveals extensive, spiny, dendritic processes. *Endocrinology* **146** 1163–1169.
- Caraty A, Fabre-Nys CJ, Delaleu G, Locatelli A, Bruneau G, Karsch FJ & Herbison AE 1998 Evidence that the mediobasal hypothalamus is the primary site of action of estradiol in inducing the preovulatory gonadotropin releasing hormone surge in the ewe. *Endocrinology* **139** 1752–1760.
- Chen WP, Witkin JW & Silverman AJ 1990 Sexual dimorphism in the synaptic input to gonadotropin releasing hormone neurons. *Endocrinology* **126** 695–702.
- Clarke IJ, Scaramuzzi RJ & Short RV 1976 Effects of testosterone implants in pregnant ewes on their female offspring. *Journal of Embryology and Experimental Morphology* **36** 87–99.
- Colborn T & Clement C 1992 *Chemically-Induced Alterations in Sexual and Functional Development: the Wildlife/Human Connection*, *Advances in Modern Environmental Toxicology*, vol XXI. Princeton, NJ: Princeton Scientific Publishing Co., Inc..
- Connolly PB & Resko JA 1994 Prenatal testosterone differentiates brain regions controlling gonadotropin release in guinea pigs. *Biology of Reproduction* **51** 125–130.
- Davis EC, Shryne JE & Gorski RA 1995 A revised critical period for the sexual differentiation of the sexually dimorphic nucleus in the preoptic area in the rat. *Neuroendocrinology* **62** 579–585.
- Davis EC, Shryne JE & Gorski RA 1996 Structural sexual dimorphisms in the anteroventral periventricular nucleus of the rat hypothalamus are sensitive to gonadal steroids perinatally, but develop peripubertally. *Neuroendocrinology* **63** 142–148.
- De Vries GJ 1990 Sex differences in neurotransmitter systems. *Journal of Neuroendocrinology* **2** 1–13.
- Dumesic DA, Abbott DH, Eisner JR & Goy RW 1997 Prenatal exposure of female rhesus monkeys to testosterone propionate increases serum luteinizing hormone levels in adulthood. *Fertility and Sterility* **67** 155–163.
- Elsaesser F & Parvizi N 1979 Estrogen feedback in the pig: sexual differentiation and the effect of prenatal testosterone treatment. *Biology of Reproduction* **20** 1187–1193.
- Fabre-Nys C & Venier G 1991 Sexual differentiation of sexual behaviour and preovulatory LH surge in ewes. *Psychoneuroendocrinology* **16** 383–396.
- Foecking EM, Szabo M, Schwartz NB & Levine JE 2005 Neuroendocrine consequences of prenatal androgen exposure in the female rat: absence of luteinizing hormone surges, suppression of progesterone receptor gene expression and acceleration of the gonadotropin-releasing hormone pulse generator. *Biology of Reproduction* **72** 1475–1483.
- Foster DL, Padmanabhan V, Wood RI & Robinson JE 2002 Sexual differentiation of the neuroendocrine control of gonadotrophin secretion: concepts derived from sheep models. *Reproduction Supplement* **59** 83–99.
- Gahr M 2001 Distribution of sex steroid hormone receptors in the avian brain; functional implications for neural sex differences and sexual behaviors. *Microscopy Research and Technique* **55** 1–11.
- Galdelman R 1986 Uterine position and the activation of male sexual activity in testosterone propionate-treated female guinea pigs. *Hormones and Behavior* **20** 287–293.
- Goldsmith PC & Song T 1987 The gonadotropin-releasing hormone containing ventral hypothalamic tract in the fetal rhesus monkey (*Macaca mulatta*). *Journal of Comparative Neurology* **257** 130–139.
- Gorski RA 1971 Gonadal hormones and the perinatal development of neuroendocrine function. In *Frontiers in Neuroendocrinology*, pp 237–290. Eds L Martini & WF Ganong. New York: Oxford University Press.
- Gorski RA 1985 Sexual differentiation of the brain: possible mechanisms and implications. *Canadian Journal of Physiology and Pharmacology* **63** 577–594.

