The Endocrinology of the Brain

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Abstract

The brain hosts a vast and diverse repertoire of neuropeptides, a class of signaling molecules often described as neurotransmitters. Here I argue that this description entails a catalogue of misperceptions, misperceptions that feed into a narrative in which information processing in the brain can be understood only through mapping neuronal connectivity and by studying the transmission of electrically conducted signals through chemical synapses. I argue that neuropeptide signaling in the brain involves primarily autocrine, paracrine and neurohormonal mechanisms that do not depend on synaptic connectivity, and that it is not solely dependent on electrical activity but on mechanisms analogous to secretion from classical endocrine cells. As in classical endocrine systems, to understand the role of neuropeptides in the brain we must understand not only how their release is regulated, but also how their synthesis is regulated and how the sensitivity of their targets is regulated. We must understand also the full diversity of effects of neuropeptides on those targets, including their effects on gene expression.

Introduction

Endocrinology is the study of hormones, secreted by endocrine glands in one part of the body, that travel in the blood and have prolonged effects on other parts of the body - effects that are determined by tissue-specific expression of their receptors. By contrast, neuroscience is the study of the brain, and particularly of neurones that release neurotransmitters at synapses with effects tightly localised in space and time, constrained by mechanisms of rapid reuptake and degradation. The schism between neuroscience and endocrinology, between interests above the neck and below it, is reflected in differences in dominant technological and methodological approaches and in different theoretical visions. *Neuroscience* has been dominated by electrophysiology and the study of information transmission by mapping neuroanatomical connectivity and by studying the spiking activity of neurones and its consequences for cognition and behaviour; *endocrinology* by the measurement of hormones and the analysis of the mechanisms by which they are produced and the signaling mechanisms by which they act.

Between these two, dangling below the brain and bathed in blood, is the pituitary gland, and dangling between neuroscience and endocrinology is neuroendocrinology, born of Geoffrey Harris' insights in the 1950's.

The birth of neuroendocrinology

In the late 1950's it was "well established" that in man and other animals that ovulated spontaneously, ovulation is controlled by the pituitary [1]. It was commonly believed that, in each ovarian cycle, increasing levels of estrogen triggered the secretion of gonadotropin hormones by direct actions on the pituitary. This "fact" was challenged by Harris, who argued that, in inducing ovulation, estrogen acted not on the pituitary but on the *brain*, where its actions resulted in the release from the hypothalamus of a substance that was

carried by blood vessels to the pituitary [2]. In Harris' theory, this was one of several 'releasing factors', each of which regulated a different pituitary hormone.

Harris and his co-workers showed that portal blood vessels in the median eminence at the base of the hypothalamus fed into a "vascular plexus" that filled the anterior pituitary, and that the direction of flow in these vessels was from the brain, not to it. He then noted that, from earlier work, transplanting the anterior pituitary gland to a different part of the body led to an irreversible loss of function and atrophy of the target tissues on which pituitary hormones were known to act. But if the anterior pituitary was removed from its normal place in the sella turcica and then replaced there or in an adjacent site, normal function often returned. Harris hypothesised that, in these cases, the portal vessels had regenerated, renewing the vascular communication between brain and pituitary [2].

To test this, Harris and Jacobsohn [3] removed the pituitary from female rats. They then grafted pituitary tissue from new-born young into the subarachnoid space below the brain, either immediately below the cut portal vessels, or to one side below the temporal lobe. Ovarian cycles returned in all rats with transplants below the cut portal vessels, but when the transplants were below the temporal lobe, the ovaries and reproductive tracts atrophied and ovarian cycles ceased. In both cases, the transplants were re-vascularised – in the first case by portal vessels, in the second case by blood vessels of a different part of the brain.

But the path to acceptance of Harris' theory was not yet clear. His theory blurred the conventionally accepted distinction between neurones and endocrine cells, and it postulated the existence of "releasing factors" whose identification seemed beyond experimental reach. It engaged the opposition of Sir Solly Zuckerman [4]. Zuckerman, as described by Lord Dainton, "*...was unique. No scientist this century can match him in the timespan and weight of his influence on governments in peace or war*" [5].

