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REVIEW ARTICLE

Investigating and Modelling Pituitary Endocrine Network Function

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Endocrine cells in the mammalian pituitary are arranged into three-dimensional homotypic networks that wire the gland and act to optimise hormone output by allowing the transmission of information between cell ensembles in a temporally precise manner. Despite this, the structure–function relationships that allow cells belonging to these networks to display coordinated activity remain relatively uncharacterised. This review discusses the recent technological advances that have allowed endocrine cell network structure and function to be probed and the mathematical models that can be used to analyse and present the resulting data. In particular, we focus on the mechanisms that allow endocrine cells to dynamically function as a population to drive hormone release as well as the experimental and theoretical methods that are used to track and model information flow through the network.

Key words: pituitary, network, modelling, imaging, neuroendocrinology.

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The pituitary gland is the master endocrine organ that is responsible for regulating many of the body's important functions, such as growth and metabolism, reproduction and stress. Thousands of individual endocrine cells must respond to stimulation by specific hypothalamic releasing-factors and release hormone in a pulsatile manner. The information encoded in the amplitude and frequency of hormone pulses is decoded by peripheral tissues to mount an appropriate physiological response. Because of the dependence of homeostasis on hormone rhythms, the investigation of pulsatile hormone release has attracted a lot of experimental and theoretical work. In terms of the latter, ordinary differential equations are usually used to capture a simplified picture of hormone pulse formation which, by virtue, cannot incorporate our growing understanding of the different control levels that are predominant throughout endocrine axes. In particular, systems-level modelling of hormone release is a daunting challenge as a result of the existence of multiple layers of feedback control that co-exist on relatively short time scales and are spatially expressed both at the local (e.g. brain) and global (e.g. organism) levels; an introduction to modelling in neuroendocrinology is provided elsewhere (1). If one considers the steps involved in the formation of a hormone pulse just at the level of the pituitary gland, it is easy to appreciate the inherent complexity that makes a systems-level mathematical approach to endocrine modelling so difficult. At the cell and tissue level, a bolus of hypothalamic secretagogue arrives at the anterior pituitary gland and binds to its specific membrane bound receptor, eliciting the activation of spatially and temporally constrained molecular signalling pathways, resulting in cell activation (2). At the tissue level, regulation occurs in the form of cell activity-dependent nutrient and oxygen supply by the pituitary vasculature, as well as directed convection that acts to propel secreted hormone through the extracellular space into the afferent blood vessels (3). Moreover, the activated cell is able to communicate with other cells in electrical (4, 5) and chemical (6) manners to temporally coordinate hormone release, the precise time-signature of which evokes a response in the target organ. The importance of this tissue-level integration is clearly demonstrated by the magnitude of differences in secretory responses to secretagogue when measured from endocrine cells in their proper tissue context compared to enzymatically-isolated cells (7, 8). Superimposed upon this, and adding yet another layer of complexity, plasticity of endocrine cell number, size, function and structure occurs in response to changing physiological needs during life (9–11).

Because of the multiscale nature of pulsatile hormone release, system- and tissue-level data must all be incorporated into a model to accurately predict hormone release profiles. However, it is often difficult to directly correlate system-level and tissue-level data without knowing the mechanisms that allow cells to act as a

population, thus linking both tiers. In particular, studies of the pituitary gland are required to help further define the mechanisms implicated in population activity because it contains thousands of individual endocrine cells that, subsequent to the arrival of hypothalamic secretagogue, must act in a temporally precise manner to drive pulsatile hormone release as an ensemble. Although the cell and systemic level mechanisms that influence activity of individual cells (e.g. clock genes) and finely regulate neuroendocrine output [e.g. somatostatin, ghrelin and growth hormone (GH)-releasing hormone control of GH release] are well understood (12–14), the mechanisms that permit pituitary endocrine cells to act as a population at the tissue level remain elusive. As such, they require further investigation before the gap between the cell and systemic levels can be successfully bridged, allowing hormone release to be modelled more precisely. To investigate population dynamics of cell activity, one can take advantage of the recent availability of various experimental techniques (from transgenic animals to multicellular calcium imaging) that allow the structure and function of cell assemblies to be explored. A key concept that emerges at the multicellular level is the network paradigm. Well implanted in neurosciences, where spatio-temporal organisation of electrically coupled cells has been a long-term preoccupation, this concept is also highly relevant for the understanding of tissue integration among endocrine cells. The goal of the present review is to detail the relevance of this concept in neuroendocrinology, to describe the experimental techniques that enable its importance to be assessed in endocrine tissues, and to comment on the specificity of neuroendocrine cell versus neural networks.

