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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

49 carried by blood vessels to the pituitary [2]. In Harris' theory, this was one of several
50 'releasing factors', each of which regulated a different pituitary hormone.
51 Harris and his co-workers showed that portal blood vessels in the median eminence at
52 the base of the hypothalamus fed into a "vascular plexus" that filled the anterior pituitary, and
53 that the direction of flow in these vessels was from the brain, not to it. He then noted that,
54 from earlier work, transplanting the anterior pituitary gland to a different part of the body led
55 to an irreversible loss of function and atrophy of the target tissues on which pituitary
56 hormones were known to act. But if the anterior pituitary was removed from its normal place
57 in the sella turcica and then replaced there or in an adjacent site, normal function often
58 returned. Harris hypothesised that, in these cases, the portal vessels had regenerated,
59 renewing the vascular communication between brain and pituitary [2].
60 To test this, Harris and Jacobsohn [3] removed the pituitary from female rats. They
61 then grafted pituitary tissue from new-born young into the subarachnoid space below the
62 brain, either immediately below the cut portal vessels, or to one side below the temporal lobe.
63 Ovarian cycles returned in all rats with transplants below the cut portal vessels, but when the
64 transplants were below the temporal lobe, the ovaries and reproductive tracts atrophied and
65 ovarian cycles ceased. In both cases, the transplants were re-vascularised – in the first case by
66 portal vessels, in the second case by blood vessels of a different part of the brain.
67 But the path to acceptance of Harris' theory was not yet clear. His theory blurred the
68 conventionally accepted distinction between neurones and endocrine cells, and it postulated
69 the existence of "releasing factors" whose identification seemed beyond experimental reach.
70 It engaged the opposition of Sir Solly Zuckerman [4]. Zuckerman, as described by Lord
71 Dainton, "*…was unique. No scientist this century can match him in the timespan and weight*
72 *of his influence on governments in peace or war*" [5].

73 Zuckerman, who had founded his career on studies of the menstrual cycle in primates,
74 recognised that Harris' theory would be disproved if *any* animal could be shown to have
75 ovulated in the absence of portal vessels. In sixteen female ferrets, Thomson and Zuckerman
76 [6] cut the neural stalk, and their case rested on results from two of them, two that had come
77 into heat in response to artificial light even though, from their histological evidence, all
78 connections between brain and pituitary had been eliminated.

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

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

93 Zuckerman did not concede, but was still maintaining his position in 1978
94 (Zuckerman 1978), a year after Schally and Guillemin had been awarded the Nobel Prize for
95 their identification of some of the releasing factors that Harris had postulated. To understand
96 Zuckerman's resistance, we might recognise the threat that Harris' theory posed to the
97 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

148 neuropeptides in the brain are neurotransmitters "is to a first approximation, a lie", at least if
149 we retain *anything* of the criteria for a neurotransmitter given above.
150 First to be dismissed must be the misperception that neuropeptides are released at
151 synapses within the brain. Many neurones produce both peptides and one or more
152 neurotransmitters, and both are packaged in vesicles, but not in the same vesicles [23].
153 Conventional neurotransmitters are packaged in small synaptic vesicles that are specifically
154 localised to synapses. Peptides are packaged in large dense-cored vesicles that are typically
155 distributed throughout the cytoplasm of a neurone, and since for most neurones the dendrites
156 comprise about 85% of the cell volume, it is in this compartment that large dense-cored
157 vesicles are often mainly found. Synaptic vesicles can only be released at specialised sites in
158 the presynaptic membrane, but large dense-cored vesicles can apparently be released from the
159 soma, dendrites, axonal varicosities and even undilated axons – the main requirement appears
160 to be that they must be close to the plasma membrane to be releasable [24-31].
161 Some large dense-cored vesicles *are* present in synapses, though not in particular
162 abundance, and generally not close to the synaptic release site, and whether any are ever
163 released into the synaptic cleft is questionable. Synaptic vesicles typically contain between
164 1,000 and 5,000 molecules of transmitter, and about one such vesicle is released when an
165 action potential invades a synaptic ending. A typical synaptic cleft has an area of ~1 μm^2, and
166 a diameter of ~50 nm. If 1,000 molecules are released into this, they will achieve a
167 concentration of ~0.3 mM, consistent with measures of quantal acetylcholine release at the
168 neuromuscular junction, and amply enough to activate the low affinity receptors at which
169 conventional neurotransmitters act [32]. However, the vesicles in which peptides are
170 packaged carry a much larger cargo. They typically contain not only the active peptide, but
171 the entire peptide precursor. In the case of oxytocin and vasopressin, the precursors have a
172 molecular weight of about 23,000; each vesicle contains about 85,000 of these molecules at a