Zuckerman, who had founded his career on studies of the menstrual cycle in primates, recognised that Harris' theory would be disproved if *any* animal could be shown to have ovulated in the absence of portal vessels. In sixteen female ferrets, Thomson and Zuckerman [6] cut the neural stalk, and their case rested on results from two of them, two that had come into heat in response to artificial light even though, from their histological evidence, all connections between brain and pituitary had been eliminated.

But Harris suspected that the portal blood vessels *had* regenerated in these ferrets, so he and Donovan [7] set about their own experiments on ferrets. In their key experiments, after cutting the stalk, they inserted a paper plate between the stalk and the pituitary to prevent revascularisation of the pituitary by the portal vessels. In all ferrets where the plate had been inserted effectively, there was no revascularisation and no ovulation. They thus concluded that the method of stalk sectioning and the histological techniques used by Thomson and Zuckerman were inadequate.

In 1954, Harris and Zuckerman presented their discrepant findings at a Conference in London. As later recounted by Reichlin [8], "*Harris won the debate, then, and in posterity.*" Harris won, not just because his evidence was more convincing, but because he could explain something that Zuckerman could not. Zuckerman's two ferrets had come into heat *in response to light* – hence the pituitaries had responded to signals from the retina despite what Zuckerman had claimed to be a complete separation of the pituitary from either nerves or blood vessels. For this, Zuckerman had no credible explanation.

Zuckerman did not concede, but was still maintaining his position in 1978 (Zuckerman 1978), a year after Schally and Guillemin had been awarded the Nobel Prize for their identification of some of the releasing factors that Harris had postulated. To understand Zuckerman's resistance, we might recognise the threat that Harris' theory posed to the community of reproductive endocrinologists among which Zuckerman was a pre-eminent

98 authority. Harris, in placing the brain as the controller of reproduction, was shifting the
99 responsibility for extending our understanding from endocrinologists to neuroscientists - to a
100 different community, one already equipped with the expertise and technical methodologies
101 that this change in focus demanded.

102 But neuroscientists, by and large, were disinclined to take up this challenge,
103 disparaging the hypothalamus as the remnants of the 'lizard brain', and they left it to a new
104 community of neuroendocrinologists. Harris himself set about identifying the releasing factor
105 for gonadotropins, and came close to doing so [9, 10], though ultimately the prize – and the
106 Nobel Prize that accompanied it - went to Schally and Guillemin, whose labs were resourced
107 at a far greater level. Nevertheless, the catalogue of Harris' research reads like a road map for
108 neuroendocrinology [2]. He pioneered the collection of portal blood for assays of releasing
109 factors [11], introduced a method of remote electrical stimulation of the hypothalamus in
110 conscious behaving animals [12], and addressed the issue of stimulus-secretion coupling in
111 oxytocin release, a key step that became important in understanding the significance of
112 pulsatile hormone secretion [13]. In these and many other ways, he carved out a distinctive
113 identity for neuroendocrinology.

114 Barricades between endocrinologists and neuroscientists remained, and these
115 barricades were manned by *definition*s. For endocrinologists, the classical definition of a
116 'hormone' was that given by Starling [14]: "Each specific hormone is manufactured by a
117 group of cells and turned into the blood, in which it travels to all parts of the body, but excites
118 definite reactions in one or a limited number of distant organs." For neuroscientists, on the
119 other hand, 'neurotransmitters' were expected to satisfy three criteria: (i) that they were
120 present at synapses within the presynaptic neurones, (ii) that they were released in a Ca^{2+}-
121 dependent manner upon depolarization of those presynaptic neurones, and (iii) that they acted
122 on specific receptors present on the postsynaptic neurone [15].

Neither endocrinologists nor neuroscientists were rigidly bound by these definitions. For endocrinologists, Starling's definition encompassed the classical peptide and monoamine hormones well, but no complaint was made by its extension to steroid hormones, which pass cell membranes freely and can reach any targets in the body by diffusion through extravascular fluid. Nor was there objection to classing as hormones many agents that act within tissues, such as prostaglandins within the uterus, or estrogens within the ovary, or the new host of "local" hormones in many tissues. For neuroscientists, the definition of a neurotransmitter encompassed the classical neurotransmitters, packaged in synaptic vesicles whose release by exocytosis is tightly coupled to action potentials, but they came to concede that neurotransmitter release at synapses can "spill over" to act at extrasynaptic receptors [16-20].