- Gorski RA, Harlan RE, Jacobson CD, Shryne JE & Southam AM 1980 Evidence for the existence of a sexually dimorphic nucleus in the preoptic area of the rat. *Journal of Comparative Neurology* **193** 529–538.
- Goubillon M-L, Caraty A & Herbison AE 2002 Evidence in favour of a direct input from the ventromedial nucleus to gonadotropin-releasing hormone neurones in the ewe: an anterograde tracing study. *Journal of Neuroendocrinology* **14** 95–100.
- Goy RW & Robinson JA 1982 Prenatal exposure of rhesus monkeys to patent androgens: morphological, behavioural and physiological consequences. Banbury Report II: Environmental Factors in Human Growth and Development, pp 355–379. New York: Cold Spring Harbor Press.
- Goy RW, Bridson WE & Young WC 1964 Period of maximum susceptibility of the prenatal female guinea pig to masculinizing actions of testosterone propionate. *Journal of Comparative and Physiological Psychology* **57** 166–174.
- Hammer RP Jr 1984 The sexually dimorphic region of the preoptic area contains denser opioid binding sites in females. *Brain Research* **308** 172–176.
- Henderson RG, Brown AE & Tobet SA 1999 Sex differences in cell migration in the preoptic/anterior hypothalamus of mice. *Journal of Neurobiology* **41** 252–266.
- Herbison AH 1995 Neurochemical identity of neurones expressing oestrogen and androgen receptors in sheep hypothalamus. *Journal of Reproduction and Fertility Supplement* **49** 271–283.
- Herbosa CG, Dahl GE, Evans NP, Pelt J, Wood RI & Foster DL 1996 Sexual differentiation of the surge mode of gonadotropin secretion: prenatal androgens abolish the gonadotropin-releasing hormone surge in sheep. *Journal of Neuroendocrinology* **8** 627–633.
- Herman RA, Jones B, Mann DR & Wallen K 2000 Timing of prenatal androgen exposure: anatomical and endocrine effects on juvenile male and female monkeys. *Hormones and Behavior* **38** 52–66.
- Jacobson BD 1962 Hazards of norethindrone therapy during pregnancy. *American Journal of Obstetrics and Gynecology* **84** 962–968.
- Kim SJ, Foster DL & Wood RI 1999 Prenatal testosterone masculinizes synaptic input to gonadotropin-releasing hormone neurons in sheep. *Biology of Reproduction* **61** 599–605.
- Kruijver FP, Balesar R, Espila AM, Unmehopa UA & Swaab DF 2003 Estrogen receptor – alpha distribution in the human hypothalamus in relation to sex and endocrine status. *Journal of Comparative Neurology* **454** 115–139.
- Masek KS, Wood RI & Foster DL 1999 Prenatal dihydrotestosterone differentially masculinizes tonic and surge modes of luteinizing hormone secretion in sheep. *Endocrinology* **140** 3459–3466.
- Matusomoto A & Arai Y 1980 Sex dimorphism in 'wiring patterns' in the hypothalamic arcuate nucleus and its modification by neonatal hormonal environment. *Brain Research* **190** 238–242.
- Matusomoto A & Arai Y 1986 Male–female differences in synaptic organization of the ventromedial nucleus of the hypothalamus in rats. *Neuroendocrinology* **42** 232–236.
- McCarthy AP & Auger TS 2002 Perrot-Sinal, getting excited about GABA and sex differences in the brain. *Trends in Neuroscience* **25** 307–312.
- Meisel RL & Ward IL 1981 Fetal female rats are masculinised by male littermates located caudally in the uterus. *Science* **213** 239–242.
- Moenter SM, Pielecka J, Quaynor SD & Sullivan SD 2005 Effects of pre- and postnatal androgens on reproductive neuroendocrine circuitry. *Biology of Reproduction*. Special issue, Abstract # 75.
- Mong JA, Glaser E & McCarthy MM 1999 Gonadal steroids promote glial differentiation and alter neuronal morphology in the developing hypothalamus in a regionally specific manner. *Journal of Neuroscience* **19** 1464–1472.
- Orikasa C & Sakuma Y 2004 Sex and region-specific regulation of oestrogen receptor b in the rat hypothalamus. *Journal of Neuroendocrinology* **16** 964–969.
