Neural transplantation in hypogonadal (hpg) mice – physiology and neurobiology

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Abstract

The hypogonadal (hpg) mouse mutant has a deletion in the region encoding the hypothalamic gonadotrophic hormone-releasing hormone decapeptide. As a consequence pituitary gonadotrophic hormone synthesis and release is severely curtailed and there is little or no post-natal gonadal development. Grafts of late fetal/early neonatal brain tissue containing the decapeptide-producing neurones into the third ventricle of hpg mice result, in a majority of animals, in a near normalisation of pituitary function with full spermatogenesis in male mice and full follicular and uterine development in females. The vast majority of positive responding females with vaginal opening and uterus growth show no evidence of spontaneous oestrous cycles, ovulation or corpora lutea. These female mice mate with normal males with many of them demonstrating reflex ovulation. In both male and female mutants with successful grafts there is an absence of gonadal steroid negative feedback upon the synthesis and secretion of pituitary gonadotrophic hormones. The releasing factor axon terminals from grafts within the third ventricle identified by immunohistochemical methods are targeted specifically to the median eminence. There is evidence for host innervation of grafts, but how specific this is for the control of gonadotrophic hormone-releasing hormone cell bodies remains to be elucidated.

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Introduction

The hypothalamic decapeptide gonadotrophic hormone-releasing hormone (GnRH) is central to the regulation of brain–pituitary–gonadal interrelationships in vertebrates (Everett 1994). Release of the hormone into the portal vessels leading from the hypothalamus to the pituitary gland results in the synthesis and secretion of the pituitary gonadotrophic hormones, luteinising hormone (LH) and follicle-stimulating hormone (FSH). These, in turn, stimulate testicular and ovarian function with feedback control from the gonads acting at the pituitary, the GnRH neurones themselves, or at central nervous system (CNS) sites controlling these neurones. With the use of antibodies raised against GnRH the preoptic area of the brain has been identified as the major site of GnRH neurones (Silverman *et al.* 1994). These neurones are actually formed within the nasal placode and migrate into the anterior hypothalamus via the cribriform plate of the ethmoid bone (Wray 2002).

A great deal of research has dealt with the neuroanatomical input into GnRH neurones and how their function can be regulated by steroid and other molecules from the peripheral circulation or locally derived factors within the

brain itself in the control of spermatogenesis in the male and ovarian function in the female (Herbison 1998, Ebling & Cronin 2000). The use of immortalised GnRH cell lines has also been central to our understanding of mechanisms controlling the synthesis and secretion of the decapeptide (Weiner *et al.* 1992). The detective work leading up to the isolation and chemical synthesis of GnRH involved the use of a variety of techniques including electrical stimulation, electrolytic lesioning, surgical ablation and the transplantation of endocrine tissues (Harris 1972). A powerful demonstration that GnRH is an essential component in controlling brain–pituitary–gonadal interrelationships (see Fig. 1) would be to disrupt the gene encoding this hormone, a technique now commonly used in gene knockout technology in mice, and to follow the physiological consequences. Nature sometimes does the experiment for us and the discovery 25 years ago of the hypogonadal (hpg) mouse in which there is a failure of post-natal gonadal development provided us with a GnRH-deficient model (see Fig. 2). The initial diagnosis of the defect in these mutants depended upon hormone assays which demonstrated that the hypothalamus contained no immunoreactive GnRH, with a consequent depletion in pituitary LH and FSH (Cattanach *et al.* 1977).

Figure 1 Diagram representing brain-pituitary-gonadal interrelationships. GnRH secretion from the median eminence to the pituitary gland is controlled by higher centres in the brain. The decapeptide stimulates synthesis and secretion of LH and FSH by the pituitary gland. These two hormones activate the gonads which, in turn, secrete steroid and peptide hormones which exert feedback control at the level of both the pituitary and the CNS.

This was confirmed in tissue sections of the median eminence in which no GnRH immunoreactive cells or fibres were found in *hpg* mice (see Fig. 3). Injections of GnRH stimulated pituitary LH and FSH synthesis and secretion and activation of the gonads (Charlton *et al.* 1983).