Neuropeptides

Following the discovery of the releasing factors and their identification as mainly peptides, came the recognition that the class of "neuropeptides" extended far beyond the class of releasing factors. They include, on current reckoning, more than 300 different peptides expressed in various combinations in distinct subpopulations of neurones throughout the brain [21]. The brain had classically been assumed to be stocked with essentially homogeneous neurones that acquire functional specificity mainly through their patterns of hard-wired connections and which shared a common language of spiking activity. Now, it seemed to comprise a vast multitude of distinct neuronal types that spoke in multiple languages.

However willing neuroscientists might have been to acknowledge peptides as an additional (though minor and supplementary) class of neurotransmitters, the facts resolutely refused to conform to this notion. As I have argued elsewhere [22], the idea that

neuropeptides in the brain are neurotransmitters "is to a first approximation, a lie", at least if we retain *anything* of the criteria for a neurotransmitter given above.

First to be dismissed must be the misperception that neuropeptides are released at synapses within the brain. Many neurones produce both peptides and one or more neurotransmitters, and both are packaged in vesicles, but not in the same vesicles [23]. Conventional neurotransmitters are packaged in small synaptic vesicles that are specifically localised to synapses. Peptides are packaged in large dense-cored vesicles that are typically distributed throughout the cytoplasm of a neurone, and since for most neurones the dendrites comprise about 85% of the cell volume, it is in this compartment that large dense-cored vesicles are often mainly found. Synaptic vesicles can only be released at specialised sites in the presynaptic membrane, but large dense-cored vesicles can apparently be released from the soma, dendrites, axonal varicosities and even undilated axons – the main requirement appears to be that they must be close to the plasma membrane to be releasable [24-31].

Some large dense-cored vesicles *are* present in synapses, though not in particular abundance, and generally not close to the synaptic release site, and whether any are ever released into the synaptic cleft is questionable. Synaptic vesicles typically contain between 1,000 and 5,000 molecules of transmitter, and about one such vesicle is released when an action potential invades a synaptic ending. A typical synaptic cleft has an area of ~1 μm^2, and a diameter of ~50 nm. If 1,000 molecules are released into this, they will achieve a concentration of ~0.3 mM, consistent with measures of quantal acetylcholine release at the neuromuscular junction, and amply enough to activate the low affinity receptors at which conventional neurotransmitters act [32]. However, the vesicles in which peptides are packaged carry a much larger cargo. They typically contain not only the active peptide, but the entire peptide precursor. In the case of oxytocin and vasopressin, the precursors have a molecular weight of about 23,000; each vesicle contains about 85,000 of these molecules at a

density so great that the contents are in crystalloid form [33, 34], which gives these vesicles their dense-cored appearance under the electron microscope. The release of just one of these vesicles into a synaptic cleft, if confined there, would yield a peptide concentration in the high molar range. As the receptors through which peptides act have affinities in the *nanomolar* range, such concentrations would not merely be massively in excess for any specific receptors present, but would also act extensively at other peptide receptors present there.

Second, is the misperception that peptide release is tightly governed by electrical activity. In the rat, about 9,000 vasopressin cells project to the posterior pituitary. Each of their axons there contains about 2,000 release sites (nerve terminals and swellings), each typically containing a few hundred vesicles – about 15 billion vesicles in all, with a total content of about 2 μg of vasopressin [35]. Ludwig and Leng estimated how often these vesicles must be secreted to achieve a basal plasma concentration of vasopressin (about 1 pg/ml) given a half-life of 2 min and a volume of distribution of vasopressin of 60 ml (i.e. the plasma volume and total extracellular fluid volume). These imply that about 2,500 vesicles/s are secreted in basal conditions [36]. This calculation was based on deliberately conservative assumptions and is likely to be an overestimate; the pituitary store would be sufficient to maintain this level of secretion for only about 6 days without replenishment, and for only a few hours in conditions of sustained demand, when the plasma vasopressin concentration is ten-fold higher. The actual distribution volume as inferred experimentally is about 20 ml in a rat, the plasma half-life is by many estimates, longer than 2 min, and to infer the rate of clearance from this requires modeling the exchange between plasma and extravascular fluid, as vasopressin is cleared from the plasma compartment alone by passage through the kidneys and liver [37]. More realistic assumptions imply a basal secretion rate of closer to 800 vesicles/s, or about one vesicle every 10 s from each cell. After two days of dehydration,