- Otten NE, Gpdwin JG, Krishnan S & Petersen SL 2004 Dual-phenotype GABA/glutamate neurons in adult preoptic area: sexual dimorphism and function. *Journal of Neuroscience* **24** 8097–8015.
- Park JJ, Baum MJ & Tobet SA 1997 Sex differences in steroidal stimulation of galanin immunoreactivity in the ferret's dorsal preoptic area/anterior hypothalamus. *Journal of Comparative Neurology* **389** 277–288.
- Parkinson TJ, Smith KC, Long SE, Douthwaite JA, Mann GE & Knight PG 2001 Inter-relationship among gonadotrophins, reproductive steroids and inhibin in freemartin ewes. *Reproduction* **122** 397–409.
- Pfeiffer CA 1936 Sexual differences of the hypophyses and their determination by the gonads. *American Journal of Anatomy* **58** 195–225.
- Phoenix CH, Goy RW, Garall AA & Young WC 1959 Organising action of prenatally administered testosterone propionate on the tissues mediating mating behaviour in the female guinea pig. *Endocrinology* **65** 369–382.
- Pillon D, Caraty A, Fabre-Nys C, Lomet D, Cateau M & Bruneau G 2004 Regulation by estradiol of hypothalamic somatostatin gene expression: possible involvement of somatostatin in the control of luteinising hormone secretion in the ewe. *Biology of Reproduction* **71** 38–44.
- Rhees RW, Kirk BA, Sephton S & Lephart ED 1997 Effects of prenatal testosterone on sexual behaviour, reproductive morphology and LH secretion in the female rat. *Developmental Neuroscience* **19** 430–437.
- Robinson JE, Forsdike RA & Taylor JA 1999 *In utero* exposure of female lambs to testosterone reduces the sensitivity of the gonadotropin-releasing hormone network to inhibition by progesterone. *Endocrinology* **140** 5797–5805.
- Robinson JE, Birch RA, Foster DL & Padmanabhan V 2002 Prenatal exposure of the ovine fetus to androgens sexually differentiates the steroid feedback mechanisms that control gonadotropin releasing hormone secretion and disrupts ovarian cycles. *Archives of Sexual Behavior* **31** 35–41.
- Robinson JE, Birch RA, Grindrod JAE, Taylor JA & Unsworth WP 2003 Sexual differentiated regulation of GnRH release by gonadal steroid hormones in sheep. *Reproduction Supplement* **61** 299–310.
- Roselli CE, Resko JA & Stormshak F 2003 Estrogen synthesis in fetal sheep brain: effect of maternal treatment with an aromatase inhibitor. *Biology of Reproduction* **68** 370–374.
- Schumacher M, Hendrick JC & Balthazar J 1989 Sexual differentiation in the quail: critical period and hormonal specificity. *Hormones and Behavior* **23** 130–149.
- Schwanzel-Fucuda M 1999 Origin and migration of luteinising hormone-releasing hormone neurons in mammals. *Microscopy Research and Technique* **44** 2–10.
- Scott CJ, Tilbrook AJ, Simmons DM, Rawson JA, Chu S, Fuller PJ, Ing NH & Clarke IJ 2000 The distribution of cells containing estrogen receptor α (ER α) and ER β messenger ribonucleic acid in the preoptic area and hypothalamus of the sheep: comparison of males and females. *Endocrinology* **141** 2951–2962.
- Scott CJ, Clarke IJ, Rao A & Tilbrook AJ 2004 Sex differences in the distribution and abundance of androgen receptor mRNA containing cells in the preoptic area and hypothalamus of ram and ewe. *Journal of Neuroendocrinology* **16** 956–963.
- Sharma TP, Herkimer C, West C, Ye W, Birch RA, Robinson JE, Foster DL & Padmanabhan V 2002 Fetal programming: prenatal androgens disrupt positive feedback actions of estradiol but does not affect timing of puberty in female sheep. *Biology of Reproduction* **66** 924–933.
- Sharma HN, Manikkam M, Herkimer C, Dell'Orco J, Welch KB, Foster DL & Padmanabhan V 2005 Fetal programming: excess prenatal testosterone reduces postnatal luteinizing hormone, but not follicle-stimulating hormone responsiveness to estradiol negative feedback in the female. *Endocrinology* **146** 4281–4291.