The GnRH precursor has been shown to consist of a 23-amino acid signal peptide, the decapeptide GnRH itself which is linked by Gly-Lys-Arg to a 56-amino acid sequence which has been called GnRH-associated peptide (GAP). Analysis of the DNA from *hpg* mice revealed a

Figure 2 Photographs of the gonads and accessory sexual tissue of normal and *hpg* mice. (A) The extremely small testes of the *hpg* male are shown (below) compared with a litter mate control (above). The atrophic nature of the seminal vesicles is also an indication that little or no testosterone is secreted by the mutant testis. (B) The ovaries and uterus of normal (above) and *hpg* (below) females are shown. Note the extremely small ovaries and the thread-like uterus.

Figure 3 GnRH immunostaining in coronal sections through the median eminence of normal and hpg mice. The intense staining for GnRH in the nerve terminals at the median eminence of a normal mouse (A) as compared with the total absence of any immunoreactive product in the hpg mutant (B). The space above the median eminence is the third ventricle.

massive 33.5 kb deletion beginning distal to the 11th amino acid in GAP. The partially deleted gene was shown to be transcriptionally active, but antigens corresponding to the precursor protein could not be detected immunocytochemically (Mason *et al.* 1986a).

Subsequent technological advances allowed the production of transgenic hpg mutants that contained copies of the normal GnRH gene. This resulted in full restoration of gonadal activity in both sexes, a powerful demonstration of the strategic importance of GnRH in reproduction (Mason *et al.* 1986b). Another method of giving back this hypothalamic factor was to transplant preoptic area (POA) neural tissue (the region of the brain rich in GnRH cell bodies) into the third ventricle of adult hpg mice.

The technique of transplantation is very simple. Using late fetal/early neonatal donors, a block of tissue is dissected from the base of the brain just anterior to the optic chiasm which includes both preoptic areas. Without further disruption of the tissue it is taken up in a plunger-in-needle syringe and, under stereotaxic surgery, ejected into the third ventricle in the mid-line (see Fig. 4). This review will describe the physiological effects of these POA grafts and discuss factors determining their survival and neurobiological interactions.

Physiology

Pituitary and gonadal activation

The first report of the physiological action of POA grafts in hpg mice was that of Krieger *et al.* (1982) in which engrafted male hpg mutants exhibited increased pituitary and plasma gonadotrophin concentrations, testicular growth and evidence of full spermatogenesis (see Fig. 5A and B). In all animals in which the gonads were stimulated,

Figure 4 Diagrammatic representation of the hypothalamus, median eminence, third ventricle and pituitary gland. On the left, the figure demonstrates GnRH cell bodies in the preoptic area (POA) sending their axons to terminate in the median eminence (ME). The third ventricle is shaded black and is the site into which neural grafts are placed. The inset shows a healthy graft occupying the space of the third ventricle. The outpushing of the median eminence is obvious at the bottom of the micrograph. CA, anterior commissure; MFB, medial fore-brain bundle; PVN, paraventricular nucleus; PEN, periventricular nucleus; F, fornix.

Figure 5 The effect of POA transplants upon the gonads of hpg mice. (A) Scanning electron micrograph of part of the testis of an adult hpg male. Note the small diameter of the seminiferous tubules and the absence of a central lumen. (B) Scanning electron micrograph, at the same magnification as (A), of part of the testis of an hpg male who received a neural graft of POA tissue from a post-natal day 1 mouse. Note the development of full spermatogenesis and the increased tubule diameter. (C) Photomicrograph of a section through the ovary of an adult female hpg mouse. Note the lack of mature follicles. (D) Section of an ovary, at the same magnification as (C), of an adult hpg female given a POA graft. Note the development of mature follicles but the absence of corpora lutea.

immunocytochemical examination indicated that GnRH neurones were present within the grafts and axon terminals reached the portal vessels draining the median eminence to the anterior pituitary gland (see Fig. 6).

Fibre outgrowth to the median eminence from POA grafts has been observed as early as 10 days post implantation. At this time there was little evidence of increased pituitary gonadotrophic content or of increased seminal vesicle weight in male hpg/POA mice. Between 26 and 40 days post grafting pituitary concentrations of gonadotrophic hormones were significantly elevated. Testicular growth was evident at 30 days in 94% of grafted males in one study (Silverman *et al.* 1985). The growth plateaued at around 90 days, with significant stimulation of the seminal vesicles in all recipients.

The results of POA grafts in hpg female mice were equally dramatic (Gibson *et al.* 1984a). In the majority of hpg female mice bearing hypothalamic transplants, vaginal opening occurred about 23 days after implantation and these animals displayed prolonged periods of oestrus

as measured by vaginal smears. Pituitary gonadotrophic hormone content was normal but GnRH receptor concentrations only reached 60% of normal values. Within the ovaries LH receptors increased nearly 100-fold (Young *et al.* 1985).

