

## Review

## Anterior pituitary cell networks

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## ABSTRACT

Both endocrine and non-endocrine cells of the pituitary gland are organized into structural and functional networks which are formed during embryonic development but which may be modified throughout life. Structural mapping of the various endocrine cell types has highlighted the existence of distinct network motifs and relationships with the vasculature which may relate to temporal differences in their output. Functional characterization of the network activity of growth hormone and prolactin cells has revealed a role for cell organization in gene regulation, the plasticity of pituitary hormone output and remarkably the ability to memorize altered demand. As such, the description of these endocrine cell networks alters the concept of the pituitary from a gland which simply responds to external regulation to that of an oscillator which may memorize information and constantly adapt its coordinated networks' responses to the flow of hypothalamic inputs.

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## 1. Introduction

Technical developments allowing a systems approach to biology have resulted in an increased focus on biological networks, in particular intracellular molecular networks involving protein–protein, protein–DNA and protein–metabolite interactions (Alon, 2003). Less attention has been focused on the organization of cells within an organ and the importance of homotypic and heterotypic cell networks in biological function, with the exception of the brain. In this review we describe the recent advances in the study of the formation and role of cell networks in a model system, the anterior pituitary gland. As it is a discrete organ containing a limited number of well-defined, tractable and tissue-restricted cell lineages, the anterior pituitary represents an excellent model for studying the roles of cell networks in endocrine function and hormone release. An additional feature which makes it an attrac-

tive paradigm for long term *in vivo* studies of endocrine function is that, whilst pituitary output has an important role in organism health and normal homeostasis, its function can be readily modified without acute effects on the animal's survival. Features of network organization in the pituitary gland are likely to be common to other endocrine tissues, from those such as the pancreas, where the organization of cells within islets has important implications for the control of hormone secretion (Jaques et al., 2008), to more diffuse endocrine organs such as the gut, where Feyrter first noted the organization of enterochromaffin cells into functional units (Champaneria et al., 2006).

## 2. An overview of the anterior pituitary gland

The anterior pituitary gland has a central role in mammalian physiology, regulating growth, metabolism, lactation, stress and reproduction. The gland is derived from oral ectoderm and in mice its development begins from embryonic day (e) 9 with the formation of Rathke's pouch, a structure which expands to form the anterior pituitary (Kelberman et al., 2009). The specialized cell types of the anterior pituitary are specified during embryogenesis by a cascade of transcription factors and signaling pathways (Brinkmeier et al., 2009), giving rise to the five endocrine cell types defined by the hormones they secrete (somatotrophs, growth hormone (GH); lactotrophs, prolactin (PRL); gonadotrophs, luteinizing and

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follicle stimulating hormone (LH and FSH); corticotrophs, adrenocorticotrophic hormone (ACTH); thyrotrophs, thyroid stimulating hormone (TSH)). During development, endocrine cells are first detected in distinct regions of the gland, leading to the suggestion that opposing signaling gradients may be responsible for cell-type specification (Ericson et al., 1998). It is also possible that terminal differentiation, identified by immunological detection of hormones, occurs at differing times in the developing pituitary, leading to a stratified localization, as occurs in the neural tube. However, elegant cell birthdating studies in mice have shown that the different hormone cell types are born concurrently, suggesting that their final location is the result of cell migration (Davis et al., 2011). The gland also contains non-endocrine folliculostellate cells, with a star shaped cytoplasm reminiscent of glial cells, and a dense capillary meshwork.

### 3. Organization of cells in the anterior pituitary

The cells of the adult mammalian anterior pituitary have previously been described as part of the “diffuse endocrine system” (initially coined by Feyrter in 1938), with two-dimensional histological studies of thin sections of pituitary tissue appearing to show a heterogeneous distribution of the different hormone-producing cells. Even in these early histological studies, some organization of pituitary cells was noted, in particular the preferential morphological association of somatotrophs with corticotrophs, thyrotrophs with somatotrophs, and gonadotrophs with lactotrophs (Nakane, 1970; Noda et al., 2001). Some large-scale organization had also been described, with lactotrophs occupying the rostral and caudal regions of the gland, and somatotrophs predominantly localized to the anterolateral wings (Sasaki and Iwama, 1988).

The most compelling evidence for a functional role of pituitary cell organization is derived from *in vitro* studies of hormone secretion from dispersed cells plated at different densities. For example, GH cells in the intact pituitary produce GH pulses of up to a thousand-fold higher than basal (Bowers et al., 1990; Clark and Robinson, 1985; Tannenbaum et al., 1976), whereas pulses are only several fold higher *in vitro* in isolated cells (Sartor et al., 1985). Increasing the density of enzymatically-dispersed cells leads to an augmented GH secretion in response to secretagogue (Sugimoto et al., 1991), as well as facilitating the inhibitory actions of dopamine on prolactin secretion (Hoefler et al., 1984). It is notable that, in these studies, the increased cell density also led to a decreased basal secretion, similar to that described for insulin-secreting beta cells cultured at different densities (Jaques et al., 2008), and which is likely mediated by gap junction coupling (Benninger et al., 2011). These observations suggest both that cell–cell contact is crucial for shaping hormone release in the pituitary and that features of pituitary endocrine cell organization may extend to other hormone systems. Whilst the pattern of secretion of pituitary hormones may vary between species (e.g. MacLeod et al., 1991; Tannenbaum et al., 1976), a high ratio of stimulated/basal hormone secretion, as opposed to peak levels *per se*, appears to be most important for mediating downstream GH receptor signaling effects (Waxman et al., 1991). This is best demonstrated by the GH system in rats, where a low basal secretion in combination with high amplitude pulses is required for the increased pattern of male growth (Robinson and Hindmarsh, 1999).

### 4. Network organization of pituitary endocrine cells

Over the last decade, our groups have challenged the concept of the pituitary as a diffuse endocrine system by combining transgenic animals possessing cell-specific fluorophore tags with multiphoton microscopy of thick tissue sections or even entire glands.

Together, these approaches have permitted in depth imaging of the gland, allowing us to reveal the large-scale three-dimensional organization of cells. Below, we describe the network organization of the four cell types that we have studied to date; studies of the thyrotroph organization are ongoing in our group.

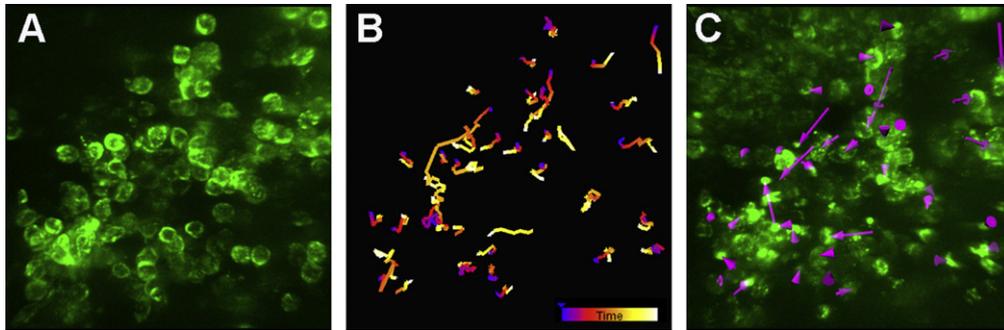
#### 4.1. Somatotroph cell organization

Of the five pituitary cell types, somatotrophs have been the most extensively studied as they were the first cell type to be targeted with fluorophore expression (Magoulas et al., 2000). Somatotrophs can be readily detected in the developing pituitary gland of mice at e15.5 (Japon et al., 1994) with strands of cells already showing signs of organization when imaged in 3D (Bonfont et al., 2005). Within 1 day, coincident with a dramatic increase in their number, the cells form a continuum. This is not simply a result of proliferating somatotrophs filling the space between the isolated clusters seen at e15.5, since birthdating studies demonstrate that only a very small proportion of GH-positive cells are dividing at this time (Davis et al., 2011). Using overnight time-lapse multiphoton imaging, we have been able to track the migration of somatotrophs in mice at e18.5. These studies demonstrate that, whilst some cells remain largely static during the imaging period, a large proportion of both nascent (with the secretable GFP chimera only present in the Golgi apparatus) and granular/secreting cells display motilities of various instantaneous speeds and directions (Fig. 1A–C and Supplemental movie 1). After birth, when the differentiated somatotrophs re-enter the cell cycle (Carbajo-Perez and Watanabe, 1990; Taniguchi et al., 2001), the organization of somatotrophs into a cellular continuum is maintained. At higher resolution, it becomes apparent that there are numerous clusters of somatotrophs which are interconnected by strands of single somatotrophs (Fig. 2A, top panel; Fig. 4B).