Experimental evidence for tissue-level regulation in the pituitary gland

Despite the importance of pituitary function for many downstream processes, scant attention has been given to modelling the tissue-level processes that occur in this organ during formation of a hormone pulse, perhaps because pituitary cells are generally considered to represent nondynamic effectors that respond to central, hypothalamic orders. The advent of multiphoton imaging, facilitating the deep imaging of tissue structures in animals expressing GH-enhanced green fluorescent protein (eGFP) (15), has allowed us to demonstrate that, rather than being a collection of randomly dispersed cells, pituitary GH secreting cells are arranged into a spatially restricted three-dimensional (3D) network. After the application of exogenous GH-releasing hormone, cells belonging to the GH-network display large-scale, repetitive and temporally coordinated calcium responses that persist after cessation of the original stimulus (10). This is in marked contrast to enzymatically dispersed cells, where the calcium response to the same secretagogue results in 10–100-fold less GH secretion (7). These responses are also vulnerable to time-scaling because the GH-network displays marked plasticity over lifespan, particularly at puberty when gross remodelling is readily detected in the male pituitary (without a notable increase in the number of GH cells) and this is well correlated with the increased physiological demand for GH, providing evidence for an important role of 3D structure in hormone secretion (10). Therefore, by virtue of its cell organisation and plasticity, the contribution of pituitary gland output to hormone secretion is likely to be more complex than a simple master–slave relationship with the hypothalamus.

Tissue level organisation of neurone activity

Although the notion of a network of endocrine cells may be novel to endocrinologists, it has been recognised for many years by neuroscientists as describing the organisation and activity of neurones throughout the brain. It is widely acknowledged that the compartmentalisation of neurones into structural and functional networks allows single neurones with basic processing elements to collectively and efficiently filter, integrate and propagate complex information both within and between brain regions, mediating important functions such as visual processing and the formation of memories (16–19). Similar to their neurone counterparts, endocrine cells are excitable and display calcium spikes as a result of action potential firing (20), participate in stimulus–secretion coupling (although not synaptic) and can communicate via gap junctions with neighbouring cells (4), as well as being able to use diffusable paracrine factors to signal to more distant cells (6). Therefore, given their organisation into distinct homotypic structures, endocrine cells may act like a neural network to integrate and propagate information in a temporally precise manner throughout the pituitary gland.

Information flow in different types of network

Neural networks represent a paradigm of a highly connected and highly correlated assembly of active units (neurones). Their connectivity and dynamic behaviour, both individually and collectively, are critical in creating complex information treatment modules throughout the brain. The physiological description of neurone populations in specific brain regions (e.g. hippocampal CA3 neurones) is currently far more advanced than their collective organisational characterisation throughout the brain, both in terms of structure and dynamics, perhaps as a result of technical difficulties in mapping large collections of neurones (21). Nevertheless, in the last decade, it has become apparent that coordinated neurone–neurone communication can be efficiently described using the 'network' paradigm, an adaptation of graph theory that maps information flow in an assembly where the coupling between individual units (or 'nodes') is mediated by specific links (k) (or connections). A large amount of theoretical work has been devoted to identifying the relation between topology and function in such assemblies, using tools from both statistical physics and graph theory, with particular interest on synchronous behaviour of the nodes. In neural science, the interest has been focused on so-called 'scale-free networks' that are characterised by a power-law distribution of the number of links per node (22, 23) (Fig. 1B). In this case, the probability for a node to have k links is defined by:

$$P(k) \sim k^{-\gamma}$$

where γ represents the scaling exponent of the power law function. Consequently, a very small number of nodes (called 'hubs') possess