The physiological actions of the grafts were not affected by the sex of the donor tissue, male or female POA tissue being equally effective. Control grafts of cortical tissue have been uniformly negative with regard to pituitary and gonadal stimulation in hpg mice.

Histological examination of the ovaries of positively responding females (i.e. those with vaginal opening and uterine stimulation) demonstrated the presence of developing and mature follicles but the absence of corpora lutea (see Fig. 5C and D), indicating that a spontaneous LH surge was not being produced. The presence of cornified cells in the vaginal smears and the stimulation of uterine growth in positive reproducing females were indications of steroid production by the ovaries. Female hpg mice treated with ovarian steroids display mating behaviour (Ward & Charlton 1981) and it was anticipated that those female mutants with a physiological response to POA grafts would mate with normal male mice. Indeed, nine out of ten females with oestrous smears mated with the male on the first night of pairing. The remaining female mated on the second night. However, what was surprising was that seven out of these ten mice became pregnant and this reflex ovulation has been observed many times over the last few years (Gibson *et al.* 1984b).

This phenomenon of reflex ovulation has been described in female rats kept under conditions of constant light for long periods. They display persistent vaginal oestrus and ovulate in response to copulation (Brown-Grant *et al.* 1973).

What might be the normal substrate for the phenomenon of reflex ovulation in hpg females with third ventricular grafts of normal POA tissue? Although normal rats and mice are spontaneous ovulators, the activation of the corpus luteum to secrete progesterone depends upon a mating-induced release of prolactin, indicating that pathways exist whereby the reflex release of factors from nerve terminals in the median eminence are involved in prolactin release (Gunnert & Freeman 1983). There is no reason to suppose that this system is not present within the brains of hpg mice.

It is difficult to envisage that the processes involved in pathway finding and axon guidance leading to a normal input to the GnRH cell body within fetal/neonatal tissue that has been dissected out and removed from its normal location and grafted to the third ventricle would be present in the grafted hpg adult. The evidence for an extensive and direct input of nerve fibres from the host into the grafts is far from robust (Silverman *et al.* 1992b).

However, in hpg/POA females that ovulate reflexly there is always evidence of GnRH fibres reaching the median eminence. This is irrespective of the random distribution of GnRH cell bodies within grafts.

Figure 6 (Left panel) GnRH fibre distribution at the level of the hypothalamus in normal and hpg mice with a POA transplant. The two micrographs are of sagittal sections through the brain at the level of the hypothalamus and pituitary stalk, seen at bottom right. (A) The distribution of GnRH nerve terminals at this site in a normal mouse and (B) the exact mirroring of GnRH innervation of the median eminence/pituitary stalk from a third ventricular POA transplant in an hpg mutant are shown. (Right panel) A comparison of hypothalamic innervation by GnRH axons in normal and POA/hpg mice. This is a series of coronal sections from just behind the optic chiasm to the pituitary stalk comparing GnRH innervation in the normal mouse (A-F) with that in an hpg mouse with a POA graft (G-M). Note the specific input of nerve terminals from the graft.

Perhaps the most parsimonious explanation for reflex ovulation is that axo–axonic contacts from fibres involved in controlling prolactin release within the median eminence might elicit, in some bystander fashion, secretion of GnRH from nerve endings from the graft. For example it has been suggested that catecholaminergic terminals close to GnRH and endorphin-containing terminals within the median eminence itself could mediate some of the actions of opioid peptides upon GnRH release (Rasmussen *et al.* 1988). Similarly, Gambacciani *et al.* (1986) have suggested that oxytocin can inhibit *in vitro* release of GnRH by a receptor mechanism operating at the level of neurosecretory terminals in the median eminence.

There is also immunocytochemical evidence of an extensive overlap of catecholaminergic and GnRH varicosities within the median eminence (McNeill *et al.* 1980) and of considerable overlap in the distribution of GnRH, tyrosine hydroxylase and glutamic acid decarboxylase fibres within the median eminence (Jennes *et al.* 1983), suggesting that dopamine and γ -aminobutyric acidergic neurones (GABA) can influence GnRH secretion via axo–axonic interactions at this site. Several other mechanisms are mentioned in the review of Kalra *et al.* (1997).

