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Abstract

The neuroendocrine systems of the hypothalamus are critical for survival and reproduction, and are highly conserved throughout vertebrate evolution. Their roles in controlling body metabolism, growth and body composition, stress, electrolyte balance and reproduction have been intensively studied, and have yielded a rich crop of original and challenging insights into neuronal function, insights that circumscribe a vision of the brain that is quite different from conventional views. Despite the diverse physiological roles of pituitary hormones, most are secreted in a pulsatile pattern, but arising through a variety of mechanisms. An important exception is vasopressin which uses bursting neural activity, but to produces a graded secretion response to osmotic pressure, a sustained robust linear response constructed from noisy, nonlinear components. Neuroendocrine systems have many features such as multiple temporal scales and nonlinearity that make their underlying mechanisms hard to understand without mathematical modeling. The models presented here cover the wide range of temporal scales involved in these systems, including models of single cell electrical activity and calcium dynamics, receptor signaling, gene expression, coordinated activity of neuronal networks, whole-organism hormone dynamics and feedback loops, and the menstrual cycle. Many interesting theoretical approaches have been applied to these systems, but important problems remain, at the core the question of what is the true advantage of pulsatility.

1. Introduction

The classical neuroendocrine systems (Figure 1), those that control hormone secretion from the pituitary gland, have been called 'windows on the brain' because of the ease with which we can relate their properties to physiological function. Many of these systems have attracted interest from modelers [1, 2], and the purpose of this review is to introduce these systems to a wider community of theoreticians, by outlining the biology and highlighting a few of the many unresolved problems.

The neuroendocrine systems generate complex patterns of hormone secretion that involve ultradian pulses of secretion (with periods of minutes to hours) superimposed upon daily (circadian) rhythms. These patterns are generated by interactions between hypothalamic neural networks, the pituitary gland and the target organ, with feedbacks at several levels on different timescales. They are modulated by changes in physiological state, and vary across the ovarian cycle in females and circannually in seasonal animals. Understanding the origin of these complex patterns, whose mechanisms involve multiple nonlinearities across diverse timescales has been a major challenge for experimental neuroendocrinologists.

Mathematical modeling has made an invaluable contribution to our understanding of these patterns, including for example through applications of geometric singular perturbation theory [3], multiple time scale analysis and the theory of mixed-mode oscillations and canards [4, 5], and has provided some persuasive and simple explanations of apparently counter-intuitive experimental observations. However, each of the neuroendocrine systems presents its own, unique questions and challenges, and many open problems remain.

The neuroendocrine systems can be divided into those of the posterior pituitary and the anterior pituitary (Figure 1). The posterior pituitary functions as a hormonal release site for the hypothalamic neurons which directly signal the body, secreting hormone through electrically signalled axonal projections. The anterior pituitary is a more complex system in which networks of endocrine cells manufacture and secrete hormones under the control of hypothalamic peptides, or 'hormone releasing hormones' secreted from hypothalamic neurons projecting to the median eminence into the portal blood supply, a network of small blood vessels that supply the anterior pituitary. This form of transport means that the anterior pituitary cells receive single combined signals from each peptide rather than many pulses from the thousands of hypothalamic neurons.

We begin here with the most fully characterised and most systematically modelled of the neuroendocrine systems – those that secrete the hormones oxytocin and vasopressin from the

posterior pituitary, hormones that are essential for reproduction and fluid and electrolyte balance respectively, and then go on to address the systems that control hormone secretion from the anterior pituitary

2. The magnocellular neurosecretory systems

Oxytocin and vasopressin are secreted from the nerve endings of neurons whose large (magnocellular) cell bodies are aggregated mainly in the supraoptic nucleus and the paraventricular nucleus of the hypothalamus [6]. The paraventricular nucleus harbors a mingled multitude of cell types, but the supraoptic nucleus seems to be designed for the convenience of the inquiring neuroscientist. This nucleus borders the ventral surface of the brain and the optic chiasm, making it easy to recognise and dissect. Each of its 3000 neurons (in the rat) synthesise either oxytocin or vasopressin, and each projects an axon to the posterior pituitary with no axon collaterals that arise or end within the supraoptic nucleus itself. The hormones are packaged in vesicles that are secreted in response to spike activity propagated down the axons, and so much hormone is secreted from the nerve endings in the posterior pituitary that, even after dilution in the systemic circulation, the concentrations of oxytocin and vasopressin are still high enough for physiologically important effects.

Every mammal makes vasopressin and oxytocin – and, in every other vertebrate, homologs are made in the hypothalamus and secreted from a posterior pituitary gland. In mammals, oxytocin is essential for milk let-down in response to suckling, and it drives the uterine contractions that deliver live young into the world [7]. Oxytocin also affects reproductive and social behaviors through its actions within the brain [8, 9]: notably, it promotes maternal behavior (in rats), bonding with offspring (in sheep), sexual behavior (in many species including male and female rodents) and mating-induced partner bonds (in monogamous rodents). It is also anxiolytic, supporting social interactions by suppressing anxiety [10, 11]. It is also involved in regulating energy balance [12]: it is anorexigenic, and suppresses voluntary intake of sweet carbohydrates; it promotes energy expenditure and thermogenesis (in mice). In some species, including rats, it promotes sodium excretion (natriuresis) by its actions at the kidneys [13] and on the heart, where it stimulates the secretion of natriuretic peptide [14]. Thus the functions of this hormone are diverse within species and vary between species, even though the anatomy, biochemistry, and pharmacology of the system are all largely conserved [15].

Vasopressin has two classical roles – at the kidney it regulates water reabsorption to help maintain a constant plasma concentration of sodium and a constant plasma volume, and it controls the dilation of peripheral blood vessels to maintain a constant blood flow as plasma volume changes. It too has behavioral effects, including on aggressive and social behaviors, and it

also regulates blood pressure, stress, circadian rhythms and thermoregulation. Vasopressin is not only expressed in magnocellular neurons, but also in neurons that regulate the stress axis, in neurons of the suprachiasmatic nucleus that regulate circadian rhythms [6], and in diverse other populations, including in the olfactory bulbs [16] and retina [17], so the diverse roles of vasopressin on behavior might partly be explained by compartmentalisation of function in these different subsets. However, oxytocin neurons are present *only* in the hypothalamus, and virtually all of them project to the posterior pituitary. Only a few oxytocin cells project exclusively within the brain [18]: in the rat, these mainly project to the dorsal vagal complex in the posterior brainstem to regulate gastric reflexes and to the spinal cord, to modulate penile erection and pain responses.

2.1 Oxytocin

In the supraoptic nucleus, every oxytocin cell projects to the posterior pituitary and is involved in both reflex milk ejection during lactation, and in regulating uterine contractions. The cells are also osmosensitive, regulating natriuresis, and are also regulated by a variety of signals that control appetite, including neural and hormonal signals that arise from the gut [6, 19-21]. All are also involved in sexual behavior, anxiety-related behaviors, and social behaviors. The challenge is thus to understand how a single population of neurons can coherently regulate such an apparently diverse set of functions – and different behavioral functions in different species.

Different physiological responses in part arise from different patterns of spike activity. In response to raised plasma osmotic pressure, oxytocin cells increase their firing rate proportionately and fire continuously, and this evokes a proportional increase in oxytocin secretion that exerts a sustained and graded effect on natriuresis [22]. In response to suckling, the same cells fire in brief, intense synchronised bursts that occur every few minutes; these bursts lead to a secretion that is massively amplified by non-linearities in stimulus-secretion coupling at the nerve terminals [23], producing a sequence of large but brief pulses of oxytocin secretion that produce milk let-down at the mammary glands (the ‘milk-ejection reflex’; Figure 2). The mammary glands, being relatively insensitive to oxytocin, are indifferent to the lower sustained concentrations induced by osmotic challenge, while the kidney is indifferent to brief intermittent pulses, requiring as it does a steady signal.

Thus oxytocin cells can simultaneously regulate milk-let down and natriuresis without conflict, because the two stimuli – from raised plasma sodium and from the suckling of hungry young – produce graded increases in firing rate [22] and bursting activity [21] respectively. Even in a suckled lactating rat exhibiting a series of intermittent milk-ejection bursts, an increase in

osmotic pressure induced for example by intraperitoneal injection of hypertonic saline will raise the basal firing rate of the oxytocin cells in the same manner that it does in virgin rats: the milk-ejection bursts will continue, superimposed on this elevated baseline, with only a modest change in the pattern of bursting – a transient reduction in burst frequency and a sustained increase in amplitude [24].