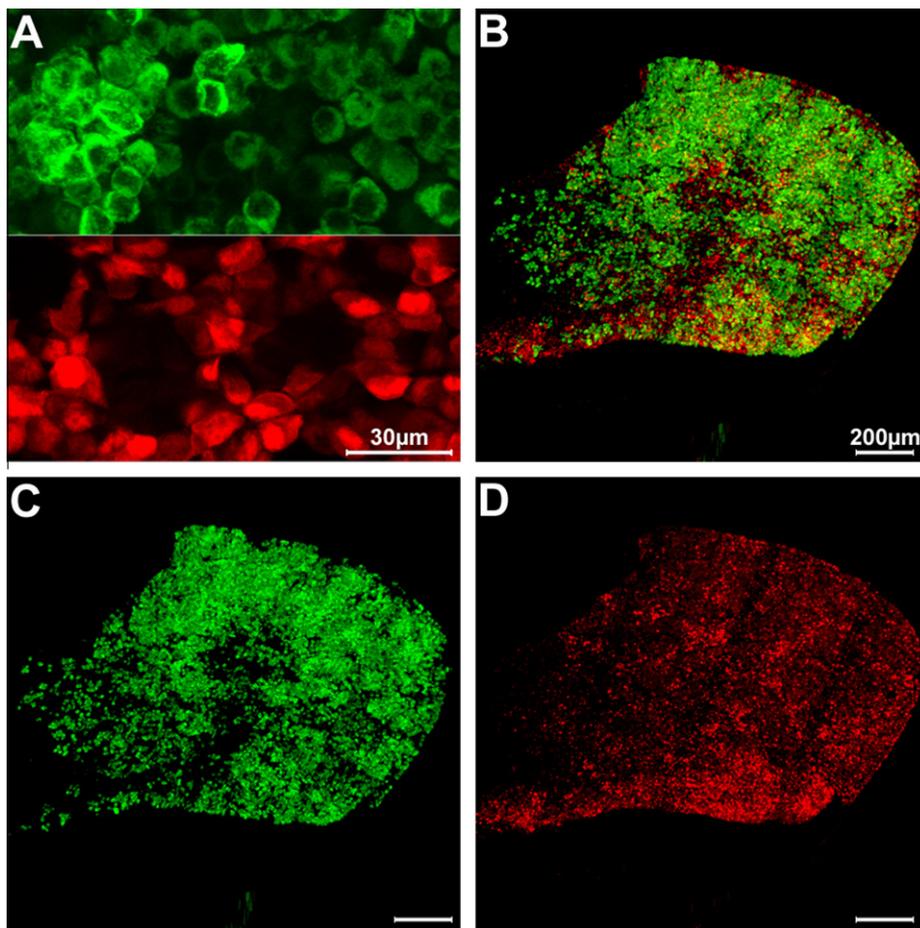
In male mice, this organization of cells is modified during sexual maturation, with a dramatic increase in the amount of cell clustering from 40 to 70 days of age, when increased pulsatile GH is critical for the post-pubertal increase in growth rate. This only occurs in the lateral regions of the gland, with strings of GH in the medial portion remaining unchanged throughout sexual maturation. This increased clustering is not observed in females (Sanchez-Cardenas et al., 2010), and castration of males prevents the increase in clustering in the lateral regions of the gland at sexual maturation (Bonfont et al., 2005). A role for steroid hormones in the dynamic modification of the network is also eluded to by long-term imaging studies in which ovariectomy leads to an increase in somatotroph cell movement, with only a subset of cells responding, as would be expected for efficient network remodeling (Schaeffer et al., 2011b). The dependence of the network on hypothalamic factors has been demonstrated using mice with genetic ablation of growth hormone releasing hormone (GHRH) neurons (Le Tissier et al., 2005), where a reduced number of somatotrophs fail to form a network and many cells appear isolated (Waite et al., 2010). This is in contrast to mice with an ablated somatotroph population, where an intact network is absent but cells still form clusters.

#### 4.2. Lactotroph cell organization

Transgenic mice with expression of the DsRed fluorophore driven by a portion of the rat prolactin promoter (He et al., 2011) have been used to determine the organization of pituitary lactotrophs (Hodson et al., 2012b). The embryonic development of the lactotroph network has not been studied as differentiated lactotrophs are the last of the pituitary hormone cell types to develop, with only very few cells detected by immunohistochemistry before birth (Featherstone et al., 2011). There is evidence that cells which will differentiate into lactotrophs are present earlier in develop-



**Fig. 1.** Remodeling of the GH cell network during embryonic developmental. (A) Still image at time 0 of a time-lapse movie of GH-GFP embryonic pituitary at e18.5 (Supplemental material, Movie 1). Dimensions  $257 \times 257 \times 26 \mu\text{m}$ , total time: 15 h41 min (10 min between each frame). Green: GH-GFP emission in somatotrophs. (B) Representative tracks followed by cells over the 15 h41 min imaging period. Time was statistically color coded using Imaris software. (C) Still image from Movie 1 at 15 h41 min, pink arrows correspond to displacement lengths and directions of cells tracked in B.



**Fig. 2.** Topological organization of both GH and PRL cell networks in adulthood. (A) Typical cell network motifs imaged in 3D using 2-photon microscopy. *Top panel:* interconnected clusters of somatotrophs in 2-month-old male GH-GFP (green) mice. *Bottom panel:* Honeycomb units of the PRL cell network in female PRL-DsRed (red) mice. (B–D) Hemi-pituitary-scale 2-photon imaging in a double transgenic GH-GFP (green)/PRL-DsRed (red) female mouse. The pituitary is orientated with the top and bottom of the image representing the dorsal and ventral sides of the pituitary, respectively.

ment (Davis et al., 2011), but in the absence of other markers for this cell type it is not possible to study the embryonic formation of the network. Multiphoton microscopy of pituitaries from adult PRL-DsRed female mice shows that, on a pituitary scale, lactotrophs are found throughout the anterior pituitary but that the distribution is not even. There is a higher density of lactotrophs in the ventral region of the gland (Fig. 2D) and the contrast with the distribution of somatotrophs is obvious in double GH-eGFP/PRL-DsRed transgenic animals (Fig. 2B and C). At higher resolution (Fig. 2A, bottom panel), the organization of lactotrophs is more

apparent, with cells arranged as honeycomb-like structures. During lactation, when there is a high demand for prolactin to support mammary gland activity, the organization of the cells into a honeycomb structure becomes more apparent, with an increase in contacts between cells (Hodson et al., 2012b). Remarkably, this increased organization remains 3 months after the lactation period, suggesting that the network is able to structurally memorize previous challenge (Fig. 3, top panels). This altered organization is not a result of the hormonal changes associated with pregnancy but of the suckling stimulus, as reducing the lactational demand by

suckling fewer pups impedes network reorganization. Notably, the network reorganization is not associated with an increase in the number of lactotrophs but with an increase in their size, as previously demonstrated by studies using PRL-Cre/ROSA-YFP (Castrique et al., 2010). It is worth noting that this sustained change in PRL network structure contrasts with the transient episode of GH cell clustering that only occurs at puberty in males (Fig. 3, bottom panels), highlighting the different facets of pituitary network plasticity in response to single/multiple physiological demands.