There is also evidence that glial–neuronal–endothelial interactions are part of a cell–cell communication system separate from transynaptic mechanisms in the control of GnRH secretion (King & Rubin 1995, Prevot 2002, Zwain *et al.* 2002). The relationship between glial elements and axon terminals from POA grafts has been reported (Silverman *et al.* 1991). GnRH axons were found adjacent to glial elements along their entire traverse from the graft–host interface, through the host basal hypothalamus to their termination on the hypophysial portal capillaries.

Whilst this reviewer considers that the main conduit for reflex ovulation in hpg/POA females is likely to be via median eminence axo–axonic contacts, this is not to say that there are no fibres crossing from the host brain to the body of the grafts themselves. Evidence for this will be discussed below in the section Neurobiology.

Pattern of hormone release induced by GnRH grafts

Gibson *et al.* (1987) demonstrated that mating induced a significant rise in plasma LH in hpg/POA females within 10 min of ejaculation by the male, falling to baseline by

30 min. Although the post-copulatory levels of LH were a fifth of those measured at the time of the pro-oestrous surge in normal mice they were sufficient to induce ovulation.

GnRH is considered to be released in a pulsatile manner with alterations in pulse frequency having important physiological consequences (Moenter *et al.* 2003). Immortalised GnRH neurones have been reported to exhibit synchronous bursts of electrical activity *in vitro* (Funabashi *et al.* 2001). Kokoris *et al.* (1988) were able to detect LH pulses in hpg/POA male mice. In ovariectomised normal females and ovariectomised hpg/POA females, blood sampling via a cardiac catheter with samples taken every 10 min for 4 h demonstrated significant LH pulses in nine out of ten normal mice and in nine out of thirteen hpg/POA females (Gibson *et al.* 1991). These experiments indicate that preoptic area grafts are capable of supporting episodic LH release and that there could be a functional GnRH pulse generator in mutant mice with grafts. The inability to detect LH pulses in all positive grafted females could be explained by technical difficulties.

Gibson *et al.* (1994) implanted osmotic mini-pumps into hpg mice delivering a constant infusion of GnRH subcutaneously. This elicited a dramatic and rapid increase in ovarian and uterine weight within 15 days. It is clear that gonadal growth in hpg/POA mice does not necessarily depend absolutely on a pulsatile pattern of GnRH release from the grafts.

Steroid feedback in hpg/POA mice

In wild-type male mice castration results in an elevation of plasma LH and a reduction in pituitary content of both LH and FSH. Subcutaneous implants of testosterone capsules for 10 days reduced pituitary GnRH receptor numbers and pituitary LH and FSH content, and induced a 50% reduction in testicular LH receptors. In positively responding hpg male mice bearing POA grafts neither castration nor testosterone capsules affected pituitary gonadotrophic hormone content (Charlton *et al.* 1987a). These experiments suggest that neither the pituitary gonadotroph nor the GnRH neurone represent major sites for the negative feedback of testosterone upon gonadotrophic hormone secretion in male mice. Gibson & Silverman (1989) extended this work to female hpg/POA mice and reported a failure of negative feedback of oestradiol benzoate in most of the mice tested.

Gibson *et al.* (1988) investigated the possibility that ovarian steroid positive feedback mechanisms could result in LH surges in hpg/POA females and found that 25% of females treated with progesterone 2 months after grafting showed increased levels of LH, but only 10% showed increased levels when animals were tested at 5 months. Sequential treatment of oestradiol benzoate and progesterone was ineffective in elevating LH. The presence of corpora lutea in the ovaries confirmed that only those

animals given progesterone ovulated. In this paper they reported the intriguing observation that a female hpg/POA mouse having mated twice, and then isolated after weaning of the second litter, displayed ovarian cyclicity and had corpora lutea in the ovaries.

Any positive feedback of ovarian steroids in stimulating LH release in physiologically active hpg/POA females could be explained by an effect upon the pituitary gland increasing gonadotroph sensitivity to a more constant GnRH input rather than the production of a surge of the decapeptide itself being released in the portal vessels. Naik *et al.* (1985) have shown that the pituitary can be a site of gonadal steroid stimulation of GnRH receptors in female mice. In the very rare case of apparent spontaneous ovulation it could also be possible that steroid feedback has sensitised the anterior pituitary sufficiently to elicit LH release without there necessarily being a GnRH surge.