198 when vasopressin concentrations in the plasma are ten-fold higher, vasopressin cells fire
199 action potentials (spikes) in long bursts at 6 to 8 spikes/s separated by silences, and each cell
200 is secreting about 1-2 vesicles/s. In these conditions, at any single release site in the axonal
201 endings of any one vasopressin cell, one vesicle is secreted, on average, for every 5,000
202 spikes or so – about once every 15 min [22]. This secretion is dependent on spike activity, but
203 the very low probability of release at any given site implies that release is a highly stochastic
204 process.

205 Third is the misconception that peptide release in the brain is governed only by
206 electrical activity. Most of the vesicles that oxytocin cells hold within the brain are located in
207 their long and voluminous dendrites. These are not normally releasable by electrical activity,
208 but are constrained by an intracellular scaffold of filamentous actin to be far from the
209 voltage-gated channels which are activated by the spikes that these dendrites conduct.
210 However, some peptides trigger oxytocin release from these dendrites by mobilising
211 intracellular Ca^{2+} stores from the rough endoplasmic reticulum that permeates the dendrites
212 [31, 38, 39]– and some, like α-MSH, stimulate dendritic release even while inhibiting spiking
213 activity [40]. This is not to say that spike activity never releases dendritic oxytocin – some
214 peptide signals can trigger a reorganisation of the filamentous actin to deliver vesicles close
215 to the plasma membrane where they can be released in response to voltage-gated Ca^{2+} entry
216 [41]. This mechanism –"priming" – underlies a change in the functional connectivity between
217 oxytocin neurones, through autocrine and paracrine actions that bind the functional activity of
218 oxytocin cells together. In lactation, this supports their ability to generate synchronous bursts
219 in response to suckling, leading to the pulsatile secretion that is essential for the milk-ejection
220 reflex [42].

221 Fourth is the misconception that communication between neurones requires physical
222 proximity between them. There is often a striking mismatch between the density of receptors

in any given brain region and the density of peptide-containing fibres in that region [43, 44]. Much is often made of the sparse peptide-containing fibres that occasionally wend their way through regions of abundant receptor expression, and these may deliver a functionally important peptide signal, as in the case of oxytocin in the amygdala [45]. But even the CSF contains oxytocin concentrations that, if present in peripheral blood, would be sufficient to activate peripheral target organs. Some brain regions distant from the sites of oxytocin synthesis contain dense plexuses of oxytocin-containing fibres, and oxytocin release from these axonal varicosities will have an important 'local' action [46] – local to the region, rather than to directly adjacent neurones. Even then we should be cautious, for every peptide-containing neurone also makes a conventional neurotransmitter, and these may often be the primary messenger of such fibres; oxytocin neurones express the vesicle glutamate transporter VGLUT2 [47], and glutamate thus appears to be a neurotransmitter used at their central synaptic projections [45, 48].

Oxytocin and vasopressin might be exceptional in the size of their vesicles. Many dense-cored vesicles in the CNS are smaller than these, with a volume only about 1/8 that of the typical oxytocin or vasopressin-containing vesicles and a correspondingly lower expected content. Thus van den Pol [49] favours a *local diffusion* hypothesis, that, given the low frequency of dense core vesicles in most CNS axons and because of the hours needed to replenish released peptides by synthesis and transport from the cell body, neuropeptides released by most neurons must act relatively locally on cells near the release site. However, any one neurone has a great many potential release sites, including all its axonal varicosities, each with a very low probability of release, thus the potential targets of peptide release even from a single peptidergic neurone will be very widely scattered and widely distributed neurones will be exposed to secreted peptide in a sparse and highly stochastic fashion. When a brain region is permeated by many axons from a population of peptide-producing cells, it

seems likely that such a projection will deliver a hormone-like signal to that region. How far such a signal will reach is hard to predict.