#### 4.3. Gonadotroph cell organization

Based on detection of hormone by *in situ* hybridization, gonadotrophs can first be identified in the pituitary of mice at e16.5 (Japon et al., 1994), although gonadotroph-specific markers can be detected at e15.5 (Barnhart and Mellon, 1994). Using a bovine LH-beta promoter to drive cerulean fluorescent protein expression, gonadotrophs of mice have been labeled with fluorophore and used for multiphoton microscopy to image their organization (Budry et al., 2011). At e17.5, the first cerulean-labeled cells are isolated as single cells on the midventral surface of the gland. By e18.5, these cells become clustered and are localized to the central mediolateral region, with some isolated cells evident in more lateral regions. In the adult, gonadotrophs are organized as strings of cells extending from the ventral surface towards the center of the gland, with a second wave of gonadotrophs appearing postnatally on the dorsal surface, again organized as cell strings but which remain close to the exterior of the gland and not extending into the center. It is possible that there is a dynamic reorganization of the gonadotroph network in response to physiological demand, similar to that described for somatotrophs. After stimulation with gonadotrophin releasing hormone (GnRH), gonadotrophs, studied in both immortalized cell lines and cells in pituitary slices, extend cellular processes and increase cell movement (Navratil et al., 2007). The authors of this study suggest that these cellular pro-

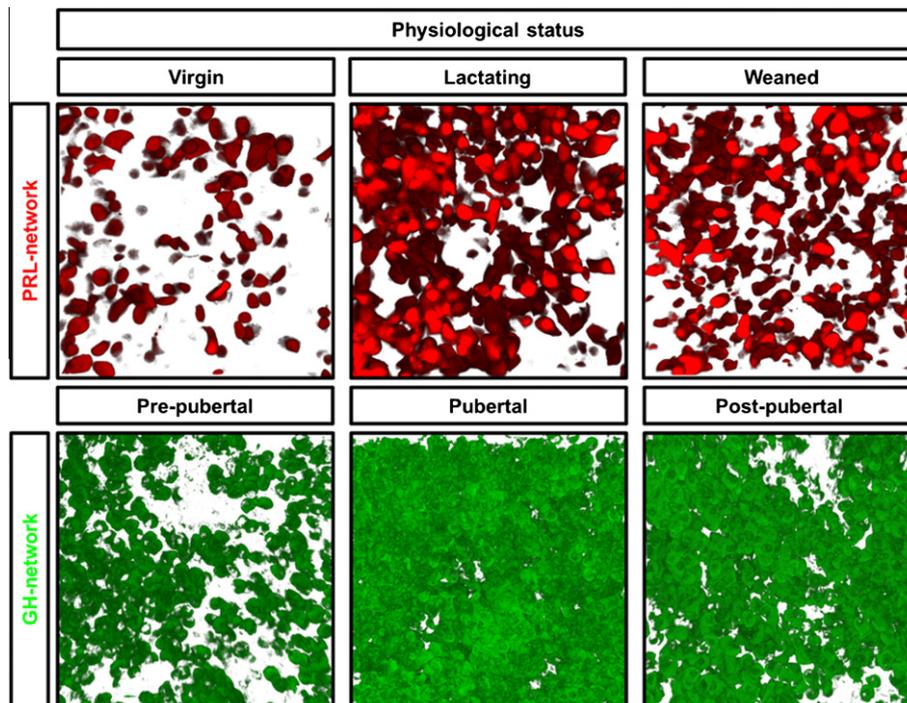
cesses and cell movements may have a role in increasing the access of gonadotrophs to the vascular endothelium, as suggested from previous studies by Childs (1985). It is also possible, however, that these processes impact network remodeling by modifying the response of gonadotrophs to stimulation, an important component of which may be the relationship of the gonadotroph network with the vasculature.

#### 4.4. Corticotroph cell organization

Corticotrophs are the first endocrine cell type to terminally differentiate in the pituitary and can be detected in mice from e13.5. Imaging of the organization of these cells in mice, identified by transgenic EGFP expression driven by a rat POMC promoter (Lavoie et al., 2008), reveals that early differentiating cells are isolated, similar to that found for other cell types in development, and localized on the ventral surface of the developing gland (Budry et al., 2011). Within 6 h (at e13.75) the cells rapidly form clusters which become strands of cells by e15.5 that extend from the surface to the center of the gland. This early organization is maintained post-natally and throughout adulthood. One interesting feature of these cells is their shape, with cytoplasmic projections, or cytonemes, developing after e15.5 in mice, and resulting in contacts between apparently isolated cells, as well as with the perivascular space lining capillaries where the projection is frequently filled with secretory granules (Childs, 1992).

### 5. Inter-network organization

All of the endocrine networks of the pituitary described above are interspersed. There are several pieces of evidence to suggest that the organization of cells into interspersed networks is not simply a consequence of pituitary development and homotypic cell adhesion:



**Fig. 3.** Adaptation of pituitary cell networks in adulthood. High-resolution snapshots of PRL- (top panels; red) and GH- (bottom panels; green) network organization under differing physiological conditions. Note the following: (1) Both PRL- and GH-cell populations form well connected three-dimensional networks even under basal conditions; (2) PRL-network structural connectivity increases in response to demand (i.e. lactation) and this does not abate following removal of stimulus (i.e. weaning); and (3) the GH-network undergoes a dramatic increase in clustering during sexual maturation (i.e. puberty) which gradually subsides in adult animals. Image size: 160 × 160 μm (top panels) and 200 × 200 μm (bottom panels).

1. Unlike mammals, the different pituitary cell types of fish are organized in zones (Wong et al., 1998), with lactotrophs localized in the rostral and somatotrophs in the distal pars distalis. Furthermore, in teleosts such as grass carp, somatotrophs surround clusters of gonadotrophs, with a functional relationship suggested by the close association between secretion of GH and gonadotrophin (Marchant et al., 1989) and role of GH in sexual maturation of fish (Le Gac et al., 1993).
2. When pituitaries are dispersed and allowed to re-aggregate *in vitro*, the cells rapidly form clumps, which coalesce over time (Denef et al., 1989). There is no evidence for sorting of specific cell types into homotypic aggregates or specific polarization within the clumps (Baes and Denef, 1987), but there is distinct organization of cell types within the cultured aggregates (Pals et al., 2006, 2008). The topographical affinities between different cell types observed *in vivo* are also recapitulated in these reaggregates (Allaerts et al., 1991), as well as in monolayer cultures (Noda et al., 2003).
3. Within mammals there are species variations in the organization and ontogeny of different cell types, with lactotrophs concentrated near the intermediate lobe of the horse and organized in clusters (Townsend et al., 2004), and monohormonal gonadotrophs being present in the developing human but not in the murine pituitary (Pope et al., 2006).

The inter-network organization of pituitary endocrine cells suggests that this may have an important role in the function of the gland. One possibility is that the organization of later differentiating endocrine cell types is directed by the endocrine cells networks formed earlier in development (Budry et al., 2011). This is supported by the observations that the gonadotroph and corticotroph networks are morphologically intertwined, and that hyperproliferation of gonadotrophs occurs in Tpit knockout mice which lack corticotrophs, leading to ventral extension of the LH-network towards the lateral extremities and gonadotroph hypotrophy. The importance of an interaction between the lactotroph and gonadotroph networks is suggested from studies of gonadotroph ablation, where lactotroph development is affected and there is a potential modification of lactotroph organization, with a selective reduction of large clusters (Seuntjens et al., 1999). A further role for inter-network organization is cross-talk between the cell networks, either through gap junctions between heterotypic cell types (that is between cells producing different hormones), which are modified in different physiological states (Hodson et al., 2012b), or by paracrine, autocrine or juxtacrine factors (Denef, 2008). The potential advantages of cross-talk between the different endocrine cell types have also been suggested by Denef (2008), who, from an evolutionary perspective, proposed that co-ordination between endocrine axes is mandatory due to the essential roles each plays in mammalian reproduction.

## 6. Network organization of non-endocrine pituitary cells

The non-endocrine cells of the anterior pituitary clearly contribute to regulation and maintenance of the hormonal cell population, as well as delivery of stimulating and inhibitory factors from the hypothalamus and periphery, and transport of secreted products from the gland. Below we will consider the organization of these cells, with a focus on their relationship with hormonal cell networks.