Transplantation of non POA GnRH neurones, or cell lines

GT-1 is an immortalised cell line synthesising and secreting GnRH (Weiner *et al.* 1992). Grafting of this tumour cell line into hpg mutants demonstrated that the cells migrated widely within the brain and in many cases stimulated gonadal growth in both sexes. The greatest growth was found in animals in which tumours or axons were present in the region of the median eminence. In the other positive responders it is possible that large quantities of GnRH peptide reached the portal vessels and pituitary by diffusion (Silverman *et al.* 1992a). The migration of GnRH neurones from the nasal placode into the brain during prenatal development has been mentioned above. Livne *et al.* (1992) placed grafts of embryonic nasal septum containing GnRH neurones into the anterior hypothalamic area and preoptic area of adult male hpg mice. In half of the experimental animals there was evidence of testicular growth with GnRH fibres extending to the median eminence.

GnRH neurones are also found in the accessory olfactory bulb (AOB), a site which does not normally innervate the median eminence. Perlow *et al.* (1987) transplanted fetal AOB tissue into the third ventricle of hpg mice with stimulation of pituitary gonadotrophic hormone synthesis and secretion and gonadal growth in 25% of grafted recipients. In all positive mice GnRH axons innervated the median eminence.

Neurobiology

The physiological effects of POA grafts in male and female mice have proved to be robust and repeatable. Only when axon terminals innervate the median eminence are there signs of increased gonadotrophic hormone synthesis and secretion and gonadal stimulation. When POA tissue is transplanted into the lateral ventricle, GnRH neurones

survive and extend processes into the host brain but never to the median eminence (Kokoris *et al.* 1987). What might be responsible for this specific innervation? The most plausible explanation is that the median eminence releases diffusible substances directing this outgrowth.

In a series of elegant experiments, Saitoh *et al.* (1992) co-grafted POA tissue either with anterior pituitary tissue or with a fetal hypothalamic block containing the median eminence. In pituitary co-grafts GnRH axon terminals entered the tissue, but also projected to the host median eminence. With the hypothalamic block the engrafted POA cells formed a mini-median eminence with a highly organised and robust GnRH innervation. In hypophysectomised hpg mice GnRH axons from POA grafts still projected to the median eminence, suggesting that the bulk of the pituitary gland was not essential for this specificity. Evidence for a diffusible chemoattractant factor has been provided in experiments *in vitro* in which GnRH axons target mediobasal hypothalamic blocks (Rogers *et al.* 1997). It must be remembered that the pars tuberalis of the anterior pituitary is closely adherent to the median eminence and could provide part of the guidance cues.

Neurons within the arcuate nucleus do not appear to play a role in directing axons to the median eminence because in hpg mice in which these cells have been destroyed by neonatal exposure to monosodium glutamate (Olney 1969) the normal pattern of GnRH innervation by grafts is maintained (Silverman *et al.* 1990).

Preoptic area grafts placed in the region of the mammillary body, which never contains GnRH cell bodies, but is nevertheless close to the median eminence, resulted in correct GnRH innervation and gonadal development. This supports the hypothesis that the median eminence releases diffusible substances directing GnRH outgrowth (Rogers *et al.* 1998).

The degree of input from the host brain into the body of the graft is more imponderable. Direct tracing methods using a fluorescent dye indicated that host innervation exists, but is limited in nature (Silverman *et al.* 1992b).

Intracellular recordings from neurones within POA grafts in brain slice preparations also indicated that there must be some input into the graft from the host (Hodgkiss & Kelly 1990). However, whether or not this represents attempts by the host brain to wire graft GnRH neurones into specific and physiologically relevant circuits remains unanswered.

It has been reported that there is an increased synaptic input onto GnRH cell bodies within grafts in hpg/POA female positive responders using immunocytochemistry for the presynaptic marker, synaptophysin (Rajendren & Gibson 2001). Galanin synapses were also increased on GnRH neurones within the grafts of mutant females exhibiting ovarian development (Rajendren 2002).

By definition, the physiologically activated hpg females will be secreting oestrogen from their ovaries. Matsumoto *et al.* (1988) have demonstrated that oestrogen stimulation of ovariectomised rats bearing preoptic area

third ventricular grafts elicits a significant increase in axo-dendritic and spine synapses on neurones within the grafts. The question remains to be answered as to whether or not increased synaptic input is from host neurones or from oestrogen-stimulated interneurons within the grafts. It really is a chicken and egg situation – does the increased synaptic input into GnRH neurones result in a greater output of gonadotrophic hormones and therefore of ovarian oestrogen, or is the increase in synapses a consequence of elevated gonadal stimulation? Oestradiol concentrating cells are present within transplants in estimated numbers ranging from 390 to 2600 (Gibson *et al.* 1989). The methodology used in these experiments did not allow for any specific relationship between GnRH cells and those taking up oestrogen, but the possibility remains that a direct action of oestrogen upon interneurons or glial elements within the graft could provide a partial explanation for increased synaptic input into transplanted GnRH neurones.