A striking demonstration of remote actions of neuropeptides comes from studies of the suprachiasmatic nucleus. Lesions of this nucleus in hamsters disrupts the circadian rhythms of behaviour that persist in constant darkness. In lesioned hamsters, circadian rhythmicity can be restored by transplanting fragments of neonatal suprachiasmatic nucleus into the third ventricle – and, remarkably, can do so even if those fragments are encapsulated in a membrane that allows substances to diffuse freely across the membrane but which permits no penetration of nerve fibres from the transplant to the host tissue [50].

Fifth, to be qualified rather than dismissed, is the notion that neuropeptides are neuromodulators, in the sense that they affect neuronal excitability, thereby altering the responses of neurones to neurotransmitters. Many neuropeptides affect gene expression in their targets: canonical examples include the effects of gonadotrophin releasing hormone on pituitary expression of gonatotropins [51, 52], the effects of thyrotropin releasing hormone on thyroid stimulating hormone expression [53], and the effects of growth-hormone releasing hormone on growth hormone expression [54]. Certainly many peptides do influence neuronal excitability, but as mentioned, some can alter functional connectivity by priming peptide release from dendrites.

For example, the splanchnic nerve terminals that innervate the adrenal medulla release PACAP (pituitary adenylate cyclase-activating polypeptide) and acetylcholine. Both regulate catecholamine release from chromaffin cells, but PACAP is released only at high frequencies, using secretory mechanisms different from those evoked by acetylcholine. During prolonged stress, PACAP maintains catecholamine synthesis via induction of tyrosine hydroxylase and PNMT (phenylethanolamine N-methyltransferase), and it enhances the transcription of other

secreted molecules found in chromaffin cells. In the words of Smith and Eiden: "*PACAP thus mediates chromaffin cell plasticity via a functional encoding of experience*"[55].

Some neuropeptides regulate local blood flow [56], some, like oxytocin may regulate glial cell morphology [57], and leptin [58, 59] and CRH [60] have been proposed to modulate synaptogenesis. The predominant attention given to electrophysiological actions of neuropeptides reflects the relative ease with which these can be determined by *in vitro* electrophysiology, and the assumption of many neuroscientists that neuropeptides are mere adjuncts to the serious business of information transfer that is conducted by neurotransmitters.

Finally, again to be qualified or at least questioned, is the notion that the roles of neuropeptides in the brain are exercised purely through activity-dependent regulation of their release. Three points should be made. (i), The level of mRNA expression for peptides in specific neuronal populations varies in different physiological states. (ii), The amount of peptide released by a given stimulus is proportional to the amount available for release, which varies with the rates of synthesis and depletion. (iii), The actions of a neuropeptide depend on the level of expression of specific receptors, which varies in different physiological conditions. These points are considered in turn below.

Regulation of mRNA expression

One of the earliest and most striking examples of this comes from studies of the effects of chronic stress on the parvocellular neurones in the paraventricular nucleus of the hypothalamus that regulate the secretion of ACTH. These neurones normally regulate ACTH secretion via release of corticotrophin releasing hormone (CRH), but after chronic stress their expression of CRH is diminished while that of vasopressin, in the same neurones, is markedly enhanced. Thus the peptidergic phenotype of these neurones is plastic – what were "CRH neurones" become "vasopressin neurones" with marked consequences for the regulation of

297 the stress axis [61]. No less striking is the recent recognition of similar plasticity in the
298 tuberoinfundibular dopamine neurones that regulate prolactin secretion. In lactation, these
299 cease to release dopamine but instead release a peptide – leu enkephalin, and this change
300 supports the stimulation of prolactin secretion in lactation [62].