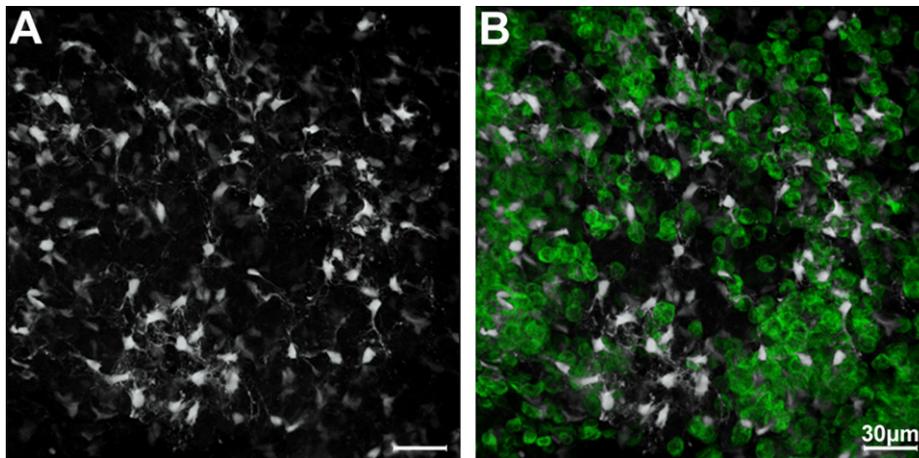
### 6.1. Folliculostellate cells

The first cells of the anterior pituitary to be shown to possess a large-scale network organization were the non-endocrine folliculostellate (FS) cells. These cells resemble astrocytes in that they have a star-shaped cytoplasmic configuration and long filamentous

processes which are intermingled between hormone producing cells (Farquhar, 1957; Rinehart and Farquhar, 1955). The cells are defined by their expression of the homodimeric calcium binding protein S-100, (Nakane, 1970), as well as uptake of the fluorescent peptide  $\beta$ -Ala-Lys-Ne-AMCA (AMCA) via cell-specific membrane-bound proton pumps (Otto et al., 1996). Very little S-100 is expressed in the embryonic or neonate pituitary (Pals et al., 2006), meaning their early development and organization has not been studied. However, by taking advantage of the selective uptake of AMCA, the organization of FS cells into a 3D anatomical network (Vila-Porcile, 1972) was confirmed (Fig. 4A). Subsequently we extended these observations to the adult gland and demonstrated that FS cells form an excitable network (Fauquier et al., 2001), capable of transmitting long range  $[Ca^{2+}]$  signals initiated by activation of TTX-sensitive  $Na^+$  channels over a distance of hundreds of microns. The inherent excitability of FS cells and the presence of connexin-43-expressing gap junctions both between themselves and with hormonal cells (Baes and Denef, 1987; Morand et al., 1996), suggests that FS cells may play a role in coordinating activity of both local and distant endocrine cell ensembles (Fig. 4B), as well as conducting signals via formation of gap-junctions with hypothalamic neurons whose fibers terminate in the pars tuberalis (Henderson et al., 2008; Mabuchi et al., 2004). A further role of FS cells in pituitary functional plasticity can be inferred from the modification of the number of gap junctions (Kurono, 1996; Soji et al., 1991), and altered morphological relationship with hormonal cells in response to physiological challenge (Shirasawa et al., 1983). FS cells also produce a range of growth factors and modifiers of pituitary hormone cell function, such as IL-6 (Correa-de-Santana et al., 2009; Gloddek et al., 2001; Lohrer et al., 2000), VEGF (Leung et al., 1989), FGF-2 (Amano et al., 1993) and annexin-1 (Theogaraj et al., 2005), as well as expressing receptors for pituitary hormones (Brokken et al., 2004), suggesting additional roles both in the regulation of hormone cells and as a short-loop feedback mechanism.

### 6.2. Adult progenitor cells

Recently a population of cells in the adult mouse pituitary with characteristics of stem cells has been described (Chen et al., 2005; Fauquier et al., 2008; Garcia-Lavandeira et al., 2009). These cells are positive for the transcription factor Sox2, which is expressed in all the cells of Rathke's pouch, the structure which eventually gives rise to anterior pituitary (Kelberman et al., 2006). In the adult, Sox2 positive cells, which may represent a distinct population from that in the embryo and neonate (Gleiberman et al., 2008), are found principally in the cleft that separates the anterior and intermediate lobes but also scattered within the anterior lobe (Rizzoti, 2010). In specific culture conditions, these cells can form self-renewing spheres which are capable of differentiating into all the anterior pituitary hormone cell types (Chen et al., 2005; Fauquier et al., 2008). Remarkably, embryonic stem (ES) cells can also be induced to differentiate into structures which both resemble Rathke's pouch and have the ability to produce pituitary hormones (Suga et al., 2011). The intrinsic self-organizing capacity of pituitary cells is demonstrated in this study as the Rathke's pouch-like cells differentiated from ES cells *in vitro* have a similar topographical organization to that found in the embryo. Using a knock-in mouse with eGFP inserted into the Sox2 gene (Taranova et al., 2006), the organization of Sox2 positive cells has been described (Mollard et al., 2012), showing that the cells lining the cleft form a continuum yet maintain contact with the dispersed cells within the lobe. This organization suggests that long-range communication between these putative stem cells may be important in either determining differentiation pathways, proliferation or maintenance of stem cell characteristics.



**Fig. 4.** Interconnections between endocrine and folliculostellate cell networks. 3D 2-photon imaging of a GH-GFP (green, B) pituitary pre-loaded with a fluorescent AMCA-labeled dipeptide (white, A and B) specifically taken up by folliculostellate cells. Note that the folliculostellate cell network can make connections between distant GH cell network units and the organization of GH cells into clusters connected by strings of cells.

### 6.3. Pituitary vasculature

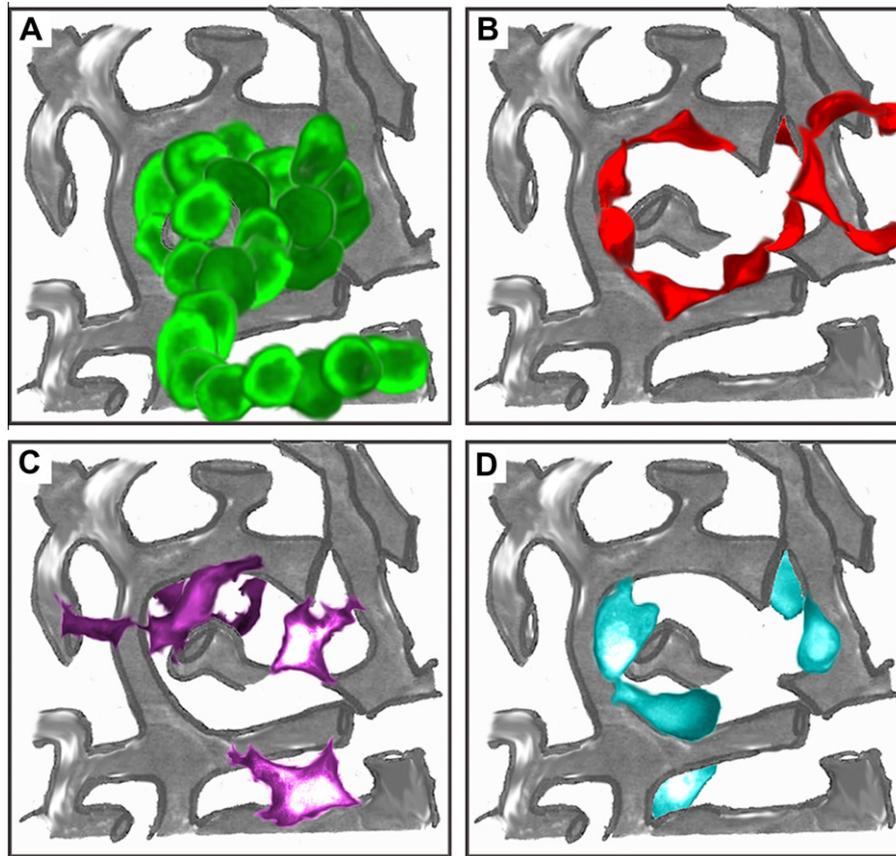
Whilst it would seem obvious that the pituitary vasculature must be organized into a network, with early studies showing the presence of abundant “capillary nets” (Page and Bergland, 1977), the relationship of its organization with the other pituitary networks is an important consideration in pituitary function. The role of the vasculature in dynamic function of the pituitary, including regulation of blood flow, partial oxygen pressure, delivery of releasing factors and transport of secreted hormone from the gland have recently been reviewed (Schaeffer et al., 2010, 2011a). Therefore, we will focus the description here on the relationship of the vasculature with hormone networks. To summarize the aspects addressed in these reviews but not covered here: blood flow is locally modified within the pituitary in response to the stimulation of secretion, meeting the increased metabolic demand of active endocrine cells and affecting clearance of secreted products from the gland.