The appearance of immunoreactivity to the transcription factor FOS in several GnRH cells following mating of hpg/POA females has been reported (Wu *et al.* 1996). This has been claimed to indicate the establishment of neuronal projections carrying somatosensory information to these cells. Again, the argument can be put forward that activation of GnRH nerve terminals in the median eminence via axo-axonic contacts could result in electrical events leading to exocytosis of granules from graft neurone terminals and a stimulation of the cell body, a consequence of which could be increased FOS gene transcription.

Saitoh *et al.* (1991), investigating the effects of N-methyl-D, L-aspartic acid on LH secretion, concluded that oestradiol was acting at the level of the pituitary in hpg/POA females. In experiments aimed at investigating potential neuromodulation of GnRH neurones in grafts, Miller *et al.* (1995) showed that β endorphin-positive axons were present within the transplants but that there were no cell bodies. However, similar fibres were also found within the median eminence in the vicinity of the outgrowth of GnRH axons from grafts. There can be no doubt that host fibres cross the ventricular wall to enter grafted tissue, but it is difficult to state categorically that a drug or hormone treatment is specific to a particular host pathway directly connected to GnRH neurones in a graft. Activation could also be at other sites, including the pituitary gland or even via the blood stream supplying the transplants or the cerebrospinal fluid bathing them.

Graft survival

The age of the grafted POA tissue is crucial to the survival and function of grafts. Grafting embryonic tissue (embryonic days 16–18) resulted in 69% of positive responders within 30 days. Post-natal day 1 (P1) tissue

was equally successful whereas P5 tissue elicited positive physiological responses in only 22% of recipients. Tissue from P10 neonatal donors failed to survive (Charlton *et al.* 1987b).

The hpg colonies are not inbred, yet there is excellent survival of grafted tissue demonstrating the relative immune privilege within the CNS. The immunology of graft survival has been extended to demonstrate that, by depleting helper and cytotoxic T-lymphocytes using monoclonal antibodies, the hpg phenotype can be reversed with grafts of rat POA tissue. With two courses of treatment, one at the time of grafting and the other at 30 days, survival of the rat tissue was extended for months (Wood *et al.* 1998).

Future perspectives

The physiological effects of POA grafts in hpg mice have been extensively reported particularly from the laboratories of Marie Gibson and Anne-Judith Silverman. What has been difficult to determine is to what extent the host brain interacts with the donor tissue, and with what specificity.

With the advent of transgenic technologies it should be possible further to investigate this aspect of neural grafting. Selected strains of transgenic mice are available in which reporter genes have been targeted to specific neuronal populations. The bacterial enzyme β galactosidase (β -gal) has the advantage that not only can the enzyme convert the x-gal substrate into a blue product in fixed tissues, there are also good antibodies and *in situ* hybridisation probes available to identify cells. It is also possible to identify living cells by fluorescent conversion of the x-gal product (Herbison *et al.* 2001).

Green fluorescent protein (GFP) has also been targeted to GnRH neurones in transgenic mice, allowing easy visualisation of the cells both in living slices and in fixed tissue. Antibodies are also available for more permanent preparations (Spergel *et al.* 2001). Grafting POA tissue from such GFP (or β -gal) transgenics will allow more precise electrophysiological studies of GnRH neurones and their responses to hormones, drugs and neurotransmitters within grafts included in tissue slices. These responses can then be compared with those of reporter/GnRH neurones from normal animals.

Transgenic mice are also available in which there is a global production of the reporter gene products, GFP and β -gal. It would be possible to breed the hpg mutation onto these backgrounds; thus by grafting GFP neurones to hpg β -gal hosts (or vice versa) it would be possible to have a host brain expressing a different transgene from the donor GnRH reporter construct. Any fibres crossing into the graft would theoretically be readily detectable and, with advances in confocal microscopy, could provide a more robust measure of host-graft interactions.

For those working in the field of seasonal breeding it might be of interest to see whether or not POA grafts can maintain gonadal stimulation even in daylengths normally associated with an inhibition of reproductive function.

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