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

180 Second, is the misperception that peptide release is tightly governed by electrical
181 activity. In the rat, about 9,000 vasopressin cells project to the posterior pituitary. Each of
182 their axons there contains about 2,000 release sites (nerve terminals and swellings), each
183 typically containing a few hundred vesicles – about 15 billion vesicles in all, with a total
184 content of about 2 μg of vasopressin [35]. Ludwig and Leng estimated how often these
185 vesicles must be secreted to achieve a basal plasma concentration of vasopressin (about 1
186 pg/ml) given a half-life of 2 min and a volume of distribution of vasopressin of 60 ml (i.e. the
187 plasma volume and total extracellular fluid volume). These imply that about 2,500 vesicles/s
188 are secreted in basal conditions [36]. This calculation was based on deliberately conservative
189 assumptions and is likely to be an overestimate; the pituitary store would be sufficient to
190 maintain this level of secretion for only about 6 days without replenishment, and for only a
191 few hours in conditions of sustained demand, when the plasma vasopressin concentration is
192 ten-fold higher. The actual distribution volume as inferred experimentally is about 20 ml in a
193 rat, the plasma half-life is by many estimates, longer than 2 min, and to infer the rate of
194 clearance from this requires modeling the exchange between plasma and extravascular fluid,
195 as vasopressin is cleared from the plasma compartment alone by passage through the kidneys
196 and liver [37]. More realistic assumptions imply a basal secretion rate of closer to 800
197 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

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

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

248 seems likely that such a projection will deliver a hormone-like signal to that region. How far
249 such a signal will reach is hard to predict.
250 A striking demonstration of remote actions of neuropeptides comes from studies of
251 the suprachiasmatic nucleus. Lesions of this nucleus in hamsters disrupts the circadian
252 rhythms of behaviour that persist in constant darkness. In lesioned hamsters, circadian
253 rhythmicity can be restored by transplanting fragments of neonatal suprachiasmatic nucleus
254 into the third ventricle – and, remarkably, can do so even if those fragments are encapsulated
255 in a membrane that allows substances to diffuse freely across the membrane but which
256 permits no penetration of nerve fibres from the transplant to the host tissue [50].
257 Fifth, to be qualified rather than dismissed, is the notion that neuropeptides are
258 neuromodulators, in the sense that they affect neuronal excitability, thereby altering the
259 responses of neurones to neurotransmitters. Many neuropeptides affect gene expression in
260 their targets: canonical examples include the effects of gonadotrophin releasing hormone on
261 pituitary expression of gonatotropins [51, 52], the effects of thyrotropin releasing hormone on
262 thyroid stimulating hormone expression [53], and the effects of growth-hormone releasing
263 hormone on growth hormone expression [54]. Certainly many peptides do influence neuronal
264 excitability, but as mentioned, some can alter functional connectivity by priming peptide
265 release from dendrites.
266 For example, the splanchnic nerve terminals that innervate the adrenal medulla release
267 PACAP (pituitary adenylate cyclase-activating polypeptide) and acetylcholine. Both regulate
268 catecholamine release from chromaffin cells, but PACAP is released only at high frequencies,
269 using secretory mechanisms different from those evoked by acetylcholine. During prolonged
270 stress, PACAP maintains catecholamine synthesis via induction of tyrosine hydroxylase and
271 PNMT (phenylethanolamine N-methyltransferase), and it enhances the transcription of other