Angiogenesis in the rodent pituitary begins as Rathke’s pouch separates from the oral ectoderm with invasion of capillaries from surrounding tissue into the developing gland (Szabo, 1987; Szabo and Csanyi, 1982). This vascular footprint then expands in parallel with the gland so that, in the neonate, a rich capillary network already extends throughout the parenchyma of the gland (Hashimoto et al., 1998). An interdependence of capillary and hormonal cell development is suggested by studies of *Prop1<sup>df/df</sup>* pituitaries where the failure of normal hormonal cell differentiation is accompanied by a marked reduction in the normal pituitary vascularization (Ward et al., 2006). A reduced expression of the angiogenic factor vascular endothelial growth factor A (VEGFA) by differentiated hormone cells and their precursors may account for the reduced capillary formation, which may in turn lead to further loss of endocrine cells.

A topographical relationship between the organization of endocrine cells and the vasculature was described in several histological studies (Baker, 1974; Moriarty, 1973; Nakane, 1970; Sasaki and Iwama, 1988). A more detailed relationship has been demonstrated using multiphoton and confocal imaging of pituitaries from transgenic mice perfused with FITC-labeled gelatin or stained for the marker CD31/PECAM. In the adult, somatotrophs are found in close proximity to the vasculature (Lafont et al., 2010), with both clusters and strands of cells surrounded by capillaries (Fig. 5A). In contrast, the relationship of lactotrophs with the vasculature is less stereotyped, with some honeycomb structures surrounded by cap-

illaries, whilst others are less closely associated (Fig. 5B). Gonadotrophs are also closely associated with the vasculature (Fig. 5D), whilst the cell bodies of corticotrophs are more distant (Budry et al., 2011). The long cytoplasmic processes, or cytonemes, of corticotrophs maintain a contact with perivascular spaces, however, and ACTH secretory granules are aligned along cytonemes (Fig. 5C). These relationships between the vasculature and endocrine cell networks may reflect the importance of the dynamic secretion of the different hormones, with shorter duration, more frequent pulsatile secretion of GH and LH required for their physiological action (Veldhuis et al., 2005; Wildt et al., 1981) compared with prolactin (Grattan and Kokay, 2008), and rapid ultradian secretion of ACTH in response to stress (Dallman et al., 1992).

The relationship of the endocrine networks with the microcirculation will obviously influence the delivery of hypothalamic and peripheral regulatory factors, as well as the clearance of secreted hormone. Using microscopes equipped with long-distance working objectives to image the surgically-exposed pituitary *in vivo* in anesthetized mice, we were able to monitor the fate of both incoming secretagogues as well as the output of hormones, simulated by the use of fluorescent dextran molecules of differing size (Lafont et al., 2010). Intravenous injection of a 4 kDa dextran, close to the size of most hypothalamic neuropeptides, revealed a heterogenic timing of detection in capillaries within the same imaging field, with diffusion of detected marker limited to a distance of <100 μm between pituitary cells. This would imply that endocrine cells may be exposed to very different secretagogue concentrations depending on their orientation versus the vasculature, and provides a role for endocrine networks in coordinating their response. Monitoring the exit of fluorescent dextran from the extracellular space surrounding endocrine cells demonstrated that, whilst the transfer of molecules of both 4 kDa and 20 kDa (approximate sizes of ACTH and GH/PRL respectively) towards the lumen of vessels was rapid, it was slower than that calculated on the basis of the theoretical passive clearance rates for similarly sized molecules. It was also noted that there was significant accumulation of the larger dextran in the perivascular region close to the capillary before slowly entering the circulation through fenestrated capillaries. This retardation of larger hormones in the perivascular space will have important implications for the shape of hormone pulses but may also have a role in the maintenance of the relationship of endocrine networks with the vasculature, as well as paracrine and autocrine interactions (see below).



**Fig. 5.** Relationships between endocrine cell networks and the pituitary vasculature. Schematic representation of the anatomical relationship of pituitary fenestrated capillaries (grey) with the GH cell (A, cells in green), PRL cell (B, red), ACTH cell (C, magenta) and LH cell (D, cyan) networks.

## 7. Role of networks in pituitary function

### 7.1. Coordination of endocrine cell stimulation

The finding that endocrine organs are topologically compartmentalized into multiple intermingled cell networks raises important questions concerning hormone release (Mollard et al., 2012). Although tissue organization of cells is clearly critical for promoting hormone release, as best evidenced by the blunted responses of enzymatically isolated cells to secretagogue (Hodson et al., 2012b; Sanchez-Cardenas et al., 2010; Sartor et al., 1985; Tannenbaum et al., 1976), deciphering the relative contribution of endocrine networks to gland function remains technically and conceptually daunting. To assess the network dynamics underlying hormone output – the functional currency of endocrine tissues – secretory activity in individual cells needs to be monitored on a population basis. Whilst exocytosis can be investigated using a range of approaches such as total internal reflection fluorescence microscopy (TIRF-M) (McGuinness et al., 2003; Ravier et al., 2008), or online measures of fluorophore-tagged hormones targeted to secretory vesicles (He et al., 2011), each is impeded by either failure to appreciate the cell population or inability to delineate events with sufficient resolution. To circumvent these issues, we instead use changes in intracellular calcium ( $\text{Ca}^{2+}$ ) concentrations as a surrogate for exocytosis, since action potential-driven increases in intracellular  $\text{Ca}^{2+}$  concentrations are intimately involved in stimulus–secretion coupling (Douglas and Poisner, 1964; Douglas and Rubin, 1961; Schlegel et al., 1987). During this process (e.g. stimulation of GHRH receptors in somatotrophs), a G-protein coupled receptor-evoked signal cascade triggers the opening of

voltage-gated  $\text{Ca}^{2+}$  channels during action potential firing, leading to extracellular  $\text{Ca}^{2+}$  entry and exocytosis of vesicles from both the readily-releasable and reserve pools (Stojilkovic and Catt, 1992; Stojilkovic et al., 2005, 2010; Van Goor et al., 2001). Due to this close relationship, and the fact that  $\text{Ca}^{2+}$  signals can be simultaneously captured from hundreds of cells, a population index of cell activity can be formed (Bonfont et al., 2005; Peterlin et al., 2000; Schwartz et al., 1998). By subjecting cell activity profiles to large-scale correlation analyses capable of simultaneously discerning multiple cell–cell interactions, a signature of network function/connectivity can subsequently be obtained (Hodson et al., 2010a; Peterlin et al., 2000).

Using these techniques, we have been able to show that two endocrine populations, namely the GH and PRL-networks, display network-driven gain of function in response to specific stimulation (Bonfont et al., 2005; Hodson et al., 2012b). Importantly, this is not due to a simple summation of unitary activity, but rather increases in coordinated behavior of a subset of cells as well as changes to population functional connectivity (i.e. the spatial distribution of correlated cell–cell activity). This gain of function is severely abrogated when cells are either plated as a monolayer or isolated *in situ* using graded cell ablation techniques, thus demonstrating a permissive role for the tissue context in intercellular communication (Sanchez-Cardenas et al., 2010; Waite et al., 2010). Despite these findings, it is nevertheless prudent to consider that changes in intracellular  $\text{Ca}^{2+}$  concentrations, whilst providing a convenient and reliable means to measure network dynamics, do not always reflect the exocytotic process. For instance, aberrant cell  $\text{Ca}^{2+}$  handling is observed immediately preceding apoptotic and necrotic cell death, and  $\text{Ca}^{2+}$  provides an important signal for

progenitor differentiation and entry into the cell cycle (Berridge et al., 1998, 2000).