301 ***Regulation of receptor expression***

302 The role of a peptide messenger may be exercised through activity-dependent
303 regulation of its release, or by regulating its synthesis, or its availability for release, or the
304 sensitivity of its targets. One classic example of the last of these is the importance of changes
305 in oxytocin receptor expression in the uterus for parturition. In all mammals, oxytocin
306 secretion is increased during parturition, and this acts on a uterus prepared by a massive
307 increase in oxytocin receptor expression [63, 64]. In rats, what is secreted from pituitary is
308 conveyed to its targets in a plasma volume of about 7.5 ml; in humans the pituitary is about
309 40 times larger than that of a rat, and the oxytocin content is proportionately greater.
310 However, what is secreted in a human is conveyed in a plasma volume of 3-4 l, at least 400
311 times larger than in a rat, and the half-life of oxytocin in rat and human are similar.
312 Accordingly circulating concentrations of oxytocin are much lower in man than in small
313 mammals [65], and the importance of the level of receptor expression in target tissues is
314 correspondingly greater. Indeed, the increase in the sensitivity of the human uterus to
315 oxytocin at term pregnancy is so great that it has been questioned whether any increase in the
316 level of oxytocin at all is involved in human parturition before the third stage of labor [64].

317 Among the best-known behavioural functions of peptides are those of vasopressin and
318 oxytocin on various facets of "social" behaviour. A feature of these is how great are the
319 species differences in these behaviours; the paradigmatic exemplars are the prairie voles, that
320 make enduring partner bonds after mating and display biparental nurturing behaviours, and
321 closely related species such as meadow voles which are promiscuous and asocial. These

behaviours critically depend on the release within the brain of oxytocin and vasopressin, but the species differences do not apparently reflect any differences in the regulation of this release, but on differences in the sites and extent of receptor expression in the brain [66, 67].

Stimulus-secretion coupling

The amount of peptide secreted from neurones in response to electrical depolarisation depends on the pattern of stimulation [37, 55, 68, 69], on the number of vesicles available for release [70], and their precise location [24]. The nerve endings of the axons that fill the posterior pituitary contain a "readily-releasable pool" of vesicles that is refilled from reserve stores as it is depleted, and the cycle of local depletion and repletion results in complex non-linearities in stimulus-secretion coupling [37, 69]. After a period of water deprivation, the gland content is severely depleted, and in these conditions electrical stimulation of the gland releases oxytocin and vasopressin in direct proportion to the gland content [70].

When a synaptic vesicle containing a conventional neurotransmitter releases its contents, there is an abundant stock of vesicles available to re-supply the releasable pool, and re-uptake mechanisms recover neurotransmitter from the extracellular fluid to refill the empty vesicle and make it available for rapid re-use. By contrast, large-dense cored vesicles cannot be re-used, they must be replaced by newly synthesised vesicles. Thus any acute activation of peptide secretion entails a cycle of depletion and repletion. Any marked increase in the rate of secretion must be compensated for by an increase in the rate of peptide synthesis and vesicle production, and the new vesicles must be transported from the cell body to the release sites – a process that can take several hours. This phenomenon will impose a temporal pattern on peptide secretion from neurones even if the signal for that secretion is unchanging.

But the availability of peptide stores is not the only factor that determines how much is secreted in response to a stimulus. As mentioned, priming of peptide stores can alter stimulus-secretion coupling in dendrites. At nerve terminals, other factors can do so.

Oxytocin neurones, for example, co-express dynorphin, which acts on kappa-opioid receptors at their nerve terminals as an inhibitory feedback regulator of stimulus-secretion coupling. In pregnancy, the expression of dynorphin is upregulated, and the enhanced negative feedback contributes to a progressive accumulation of oxytocin stores in the pituitary in preparation for parturition – the gland content increases by about a third without any apparent increase in the level of oxytocin mRNA expression [63].

In β-pancreatic cells, insulin secretion in response to glucose is elicited by an increase in intracellular [Ca^{2+}]. This "triggering" pathway depends on the suppression of K_{ATP} channels in the plasma membrane [71]. But after the first phase of insulin secretion, a metabolic amplifying pathway is engaged which depends on the initial triggering signal but is independent of K_{ATP} channels and involves cAMP signalling. This pathway enhances the sensitivity of the insulin-containing secretory vesicles to a given Ca^{2+} influx, and it can be engaged by peptide signals such as GLP-1 and GIP from gastrointestinal endocrine cells [72].