The sensory stimulus, from a litter of suckling young, is continuous and unpatterned – there is no pattern in this that can explain the bursting pattern of the oxytocin cells. So how can the oxytocin cells encode some stimuli (such as plasma sodium concentration) linearly but encode others (suckling) as a pattern of intermittent pulses?

In a non-lactating rat, oxytocin cells fire sparsely and independently. These cells are not spontaneously active in the absence of synaptic input; the spikes are triggered by summating excitatory postsynaptic potentials (EPSPs) from afferent neurons in a wide diversity of locations. Spikes are followed by a large hyperpolarising afterpotential (HAP) that produces a relative refractory period of about 30 ms, and by a much smaller but much longer-lasting afterhyperpolarisation (AHP) which markedly suppresses activity after a burst of spikes [25]. These cells are also *osmoreceptive* –a rise in osmotic pressure leads to a graded depolarisation that is amplified by synaptic inputs from other osmoreceptive neurons in the forebrain [26]. This spiking behavior of oxytocin cells in basal conditions can be closely matched by a leaky integrate-and fire model modified to incorporate a HAP, and an AHP [27]. Though simple, this model has a very close match in its spiking behavior to a Hodgkin-Huxley type model that incorporates the known biophysical properties of oxytocin neurons [28]. Because we have a detailed understanding of the complex, non-linear relationship between spike activity and oxytocin secretion from the axon terminals in the pituitary, it is possible to combine the spiking model with a quantitative model of stimulus-secretion coupling, and because the pharmacodynamics of oxytocin in the blood are well characterised, it is also possible to use this framework to simulate the changing plasma concentrations of oxytocin [29].

To model milk-ejection bursting however, we need to model interactions between the oxytocin cells. During suckling oxytocin cells they communicate via dendritic release of oxytocin. The dendrites contain abundant neurosecretory vesicles, and in lactating rats the dendrites are wrapped together in bundles of 8-15 directly apposed dendrites, each bundle ensheathed by the sheet-like processes of specialised glial cells. This anatomical rearrangement is dynamic and is itself governed by oxytocin release from the dendrites [30].

Within the dendrites, neurosecretory vesicles are constrained by an intracellular network of actin filaments. Dendritic release is not always regulated by spike activity because the vesicles

are not docked at the plasma membrane, but the vesicles can be released by peptides that mobilise intracellular Ca^{2+} stores, and, importantly, such signals can 'prime' activity-dependent dendritic release[31]. *Priming* involves a re-organisation of the filamentous actin network [32] to translocate vesicles to sites close to the membrane, where voltage-gated Ca^{2+} channels are densely expressed [33]. Primed vesicles can thus be released in response to spikes, propagated down the dendrites, that trigger voltage-gated Ca^{2+} entry. In lactating rats, oxytocin cells express oxytocin receptors and oxytocin is excitatory, hence priming reconfigures the network to introduce positive feedback, and this underlies the capacity of oxytocin cells to display synchronised bursts [34].

To model that behavior, Rossoni *et al.* [35] generated a population of integrate-and-fire neurons, each with a HAP and an AHP, receiving independent, random synaptic inputs (Figure 2). The patterns of spike activity in this model are quantitatively indistinguishable from the observed spontaneous spiking patterns of oxytocin cells. The model neurons were sparsely coupled by simulating weak dendro-dendritic interactions that involve priming-dependent excitation (mediated by oxytocin release that is a function of spike activity with the same non-linearity of secretion as observed in axonal terminals) together with a slower, activity-dependent suppression of secretion mediated by endocannabinoids acting presynaptically on afferent inputs, a phenomenon identified in electrophysiological studies. The model displays intermittent synchronised bursts that arise as an emergent property of a complex system, and the shape of the bursts is moulded by the characteristics of the AHP [35-37]. The bursts in the model are quantitatively indistinguishable from observed bursts in their temporal profile, and the model shows striking quantitative concordance with a number of experimentally observed "paradoxical" behaviors – including an apparently paradoxical stimulation of bursts by inhibitory signals.

This is a complex model with many variables and complex network topology, but it can be reduced through bifurcation analysis to a tractable model with two variables, which retains the key qualitative features of the original model. This approach indicates how emergent synchronous bursting can arise from a neuronal network which embodies known biological feature, and suggests a generic way to exhibit emergent and multiple time scale oscillations [36].

Still unexplained by this model are two important things: exactly what signal from the suckling input primes dendritic oxytocin release to initiate a sequence of suckling-induced bursts, and how the bursts are synchronised between the different hypothalamic nuclei. The priming

signal seems likely to be a peptide secreted from neurons in the brainstem that receive inputs from the spinal pathways activated by suckling, while bursting might be co-ordinated between nuclei by a small population of oxytocin neurons that project from the paraventricular to the supraoptic nucleus [18], but these speculations remain to be confirmed experimentally.

This explanation of the milk-ejection burst was derived from the discovery of priming and its implementation in a mathematical model. It contains an implicit explanation of how the oxytocin cells can regulate central behaviors independently of their peripheral functions, and also an implicit explanation of how a transient oxytocin signal can have prolonged behavioral consequences – including on maternal behavior and social behavior. Whereas oxytocin secretion from nerve terminals is governed only by spike activity (via voltage-gated Ca^{2+} entry), oxytocin release from dendrites can be evoked by agents that mobilise intracellular Ca^{2+} – the cells are 'hybrids' of classical neurons and classical endocrine cells. Considerable amounts of oxytocin can be released from the dendrites, and because oxytocin has a relatively long half-life within the brain and acts at high affinity receptors, what is released can have extended 'hormone-like' actions on relatively distant sites [38, 39]. In the brain, oxytocin can act on glial cells in the hypothalamus to re-organise their architecture, and can prime peptide release from its neuronal targets, thereby altering their functional connectivity. Oxytocin, like other peptides, may also have extended effects on gene expression in its targets. Accordingly, the actions of oxytocin in the brain are akin to a re-programming of neuronal networks – and it is in this re-programming we can glimpse an understanding of how oxytocin can exert long term effects on complex behaviors[40].

2.2 Vasopressin

Vasopressin secreted from the posterior pituitary is essential for maintaining fluid and electrolyte balance. Its principal action is at the kidney: when water loss from the body exceeds replenishment through drinking, increased vasopressin secretion will restrict water loss by concentrating the urine – hence its older name, 'antidiuretic hormone'. Loss of body fluid also requires compensatory mechanisms in the vasculature –the vasoconstrictive actions indicated by the name 'vasopressin'. Vasopressin cells, like oxytocin cells, are regulated by an osmoreceptive mechanism – they respond directly to the osmotic pressure of their external environment, and they receive synaptic inputs from other osmosensitive neurons [41]. As far as is known, the mechanisms of osmosensitivity are the same for vasopressin cells, oxytocin cells, and for the other osmoreceptors in the forebrain. [42]

As well as receiving osmotic pressure encoding synaptic input, vasopressin cells, like oxytocin cells, are intrinsically osmoreceptive. When the external osmotic pressure rises, vasopressin cells, shrink as water leaves them, activating a depolarising current. This small voltage change increases the probability that any given synaptic input event will trigger a spike: in the presence of a vigorous level of afferent input, even a small increase in osmotic pressure will produce a depolarisation that changes the mean firing rate [43]. Thus noise, in the form of random synaptic input, enhances the sensitivity of vasopressin and oxytocin cells to osmotic pressure changes.

Whereas oxytocin cells fire continuously in response to raised osmotic pressure, vasopressin cells fire *phasically*, in bursts of spikes at 4-10 spikes/s that typically last for 20-60 s, separated by silent intervals of 15-30s. However, vasopressin cells fire asynchronously, so the output from the population as a whole is *not* phasic but continuous. This leaves an interesting question of what functional purpose is served by phasic firing in vasopressin neurons.

How these bursts arise and their consequences for secretion have been extensively studied and modeled. In vasopressin and oxytocin neurons spike patterning is shaped by activity dependent afterpotentials such as the HAP and the AHP. Most of these afterpotentials are driven by Ca^{2+} dependent ionic currents. Activity-dependent mechanisms that act on longer timescales than the very short lived spikes (a few ms) require a longer lasting intermediary signal. In vasopressin (and oxytocin) cells, each spike triggers a rapid entry of Ca^{2+}, and during repeated spike activity the intracellular Ca^{2+} concentration rises to a mean level that effectively encodes the recent history of spike activity. This Ca^{2+} signal is critical for generating the bursts in vasopressin cells, but exactly what type of current is involved is still uncertain: it seems either that the raised intracellular Ca^{2+} activates a slow depolarising afterpotential (DAP), or that it suppresses a hyperpolarising K^+ 'leak' current. Both the DAP model, and the K^+ leak current model's (Figure 3a) double negative of switching off an inhibition, result in a positive feedback, generating a self-sustaining prolonged plateau potential which raises the probability that EPSPs will exceed the spike threshold and generate a burst of spikes. . Bursts begin with relatively intense firing that subsides, as a result of activation of a slow AHP, to a stable firing rate [25, 44] (Figure 3b). The AHP must be sufficient to stabilise the spike rate but not terminate the burst. The bursts are terminated by a more unusual mechanism: sustained spike activity leads to sparse release of vesicles from the dendrites of vasopressin cells, and these vesicles contain not only vasopressin but also the opioid peptide dynorphin, which acts back at kappa opioid receptors on the cell of origin to oppose the Ca^{2+} signal's activation of the burst-sustaining mechanism.