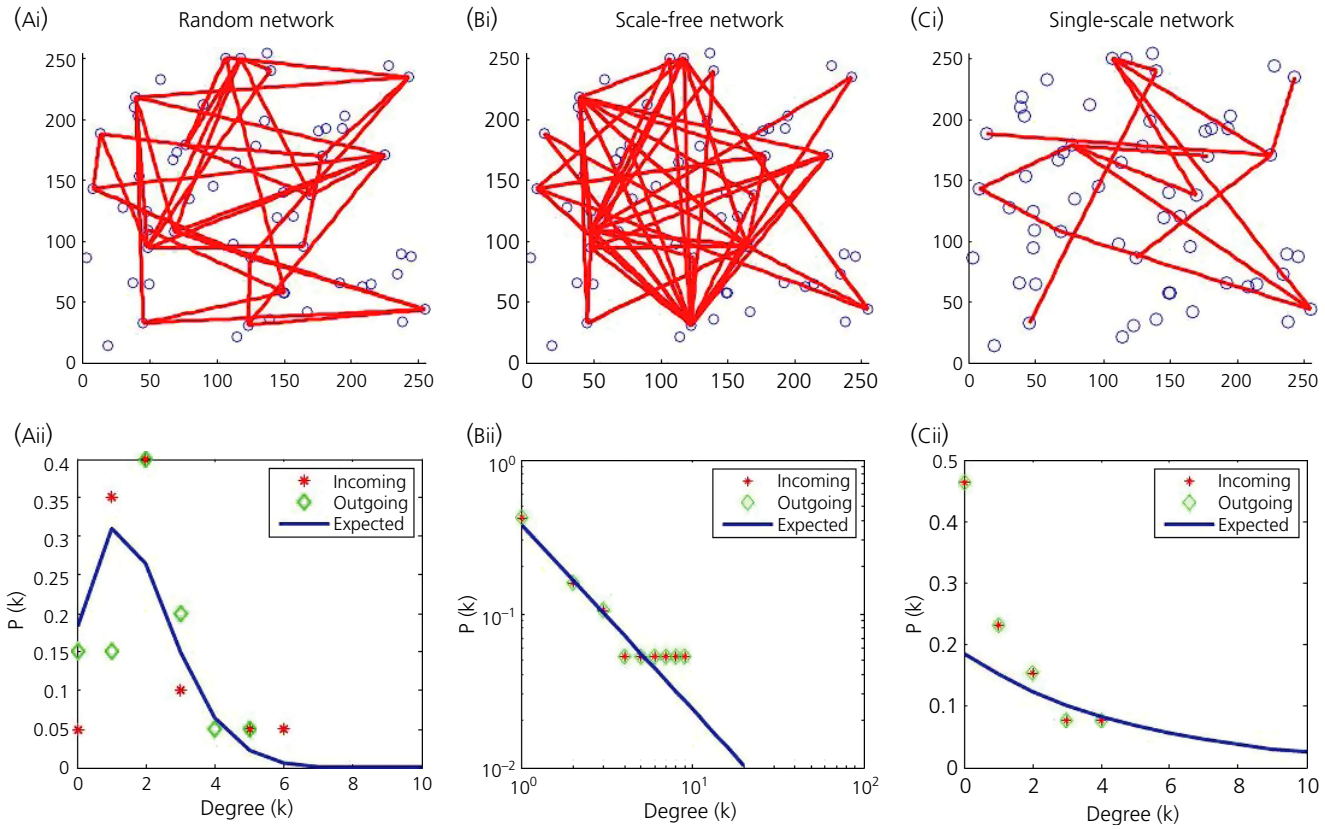

Fig. 1. Schematic representation of characteristic network structures. A random network (Ai) follows a Gaussian (or Poisson) link/degree (k) distribution with a high probability (P) of each node (* and ◇) possessing an average <I> number of links (Aii). A scale-free network (Bi) obeys a power law (Bii), such that a minority (in this example, 0.005%) of superconnected nodes possess the majority of functional links, thus acting as communication 'hubs'. Note that, in (Bii), the *x*- and *y*-axes are displayed on a logarithmic scale to better delineate the relationship between connection/degree (k) and probability (P). A single-scale network (Ci) contains nodes that have a similar degree (i.e. the probability of two unrelated nodes having the same number of links is high) (Cii) forming a lattice-like structure with vertices that are not well connected. After a random attack, communication flow in a scale-free network is maintained as a result of the presence of hubs but is severely disrupted in single-scale and random networks. By contrast, a targeted attack can completely disrupt a scale-free network, whereas random and single-scale networks will emerge largely intact. Graphs were produced using the Complex Networks Analysis Package (http://www.levmuchnik.net/Content/Networks/ComplexNetworksPackage.html) and previously described algorithms (52).

the majority of functional connections and, as such, are directly linked to a large number of other nodes. These 'conductor' or 'communication' hubs represent an efficient way of synchronising the information flow through a whole assembly via their connections. Another aspect of networks obeying a power-law concerns the so-called small world property: the average number of steps (i.e. links) between two nodes of the network is drastically reduced by the existence of the hubs, which are shortcuts between otherwise 'distant' regions of the network (24, 25). As a real-world example, flight paths are both scale-free and small world because a minority of major hub airports (e.g. London Heathrow) receive inputs from multiple smaller 'feeder' airports (e.g. Liverpool and Bristol) before conveying passengers onwards to more far-flung destinations (e.g. the Caribbean). Scale-free and small-world networks have evolutionary advantages in terms of robustness because a random attack (e.g. by a trojan virus on a home PC) will only disrupt a small proportion of links, thereby maintaining information propagation. However, should a hub be targeted (e.g. a server), the effects are catastrophic, and the whole network effectively disintegrates.

Scale-free topology is not the only structure to be relevant for network applications. So-called 'single-scale networks' have an exponential form of the probability P(*k*):

$$P(k) \sim e^{k/k_0}$$

Hubs are not relevant here, and the system possesses a typical scale k_0 for the number of links, meaning that the nodes all have the same degree (number of connections), forming a network with a lattice-like structure (Fig. 1C). The final structure is the 'random network', whose nodes all possess on average <I> of links with Gaussian fluctuations, giving rise to a random number of connections for each node (Fig. 1A). Scale-free and single-scale networks do have the small-world property but not the random networks; detailed descriptions of network structures in biology are provided elsewhere (24, 26, 27). In comparison with scale-free networks, single-scale and random networks are less robust when faced with a random attack because each node is more likely to host a high percentage of the total connections. However, when faced with a targeted attack, they are more resistant because no node hosts

a critical number of connections, unlike hub cells that comprise scale-free networks.