Three modes of action of neuropeptides

When considering the actions on neurones of neuropeptides released from neurones in the brain, we can recognise three common modes of action. Neuropeptides act as autoregulators of neuronal activity, as paracrine regulators of aggregated populations of neurones, and as neurohormonal regulators of distant populations of neurones.

Autocrine regulation

Commonly, neurones express autoreceptors for the peptides that they release. As discussed above, in the case of oxytocin cells, activity-dependent release of dynorphin, a peptide co-packaged with oxytocin in neurosecretory vesicles but in very much lower abundance, is a negative feedback regulator of secretion from nerve terminals in the pituitary. Magnocellular vasopressin cells also express dynorphin, co-packaged in vasopressin-

containing vesicles. For these cells, dynorphin is again an autoregulator, but in this case of electrical activity – in vasopressin cells, sparse, activity-dependent release of dynorphin from the soma and dendrites has a critical role in sculpting the phasic pattern of electrical activity [73].

Paracrine regulation

Oxytocin cells also express oxytocin receptors and vasopressin cells also express vasopressin receptors, but in both cases their functional activity is quite elusive; because the receptors are internalised after ligand binding, and because there is a high concentration of these peptides in the extracellular space around the magnocellular cells, at any given time there are normally few free receptors available for binding on the cell surface. For oxytocin cells therefore these receptors are functionally effective only when very large amounts of oxytocin are released. During lactation, dendritic oxytocin release in response to suckling binds the population of oxytocin cells together, supporting their ability to generate synchronous bursts of activity [42]. This can be considered as an example of positive feedback, but *negative* feedbacks can also bind a population together. In magnocellular vasopressin cells, dendritic vasopressin release is an inhibitor of neuronal activity – it acts as a "population signal" allowing each cell to be aware of the level of activity amongst the whole population, and this feedback serves to equalise the average level of activity in the population, spreading the load of activity equitably [22, 74].

Neurohormonal actions

Neuropeptides in the brain are not generally scoured from the extracellular space by uptake mechanisms, and enzymatic degradation is relatively slow. They travel within the brain not by diffusion, but by the continuous flow of extracellular fluid, ending up in the CSF from which they are ultimately cleared. How much reaches the CSF varies considerably. Oxytocin and vasopressin are degraded within brain tissue by specific aminopeptidases,

notably the membrane-bound enzyme PLAP [75]. Oxytocin and vasopressin are released in equimolar amounts with their respective neurophysins, which are large fragments of their precursor molecules, and the neurophysins are not enzymatically degraded within the brain. By comparing the concentrations of neurophysins in CSF with those of oxytocin and vasopressin, and given the rate of clearance from CSF, we can deduce that only about 5% of the oxytocin and vasopressin that is released within the brain actually reaches the CSF [36]. Yet their concentrations in CSF are still about ten-fold higher than the basal concentrations in plasma, and at levels that, when present in plasma, are amply sufficient to exert physiological effects. Peptide concentrations must vary considerably in different brain regions, as the result of differential degradation, the inhomogeneous flow of extracellular fluid and the variations in levels of local release. Neurohormonal signalling in brain is not homogeneous and indiscriminate. Nevertheless, such signaling reflects not a rapid and specific system of communication from neurone to neurone, but a prolonged communication between one population of cells and another – the difference between a 'whispered secret' and a 'public announcement' [36].

The potential impact of such neurohormonal signals might be glimpsed from studies in simple organisms such as *Drosophila* [76] and *C.elegans* [77]. It seems that the connectome – the wiring diagram of connectivity amongst the 302 neurones of *C.elegans* - allows multiple potential behaviours for any given neuronal network. Which of these behaviours is expressed in a given circuit at a particular time depends on what Cornelia Bargmann called "*the dark energy of the nervous system."* The *C. elegans* genome encodes over 200 peptides, and these, she argues, along with biogenic amines such as serotonin and dopamine, sculpt the functional connectivity between neurones – defining which of the set of latent circuits in a neuronal network is engaged at a given time [77].