### 7.2. Plasticity of endocrine cell output

The greatest demand for GH secretion is observed during puberty when nocturnal levels must double to support continuing growth and maturation of the reproductive and metabolic axes (Rose et al., 1991). These changes are widely believed to emanate from alterations at the level of hypothalamic GHRH neurons and, as such, the pituitary somatotroph population is considered to be relatively non-plastic (Gouty-Colomer et al., 2010). However, as described above, there is considerable plasticity of somatotroph network organization, which is most obvious at puberty. The transient but large increase in GH-cell clustering noted in male but not female animals during puberty (Fig. 3, bottom panels) (Sanchez-Cardenas et al., 2010) is mimicked at the functional level. Indeed, multicellular  $Ca^{2+}$  imaging revealed the presence of sexually-divergent network responses to the same secretagogue, and online measures of GH-GFP secretion directly from pituitary slices confirmed the presence of larger amplitude GH pulses in males compared with females (Hodson et al., 2010a). The demand for prolactin secretion is also dramatically altered in the adult, with an altered pattern of secretion during pregnancy and lactation (Grattan, 2002). This altered demand for lactotroph function is associated with changes in the lactotroph cell network, as eluded to above, and this was directly correlated with increased functional connectivity using multibeam two-photon  $Ca^{2+}$  imaging in combination with systems analyses to depict multiple cell–cell interactions (Hodson et al., 2012b). During lactation, increases in functional connectivity are not only associated with suckling-induced improvements to structural connectivity but also heightened gap junction signaling.

These studies demonstrate that both somatotroph and lactotroph network plasticity contributes to the differences in secretion which underlie their physiological roles. Similar roles of the corticotroph and gonadotroph networks have yet to be described but seem likely.

### 7.3. Memory of functional demand

To study whether the function of endocrine cell networks could be permanently altered by previous experience, we employed lactotrophs as a physiologically relevant model of a mammalian cell population whose function can be specifically and repeatedly interrogated by imposing lactation. Since PRL induces a range of adaptive responses in the female, including changes in maternal behavior (Bridges et al., 1985; Larsen and Grattan, 2010; Shingo et al., 2003) and improved spatial memory (Gatewood et al., 2005; Kinsley et al., 1999), we hypothesized that functional adaptation of the PRL-axis would be present in dams that have experienced one or more lactations. Remarkably, we found that the changes in the lactotroph network associated with lactation were still present 3 months post-weaning and were stored through permanent structural and gap junction remodeling (Fig. 3, top panels). Although the exact mechanisms by which the suckling stimulus upregulates network structure/function remain elusive, they are unlikely to involve dopamine, the inhibitory catecholamine neurotransmitter whose release from hypothalamic neurons is principally tasked with suppressing PRL release (Anderson et al., 2006; Demarest et al., 1983; Selmanoff and Wise, 1981). Indeed, treatment of female mice with a dopamine D2-receptor antagonist was without effect, suggesting that pregnancy- and lactation-induced decreases in dopamine tone probably do not contribute to the observed PRL-network plasticity. In an attempt to uncover adaptive features of the PRL-network, dams were weaned before

being subjected to a second lactation. In response to repeat demand, the PRL-network mounted temporally synchronized cell responses which improved tissue function by driving higher levels of PRL secretion. Therefore, similarly to cells comprising the immune (Sprent and Surh, 2002; Sun et al., 2009) and nervous systems (Bliss and Collingridge, 1993; Chklovskii et al., 2004), endocrine cell networks appear to possess emergent experience-dependent responses (or memory) which lead to heightened tissue function in response to repeat demand (Hodson et al., 2012b; Mollard et al., 2012).

### 7.4. Intrapituitary regulation of endocrine cell network function

Perturbation of pituitary network function, whether due to secretagogue bolus or withdrawal of an inhibitory factor, results in generation of spatiotemporally complex activity patterns by the resident endocrine cell population (Hodson et al., 2012b; Sanchez-Cardenas et al., 2010; Sanchez-Cardenas and Hernandez-Cruz, 2010). Whilst the initial stimulus clearly provides the initiating factor, it does not explain the presence of autoregenerative cell responses which amplify and propagate the signal, even following cessation of stimulus (Bonfont et al., 2005; Sanchez-Cardenas et al., 2010). Therefore, as well as exogenous factors, the pituitary gland must also be furnished with an endogenous signal processing 'toolbox'. Based upon morphological observations, as well as findings from re-aggregation studies, an important role for cell–cell communication for pituitary function and hormone release has long been suspected. For example, cup-shaped lactotrophs embrace and surround gonadotrophs in multiple species (Gregory et al., 2000; Hovarth et al., 1977; Tortonesi et al., 1998), gonadotroph-conditioned medium evokes PRL secretion from cultured lactotrophs (Andries et al., 1995; Defen and Andries, 1983), application of a gonadotroph-specific secretagogue (GnRH) upregulates lactotroph output (Henderson et al., 2008), and gap junction-signaling is positively correlated with circulating PRL levels (Vitale et al., 2001). Whilst it is beyond the scope of the current review to discuss intrapituitary communication in detail (please refer to recent reviews from ours and other groups (Defen, 2008; Hodson et al., 2012a)), the important routes will nonetheless be highlighted within the context of endocrine cell networks.

Endocrine cells are capable of synthesizing and secreting myriad substances which diffuse locally to elicit effects upon target cells; as such, paracrine and autocrine signaling comprise arguably the most diverse intercellular communication pathway within the pituitary (Defen, 2008; Vankelecom and Defen, 1997). Paracrine and autocrine molecules are not limited to peptides, but can also encompass nucleotides (e.g. ATP), neurotransmitters and gases (e.g. NO) (Defen, 2008; Hodson et al., 2012a). It is possible that the hormone secreted by the pituitary endocrine cell may have paracrine effects, as well as potential ultra-short feedback autocrine effects if the cell expresses the receptor for its own hormone, most likely leading to short term suppression of secretion. It is noteworthy that signaling events in pituitary networks can be rapid and are spatiotemporally precise. Unless preferential diffusion pathways and/or active transport mechanisms exist, it is difficult to conceive how paracrine and autocrine factors, rate-limited by diffusion gradient/coefficient and susceptible to random Brownian motion, can distribute specifically to account for such network dynamics. Albeit unlikely implicated in rapid cell–cell exchanges, a more exact communication may be achieved by the annexins, a class of juxtacrine factors. These membrane-bound phospholipid-binding proteins require close cell–cell apposition for activity, exhibit  $Ca^{2+}$ -dependency (John et al., 2004), and underpin the suppressive effects of glucocorticoids upon corticotroph, somatotroph and lactotroph function most likely through effects upon PKC and cAMP (Chapman et al., 2002; John et al., 2004; Taylor

et al., 2000a,b; Tierney et al., 2003). Finally, gap junctions may constitute the most cell-specific communication mode. These intercellular channels are expressed throughout the pituitary gland, provide electrical and chemical cell–cell linkages, display a high degree of plasticity in response to both internal and external stimuli, and synchronize responses between pituitary endocrine cell ensembles (Guerineau et al., 1998; Hodson et al., 2010b, 2012b; Vitale et al., 2001). Crucially, gap junctions are permeable to both  $\text{Ca}^{2+}$  ions and intracellular  $\text{Ca}^{2+}$ -mobilizing agents such as IP<sub>3</sub>, providing a means for propagation of  $\text{Ca}^{2+}$  events in a network-restricted manner (Foskett et al., 2007; Straub et al., 2000; Zimmermann and Walz, 1999).

### 7.5. Network support of transcription

As well as the  $\text{Ca}^{2+}$ -spiking activity underlying hormone release, endocrine cell networks are host to longer term signaling processes such as gene transcription. By contrast to hormone secretion, transcriptional dynamics evolve over dozens of hours to days and, perhaps as a consequence of their  $\text{Ca}^{2+}$ -dependency (Berridge et al., 2000), are equally reliant upon the tissue context for proper manifestation. Using luciferase- and destabilized eGFP-based whole-gland assays, heterogeneous cycles of lactotroph gene transcription have been shown to summate at the population level to produce coordinated responses which disappear in dispersed cells (Harper et al., 2010; Semprini et al., 2009). Although the functional consequences of such behavior remain unclear, it may constrain uncontrolled and undesirable transcriptional responses (e.g. tumorigenesis) at the individual cell level whilst maintaining adequate hormone release at the tissue level by facilitating continued responsiveness to stimuli (Harper et al., 2011; Paszek et al., 2010). Using similar techniques, it has also been demonstrated that, during embryonic development, lactotroph transcription is pulsatile before stabilizing in neonates; this transition may coincide with terminal differentiation when non-lineage specific genes become silenced (Featherstone et al., 2011).