- Short RV 1974 Sexual differentiation of the brain. *INSERM* **32** 121–142.
- Simerly RB 2002 Wired for reproduction: organization and development of sexually dimorphic circuits in the mammalian forebrain. *Annual Review of Neuroscience* **25** 507–536.

- Simerly RB, Swanson LW & Gorski RA** 1984 Demonstration of a sexual dimorphism in the distribution of serotonin-immunoreactive fibres in the medial preoptic nucleus of the rat. *Journal of Comparative Neurology* **225** 151–166.
- Simerly RB, Swanson LW & Gorski RA** 1985 The distribution of monoaminergic cells and fibres in a periventricular nucleus involved in the control of gonadotropin release: immunohistochemical evidence for a dopaminergic sexual dimorphism. *Brain Research* **330** 55–64.
- Skinner RA, Caraty A & Allingham R** 2001 Unmasking the progesterone receptor in the preoptic area and hypothalamus of the ewe: no colocalization with gonadotropin releasing hormone neurons. *Endocrinology* **142** 573–579.
- Steiner RA, Clifton DK, Spies HG & Resko JA** 1976 Sexual differentiation and feedback control of luteinizing hormone secretion in the rhesus monkey. *Biology of Reproduction* **15** 206–212.
- Sumida H, Nishizuka M, Kano Y & Arai Y** 1993 Sex differences in the anteroventral periventricular nucleus of the preoptic area and in the related effects of androgens in prenatal rats. *Neuroscience Letters* **151** 141–144.
- Swaab DF & Hofman MA** 1984 Sexual differentiation of the human brain. A historical perspective. *Progress in Brain Research* **61** 361–374.
- Tobet SA** 2002 Genes controlling hypothalamic development and sexual differentiation. *European Journal of Neuroscience* **16** 373–376.
- Unsworth WP, Taylor JA & Robinson JE** 2005 Prenatal programming of the reproductive neuroendocrine axis: the effect of prenatal androgens on the development of estrogen positive feedback and ovarian cycles in the ewe. *Biology of Reproduction* **72** 619–627.
- Van Vugt HH, Swarts HJM, Van der Heijning BJM & Van der Beek EM** 2004 Centrally applied somatostatin inhibits the estrogen-induced luteinising hormone surge via hypothalamic gonadotropin releasing hormone cell activation in female rats. *Biology of Reproduction* **71** 813–819.
- vom Saal FS** 1989 Sexual differentiation in litter bearing mammals: influence of sex of adjacent fetuses *in utero*. *Journal of Animal Science* **67** 1824–1840.
- West C, Foster DL, Evans NP, Robinson JE & Padmanabhan V** 2001 Intra-follicular activin availability is compromised in prenatally-androgenised sheep. *Molecular and Cellular Endocrinology* **185** 51–59.
- Wilkins L** 1960 Masculinization of female fetus due to orally given progestins. *Journal of the American Medical Association* **172** 1028–1032.
- Wolfe CA, Van Doren M, Walker HJ, Seney ML, McClellan KM & Tobet SA** 2005 Sex differences in the location of immunochemically defined cell populations in the mouse preoptic area/anterior hypothalamus. *Developmental Brain Research* **157** 34–41.
- Wood RI, Ebling FJP, I'Anson H & Foster DL** 1991 Prenatal androgens time neuroendocrine sexual maturity. *Endocrinology* **128** 2457–2468.
- Wood RI, Newman SW, Lehman MN & Foster DL** 1992 GnRH neurons in the fetal lamb hypothalamus are similar in males and females. *Neuroendocrinology* **55** 427–433.
- Wood RI, Mehta V, Herbosa CG & Foster DL** 1995 Prenatal testosterone differentially masculinizes tonic and surge modes of luteinizing hormone secretion in the developing sheep. *Neuroendocrinology* **62** 238–247.
- Wray S & Hoffman G** 1986 Postnatal morphological changes in the rat LHRH neurons correlate with sexual maturation. *Neuroendocrinology* **43** 93–97.

Received 13 June 2006

First decision 20 July 2006

Accepted 14 August 2006