In neural systems, both scale-free and single-scale topologies have been identified. In the rat hippocampus, high-connectivity cells were identified as a first step towards demonstrating the existence of scale free structure (28). By contrast, the neural network of the nematode, *Caenorhabditis elegans*, possesses a single-scale connection distribution, probably reflecting its lower complexity than the vertebrate central nervous system (29). We should note that the existence of hubs is only possible if a given node can accumulate a very large number of links. This is no problem for an internet router, although it can become a stringent constraint in space for a cell of a given shape. In the case of neuronal circuits, the capacity of individual cells to send thin extensions (axons) to create distant cell–cell contacts is a key property in forming numerous links. Thus, scale-free networks should not be expected to be widespread in biological systems that are comprised of space-restricted physical and chemical cell–cell connections. Another issue concerns the network growth processes that are susceptible to promote the emergence of scale-free or single-scale networks. The main mechanism invoked in the case of scale-free networks is the preferential attachment scenario, through which new links added to the network have a greater probability to attach to the most richly connected vertices or hubs ('rich get richer' or 'cumulative advantage' theories) (23, 30). However, the physiological relevance and advantage of such a scenario during central nervous system development remains unclear.

From neural networks to endocrine networks

Endocrine network function is arguably easier to probe than neural network function for a number of reasons: (i) unlike neurones that possess axonal projections of tens to hundreds of micrometres and, as a consequence, can possess many connections, endocrine cells can only form contacts with a few other neighbouring cells, simplifying the process of identifying the structure–function relations that influence network function; (ii) the murine pituitary gland is much smaller than the brain, allowing simultaneous mapping and functional imaging of entire networks as opposed to portions of specific brain regions, such as the optical cortex; (iii) alterations in network function after structural changes in response to physiological demand can be easily assessed (e.g. changes in the GH-network during puberty); (iv) endocrine network function can be directly correlated with an output (i.e. secretion) compared to neural network function, which must be correlated with other neural systems (upwards or downwards) and which can possess multiple and complex physiological outputs; and (v) the effects of *in situ* cell-type specific graded ablation on network structure and function can easily be investigated using genetic approaches (31). Therefore, compared to neural networks, detailing the collective behaviour of endocrine cells in the pituitary may represent a more straightforward process because of the possibility of detailed spatio-temporal mapping, structural manipulation and output monitoring. However, caution must be exercised because extrapolating neural network processes from neurones to a population of endocrine cells assumes that both systems share similarities in input/output and spatial characteristics, which is clearly not the case. Firstly, whereas neurones for the most part communicate via synapses, communication in the pituitary gland can consist of electrical (4, 5), paracrine (6) and juxtacrine modalities (32). Therefore, the relative contribution of communication type to pituitary network function is not easy to define. Moreover, neurone–neurone communication depends not only on the number of physical (synaptic) connections present, but also on the relative balance between well identified excitatory and inhibitory post-synaptic inputs. In cybernetic terms, this constitutes a private network where only cells possessing axonal connections are actually coupled and, as such, the topology has a clear morphological sub-organisation. Similarly, endocrine cell communication via gap junctions and juxtacrine factors would also represent private networks where information propagation would be far-reaching and rapid, at least regarding the former signalling modality. Indeed, we have shown that non-endocrine folliculostellate cells electrically coupled by gap junctions can communicate over long ranges (in excess of 1 mm), providing a possible mechanism by which the activity of distant assemblies of endocrine cells could be synchronised in a manner reminiscent of interneurone function in the brain (5). By contrast, communication via paracrine factor release comes under an entirely different concept, which is typically characteristic of endocrine systems: a public network where the interaction between modules is mediated by diffusable or circulating factors. Hence, all cells are susceptible to shared excitatory/inhibitory inputs. In this context, space filling constraints become all the more important because the diffusion process itself acts as a rate-limiting step, in terms of coupling time and the range of interaction. Therefore, the number of cells that are able to effectively communicate would be solely dependent on particle diffusion dynamics. To address this, we have recently started to define pituitary diffusion parameters (e.g. dextrans with similar sizes to some pituitary peptides travel through the pituitary parenchyma at approximately 11 μm/s to a maximum extent of 82 μm) (3). Nevertheless, the variety of cell coupling mechanisms that may exist in endocrine cell networks, together with their underlying structural constraints, requires careful consideration when attempting to directly import network concepts from neuroscience. Therefore, to accurately describe endocrine cell-network physiology, we require both a quantitative assessment of the 3D structural motifs that may underlie cell–cell communication, as well as a detailed characterisation of the functional relationships in terms of population activity dynamics.