## 8. Mathematical modeling of network function

Mathematical models are a valuable tool for the study of biological networks, in particular those which are complex multi-modular systems like pituitary networks. Whilst several models of the pituitary endocrine axes have been developed, such as the rhythmic secretion of prolactin induced by vaginal stimulation (Bertram et al., 2010) and the pulsatile secretion of the hypothalamic–pituitary–adrenal axis (Walker et al., 2010), few have included the network organization of pituitary cells as a key variable of the model. Recently, Lyles et al. (2010) described a model of the LH surge which included pituitary network organization. In this model, GnRH stimulates the gonadotroph network directly and indirectly through a second pituitary cell network (using the FS cell network as an example), with oestradiol inhibiting gonadotrophs but increasing the network connectivity of the second pituitary cell type. This model successfully recapitulates an LH surge using known physiological parameters but, importantly, also succeeds in reproducing the LH surge observed during secretagogue bolus treatment of Kallman's patients lacking functional GnRH neurons.

Mathematical modeling of secretion from the somatotroph network (modified from Thomas and Waring (1997)), based on the calcium activity of cells measured in pituitary slices from the studies of Sanchez-Cardenas et al. (2010) (Fig. 6), shows that recurrent GH pulses occur from male but not female glands, due to network organization. This model of pituitary secretion in slices isolated from hypothalamic input does not imply that GHRH is not the major regulator of pulsatile secretion, but does indicate that recurrent

pulses of secretion could occur in response to a single GHRH stimulation in males. The fact that a single, yet prolonged calcium/exocytotic response can also be triggered in males in response to a GHRH bolus suggests that a finely tuned balance between recurrent and single GH secretion from GH network units may participate to the variability of amplitude/shape of GH pulses detected *in vivo*. By contrast, the single, sharp calcium/exocytotic response consistently observed in GH network units demonstrates the sexually dimorphic response of the gland to stimulation and fits well with the shorter-lived GH pulses described in female rats. Hypothalamic activity, or features of the pituitary microenvironment which modify GHRH delivery to, or GH exit from the gland, may translate the responses we have modeled to the differences in frequency, rather than duration, that have been described in mice (MacLeod et al., 1991). Further detailed studies of the GH secretory profile in female mice using the elegant technique developed by Steyn et al. (2011) are ongoing and are required to further validate and develop this mathematical model of GH secretion.

## 9. Perspective/questions for future studies

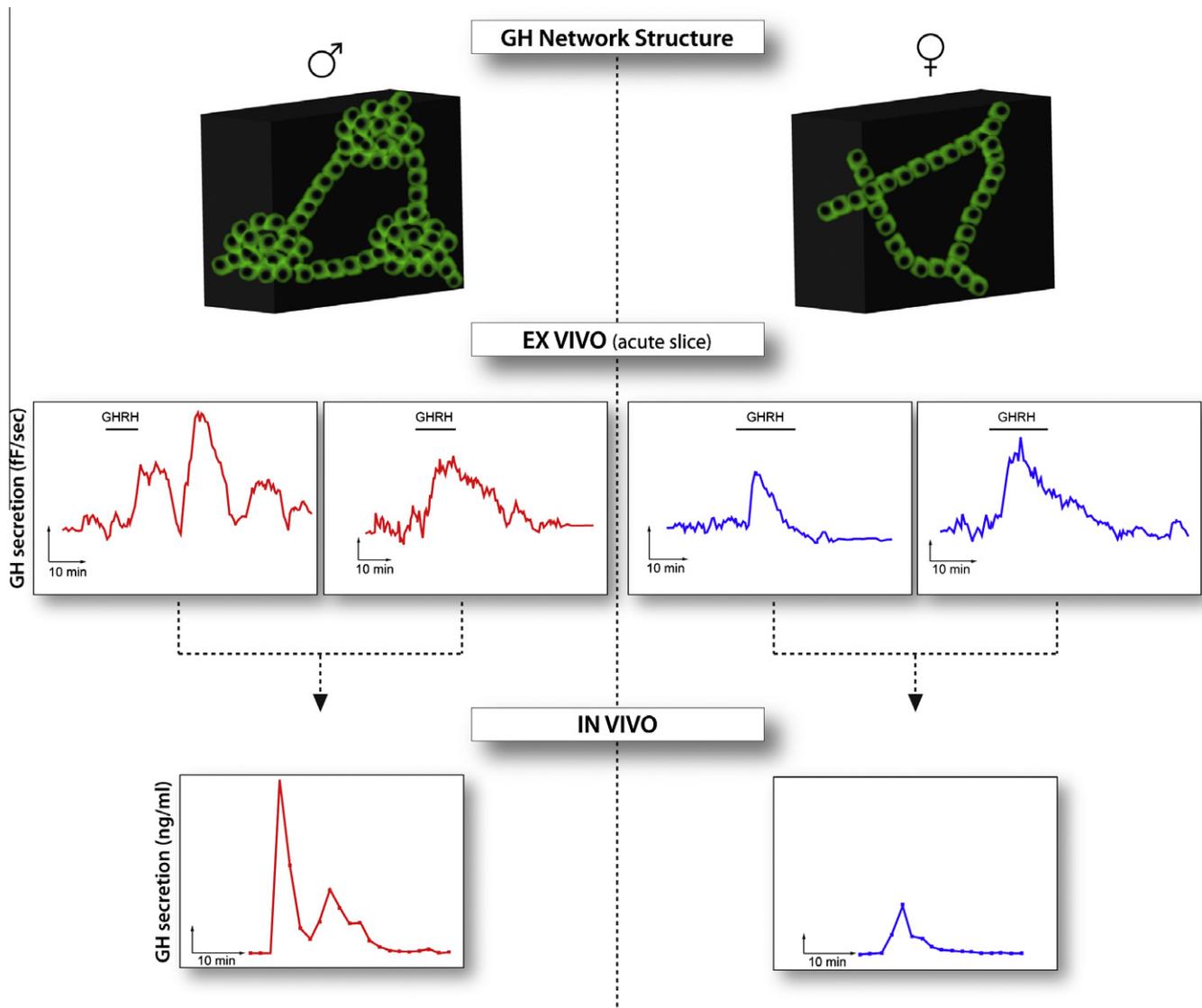
### 9.1. Mediators of network formation

The intrinsic ability of the pituitary endocrine cell types to aggregate after dispersion and form connections suggests that they express a range of adhesion molecules which leads to cell-autonomous organization. Previous studies have demonstrated cell-type specific expression of members of the cadherin family in the pituitary (Chauvet et al., 2009), but a more detailed analysis is required, such as conditional knockout or blocking adherence *in vitro* to define their role in network formation. Of the myriad of other potential mediators of network formation, ephrins and their cognate receptors are obvious candidates, as they have a clear role in the organization of cells in a large number of systems through mechanisms of both attraction and repulsion (Murai and Pasquale (2011) and Solanas and Batlle (2011) for examples). A role for ephrins in beta cell communication and the regulation of insulin secretion (Konstantinova et al., 2007) demonstrates that they have a role in endocrine cell function and warrants further study in the pituitary. Studies utilizing hydrogels conjugated with ephrin and Eph receptor to model  $\beta$ -cell function (Lin and Anseth, 2011) demonstrate the power of identifying the molecules mediating cell–cell communication, and opens the possibility of building 3D cell networks *in vitro* to further analyse their function.

As well as the molecular basis for the adhesion and sorting of endocrine cells, the factors mediating the remodeling of both endocrine networks and the extracellular matrix require further study. Members of the matrix metalloproteinase (MMP) family are expressed in gonadotrophs and are released after GnRH treatment; they may contribute to cytoneme extension as well as gonadotroph network remodeling (Navratil et al., 2007), although the latter requires investigation. MMP expression has also recently been described in folliculostellate cells (Ilmiawati et al., 2012), suggesting that these cells may be also be capable of matrix remodeling, with implications for both the dynamics of secreted hormone transport to the vasculature and network remodeling.