Reflections

The original schism between endocrinology and neuroscience was bolstered by the apparently separate embryological origins of neurones and endocrine cells. However if we look to contemporary understanding of the molecular determinants of cell fate and to comparative genomics, we see a different story [78, 79]. In *Urbilateria*, the marine organism proposed to be the last common ancestor of vertebrates, flies, and worms, cells that secreted a peptide ancestor of vasopressin and oxytocin combined properties that we have thought of as separate properties of endocrine cells and neurones. They used diverse signaling mechanisms, made both neurotransmitters and peptides, and had a wide range of specialized senses, linking feeding, reproduction and internal homeostasis to environmental conditions [80].

Given the many commonalities between, for example, β-pancreatic cells or any of the pituitary cell types and neurones, it seems clear that if these cells were embedded in the brain we would not hesitate to call them neurones. Yet although information flows in both directions between the brain and endocrine glands, we still cleave to a hierarchical view in which the brain, and the higher centres of the brain in particular, are credited with particular cognitive agency, as though neurones were clever in ways that endocrine cells are not.

We can extend this argument to encompass all endocrine cells in the body. For example, the adipocytes that store our fat not only sense the environment in which they reside, they communicate bidirectionally with other endocrine cells in the pancreas [81, 82] and elsewhere, express intrinsic circadian rhythmicity[83], and are innervated by neurones [84, 85]. Through the actions of their product leptin on the brain, they not only regulate appetite by their effects on diverse populations of peptidergic neurones [86, 87], but also modulate energy expenditure [88] and food reward [89, 90]. As I have argued elsewhere, "*from the perspective of an adipocyte […] the brain is just something that follows its instructions to keep it supplied with lipid."*[22].

There are two distinct ways to conceive of intelligence. We can conceive it as the ability of a cell to sense both the external environment and its internal state combined with the ability to respond adaptively to changes in the external environment. This would be to follow the sense in which Barbara McClintock, in her Nobel Prize lecture wrote *"a goal for the future would be to determine the extent of knowledge the cell has of itself and how it uses that knowledge in a "thoughtful" manner when challenged*" [91]. Or we can conceive it as an *emergent* phenomenon, a property specific to highly complex multicellular systems that embraces the abilities to learn from past experience, to anticipate future challenges, and to select from a range of possible strategies one that will appropriately meet those challenges. Intelligence in the latter sense is embodied not in a discrete location but in the whole complex network. In neither sense can we accord neurones greater cognitive capacity than, say, adipocytes.

Hierarchical metaphors of the organisation of brain and body have run their course. It is time to abandon them, and abandon too the conceit that will understand the brain through studies of neuronal connectivity and electrical activity alone. New technological advances, such as optogenetics and chemogenetics, have given unprecedented opportunities for understanding the role of electrical activity in information processing in the brain, but we are desperately in need of comparable advances in studying the functional regulation of neuropeptide release in the brain and its behavioral and physiological consequences.

It is time for endocrinologists to claim the brain as one of their own, and take up the challenge of understanding the hormones of the brain. At present, we have the technical ability to measure only oxytocin and vasopressin release by radioimmunoassay in brain areas in a functional context, and only with a relatively poor spatial and temporal resolution. Nanoflow liquid chromatography-mass spectrometry offers a potentially powerful alternative to immunoassay for peptide detection because of its high sensitivity and specificity, and a recent paper has used this to measure opioid peptide release in discrete brain areas using microdialysis in fractions collected at 15-min intervals [92]. This is clearly a step forward, but temporal resolution remains a challenge. Important advances have recently been made in the ability to measure the release of some neurotransmitters with high spatial and temporal resolution through the use of genetically encoded fluorescent sensors [93, 94]. It seems

possible that similar approaches may yet provide the ability to measure neuropeptide release with similar precision, but there are considerable technical barriers [95].

While chemogenetic approaches directly target G-protein coupled receptors, optogenetic approaches have mainly been used to activate or inhibit neurones through regulation of ion channels (e.g. [45, 96]). However, optogenetic tools have also been developed to target intracellular signaling cascades [97]. Thus, both optogenetic and chemogenetic approaches should be capable of being adapted to target the non-spike dependent pathways that regulate peptide release – if the problems with measuring this release on an appropriate timescale can be overcome.

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