272 secreted molecules found in chromaffin cells. In the words of Smith and Eiden: "*PACAP thus*
273 *mediates chromaffin cell plasticity via a functional encoding of experience*"[55].
274 Some neuropeptides regulate local blood flow [56], some, like oxytocin may regulate
275 glial cell morphology [57], and leptin [58, 59] and CRH [60] have been proposed to modulate
276 synaptogenesis. The predominant attention given to electrophysiological actions of
277 neuropeptides reflects the relative ease with which these can be determined by *in vitro*
278 electrophysiology, and the assumption of many neuroscientists that neuropeptides are mere
279 adjuncts to the serious business of information transfer that is conducted by
280 neurotransmitters.
281 Finally, again to be qualified or at least questioned, is the notion that the roles of
282 neuropeptides in the brain are exercised purely through activity-dependent regulation of their
283 release. Three points should be made. (i), The level of mRNA expression for peptides in
284 specific neuronal populations varies in different physiological states. (ii), The amount of
285 peptide released by a given stimulus is proportional to the amount available for release,
286 which varies with the rates of synthesis and depletion. (iii), The actions of a neuropeptide
287 depend on the level of expression of specific receptors, which varies in different
288 physiological conditions. These points are considered in turn below.
289 ***Regulation of mRNA expression***
290 One of the earliest and most striking examples of this comes from studies of the
291 effects of chronic stress on the parvocellular neurones in the paraventricular nucleus of the
292 hypothalamus that regulate the secretion of ACTH. These neurones normally regulate ACTH
293 secretion via release of corticotrophin releasing hormone (CRH), but after chronic stress their
294 expression of CRH is diminished while that of vasopressin, in the same neurones, is markedly
295 enhanced. Thus the peptidergic phenotype of these neurones is plastic – what were "CRH
296 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

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

325 ***Stimulus-secretion coupling***

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

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

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

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

353 In β-pancreatic cells, insulin secretion in response to glucose is elicited by an increase
354 in intracellular [Ca^{2+}]. This "triggering" pathway depends on the suppression of K_{ATP}
355 channels in the plasma membrane [71]. But after the first phase of insulin secretion, a
356 metabolic amplifying pathway is engaged which depends on the initial triggering signal but is
357 independent of K_{ATP} channels and involves cAMP signalling. This pathway enhances the
358 sensitivity of the insulin-containing secretory vesicles to a given Ca^{2+} influx, and it can be
359 engaged by peptide signals such as GLP-1 and GIP from gastrointestinal endocrine cells [72].
360

361 **Three modes of action of neuropeptides**

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

366 *Autocrine regulation*

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

372 containing vesicles. For these cells, dynorphin is again an autoregulator, but in this case of
373 electrical activity – in vasopressin cells, sparse, activity-dependent release of dynorphin from
374 the soma and dendrites has a critical role in sculpting the phasic pattern of electrical activity
375 [73].

376 *Paracrine regulation*

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

391 *Neurohormonal actions*

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

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

412 The potential impact of such neurohormonal signals might be glimpsed from studies
413 in simple organisms such as *Drosophila* [76] and *C.elegans* [77]. It seems that the
414 connectome – the wiring diagram of connectivity amongst the 302 neurones of *C.elegans* -
415 allows multiple potential behaviours for any given neuronal network. Which of these
416 behaviours is expressed in a given circuit at a particular time depends on what Cornelia
417 Bargmann called "*the dark energy of the nervous system."* The *C. elegans* genome encodes
418 over 200 peptides, and these, she argues, along with biogenic amines such as serotonin and
419 dopamine, sculpt the functional connectivity between neurones – defining which of the set of
420 latent circuits in a neuronal network is engaged at a given time [77].
421

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].

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

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

466 It is time for endocrinologists to claim the brain as one of their own, and take up the
467 challenge of understanding the hormones of the brain. At present, we have the technical
468 ability to measure only oxytocin and vasopressin release by radioimmunoassay in brain areas
469 in a functional context, and only with a relatively poor spatial and temporal resolution.
470 Nanoflow liquid chromatography-mass spectrometry offers a potentially powerful alternative
471 to immunoassay for peptide detection because of its high sensitivity and specificity, and a
472 recent paper has used this to measure opioid peptide release in discrete brain areas using
473 microdialysis in fractions collected at 15-min intervals [92]. This is clearly a step forward,
474 but temporal resolution remains a challenge. Important advances have recently been made in
475 the ability to measure the release of some neurotransmitters with high spatial and temporal
476 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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