Modeling was used to understand how this might work, and predict which might be the

real mechanism. Models show that both mechanisms are capable of generating bursts but that only the K^+ leak current mechanism can also explain the long silent periods between bursts. Both the Ca^{2+} signal and the dynorphin signal are spike activity dependent, but with a slower decay rate (typically a 2.5s half-life for Ca2+ and 10s half-life for dynorphin) and smaller increment per spike, the dynorphin signal accumulates more slowly. A burst is initiated by a randomly rapid cluster of spikes, sufficient to activate a self-sustaining Ca^{2+} signal. The dynorphin signal accumulates during a burst, progressively limiting the intra-burst spike rate in a similar manner to the AHP, but its negative effect also limits its own accumulation. To match the long bursts we observe the dynorphin parameters must be tuned such that the dynorphin signal is able to terminate a burst only in conjunction with another randomly rapid burst of spikes. Thus the dynorphin signal does not determine burst termination, but merely makes it progressively more likely. The mechanism can only function when subject to a noisy synaptic input signal. In the following silent period, when the burst sustaining plateau and the Ca^{2+} signal have rapidly collapsed, the leak current is fully active, sustained by the residual, slow decaying, dynorphin signal. Thus the K^+ leak current model explains both the bursts and the silences. The depolarising-afterpotential based mechanism requires some other hyperpolarising mechanism or the suppression of EPSPs to explain the silent period. The AHP, which regulates spike rate during the burst, is necessarily too small to completely silence the neuron. In summary, the phasic firing is generated by competing positive and slower negative feedback mechanisms (Figure 3) that combined with a noisy input signal result in emergent bistability. The unusual mechanism would make an interesting and possibly challenging case for dynamical systems analysis. As *bistable oscillators* the cells have some intriguing signal-processing properties: most obviously, impinging transient excitatory signals will trigger bursts if they arrive during the silent period of a cell, but can truncate bursts if they arrive when the cell is active [45]. Because the vasopressin cells fire asynchronously, this property ensures that the population can filter out transient signals while remaining responsive to small sustained signals [46-48].

The remaining open question is what is the purpose of this phasic firing? The spiking activity of vasopressin cells is coupled to secretion by highly non-linear mechanisms: secretion is subject to frequency-facilitation: at increasing spike frequencies, more vasopressin is secreted on average per spike up to about 13 spikes/s. However continuous activation at this frequency results in fatigue of secretion: thus the vasopressin cells fire in a phasic pattern that optimises the efficiency of secretion, with bursts of optimal fast spiking separated by silent periods that allow recovery from fatigue. However, it would be injudicious to see this as a full explanation for why vasopressin cells fire phasically. Phasic firing is efficient for secreting vasopressin because the

particular properties of the vasopressin axon terminals make it so. But the axon terminals of oxytocin neurons have different properties, so the electrical properties of the vasopressin cells and the properties of the terminals have co-evolved.

Thus vasopressin cells exhibit complex non-linearities in their spike-generating mechanisms, and these are coupled to complex non-linearities in their stimulus-secretion properties. For any individual vasopressin cell, there is thus a highly non-linear relationship between afferent stimulation (the mean rate of EPSPs) and secretion, and this relationship has a quite narrow dynamic range. To understand the relationship between mechanisms and function, we need to look not at individual cells, but at a population. Vasopressin cells are highly heterogeneous in their firing rates and spike patterning, thus the varying non-linear responses of individual cells are spread over the input range. Because the functional signal is the summed secretion over the neural population, this heterogeneity linearises the population response and increases the dynamic range, producing a linear secretion response very close to that observed *in vivo* [49].

Vasopressin cells must also sustain their response during prolonged and progressively increasing osmotic challenge, over hours and days. They must balance immediate response to osmotic challenge with preserving vasopressin stores for as long as possible, as depleted vasopressin stores combined with lack of access to water rapidly lead to fatal dehydration. In a model of vasopressin secretion that links electrical activity to secretion, fatigue of stimulus-secretion coupling helps to maintain a consistent response to stimulation in the face of progressive depletion of the pituitary store of vasopressin. In the model, the presence of a fatigue mechanism enables a consistent response to osmotic stimulation to be maintained until the stores have reached about 50% depletion. In a heterogeneous population, individual cells will become depleted at different times: the slowest cells maintain their response for 24 h, but the most active cells become depleted, and their responses become proportional to their reserve store levels much sooner. However, although the decline in the overall secretion of the population begins at a time determined by the most active cells, heterogeneity in the vasopressin cells reduces the rate of the decline in the population signal [49].

To summarise, the adaptive value of the properties of vasopressin cells only become apparent when we consider the population as a whole. Then we see a system that (i) operates with extremely high threshold sensitivity; (ii) sustains a constant response to a constant stimulus that is linearly proportional to the stimulus intensity over a wide dynamic range; (iii) filters out transient fluctuations; and (iv) maintains output over relatively prolonged stimulation. These

characteristics depend respectively on (i) noise; (ii) heterogeneity; (iii) bistability in the neurons that constitute the system; and (iv) properties of stimulus-secretion coupling.

While we can thus construe a hypothesis about the adaptive value of phasic firing, this remains to be fully tested. We can infer the benefits of phasic firing – but have a poor understanding of the energetic costs of the mechanisms involved. On the face of it, the vasopressin neurons seem remarkably profligate in their expenditure on action potentials. At conventional synapses, typically about one synaptic vesicle is released, on average, for every spike that invades the synaptic ending. At each of the nerve endings and swellings of the axons of vasopressin neurons, it takes on average, *several thousand* spikes for the release of a single neurosecretory vesicle.

Neurosecretory vesicles contain a cargo far more precious and potent than that of synaptic vesicles [39]: a typical synaptic vesicle contains about 5,000 molecules of glutamate, which acts with micromolar affinity at postsynaptic ionotropic receptors, and is rapidly cleared from the synaptic cleft by glutamate transporters that recycle the glutamate, allowing synaptic vesicles to be quickly refilled and available for re-use. Thus the actions of a synaptic vesicle are localised in space – normally just to the postsynaptic site, are very restricted in time – with a half-life of just a few milliseconds, and synaptic release can follow spike activity relatively faithfully. By contrast, the large dense-cored neurosecretory vesicles that contain oxytocin or vasopressin contain about 85,000 peptide molecules that have nanomolar affinity for their G-protein coupled receptors. These are neither rapidly inactivated by enzymes nor are they actively transported into cells; in the circulation oxytocin and vasopressin have a half-life of 2-5 min, and in the CSF a half-life of about 20 min. Hence these signals are not restricted to synapses – indeed, whereas small synaptic vesicles are specifically targeted to vesicles, peptide-containing vesicles are typically distributed in all compartments of a neuron, and can be released from any compartment. Very few seem to be released at synapses; the common sites of release appear to include axonal varicosities – swellings that stud the axons of peptidergic neurons, and the dendrites. Each vesicle that is released is likely to have a large sphere of action [38], encompassing many potential target neurons. These vesicles cannot be recycled – to replace a vesicle, a new vesicle must be constructed, filled with newly synthesised peptide, and transported from the cell body to the release site, a process that takes several hours. Thus vasopressin and oxytocin, and probably peptides generally in the brain, are more like ‘hormones of the brain’ than neurotransmitters: acting at a distance from their site of release, at targets that selectively express high affinity receptors rather than at targets defined by anatomical connectivity.

3. The anterior pituitary gland and the hypothalamus

Each of the systems that regulate the anterior pituitary comprises a network of hypothalamic neurons that generate co-ordinated bursts of activity. This produces patterned secretion of a releasing factor or factors; these act on endocrine cells that filter and amplify the hypothalamic signal. The final result is pulsatile secretion of a hormone into the systemic circulation, which acts on a target organ in the periphery to induce secretion of other hormones which feedback on both the pituitary and hypothalamus (Figure 1)[40].