In terms of pituitary network function, it is still unclear what types of information flow exist, and this task is complicated by the need to account for both basal and secretagogue-driven conditions, the absence of quantitative data on the 3D-network structures encountered, and the historic lack of techniques with which to image large populations of endocrine cells in acute pituitary slices (see above). It has previously been reported using two-photon imaging that cells comprising the GH-network are topologically arranged into distinct and interconnected clusters in close proximity to the vasculature (10). This is entirely consistent with information propagation through the network, which, as a result of its scale-

free connectivity, displays a high degree of node clustering, allowing cells both within and between clusters to effectively communicate, even under spontaneous conditions (Fig. 3Biii). Although the exact signalling modalities that contribute to information propagation in the GH-network remain uncharacterised, gap junction communications and paracrine factor release have been demonstrated to be critical for coordinated GH-cell function (4, 6, 33), and a secondary cell type, such as electrically excitable folliculostellate cells, may participate in signalling over long ranges (5, 34).

Quantitative characterisation of pituitary gland 3D network structure

A first step in understanding the collective behaviour of endocrine cells is to reach a quantitative description of its 3D structure, which is an unenviable task. However, the recent advent of transgenic animals possessing fluorescently-tagged endocrine cells; for example, GH-eGFP (15), pro-opiomelanocortin-eGFP (35), prolactin-dsRED (3) and gonadotrophin-releasing hormone receptor-yellow fluorescence protein strains (36), will make the process of detailed cell mapping possible. Using an apotome microscope coupled directly to a vibratome, so that images can be sequentially acquired at confocal-like resolution every 30 μm, image stacks can be registered and a full 3D image of the gland at cellular resolution is obtained. State of the art computational techniques (currently under development in our laboratory) are then used to segment the images to identify the hormonal nature of the cells, as well as their positions. Using this technique, certain parameters can be described in a quantitative manner: (i) topological markers inspired by network modelling, such as the average number of neighbours and the shortest path length between two given cells belonging to a homotypic network (i.e. the GH-cell network); (ii) metric markers more relevant for paracrine interactions because they involve distances between cells, such as 3D correlation functions; and (iii) distances between the cells and the capillaries for different cell types, the organisation of cells in clusters, the differences between physiological conditions and the emergence of the cell network during development. Finally, the physiological relevance of topological versus metric markers can provide an indication about the relative importance of paracrine (long distance) versus nearest neighbour interactions, and thus about the relevant network description. Hence, quantitative structural work is crucial for providing the foundations upon which a functional understanding of cell networks can be formed.

Measuring endocrine cell network function

Following characterisation of the cell-network structure, how can network function be reliably assessed? In most cases, the measurement of intracellular calcium rises provides the best method to assess the dynamics and organisation of network activity for a number of reasons. First, calcium is a critical second messenger for hormone release (37, 38) and is also involved in information transfer between connected cells (39). Second, calcium fluctuations can be simultaneously monitored in hundreds of cells using either commercially available calcium indicators (e.g. Fura-2 and Fluo-4) (40) or mouse strains expressing genetically encoded calcium indicators in combination with rapid-scan microscopes (41, 42). By contrast, although secretory events can be observed in a small numbers of endocrine cells using secreted dyes or total internal reflective microscopy (43), they cannot yet be reliably recorded *in situ* in populations large enough to provide an accurate representation of network activity over time. Lastly, in combination with transgenic animals possessing fluorescently-tagged endocrine cells, the network-activity of specific subpopulations of endocrine cells can be determined. Using these methods, the distribution of calcium spiking events over time, which can be assumed to be a reasonable estimate of secretory activity of a cell, can be assessed for a large number of individual cells/neurones belonging to a large proportion of a given endocrine/neural network. By comparing the profiles of calcium activity of all the recorded cells, the ability of cells to display temporally correlated behaviour, and hence simultaneous secretory behaviour, can be calculated. By contrast to neuronal network events, which typically occur over time scales of seconds to minutes, network events in endocrine cells occur over the range of minutes to hours (over a time frame similar to the associated hormonal output). This requires special consideration of the techniques used because calcium activity from a large population of cells must be stably recorded for long periods of time (60–90 min). Furthermore, because endocrine, paracrine, juxtacrine and autocrine interactions can alter aspects of cell function unrelated to secretion through effects on intracellular calcium concentrations (6), analysis readout must be interpreted carefully; a review of the multitude processes controlled by calcium is provided elsewhere (44).