### 9.2. Heterogeneity of cell types

Although the pituitary endocrine cell types are generally defined by the hormones that they produce, there is considerable evidence for heterogeneity within these cell types (Denef et al., 1978a,b; Huerta-Ocampo et al., 2005; Villalobos et al., 2004). This may be reflected in the expression of fluorophores in some of the transgenic animals used in studies of network organization, with



**Fig. 6.** Modeling of the generation of sex-dependent GH pulses from sexually dimorphic-GH network responses. *Top panels:* schematic representation of sex-dependent GH cell network motifs. *Middle panels:* simulated exocytosis of GH granules (using equations derived from Thomas and Waring (1997)) due to GHRH-triggered coordinated calcium responses in acute GH-GFP pituitary slices (calcium responses from Sanchez-Cardenas et al. (2010)). *Bottom panels:* schematic representations of sex-dependent GH pulse profiles in young mature animals.

the POMC-GFP and PRL-DsRed transgenes not being fully penetrant (He et al., 2011; Lavoie et al., 2008), and gonadotroph identification in LH-cerulean transgenic mice potentially lacking labeling of some FSH-expressing cells (Wen et al., 2010). Heterogeneity is a challenge in the analysis of cell organization, as the complete network may not be revealed and the potential heterogeneous function will complicate activity studies. It should also be noted that altered cell activity resulting from network organization may modify transgene expression of fluorophores. This could mask the full extent of a network or, conversely, provide an exaggerated depiction of population organization by only providing information on a subset of cells. Nonetheless, if there is sufficient modification of hormone-promoter driven transgene expression, this would imply that important cell traits, such as transcription, are dictated by the large-scale organization of distinct functional cell sub-populations, lending weight to the argument that endocrine networks are of physiological relevance. Identification of markers specific for sub-population of cells and better definition of the function of those sub-populations are required to clarify the network organization and function within the pituitary. One particular sub-population

of cells which may have an important role in pituitary network function are those producing multiple hormones, for example mammosomatotrophs, which may change in proportion and function in response to altered physiological demand (Porter et al., 1990, 1991). The role of multihormonal cells can be addressed using double transgenic animals and studies of their interaction with mono-hormonal cells are likely to reveal interesting facets of the mechanisms underlying network function.

### 9.3. Modeling of network function

Studies of network organization and function have demonstrated the importance of analysing endocrine cell activity within the context of the intact tissue and have yielded insights into pituitary function. The development of *in vivo* pituitary imaging will allow parameters such as blood flow rate, oxygen tensions, intravital cell electrical behavior and various microenvironment parameters (e.g. diffusion rates/kinetics) to be incorporated into theoretical models of pituitary hormone release. Detailed analysis of network function, and the impact of complex *in vivo* inputs upon this, re-

quires the development of tools such as optogenetics to precisely and remotely control the gland environment (Fenno et al., 2011; Madisen et al., 2012). Development of genetically modified mice with Cre-recombinase activatable optogenes (Madisen et al., 2012) means lines of mice with pituitary and hypothalamic specific expression of Cre can be utilized for these studies. By isolating different components of the hypothalamic/pituitary axes, data suitable for mathematical modeling will be produced which is likely to generate new questions and paradigms for further studies. As an example, selective stimulation of the GHRH neuron population could be used to trigger one or several GHRH boluses in the portal system. This may be an invaluable tool with which to understand whether a complex multi-peak GH pulse (as frequently observed in young mature males) depends on a primary GHRH oscillator and/or recurrent GH network responses.

#### 9.4. Plasticity

The finding that somatotroph and lactotroph network organization mediates the plasticity of output in response to physiological demand (Fig. 3) may be a common feature of the other pituitary networks and warrants further study. Mapping the organization of thyrotrophs will be particularly interesting as these cells display considerable functional plasticity in response to thyroid challenge (Castinetti et al., 2011) but the network organization remains to be described. The molecular mechanisms underlying plasticity also remains poorly understood, especially in the case of lactotrophs which display long-term functional adaptation in response to stimulation. Preliminary data from our labs (Le Tissier, Mollard and Drouin, unpublished) shows that lactotroph gene expression undergoes limited alterations during lactation, suggesting that mechanisms such as hysteresis, where bistability of systems results from persistent signaling even in the absence of persistent stimulation, may have a role. Hysteretic mechanisms have been implicated in cell proliferation (Tyson and Novak, 2001), embryonic development (Balaskas et al., 2012) and modification of signaling in neural networks controlling metabolism (Yang et al., 2011). The study of potential mechanisms will again be aided by the identification of the molecules mediating plasticity, and the use of cell-type specific recombinases to determine the effects of their modification on pituitary function.

#### 9.5. Cell turnover, recovery after injury and the role of stem cells

There is a considerable basal cell-turnover of pituitary cells, as well as cell population changes in response to some physiological challenges (Nolan et al., 1998). Even in cases where there is no increase in cell number in response to challenge, such as lactation (Castrique et al., 2010), it is possible that an increased turnover of cells exists due to a balanced combination of mitosis and apoptosis. Although some of this cell renewal and proliferation may be a result of mitosis of differentiated cells (Sakuma et al., 1984), it is clear that a large proportion results from division and differentiation of hormone-negative cells (Nolan and Levy, 2006), which are likely to have an origin in the recently identified pituitary stem cell populations (Chen et al., 2005; Fauquier et al., 2008; Garcia-Lavandeira et al., 2009). As well as being responsible for the renewal of cells required to maintain the pituitary and respond to physiological challenge, these stem cells are also likely to be required for pituitary regeneration after injury, as suggested by the recent paper of Fu et al. (2012). The existence of functional cell networks in the pituitary implies that these newly differentiated cells must potentially move large distances and become integrated into the existing network. Cell lineage tracing is required to determine the contribution of these stem cells to the different hormonal cell networks and the development of mice similar to the recently de-

scribed model with a tamoxifen inducible Sox2-Cre (Arnold et al., 2011) will be invaluable in answering these questions. An additional question is the nature of the signals that drive stem cell proliferation and differentiation into the different cell types. The development of a system for *in vitro* differentiation of ES cells into pituitary cell types (Suga et al., 2011) will aid the study of the factors required for normal differentiation and the proliferation of different pituitary cell types, including the role of secreted output from endocrine cells. The seminal studies of Levy in the rat (Nolan and Levy, 2006) suggest that the differentiated cells are capable of assessing the size of their population, as well as prioritising the stem cell differentiation choice, but the mechanism underlying this is unclear. An intriguing possibility is that the network organization is responsible for the monitoring of cell populations and that their intermingling may provide a mechanism for determining choice of stem cell differentiation pathway. In this context, the POMC cells which form the first cell network during development may serve to control the extent of other cell networks, as was recently reported for the gonadotroph network (Budry et al., 2011).

#### 9.6. Pathology

The role of pituitary cell networks in pituitary gland pathology, in particular tumour formation, is unclear but it is possible that somatic mutations leading to a loss of normal cell-cell communication would lead to an aberrant proliferation of cells, especially in cases where juxtacrine signaling is a trophic factor. Vascularization is an important factor in the generation of tumours and the clear interaction between pituitary cell networks and the vasculature hints at a potential role for endocrine cell organization in stimulation of angiogenesis or vice versa. Screens for altered expression of mediators of cell-cell communication in adenomas may provide insight into the role of networks in the aetiology of tumour formation, as well as potentially identifying targets for therapy. The detection of altered expression of MMPs in adenomas, in particular in those which are invasive (Qiu et al., 2011), suggests a role for remodeling of the organization of cells and interaction with the extracellular matrix in tumour formation and is one exciting avenue for this research.

### 10. Concluding remarks

The description of the organization of pituitary cells into networks and studies of their role in the function of the gland has defined their importance in endocrine organ function. Consequently, the gland can now be considered to be an oscillator which may memorize information and dynamically adapt its coordinated network responses to the flow of hypothalamic inputs. The pituitary will continue to provide an excellent model for the study of the role of networks in other endocrine glands, particularly with the development of new tools for *in vivo* imaging and manipulation of cell function. Network organization has important implications for studies of all endocrine systems, especially the reassessment of those using isolated cells in culture, and the pituitary model may act as a paradigm for the dissection of the role of cell networks in the control of hormone output.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.yfrne.2012.08.002>.

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