Six hormones are secreted from the anterior pituitary, made by subpopulations of endocrine cells that form interconnected networks, communicating with each other and with the permeating blood vessels. Each of these hormones is a peptide, stored in vesicles that are secreted by Ca^{2+}-dependent exocytosis either in response to Ca^{2+} entry via voltage-dependent membrane channels, or in response to mobilisation of intracellular Ca^{2+} stores. Their secretion (and also gene expression) is regulated by hypothalamic neurons that secrete factors into the hypothalamo-hypophysial portal vessels of the median eminence that connect the hypothalamus to the anterior pituitary, factors which bind to specific receptors on the endocrine cells. These portal blood vessels are *fenestrated*: the fenestrations allow large molecules to pass between the blood and the extravascular fluid, so factors secreted from neurosecretory endings that terminate there can enter these vessels freely.

The endocrine cells form networks involving autocrine and paracrine interactions, and intercellular communication via gap junctions [50-52]. Each endocrine cell type is heterogeneous [53-55] and may exhibit either a broad spectrum of properties or contain multiple sub-populations [56]. The cells generate Ca^{2+}-dependent action potentials, and a diverse family of Ca^{2+}-activated K^+ channels regulate both electrical activity and activity-induced secretion from these cells [57, 58]. Common electrical behaviors include continuous spiking and ‘pseudo-plateau’ bursting: the amplitude of fluctuations in intracellular Ca^{2+} is greater in bursting cells, leading to the hypothesis that bursting cells release more hormone than spiking cells [59]. Gene transcription in endocrine cells also occurs in bursts or pulses of activity, and these seem to be linked to the bursts of electrical activity [60-62], raising the possibility that bursts of spikes are also more efficient in stimulating synthesis.

Gonadotrophs, the endocrine cells that synthesise LH and FSH, display spontaneous activity consisting of a continuous train of action potentials. This behavior that has been modeled as an interaction between Ca^{2+} entry through voltage-gated channels and Ca^{2+} release from the endoplasmic reticulum [63]. In the unstimulated case, the endoplasmic reticulum is a Ca^{2+} buffer, taking up the Ca^{2+} that enters the cell during the upstroke of each spike, and releasing it back to

the cytoplasm during the downstroke [64, 65]. By contrast, somatotrophs typically fire bursts of spikes during their basal activity. To investigate why their spontaneous activity is so different, Van Goor *et al.* [66] adapted a model of the gonadotroph by adding another type of Ca^{2+}-activated K^+ current, the BK (big conductance) current, which is present in somatotrophs but not in gonadotrophs: if these channels are blocked, the cells switch from bursting to continuous spiking. This conductance activates rapidly during the upstroke of a spike; as a result, the spikes are wider, and single spikes can be converted into bursts of spikes. Thus, paradoxically, increasing a hyperpolarizing K^+ current *increases* intracellular Ca^{2+} concentration and so stimulates hormone secretion.

3.1 Gonadotrophins

Two anterior pituitary hormones, LH and FSH, regulate the gonads, controlling production of the sex steroid hormones (oestrogen and progesterone in females, testosterone in males). LH and FSH are made in gonadotroph cells; they are packaged in separate vesicles, but only one hypothalamic factor regulates their secretion – GnRH - made by a few hundred neurons whose cell bodies are scattered in the rostral hypothalamus. The patterns in which LH and FSH are secreted can be very different: the synthesis of LH and FSH is affected differentially by feedback from sex steroids and the ovarian peptide hormone inhibin, and the changing pattern of activation by GnRH affects the synthesis and secretion of LH and FSH differentially This is further complicated by the fact that the pattern of GnRH release is regulated by both negative and positive feedbacks from sex steroids [40]. The “decoding mechanism” that underlies the differential response of gonadotrophs to GnRH has attracted theoretical attention: models that involve either activation of a signaling component with a refractory period or inactivation of a factor needed for induction of FSH expression display true pulse-frequency sensitivity [67-70].

In males, the GnRH neurons generate GnRH pulses that maintain a pulsatile secretion of LH that is necessary for spermatogenesis. In females, pulsatile LH secretion varies in frequency and amplitude across the ovarian cycle, and ovulation, the climactic event of the female reproductive cycle, is triggered by a surge in GnRH that arises in still mysterious ways after prolonged exposure to high levels of estrogen. The (relatively small) GnRH surge triggers an event so cataclysmic that it might fairly be called an LH tsunami, but which, with admirable understatement is known as the *LH surge*. At the pituitary, the GnRH surge is amplified to produce the LH surge by an intriguing phenomenon of ‘self-priming’ [71, 72] (modeled in [73, 74]), whereby successive GnRH pulses release more and more LH as they recruit vesicles into

release sites at the plasma membrane, resulting in LH pulses that merge into a wave of secretion that diminishes only when the pituitary stores of LH are exhausted.

Stimulation by GnRH results in the production of an intracellular messenger, IP_3, which releases Ca^{2+} from the endoplasmic reticulum, which then inactivates the IP_3 receptors, giving rise to Ca^{2+} oscillations [75]. Li et al. (1997) developed a model combining the electrical activity of the gonadotroph with the endoplasmic reticulum oscillator. During the upstroke of an oscillation, the release of Ca^{2+} hyperpolarizes the gonadotroph by activating a K^+ conductance, turning off the spiking; when the intracellular Ca^{2+} subsides at the end of an oscillation, the K^+ conductance is deactivated, allowing the cell to spike again. Thus the model produces bursts of electrical activity, but, unlike most bursting cells, the intracellular Ca^{2+} concentration is low during bursts and high between them. The bursts produce pulsatile secretion of LH and FSH, and this output is sensitive to the frequency, amplitude and width of the GnRH pulses [76-78].

The coarse features of the menstrual cycle in women – the mean daily concentrations of LH, FSH, estrogen, progesterone and inhibin that vary in orchestrated rhythms across the cycle- can be well simulated by a system of delay differential equations that expresses the rates of synthesis and secretion of LH and FSH as functions of estrogen, progesterone and inhibin, and the production of estrogen and progesterone and inhibin as functions of LH and FSH secretion, and such models can give insight into abnormal ovarian cycles such as that which characterises polycystic ovarian syndrome – a common cause of infertility. [79, 80].

An alternative model of the ovarian cycle that does not involve delay equations introduces a simple deterministic model of the GnRH pulse pattern [81], and this can serve as a good starting point for incorporating models of the GnRH pulse generator. To generate pulses of secretion, the activity of the GnRH neurons must be co-ordinated, presumably with quasi-synchronous bursts of electrical activity [82]. Some direct interaction seems likely to be involved, but the neurons are not clustered together - they are scattered sparsely across the anterior hypothalamus. However, these cells have long dendrites, at least some of which are intertwined [83], so they may be connected via dendro-dendritic contacts in a similar way to the oxytocin cells. The presumption that the activity of the GnRH neurons is closely co-ordinated, leads to the presumption that the GnRH neuronal population behaves as a single excitable element. Because GnRH neurons are not directly sensitive to estrogen, models have been developed treating the GnRH network as a single element that interacts with an estrogen-sensitive element, and these can concisely capture qualitative features of GnRH secretion and the influence of the changing steroid environment [84].

The key estrogen-sensitive elements appear to be two populations of neurons that express kisspeptin, a potent activator of GnRH neurons [85, 86]. One of these populations is in the rostral hypothalamus, close to the cell bodies of the GnRH neurons, and one is in the arcuate nucleus, close to the GnRH neurosecretory nerve terminals – the arcuate nucleus is directly adjacent to the median eminence. The arcuate kisspeptin neurons are inhibited by estrogen, and the rostral kisspeptin neurons are activated by it. Intriguingly, the arcuate kisspeptin neurons seem to be essential for pulsatile GnRH secretion, while the rostral neurons are essential for the surge. The two populations differ in other ways too – the arcuate neurons co-express two peptides that the rostral neurons do not, neurokinin B and dynorphin, leading them to be rather coyly called KNDy ('candy') neurons.

It seems possible that the KNDy neurons affect GnRH secretion by an action at the neurosecretory endings of the GnRH neurons. This influence, being excitatory, must be of a type without clear precedent. Prevot et al. have proposed that the GnRH neurosecretory endings and the tanycytes (a population of specialised glial cells with which they are intimately associated) form a functional unit whose morphology is regulated by the arcuate kisspeptin neurons [87]. They propose that pulsatile GnRH secretion does not need the spike activity of GnRH neurons to be co-ordinated– rather, it involves the sequestration of erratically released GnRH, and its periodic liberation as a bolus into the blood vessels that connect the hypothalamus to the pituitary. This sequestration, it is proposed, arises because the neurosecretory endings do not discharge their contents directly into blood vessels, but into an extracellular compartment enclosed by the sheet-like processes of the tanycytes – processes that rapidly change their configuration in response to neuropeptide signalling.