To address these issues, we have recently adapted two-photon calcium imaging techniques for use on acute pituitary slice preparations in which cell/tissue architecture is preserved (Fig. 2). Because two-photon excitation results in less emitted photons than single-photon (i.e. confocal) excitation, long acquisition times are generally required to resolve an image using a single raster-scanning beam. To overcome this problem, we use a multiplexed femtopulsed laser, which splits the beam into 64-beamlets, each with a small scan-field, allowing tissue to be imaged at low laser powers with a pixel dwell time (approximately 20 ms) that would not normally be compatible with emission of a sufficient number of photons to successfully resolve an image. Importantly, this means that a large section of pituitary gland (up to 500 $\times$ 500 μm; approximately 400–800 cells) can be rapidly scanned for each acquisition, such that events occurring at one edge of the pituitary gland are not temporally dissociated with events occurring at the opposite side of the tissue, as would be the case with conventional line-scanning microscopes (45). In addition, because of the use of infrared wavelengths coupled with the short exposure of tissue to each beamlet (and hence the damaging laser), multicellular calcium recordings can be obtained in a 3D cell context for long periods of time at the same time as reducing phototoxicty, photobleaching and artefacts as a result of recording cells near the cut surface, which are problems typically associated with using either standard epifluorescence and confocal techniques; examples of neural network studies using this setup are provided elsewhere (28, 46). Currently, two-photon multibeam imaging depth (approximately

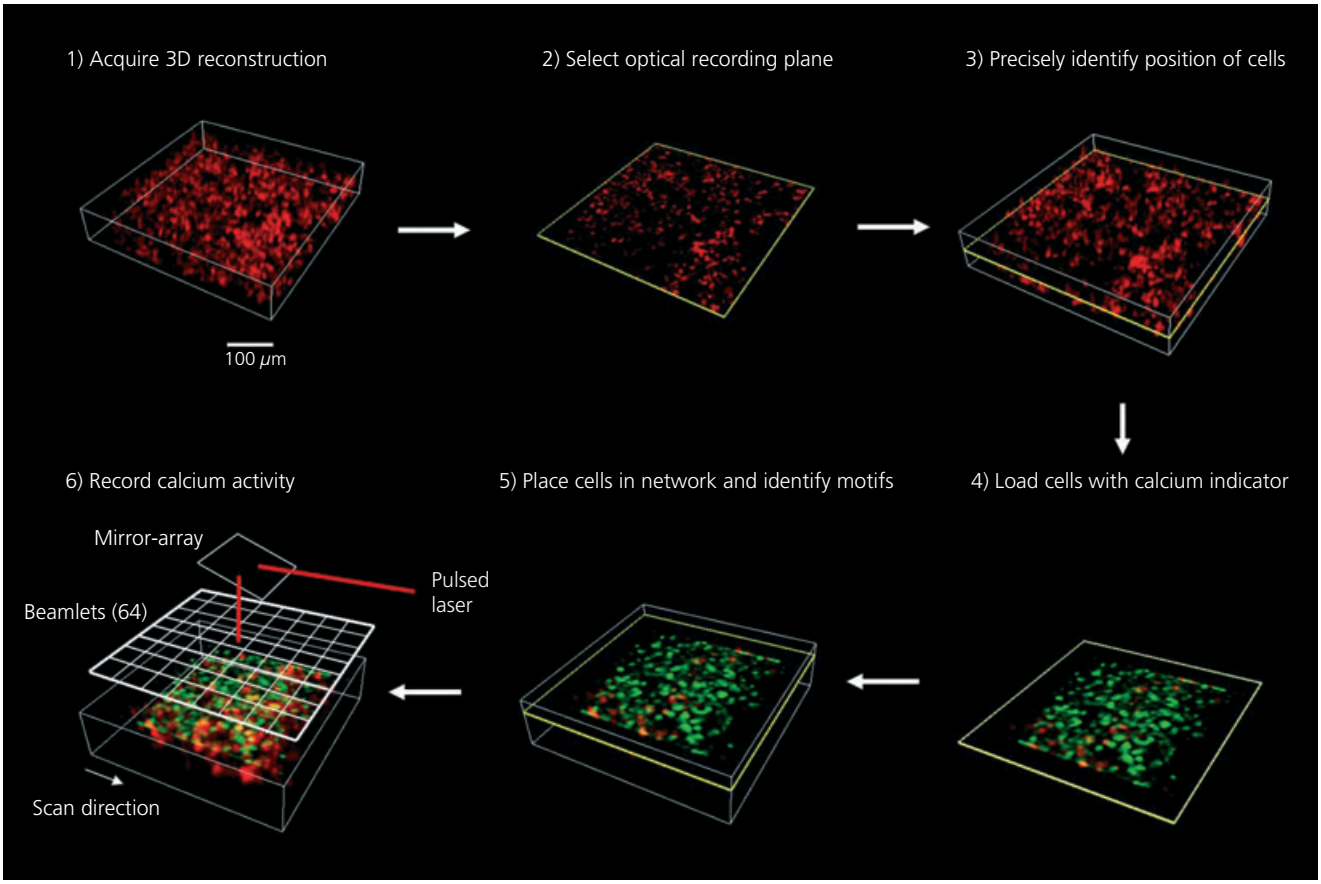

Fig. 2. Two-photon calcium imaging of endocrine network function. Schematic depicting calcium imaging of acute pituitary slices, in this case from animals expressing dsRED in pituitary lactotrophs (red). Tissue is subjected to 64-beam multiphoton imaging, which allows a large area of calcium-indicator loaded tissue (green) to be rapidly scanned during each acquisition. Hence, a 'snapshot' of network function can be obtained at depth (40 μM) within the endocrine tissue of choice.