An alternative possibility is that KNDy neurons influence GnRH neurons by an apparently perverse axonal projection (perverse because the KNDy neurons lie close to the GnRH terminals but far from the GnRH cell bodies) for which there is no clear evidence. It seems that each GnRH neuron has one long process, termed a 'dendron', that extends from the preoptic area to the arcuate nucleus and which bears features of both dendrites and axons [88]. Chen and Sneyd [89] constructed a computational model of the dendron, concluding that synaptic inputs anywhere along its length can influence spike initiation, but the effects are greatest for inputs close to the soma. This conclusion follows from the identification of a spike initiation zone in the proximal dendrite, about 100um from the soma. There is little information about the membrane properties of more distal regions of the dendron, so it seems possible that there are additional spike initiation sites close to the neurosecretory terminals. However, current evidence indicates that Ca^{2+}-induced Ca^{2+} release from intracellular stores is important in the bursting activity that is presumed to

underlie GnRH pulsatile release [90-93]. This seems to be confined to the soma; if so, it seems unlikely that a distal spike initiation site would be compatible with bursting [94].

If rostral kisspeptin neurons are the surge generators, this does not resolve the question of how the GnRH/LH surge is generated. The problems of understanding the surge are not trivial. While high levels of estrogen are needed for an LH surge, they appear not to be sufficient – estrogen may be permissive but not decisive. In different species, the LH surge is triggered by different stimuli: cats are reflex ovulators and a surge is triggered by coitus; in seasonal hamsters, the trigger is daylength; in rats it is the time of day; in herd animals, the trigger is a pheromone. So if estrogen actions on kisspeptin neurons are essential for an LH surge, is the kisspeptin signal itself a trigger, or is it a 'gate' for the trigger?

It remains unclear how the bursts of activity in GnRH neurons are regulated and how the GnRH surge is generated, but we do understand why pulsatile secretion of LH is important. We have a reasonably good understanding of how individual gonadotrophs respond to GnRH, but the gonadotrophs interact with each other and with other pituitary cell types, and these interactions are also likely to be important [95].

When the target cell for a hormone has a receptor that desensitizes during sustained activation, then continuous hormone secretion will not be optimally effective. However, a pulsatile pattern is not *necessarily* any better– what matters is the *duty cycle* of the pattern, the ratio of the duration of the active secretory phase to the intervening quiescent phase, and this depends on the rate at which the receptors desensitize and resensitize. Li and Goldbeter showed that there is an optimal duty cycle for which pulsatile hormone secretion has a maximum impact on the target cell. [96, 97]. Unusually, the GnRH receptor on gonadotrophs does not desensitize, but LH receptors on the gonads do, and continual activation of these leads to infertility – a property exploited in the use of GnRH analogs as contraceptive agents [98].

The pulsatile patterning of GnRH is important not only for the secretion of LH and FSH but also for gene transcription. The effects of GnRH on transcription of LH and FSH display a bell-shaped frequency–response relationship, and a recent model suggests that this frequency decoding arises from the interplay of two transcription factors that interact co-operatively, - a phenomenon that may commonly arise as an emergent feature of signalling networks , 60,[99, 100].

3.2 Growth hormone

While one regulatory factor, GnRH, orchestrates the patterns of secretion and synthesis of two gonadotrophin hormones, the secretion of growth hormone requires two factors: growth

hormone secretion is promoted by neurons that release GHRH, and is inhibited by others that release somatostatin [101]. At first sight, the biggest mystery about growth hormone secretion is not *why* two factors are needed, but why it needs a brain at all. The 'classic' answer is that it is not *how much* growth hormone is secreted that matters, but the pattern in which it is secreted – and neural networks are good at pattern generation. Certainly the pattern does matter: in humans, growth hormone is secreted in large pulses at intervals of about 3 h, and the magnitude of these declines inexorably with age after puberty. In male rats the pattern is very like that in humans, but in female rats the pulses are smaller and more frequent, and inter-pulse levels are higher. This difference in pattern accounts for the marked sexual differences in growth rate and body composition.

In the absence of growth hormone, GHRH, or either of their receptors, rats and mice are born of normal weight but grow slowly, and inbred lines of small rats or mice with one or other of these deficiencies have been used to establish the importance of growth hormone patterning, for example by showing that dwarf male rats that do not synthesise growth hormone grow at a normal rate if given an injection every 3 h, but grow more slowly if the same amount of growth hormone is given by more frequent or less frequent injections, or as a continuous infusion [102].

Pulsatile growth hormone secretion is orchestrated by alternating stimulation by GHRH and inhibition by somatostatin [103], an alternation that arises because of neural interactions between these two populations of neurons – somatostatin directly inhibits GHRH neurons. The periodicity of the pulses is set by negative feedback from growth hormone and from IGF-1, which is secreted from the liver in response to growth hormone. Models suggest that the dynamics of this system require a delay between the arrival of the feedback signal (growth hormone or IGF-1) and activation of somatostatin release [104] [105-108] (Figure 4)[109]. At present, there is no explanation for this delay, or direct evidence that it exists. All models agree on the need for a delay of about 30 min: this is too long to be explained by the transport of growth hormone across the blood-brain barrier, and too short to reflect the *de novo* synthesis of somatostatin. One mechanism that operates on the right timescale is *priming*, as characterised in vasopressin and oxytocin cells (see above). This postulated explanation proposes that the actions of growth hormone and/or IGF-1 on somatostatin neurons involve a reconfiguration of the cytoskeleton of somatostatin neurons to enhance the activity-dependent releasability of somatostatin. An alternative possibility is that the classical view of regulation of growth hormone secretion is incomplete, and a third hypothalamic factor, perhaps neuropeptide Y, might be involved [110].

The mechanism underlying pulsatile secretion of growth hormone is not quite the pernickety detail that it might appear. The name 'growth hormone' is misleading, for growth

hormone has important actions throughout life, not only in the pre-pubescent growth period. In humans, growth hormone secretion declines with age, and this decline is a cause of the increased adiposity, increased bone fragility, reduced muscle strength, and impaired glucose homeostasis that accompanies aging. However, correcting this with exogenous growth hormone is impractical because of the expense and the need for multiple daily injections.

The age-related decline in growth hormone secretion does not reflect a loss of secretory capacity at the pituitary, but a progressive enfeeblement of the hypothalamic systems. In the 1990's, considerable excitement was generated by the finding that a class of synthetic peptides, called growth hormone secretagogues, could reverse this enfeeblement, restoring the robust juvenile pattern of growth hormone secretion [109]. Exactly how they achieve this is still not clear. They act on both the GHRH cells [111] and on the somatotrophs [112], and the combined effects of GHRH and growth hormone secretagogues show a striking synergy *in vivo* [113]. From a unified phenomenological model it appears likely that this synergy arises by a combination of effects: a direct action of secretagogues at the pituitary, a potentiation of GHRH release, and an inhibition of somatostatin release [114]. However, exactly how this combination produces the observed change in pattern of growth hormone secretion remains unclear.

The discovery of growth hormone secretagogues led to the identification of a specific receptor for them, which was mainly localised to the hypothalamus and anterior pituitary gland [115]. In 1999, the endogenous ligand for this receptor was discovered [116], and named ghrelin (for *GH-REL*easing). But ghrelin is now best known for actions unrelated to growth hormone. Ghrelin is released from the stomach, and stimulates appetite by its actions on neurons in the arcuate nucleus that make two peptides, neuropeptide Y and agouti-related peptide, that are powerful stimulators of appetite in their own right. This discovery, that ghrelin can promote obesity by stimulating appetite, killed interest in growth hormone secretagogues as potential anti-aging elixirs. Nevertheless, there remains considerable therapeutic potential in the possibility of enhancing pulsatile secretion of growth hormone in the elderly.

3.3 Adrenocorticotrophic hormone (ACTH)

ACTH is necessary in mammals for anything approaching a normal life. It is secreted from anterior pituitary corticotroph cells in response to two releasing factors, CRH and vasopressin, and it acts on the adrenal glands to control the production of glucocorticoid hormones (mainly cortisol in man, corticosterone in rodents). The corticotrophs are electrically excitable [117-119] and they display bursts of spikes in response to CRH and vasopressin.

Exposure to corticosterone reduces their spontaneous spiking activity and prevents the emergence of bursting in response to stimulation [120].

Llike all of the anterior pituitary hormones, ACTH is secreted in a complex pattern, with a circadian rhythm superimposed on which are 'ultradian' pulses at intervals of about 20 min [121]. The hypothalamo-pituitary-adrenal axis (HPA axis), the 'stress' axis, is activated by both physical and mental stressors. Glucocorticoid hormones have potent actions throughout the body: they mobilize energy reserves by promoting fat breakdown and by stimulating glucose production by the liver, reorganize blood flow to muscles and away from superficial vessels and deep organs, and reduce inflammatory responses.

A number of models have been developed of the HPA axis where ultradian pulses are driven by CRH release subject to negative feedback from glucocorticoids [122-127]. However, a simple and elegant mathematical model suggests that the ultradian pulses can arise without the hypothalamus [128, 129]. These authors noted (i) that there was a delay in the production of glucocorticoids in response to ACTH (steroid hormones cannot be stored, and so are produced on demand), and (ii) that glucocorticoids have negative feedback effects on the corticotrophs. Combining just these two, they showed that ultradian pulses of the type seen *in vivo* can arise without any patterned CRH stimulation.

This was an uncomfortable conclusion: if correct, then the ultradian pulsatile secretion of ACTH and glucocorticoids is accompanied by changing sensitivity of the pituitary to CRH [130]. Thus, a given stressor, eliciting a given release of CRH, will evoke different amounts of ACTH and glucocorticoid secretion depending on when it occurs relative to the last ultradian pulse of ACTH. Do the ultradian pulses have any intrinsic adaptive value, or are they merely incidental consequences of feedforward simulation of glucocorticoid production by ACTH together with the delayed negative feedback from glucocorticorticoids on ACTH secretion? This is hard to answer: for optimal transcriptional responses, tissues need oscillating concentrations of glucocorticoids [131], but just as the properties of vasopressin cells and axonal terminals have co-evolved to achieve efficiency, so glucocorticoid- responsive pathways might simply have evolved to use signal properties efficiently.

The HPA axis has other perversities. Two hypothalamic factors regulate ACTH secretion, CRH and vasopressin, and both of these are stimulatory (through separate receptors). Vasopressin secreted from the posterior pituitary does not affect ACTH secretion, at least in most species: vasopressin from this source enters the systemic circulation without travelling to the anterior pituitary, and the concentrations reached in the circulation are too low to activate the

corticotrophs. However other 'parvocellular' vasopressin neurons in the paraventricular nucleus project to the median eminence, and it is this vasopressin that regulates ACTH secretion.

CRH and vasopressin are produced in the same neurons in the paraventricular nucleus. CRH is more potent at releasing ACTH than vasopressin, but, in combination, their effects synergise [132]. Curiously, in response to chronic stress, CRH synthesis is down-regulated by negative feedback effects of glucocorticoids, but vasopressin synthesis is upregulated. Accordingly, chronic stress changes the biochemical phenotype of the hypothalamic part of the HPA axis – neurons that were CRH neurons become vasopressin neurons. [133, 134]. Thus the declining secretion of ACTH and corticosterone with repeated stress is a consequence of the loss of CRH signalling (through depletion) and its replacement by vasopressin, a less potent secretagogue. The significance of this is not known. However, while the HPA axis shows habituation to a repeated stress, there is no cross-habituation – a different and novel stress will still evoke a robust activation of ACTH and corticosterone secretion. It therefore seems possible that habituation might be confined to a subpopulation of CRH/vasopressin neurons that are activated by a particular stressor, but this has yet to be demonstrated.

3.4 Prolactin

The classical role of prolactin, which is secreted from lactotroph cells, is to stimulate milk production by actions at the mammary gland. However, prolactin is present in all vertebrates, and has a diverse repertoire of functions including (in fish) osmoregulation, regulation of reproduction and bodyweight in pregnancy in mammals, and regulation of pelage in seasonal mammals [135]. In some mammals prolactin is important in maintaining pregnancy. A normally-cycling female rat has low levels of circulating prolactin except on the afternoon of proestrus, when there is a surge of prolactin coincident with the LH surge. However, after mating, female rats show a surge every morning and another every afternoon for the first ten days of pregnancy [136, 137]. This mating-induced pattern is not a consequence of pregnancy, but of that single episode of coitus: if the male has been vasectomised, the mating will, by this mechanism, trigger a 'pseudo-pregnancy.'It seems possible that the effect is mediated by oxytocin: in rats, copulation triggers a pulse of oxytocin secretion, oxytocin can trigger prolactin secretion, and mimicking the copulation-induced secretion of oxytocin can trigger a persistent rhythm of prolactin secretion like that seen in pregnant rats [137]. The prolactin that is secreted in this mating-induced pattern helps to maintain the corpus luteum, which continues to produce progesterone and suppress ovarian cyclicity; without this, the pregnancy will fail and the fetuses will be reabsorbed. After about day 12 of pregnancy, the placenta is secreting large amounts of

placental lactogen – a prolactin-like hormone that maintains the progesterone production that is essential for the continuation of pregnancy, whose action in the brain terminates the rhythm of prolactin secretion seen in early pregnancy [138].

Alone amongst the anterior pituitary cell types, lactotrophs are spontaneously active *in vivo* as well as *in vitro* [139], and disconnecting the pituitary from the hypothalamus results in elevated prolactin secretion. Thus the dominant hypothalamic influence on prolactin secretion is inhibition, mediated by dopamine released from neuroendocrine neurons in the arcuate nucleus – the tuberoinfundibular dopamine neurons (TIDA neurons). Whereas dopamine inhibits prolactin secretion, prolactin feeds back with a time delay to stimulate dopamine synthesis and secretion (see). Bertram *et al.* [140] developed a mathematical model for the interaction between TIDA neurons and lactotrophs to explain the mating-induced rhythm. This model represented the activity of the TIDA neurons and that of the lactotrophs as single variables, with a direct inhibitory effect of dopamine on prolactin secretion and a delayed inhibitory effect of prolactin on dopamine release. In this model, mating-induced activation of oxytocin neurons activates a population of bistable hypothalamic neurons that innervate and inhibit the TIDA neurons, and the autonomous rhythm generated by this interaction is entrained to the light-dark cycle by a circadian input from the suprachiasmatic nucleus [140-142].

However, the actions of dopamine are complex: at micromolar concentrations it blocks electrical activity in lactotrophs and lowers the intracellular Ca^{2+} concentration, but at nanomolar concentrations it has the opposite effect and increases prolactin secretion. To explain this, Tabak *et al.* [143] showed that dopamine can be stimulatory in lactotrophs if it activates either a BK current or an A-type K^+ current. They went on to show that the electrical activity of lactotrophs is characterized by spikes superimposed on an intermittently elevated voltage plateau, a style of oscillation that has been called “pseudo-plateau bursting”. They showed that this behavior is a canard-induced mixed mode oscillation, and used canard theory to characterize the dynamics of the oscillation and bifurcation analysis of the full system of equations to extend the results to the physiological regime [3, 144].

While current dogma thus holds that prolactin is under inhibitory control by dopamine and that the major physiological stimulus for prolactin secretion is suckling, even complete removal of dopamine will not account for the very high levels of secretion seen during lactation [145]. Recently, our understanding of the regulation of prolactin secretion has been thrown into confusion by a paper which suggests that the TIDA neurons, in lactation, stop making dopamine [146-148]. This only partly explains why prolactin levels are high in lactation, but it does not itself explain why suckling elevates prolactin release. It appears that, in place of dopamine, the

TIDA neurons secrete leu-enkephalin, which stimulates prolactin secretion, and this might explain suckling induced stimulation of prolactin secretion if, against expectation, the TIDA neurons are *activated* by suckling. Prolactin acts back on the TIDA neurons to activate them, normally as a negative feedback regulator of prolactin secretion, but if these neurons switch from inhibiting prolactin secretion to being stimulatory, then this will become a positive feedback signal.

3.5 Thyroid-stimulating hormone (TSH)

The last of the anterior pituitary hormones, TSH, is probably the least well understood. It is secreted from thyrotroph cells in response to TRH, which is released from yet another population of neuroendocrine cells in the paraventricular nucleus[149]. The relationship between the pituitary and the thyroid glands has been modeled phenomenologically [150].There is evidence for hormone oscillations in this axis, including circadian oscillations in TSH [151] which have been modelled by Berberich *et al.* [152], and evidence of pulsatile secretion of TSH [153].