40–60 μm) is limited by a high degree of beamlet scatter in pituitary tissue, limited uptake of the AM-ester calcium dyes into the first few cell layers and infrared laser penetration at calcium indicator excitation wavelengths of 780 nm (i.e. for Fura-2AM). However, the use of animals possessing genetically encoded calcium indicators, far infrared lasers (47) and the development of spatio-temporal excitation–emission beam multiplexing (i.e. decreasing scatter and allowing use of highly sensitive hybrid photomultiplier tubes) will mean that resolvable imaging depths of up to 200 μm should be achievable in the future. Until the more flexible and rapid spatial light modulator 'scanless' multiphoton microscopes become commercially available (48), multibeam rapid-scan imaging and, to some extent, Nipkow spinning disk confocal setups (49), remain the benchmarks for assessing network activity.

Correlation analysis and cell network mapping

After data acquisition, appropriate analyses of cell–cell correlation must be performed. For long time-scale recordings, cell activity (Fig. 3Ai) can be binarised such that any time-point presenting with a deflection of 20% over the baseline (to account for the signal-noise ratio) is represented with a '1', whereas inactivity is represented with a '0'. Because this analysis assumes a stable baseline, we have developed a specific tool using empirical mode decomposition algorithms (http://perso.ens-lyon.fr/patrick.flandrin/emd.html) embedded in a semi-automatic user-friendly environment in which the operator can retrieve the baseline trend and identify the activity events present in the calcium traces. Once the signal has been binarised, the activity of all the cells in the field can be represented as a raster plot (Fig. 3Aii), and correlations of activity measured as:

$$C_{ij} = \frac{T_{ij}}{\sqrt{T_i T_j}}$$

where T_{ij} is the total coactivity time and T_i and T_j are the total activity time for the two cells considered. It should be noted that this analysis measures correlation as a function of temporal synchronisation and is unable to discriminate the spatial distribution of correlation events making recurrent and temporally complex network correlation events difficult to assess; a recent example of a sophisticated analysis that can account for the spatial nature of correlation is provided elsewhere (50). Moreover, because the directionality of correlation requires the presence of systematic time lags between similar events in signal pairs (Granger causality), and this is not the case in our experiments, analysis is solely limited to

observations of synchronous activity (51). After analysis of the calcium traces, a correlation matrix for all the cell pairs can be obtained, and the significance of the measured correlations is assessed against chance by subjecting the activity events of each cell to random Monte Carlo shuffling (Fig. 3Aii). Using the significant correlations, a weighted graph that displays the distribution of connections (k) between nodes (n) is constructed, allowing the cell network to be depicted (Fig. 3Biii). Subsequently, typical P(k) plots (averaged for many independent samples), clustering coefficients, network diameter, path length and the correlation value can all be