TSH controls the secretion of thyroid hormones and hence regulates the basal metabolic rate; accordingly, TSH secretion is powerfully influenced by body temperature and energy stores. Temperature regulation of secretion involve thermosensitive neurons in the ventromedial nucleus of the hypothalamus, while regulation in response to energy stores is achieved in two ways. The adipocytes, cells which store body fat, secrete leptin, which circulates in proportion to body fat mass and which acts at the hypothalamus, including on the TRH cells. A second system involves hypothalamic neurons that express intrinsic energy-sensing systems [154]. Feedback from thyroid hormones to the hypothalamus involves thyroid hormone uptake from blood vessels within the paraventricular nucleus, and uptake from the cerebrospinal fluid in the third ventricle followed by transport to TRH neurons, and thyroid hormone sensing in the arcuate hypothalamus by neurons that project to TRH neurons [155]. Axons containing TRH project to the median eminence to terminate on hypothalamic tanycytes, and stimulation of the TRH receptor 1 on tanycytes increases intracellular Ca^{2+} in the tanycytes, increases the size of tanycyte endfeet that shield pituitary vessels and induces the activity of the TRH-degrading ectoenzyme, mechanisms likely to restrain TRH release to the pituitary [156]. At present, this intriguing hypothesis has not, to our knowledge, been modeled.

4. Conclusions

To fully understand a biological system, we must understand how it works and to what ends, but also how it has developed – the rules by which the system was built or by which it built itself. Indeed, it might be easier to build a working model of a biological system by simulating its development than by attempting to simulate its function. We must also understand how it evolved: the evolution of every system follows just one of many possible paths, and while each path offers access to some possible solutions to problems that arise, not all solutions are available for all paths [157]. Importantly, signal coding mechanisms (such as pattern generator systems) have co-evolved with decoding mechanisms at the targets.

Often, the important behavior of neuroendocrine systems- systems that comprise many heterogeneous neurons that interact with a heterogeneous population of endocrine cells to generate complex patterns of hormonal secretion – can be well approximated by simple dynamical systems models. It is equally true that the behavior of individual neurons or individual endocrine cells can be closely modelled by complex biophysical models that can themselves be well approximated by much simpler models. But models of single cell behavior cannot generally substitute for the elements of models of the system as a whole: the heterogeneity of cells is not some inconvenient detail that can be ignored because it is filtered out by averaging – the heterogeneity fundamentally alters the signal processing characteristics of the system, as illustrated here in the case of vasopressin cells. Nor can noise be safely neglected: stochasticity arises in every element of a biological system.

The ability of simple dynamical systems models to mimic complex behaviors reflects the robustness of the emergent behavior of noisy and heterogeneous populations that arises from their complex interactions [158]. The miracle of biology is not that complex behaviors arise from perfectly designed complex elements, but that they arise from complex, noisy and heterogeneous elements, elements that are individually erratic and unpredictable. Understanding this miracle seems tractable through the prism of neuroendocrine systems.

But in each of the neuroendocrine systems, major problems remain. For example, the oxytocin system is perhaps the most fully understood, but it remains the initiation of the milk-ejection reflex remains unclear. This has some distinctive features: the first bursts in a suckling episode increase progressively in magnitude, not something that is a feature of the present model[159]. For the vasopressin system, it remains unclear what is the adaptive value of dendro-dendritic communication between these neurons, especially communication via vasopressin itself [160]. For the gonadal axis, the cellular mechanisms in GnRH neurons that underlie pulsatile secretion and surge secretion are both still poorly understood. For the growth hormone system, understanding how ghrelin modifies pulse patterning may lead to important advances in

therapeutic interventions. These, and many other outstanding problems seem to invite the attention of modelers.

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Figures and Figure Legends

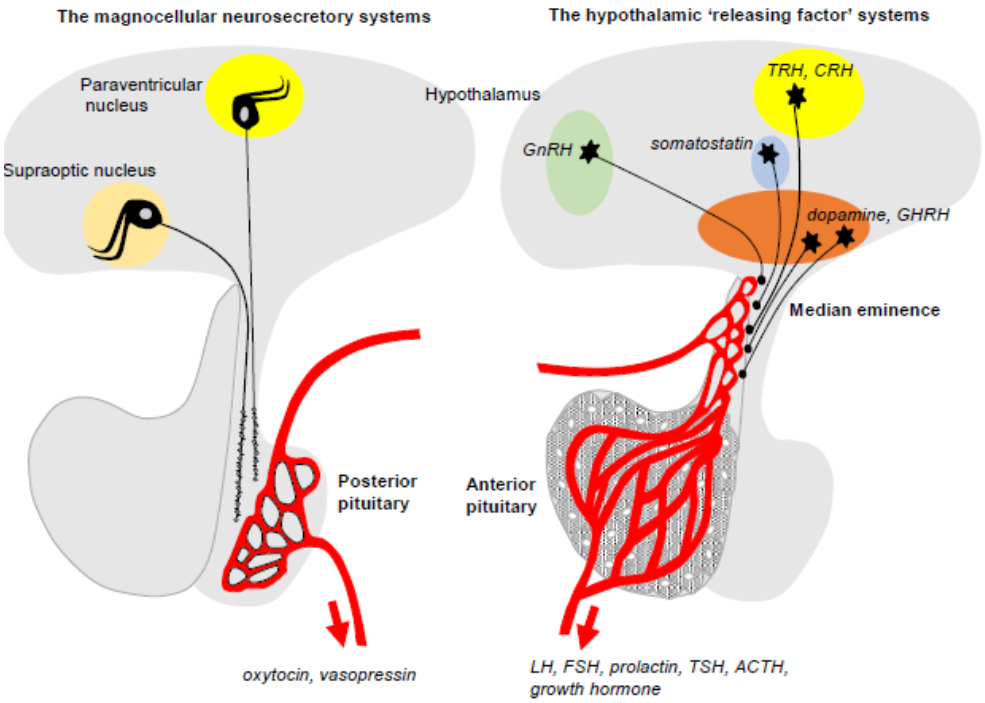

Figure 1. **The neuroendocrine systems of the hypothalamus.**

Left: The posterior pituitary, in the rat, contains the axons of about 10,000 magnocellular vasopressin neurons and a similar number of magnocellular oxytocin neurons, whose cell bodies are mainly aggregated in the supraoptic and paraventricular nuclei of the hypothalamus (indicated in green and yellow respectively).. At the pituitary, these axons each give rise to about 2,000 nerve endings and swellings filled with the neurosecretory vesicles that contain oxytocin or vasopressin. These are secreted by calcium-dependent exocytosis in response to action potentials generated in the cell bodies and propagated down the axons.

Right: The anterior pituitary contains endocrine cells that produce six hormones:

- *Gonadotroph*s: produce *luteinising hormone* (LH) and follicle stimulating hormone (FSH) that regulate the gonads;
- *Thyrotrophs*: produce *thyroid stimulating hormone* (TSH) that regulates the thyroid gland
- *Corticotrophs*: produce *adrenocorticotrophic hormone* (ACTH) that regulates the secretion of glucocorticoid hormones from the adrenal gland;
- *Lactotrophs*: produce *prolactin* that regulates milk production by the mammary glands;
- *Somatotroph*s: produce growth hormone that acts at the liver to control the production of growth factors (mainly *insulin-like growth factor 1*, IGF-1) that control bone growth and muscle development.

The secretion of these is controlled by 'releasing factors' produced by neuroendocrine neurons in different regions of the hypothalamus. Gonadotrophs are regulated by *gonadotrophin releasing hormone* (GnRH), made by about 700 neurons scattered across the anterior hypothalamus (green). Thyrotrophs are regulated by *thyrotropin releasing hormone* (TRH), produced by neurons in the paraventricular nucleus. Corticotrophs are regulated by *corticotropin releasing hormone* (CRF) and vasopressin, co-produced by another subset of neurons in the paraventricular nucleus that are distinct from the magnocellular vasopressin neurons. Lactotrophs are primarily regulated by an inhibitory factor, *dopamine*, released by neurons in the arcuate nucleus (orange). Somatotrophs are regulated by a stimulatory factor (*GH releasing hormone*, GHRH), released by another population of neurons in the arcuate nucleus and by an inhibitory factor (*somatostatin*) released by neurons in the periventricular nucleus (blue). See Leng[40] for a full account of the hypothalamus and its hormones.

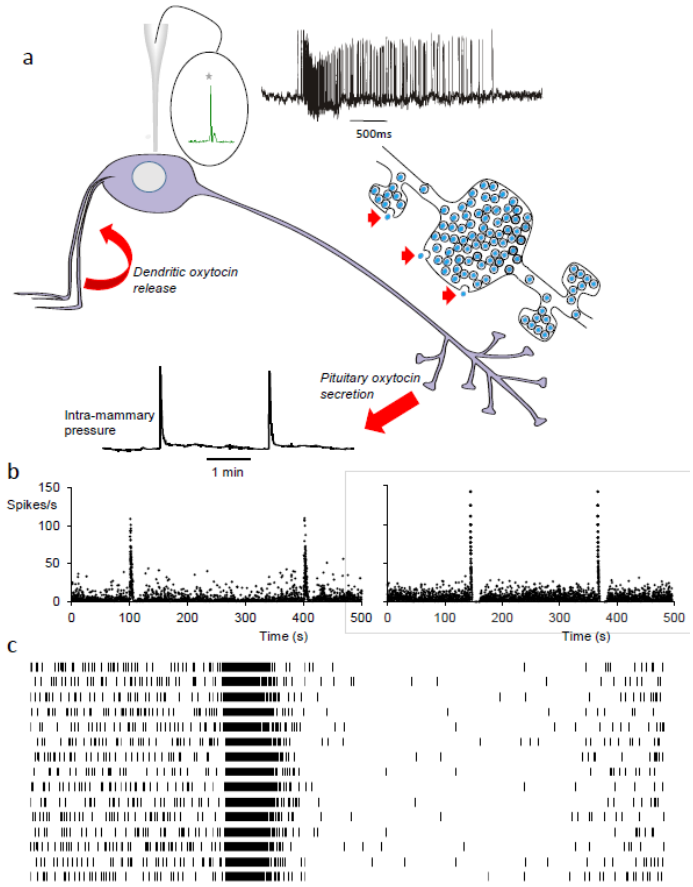

Figure 2.**The milk-ejection reflex**

(a) In response to suckling, oxytocin cells discharge in brief synchronised bursts that evoke secretion of pulses of oxytocin that induce abrupt episodes of milk let-down that induce abrupt increases in intramammary pressure. The bursts are brief (about 2 s in duration) and intense, typically containing ~ 100 spikes (the example shown here is from [161]), and the resulting Ca^{2+} influx at the pituitary axons causes exocytosis of neurosecretory vesicles from axonal swellings and nerve terminals (small red arrows). This secretion is massively potentiated at high frequencies of spike discharge, resulting in large intermittent pulses of secretion. The bursts occur approximately synchronously throughout the population of oxytocin neurons, and are dependent on activity-induced dendritic release of oxytocin that acts as a positive-feedback signal. This activity-dependent dendritic release occurs only when the dendritic stores have been primed by a mechanism that translocates dendritic stores of vesicles to juxta-membrane sites where they can be released in response to spike-triggered Ca^{2+} entry.

(b) A network model of oxytocin cells incorporating dendron-dendritic interactions and priming generates bursts in model cells (right) that closely match bursts observed in oxytocin cells in vivo (left); plots show instantaneous firing rates.

(c) Raster plot showing near synchronous bursts in 15 cells in a network model [35].

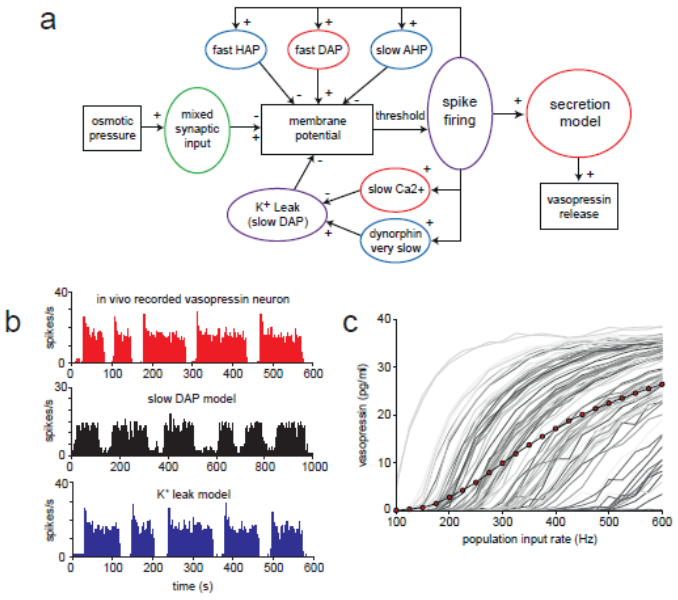

Figure 3. **A coupled spiking and secretion model used to investigate population heterogeneity in vasopressin neurons.**

(a). The integrate-and-fire based spiking model sums together a randomly timed mix of input EPSPs and IPSPs with a set of activity-dependent afterpotentials (a fast HAP, a slow AHP and a depolarising afterpotential (DAP)), generating a spike when the sum causes the membrane potential to exceed the spike threshold. The mix of afterpotentials modulates the spike patterning. The K^+ leak current, which is subject to competing fast positive feedback from activity-dependent Ca^{2+} influx and slow negative feedback from activity-dependent dynorphin secretion, generates an emergent bistability that is responsible for phasic firing. The spiking model is coupled to a secretion model to simulate the full response from the synaptic input signal to vasopressin plasma concentration.

(b) Recorded spike patterning of a single vasopressin neuron compared with two alternative models. A model with a slow DAP mechanism cannot match the silent periods or the sharp changes in spike rate at the onset of bursts, whereas the K^+ leak current based model almost indistinguishably matches in vivo phasic spike activity.

(c) The single neuron secretory response to an increasing input signal is very non-linear, but simulating a population with varied input rates, shows that the heterogeneous non-linear responses of individual neurons (black lines) sum together (red dots) to make a population signal response which is both much more linear and with a greater dynamic range, matching the linear response observed *in vivo*[49].

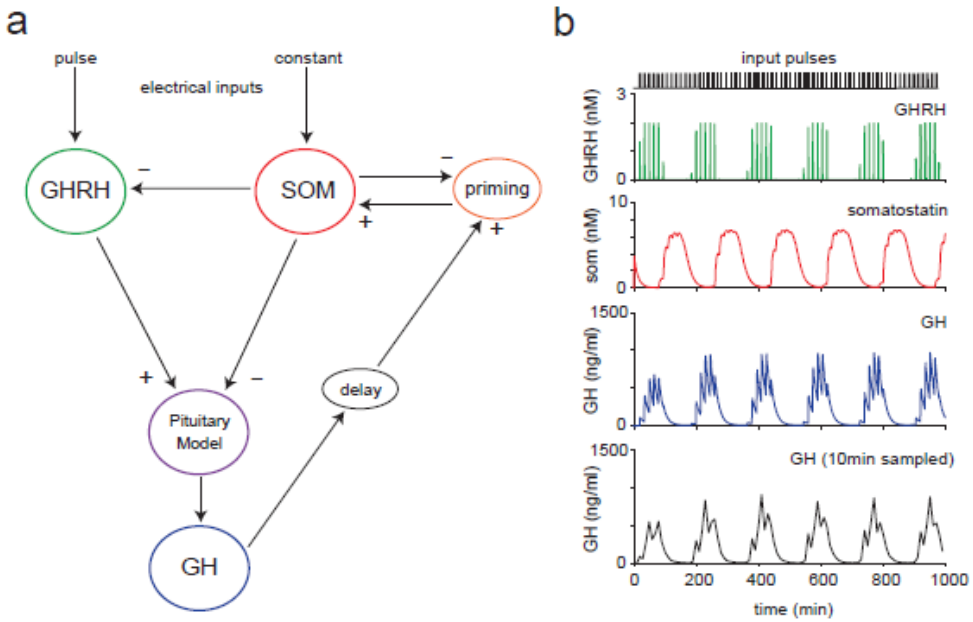

Figure 4. **A growth hormone (GH) pulse generating model of hypothalamic signal generation and pituitary response**.

(a) GHRH and somatostatin (SOM) signals generated by neurons in the hypothalamus act at the somatotrophs in the pituitary to regulate pulsatile growth hormone (GH) secretion. A delayed negative feedback from GH to the somatostatin neurons is responsible for the pulsatile pattern of release. Part of the delayed control may be due to GH acting through a release priming mechanism rather than directly controlling somatostatin activity.

(b) In the model short pulses of GHRH activity are modulated by somatostatin. Somatostatin neurons receive a constant input signal, modulated by GH feedback through a priming mechanisms to generate an oscillating signal. These signals act together at the pituitary to generate to 3-hourly multiple peaked pulses of GH secretion, matching pulsatile secretion observed in the male rat. The match to experimental data is enhanced by simulating the effect of the 10-min sampling used to measure GH plasma concentration *in vivo*[104].

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