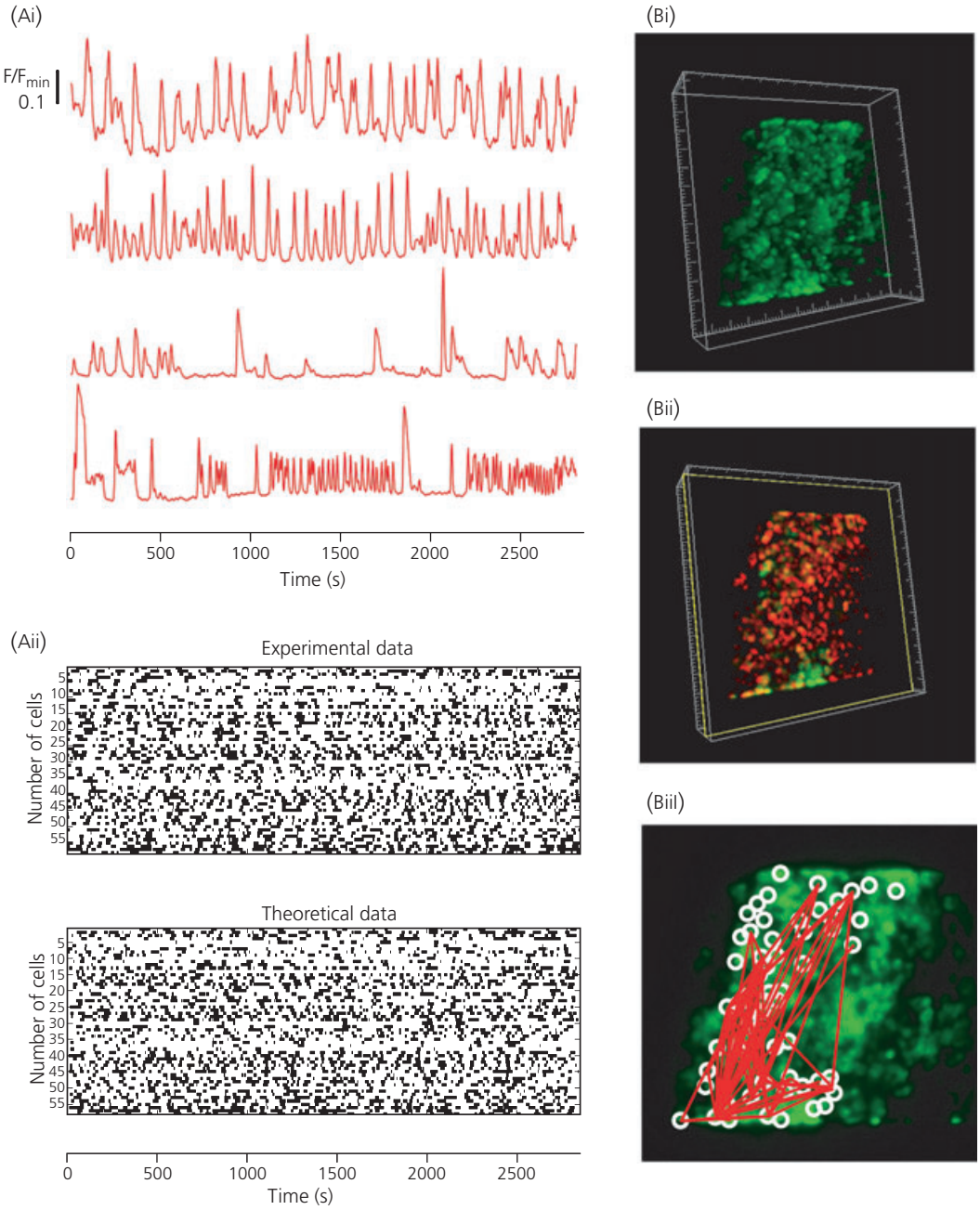

Fig. 3. Analysis and mapping of endocrine network activity. Example of correlation analysis from a 45 min calcium recording of spontaneous growth hormone (GH)-cell network function from pituitary glands of GH-enhanced green fluorescent protein mice. Representative raw calcium traces are shown for four cells (Ai). Experimental and simulated/theoretical (Aii) (subjected to Monte Carlo random shuffling) raster plots display cell activity (black) as a function of time. Subsequently, significantly correlated ($P < 0.05$) cells belonging to the GH-network (Bi) and loaded with calcium indicator (red) (Bii) are connected by a solid line (Biii), allowing functional mapping of cell connectivity. Note that: (i) spontaneous GH-cell calcium spiking activity is reasonably stochastic as a result of the requirement for the presence of secretagogue to display high levels of coordinated activity (10); (ii) despite this, cell–cell communication under spontaneous conditions is scale-free and highly clustered, reflecting the topology of the network; and (iii) cells are able to communicate over long distances, reflecting the existence of either specialised long-range signalling pathways or short-range mechanisms, such as gap junctions, which may couple multiple chains of cells that are out of view.

calculated (algorithms are freely available as Matlab code with graphic user interfaces at: http://ipam.igf.cnrs.fr/). Because the outright correlation value is not illuminating in the context of system spontaneous activity, it is better to set a significance threshold and plot all the cells that surpass this. However, significance value may become relatively more important when looking at network perturbation after, for example, the application of secretagogue. After mapping of the functional network in 2D, the contribution of structural motifs to correlation events can be assessed using the 3D homotypic network structure data obtained from the same sample (Fig. 2).

Conclusions and perspectives

The detailed study of the organisation and function of the pituitary gland demonstrates that concepts from the neurosciences are excellent candidates for the understanding of cell population level regulation in neuroendocrine tissues. Hormone-secreting cells, although different from neurones in many respects, share electrical excitability and strong coupling mechanisms, leading to network-level response integration. The constraints specific to their physiology, both temporally and spatially, have to be incorporated, and the concepts relevant for neural networks must be extended, to account for paracrine and juxtacrine biochemical coupling. Nonetheless, the main tools from network science are applicable, and the pituitary endocrine system can even claim to possess significant advantages in terms of structure–function coupling. The network structure is far simpler than in the brain because only five secretory cell types are present and cell shapes are typically compact, allowing only neighbour–neighbour direct coupling or long distance factor exchange via diffusion (for a glimpse of neural network structure complexity, see the Blue Brain Project: http://bluebrain.epfl.ch/). The relative weight of these mechanisms on different time scales can be assessed using currently available methods and the effects of manipulating network activity on hormone secretion, an output of direct physiological relevance, can be assessed. This description of the collective behaviour of cells in the pituitary gland constitutes an important step in a full multiscale description of the complete neuroendocrine function, which must further be coupled to other mechanisms known to exist at different levels, such as the cell/brain/whole body. It is envisaged that studies of this type will be perfectly suited for integration into a global project of multilevel modelling and the analysis of physiological functions such as the IUPS physiome project (http://www.physiome.org.nz/).

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